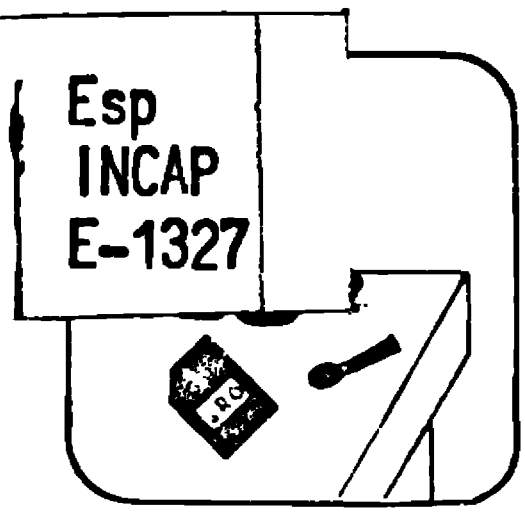
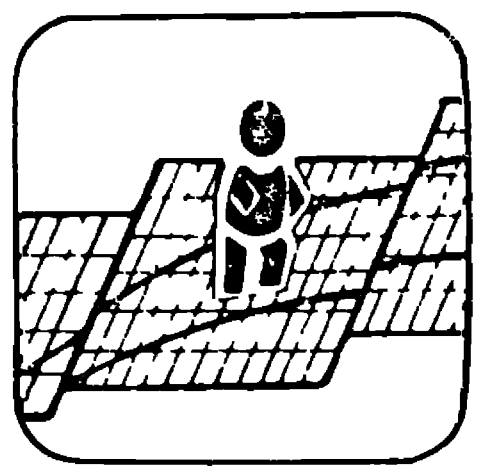
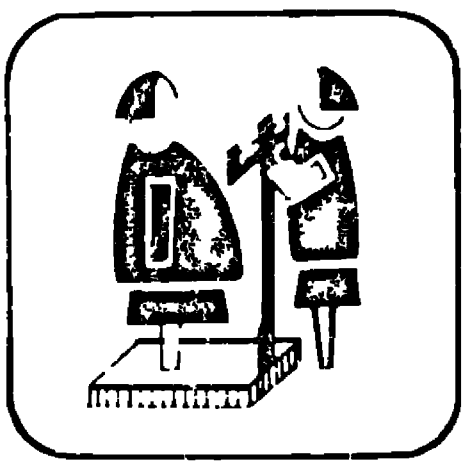
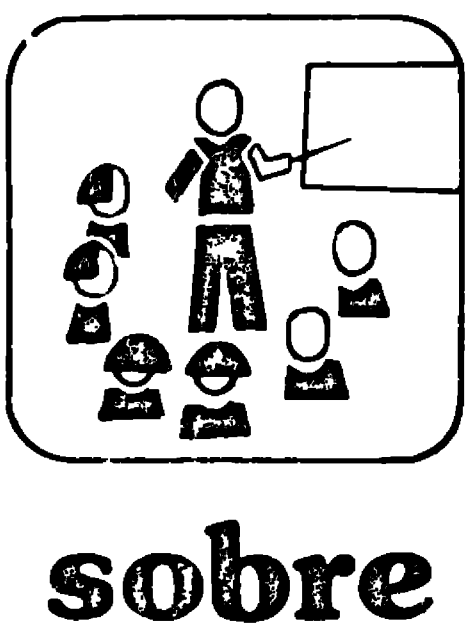








# Centro Regional de Documentación sobre Supervivencia Infantil

BIBLIOGRAFIA ESPECIALIZADA No. 3

PUBLICACIONES INCAP RELACIONADAS CON DIAGNOSTICOS E INDICADORES DE DESNUTRICION

Diciembre, 1989

Instituto de Nutrición de Centro América y Panamá
Apartado Postal 1188 [] Guatemala, Guatemala, C.A.

La reproducción de estos documentos ha sido financiada por la Agencia Internacional para el Desarrollo (Donación ROCAP Nos. 596-0104 y 596-0115) de los Estados Unidos.

# Centro Regional de Documentación sobre Supervivencia Infantil

Esta bibliografía especializada compila las publicaciones sobre diagnóstico e indicadores de desnutrición en general, realizadas por profesionales del INCAP de 1966 a 1987.

Los artículos se han clasificado en orden correlativo y por tipo de publicación: E = publicaciones en español, I = publicaciones en inglés y C = trabajos colaborativos.

Esta bibliografía especializada complementa a otras cuatro bibliografías especializadas de publicaciones INCAP relacionadas con desnutrición:

No. 2 Desnutrición proteínico-energética

No. 4 Nutrición e infección

No. 5 Tratamiento de la desnutrición proteínico-energética

No. 6 Desnutrición, planificación y desarrollo

El propósito de esta serie de cinco documentos es proporcionar información básica a docentes, investigadores y planificadores sobre las investigaciones realizadas por el INCAP en el campo de la desnutrición.

Para sugerencias, solicitudes o comentarios, favor dirigirse a:

Unidad de Documentación e Información
INCAP
Apartado Postal 1188
01901 Guatemala, C.A.

# BIBLIOGRAFIA ESPECIALIZADA No. 3

## PUBLICACIONES INCAP RELACIONADAS CON DIAGNOSTICO E INDICADORES DE DESNUTRICION

| Serie | Referencia | Separador |
|---|---|---|
| E-455 | Alvarado, J.; et al. "Desnutrición proteínico-calórica: El uso de la biopsia muscular percutánea en la valoración de la recuperación nutricional". Rev. Col. Med.(Guatemala), 21:100-110. 1970. | 1 |
| E-660 | Viteri, F.E.; et al. "Métodos de evaluación del estado nutricional proteínico-calórico en pre-escolares de condiciones socio-económicas diferentes". Arch. Latinoamer. Nutr. 23: 13-31. 1973. | 2 |
| E-1018 | Valverde, V.; et al. "Clasificación funcional de poblaciones desnutridas en la República de El Salvador". Alim. Nutr. 4(3/4):8-14. 1978. | 3 |
| I-421 | Burk, R.F.; et al. "Blood-selenium levels and in vitro red blood cell uptake of Se in kwashiorkor". Am. J. Clin. Nutr. 20(7): 723-733. 1967. | 4 |
| I-527 | Alvarado, Jorge y David G. Luthringer. "Serum immunoglobulins in edematous protein-calorie malnourished children". Clin. Pediatr. 10(3): 174-179. 1971. | 5 |
| I-547 | Alleyne, G.A.; et al. "Indices of body composition in infantile malnutrition: Total body potassium and urinary creatinine". Am. J. Clin. Nutr. 23(7):875-878. 1970. | 6 |
| I-606 | Béhar, M. "The importance of accurate measures of malnutrition". In: Nutrition, National Development and Planning. Cambridge, The MIT Press, 145-149. 1973. | 7 |

| Serie | Referencia | Separador |
|---|---|---|
| I-662 | Guzmán, M. A. "Bone maturation in children recovered from malnutrition". In: Nutrition. Proc. 9th Int. Congr. Nutr. (México) 2:276-279. 1975. | 8 |
| I-698 | Contreras, C. and F. Viteri. "Sialic acid content of red blood cells from protein-calorie malnourished children and during recovery, and from normal children and adults". Can. J. Physiol. Pharmacol. 51:853-858, 1973. | 9 |
| I-780 | Martorell, R.; et al. "The head circumference/chest circumference ratio in mild-to-moderate protein-calorie malnutrition". Env. Child Health. 203-207. 1975. | 10 |
| I-822 | Himes, J. H.; et al. "Patterns of cortical bone growth in moderately malnourished preschool children". Hum. Biol. 47(3):337-350. 1975. | 11 |
| I-923 | Martorell, R.; et al. "Sibling similarities in number of ossification centers of the hand and wrist in a malnourished population". Hum. Biol. 50(1):73-81. 1978. | 12 |
| I-1063 | Valverde, V.; et al. "Functional classification of undernourished populations in the Republic of El Salvador". Food Nutr. Bull. 4(3-4): 4-14. 1978. | 13 |
| I-1472 | Cruz, J. R.; et al. "Effects of nutritional recuperation on E-Rosetting lymphocytes and in vitro response to thymosin in malnourished children". J. Pediatr. Gastr. Nutr. 6: 387-391. 1987. | 14 |
| I-1284 | Solomons, N. W.; et al. "The effect of dietary lactose on the early recovery from protein-energy malnutrition. I. Clinical and anthropometric indices". Am. J. Clin. Nutr. 40: Sept.591-600. 1984. | 15 |

| Serie | Referencia | Separador |
|---|---|---|
| I-1285 | Torún, B.; et al. "The effect of dietary lactose on the early recovery from protein-energy malnutrition. II. Indices of nutrient absorption". Am. J. Clin. Nutr. 40:Sept. 601-610. 1984 | 16 |
| C-123 | Velzeboer, M.; et. al. "Evaluation of arm circumference as a public health index of protein energy malnutrition in early childhood". J. Trop. Pediatr. 29:135-144. 1983. | 17 |

# DESNUTRICION PROTEINICO-CALORICA: EL USO DE LA BIOPSIA MUSCULAR PERCUTANEA EN LA VALORACION DE LA RECUPERACION NUTRICIONAL*

**Dr. Jorge Alvarado**, Dr. Buford L. Nichols*** Dr. Carlton F. Hazlewood, Dr. Humberto Mansylla**** Dr. Fernando Viteri****

*Instituto de Nutrición de Centro América y Panamá (INCAP), Guatemala, C. A.*

## *I. INTRODUCCION*

En el curso de los últimos años se han llevado a cabo trabajos sobre diversas alteraciones electrolíticas en el niño desnutrido (1-5), así como estudios relacionados con cambios enzimáticos a nivel muscular (6) utilizando la técnica de la biopsia quirúrgica. Este procedimiento presenta inconveniencias y riesgos para el paciente, lo que limita la obtención de muestras musculares en forma seriada. la aguja pediátrica Baylor para biopsias musculares por vía percutánea (7), cuyo uso se introdujo recientemente, ha resuelto estos problemas en gran parte, ya que por ser un procedimiento sencillo, inocuo y poco doloroso, permite el estudio seriado de las alteraciones de este compartimiento en una forma dinámica.

La masa muscular de un niño normal representa aproximadamente el 43% del total de la masa proteínica del organismo. Es, por lo tanto, el tejido que contiene la mayor cantidad de proteína de todo el cuerpo. Aun cuando se sabe que en la desnutrición proteínico-calórica existe una disminución de la masa muscular, éste ha sido un compartimiento relativamente poco estudiado. Desde hace ya algún tiempo se cuenta con estudios descriptivos de los cambios anatomopatológicos (post mortem) que ocurren en el músculo de niños desnutridos (8-10) los cuales subrayan la importancia de relacionar los cambios estructurales con cambios funcionales. Investigaciones efectuadas en Jamaica (11), México (2), y el Congo (4) han señalado que en los niños "desnutridos" existe depleción de potasio muscular. El grupo de investigadores de Jamaica (12, 13) ha informado que existe también depleción del potasio total del cuerpo en base a su concentración por kg.

---

* Este trabajo se llevó a cabo con ayuda financiera de los Institutos Nacionales de Salud, Servicio de Salud Pública de los Estados Unidos de América (Subvención No. AM-0981) y de la Escuela de Medicina, Universidad de Baylor, Houston Texas, E. U. A.

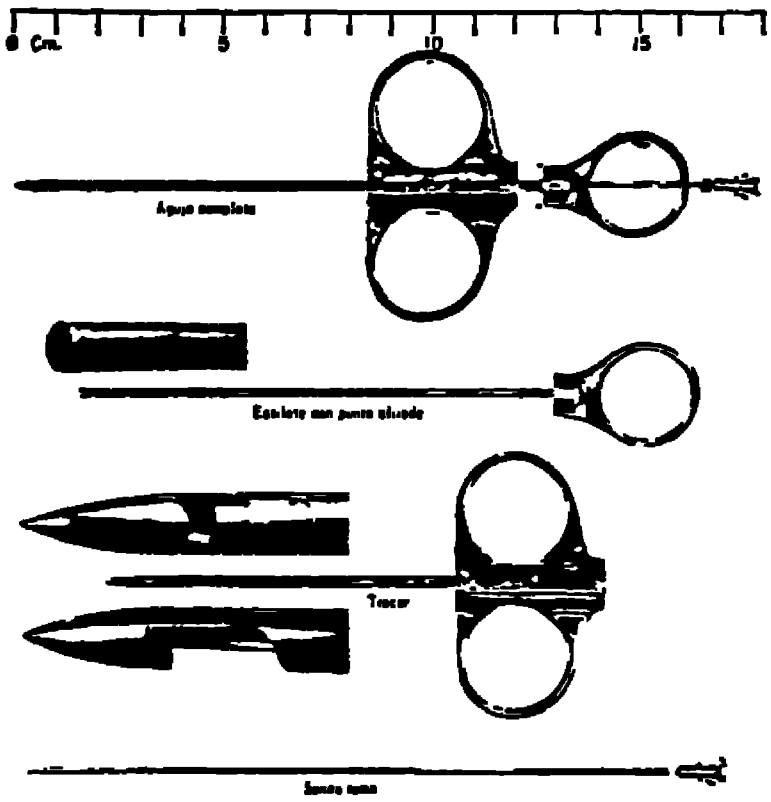
** Jefe Asistente y Jefe, División Biomédica del Instituto de Nutrición de Centro América y Panamá, respectivamente.

*** Profesor Asociado de Pediatría, Universidad de Baylor, Houston, Texas, E. U. A.

**** Cuando se llevó a cabo este trabajo, el Dr. Mansylla servía el cargo de Médico Residente del Centro Clínico de la División Biomédica del INCAP. Publicación INCAP E-455.

de peso corporal. Por otra parte, estudios más recientes utilizando la vía percutánea para obtener la biopsia (7) han demostrado una correlación significativa entre la concentración de potasio muscular y la del potasio total del organismo, por kg de peso corporal, en niños que padecen de desnutrición proteínico-calórica (14). Esta última observación es de particular relevancia, ya que con la obtención de una pequeña muestra pueden obtenerse datos que reflejan la deficiencia de potasio en todo el organismo.

**Figura 1:**




**Aguja pediátrica Baylor para biopsias musculares y sus distintos componentes**

Estudios comparativos entre el análisis de la concentración de electrolitos en biopsias musculares obtenidas tanto por el método quirúrgico como por el procedimiento percutáneo (7) han demostrado que no existe diferencia significativa entre los resultados que se logran utilizando uno u otro método. Esta observación sustenta aún mas el uso de la biopsia muscular percutánea para estudios de esta índole

Los objetivos del presente trabajo son exponer la metodolcgía de la biopsia percutánea con este tipo de aguja, y valorar el significado fisiológico de la depleción de potasio y su correlación con algunos cambios en la proteína estructural del músculo o con otros electrolitos, tanto en el niño desnutrido como durante el proceso de recuperación nutricional. La aplicación práctica del estudio se relaciona directamente con el tratamiento, ya que hasta la fecha se desconoce cómo debe tratarse la depleción de potasio, y más aún, sabiéndose que dosis elevadas de este catión pueden ser tóxicas y hasta fatales.

## *II MATERIAL Y METODOS*

Un total de trece niños con desnutrición proteínico-calórica severa, y con las características clínicas descritas en estudios previos (15), fueron admitidos al Centro Clínico del Instituto de Nutrición de Centro América y Panamá (INCAP). Durante los primeros 14 a 18 días de hospitalización recibieron una "dieta de mantenimiento" o de estabilización a base de caseína suplementada con metionina que aportaba 0 7 g de proteína por kg de peso corporal, por día. Con este tipo de dieta se logra mantener al niño desnutrido en equilibrio nitrogenado. La ingesta calórica fue de 70 calorías por kg por día, el 20% de éstas en forma de grasa vegetal. Además se les dio un suplemento de vitaminas, hematínicos y potasio por la vía oral (3-4 mEq/kg/día). Por esta misma vía se les proporcionó también diariamente una mezcla multielectrolítica* (30-40 q). Durante la segunda fase del estudio la ingesta proteínica fue incrementada paulatinamente en un lapso de cinco días a 3 0 g/kg/día, y la ingesta calórica se aumentó a 120 Cal/kg/día. La suplementación de potasio, vitaminas, hematínicos, electrolitos y minerales se continuó en la misma forma durante todo el estudio.

Se obtuvieron especímenes musculares en forma seriada cada 10 a 12 días en los que se determinó potasio, sodio, cloruros, magnesio, nitrógeno y agua. Simultáneamente se recolectó una muestra de sangre para análisis de potasio, cloruros, sodio magnesio, proteínas totales y albúmina La

*Lytren (Mead Johnson).

masa muscular se estimó en forma indirecta utilizando la excresión urinaria de creatinina en 24 horas. Este metabolito, proveniente de la creatina muscular, se relacionó con la excresión urinaria de creatina de un niño normal de la misma talla que el paciente, independientemente de la edad. A esta razón se le ha denominado índice de creatina/talla (ICT) (16, 17) y permite valorar de manera indirecta el grado de depleción y recuperación de la masa muscular. Se expresa como sigue:

$$ICT = \frac{\textit{Excreción de creatinina del paciente en 24 horas.}}{\text{Excresión de creatinina de un niño normal de la misma talla en 24 horas.}}$$

**Procedimientos**

**1. Biopsia muscular***

Según se dijo, se utilizó la aguja pediátrica Baylor para biopsias musculares (Figura 1) y el sitio estandarizado por la mayoría de los investigadores en este campo, esto es, el tercio inferior y medio del músculo cuadriceps (Figura 2). Primero se fija la pelvis y la pierna con ayuda de un asistente y luego se aplica una solución germicida sobre la piel del muslo. Empleando 0.1 ml de procaina al 2% se produce un botón de anestesia intradérmica e inmediatamente después se practica una incisión de 3 mm de largo en la piel y tejido celular con un bisturí No 11 (BP). Luego se introduce la aguja siguiendo una trayectoria perpendicular al muslo manteniendo las relaciones anatómicas que ilustra la Figura 2. La ventana del trocar debe quedar en posición opuesta al fémur Para facilitar la operación se sostiene la porción externa del cuadriceps entre el pulgar y el índice de la mano libre del operador Después de penetrar la facia que presenta resistencia, se calcula que la ventana quede en el centro del manojo muscular, entonces se retrae el estilete cortante para abrir la ventana y permitir así que las fibras musculares queden dentro de ella. El estilete se empuja hasta el tope y en esta posición se retira toda la aguja. Si no se obtiene una muestra satisfactoria se repite la operación a través de la misma incisión, sin que esto represente problema o mayor trauma. Los bordes de la herida se aproximan con una pequeña banda adhesiva y gasa que cubre directamente el sitio de la incisión. Esto basta para lograr una buena cicatrización aún en los pacientes con desnutrición severa.

La biopsia obtenida en la forma descrita está así lista para establecer su peso húmedo y proceder a los diferentes análisis. Según se ha determinado, el peso de cada muestra oscila entre 5 y 20 mg, y con este método los autores han logrado obtener más de 300 especímenes sin ninguna complicación.

**2. Determinaciones de potasio muscular.**

Estas se practicaron en un fotómetro de llama utilizando un estándar interno de litio (7).

**3. Contenido de nitrógeno.**

Se determinó por medio de Nesslerización y el de nitrógeno no colágeno a nitrógeno sarcoplásmico (NNC) fue establecido después de agregar NaOH, con lo cual se obtuvo una fracción soluble en alcali (18).

Las determinaciones restantes enumeradas en un párrafo previo serán comentadas en otras publicaciones

## III RESULTADOS

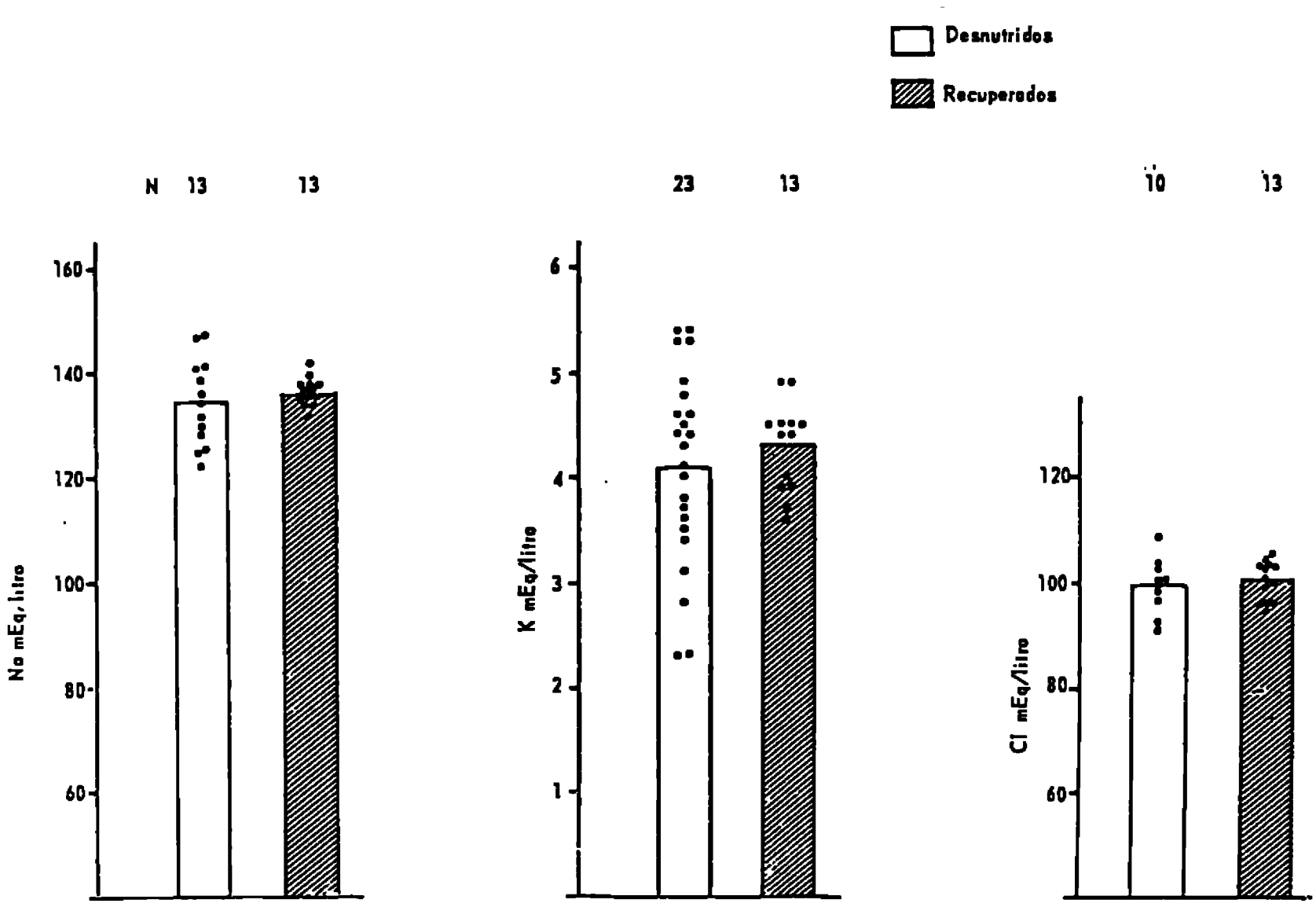
La concentración de electrolitos séricos ha sido objeto de amplias investigaciones (2, 19-21) y los hallazgos del presente estudio son muy similares a los notificados

*Una película sonora de 16 mm que ilustra esta técnica está a disposición de los interesados en la División Biomédica del INCAP.

a ese respecto. La Gráfica 1 ilustra los valores que acusaron los niños, a su ingreso al Centro Clínico y ya recuperados por completo. Según se observa, los niveles de potasio sérico muestran gran dispersión (promedio: 4.1 mEq/litro y una desviación estándar de 0.92). Sin embargo, estas cifras se normalizaron durante la fase inicial del tratamiento. La hipocaliemia (deficiencia de potasio en la sangre) está relacionada a procesos diarreicos y/o vómitos severos (22). En algunos países, esto último es de observación casi constante en lactantes severamente desnutridos (20). Los únicos dos niños que fallecieron durante el desarrollo de este trabajo tenían valores de potasio sérico por debajo de 3 mEq/l, y esta alteración se corrigió rápidamente al administrarles potasio en forma terapéutica. La causa de muerte de ambos niños fue acidosis láctica y bronconeumonía, en uno de ellos, y bronconeumonía e insuficiencia cardiaca, en el otro. Según la experiencia de otros investigadores (23) y la de los propios autores, la hipocaliemia de esta magnitud es un signo de mal pronóstico. En cuanto al sodio sérico, los datos revelan que el contenido promedio fue de 134.6 mEq/l, con una desviación estándar de 8.2. En esta serie, cuatro de los 13 casos estudiados acusaron valores por debajo de dos desviaciones estándar del promedio normal, pero ninguno fue inferior a 120 mEq/l, cifra que se considera

**Gráfica 1:**

ELECTROLITOS SERICOS EN NIÑOS CON DESNUTRICION PROTEICO-CALORICA AL INGRESO Y COMPLETAMENTE RECUPERADOS




**Electrolitos séricos en pacientes desnutridos y ya recuperados por completo.**

ya de pésimo pronóstico. Este fenómeno se debe en parte a hemodilución provocada por una ingesta pobre en sodio (dieta a base de atoles), a pérdidas exageradas de ese electrolito en casos de diarrea severa (de 30 a 50 mEq/1 de heces) y, en algunos casos, a hemodilución secundaria a terapia endovenosa. La hiponatremia contribuye en gran medida a la hipotonicidad del plasma que caracteriza a estos enfermos, y constituye un reflejo de adaptación metabólica. Los datos relativos a la concentración sérica de magnesio serán comentados en otro trabajo. Sin embargo, cabe mencionar que según pudo observarse, los valores están dentro de los límites normales y coinciden con los descritos para niños normales en otros estudios (5,24-26).

En el Cuadro No. 1 se comparan las concentraciones de potasio en el suero y en el músculo, así como la relación entre éstos y el grado de depleción proteínica a partir del índice de creatinina/talla (ICT), notándose que no existe correlación alguna entre estos parámetros.

La Gráfica 2 es una representación del estudio longitudinal de 13 niños con desnutrición proteinico-calórica tipo edematoso. Para propósitos de comparación, el estado del niño en cuanto a potasio muscular se expresa tanto por kilogramo de tejido húmedo, como por gramo de nitrógeno no colágeno. Se observa así que la concentración de potasio por peso húmedo es baja, y que a pesar de la suplementación oral de este catión, no se altera durante la fase inicial de estabilización. Al comenzar la recuperación aún se observan niveles más bajos, no siendo sino hasta después de un tiempo relativamente largo que estos valores se normalizan. Cuando el potasio se expresa en base al nitrógeno no colágeno, la relación se mantiene casi constante durante las dos fases del estudio, sin que los valores que acusan los niños a su ingreso difieran significativamente de los que presentan al estar ya recuperados por completo. El promedio normal de esta última relación es de 3.5, considerándose como valores deficientes todos aquéllos por debajo de 2.8. En esta serie de niños únicamente dos tenían razones anormalmente bajas, siendo posible que hayan sido sólo dos los que tenían una "verdadera deficiencia de potasio"; el resto, en cambio, mantuvo una relación normal a pesar de que su estado nutricional se encontraba alterado.

Para facilitar la interpretación de esta terminología, se incluye la Figura 3, la cual esquematiza la histología del músculo normal y de niños desnutridos. Según se observa, en el sarcoplasma es donde se encuentra el nitrógeno no colágeno (NNC), mientras que entre los haces de fibras musculares se aprecia el tejido colágeno que contiene el nitrógeno colágeno (NC).

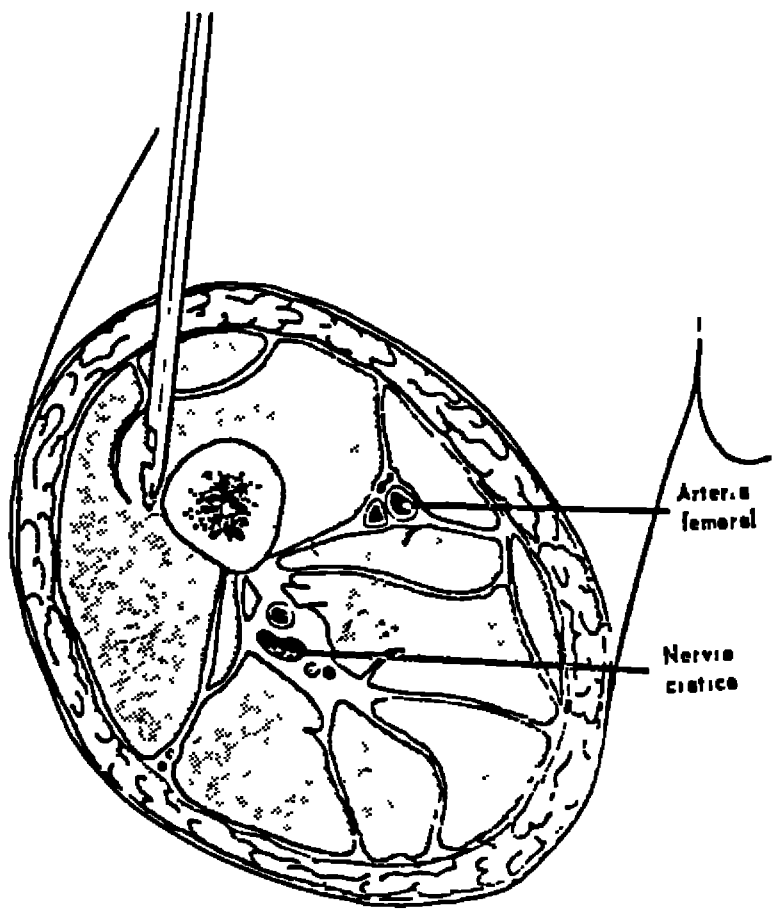
En la Gráfica 3 se exponen los resultados de la razón de nitrógeno no colágeno (NNC) a nitrógeno total (NT) en biopsias musculares obtenidas de pacientes seguidos longitudinalmente, comparados con el índice de creatinina/talla ICT). Según se aprecia, durante la fase de estabilización hay un ligero descenso de la masa proteínica y la razón de NNC/NT se mantiene estable. En cambio, en la segunda fase del estudio, es decir, cuando estos niños estaban recibiendo una dieta adecuada en su contenido de proteínas, ocurre una recuperación lenta y casi paralela de ambos parámetros.

## *V. DISCUSION*

Los autores han observado cierta discrepancia y poca correlación entre los valores de potasio sérico y el contenido de potasio muscular, cualquiera que sea el parámetro que se utilice para expresarlo (Cuadro No. 1). La hipocaliemia ha sido explicada como fenómeno secundario a una pérdida aguda de este electrolito a través de diarrea o vómitos severos (22). Así, tanto en el caso de niños desnutridos como en el de niños normales que padecen de diarreas severas, el potasio sérico retorna rápidamente a valores normales cuando se les proporciona el potasio que han perdido.

La baja concentración de potasio muscular por unidad de peso de tejido húmedo y libre de grasa (Gráfica 2) puede deberse no sólo a una deficiencia "real" sino a fenómenos de dilución inducidos en parte por un aumento del agua tisular y por cambios en la composición proteínica de la muestra Frenk y colaboradores (2) postularon que la sobrehidratación muscular (aumento del agua intracelular) era el factor responsable en gran medida del descenso en la concentración de potasio a nivel del músculo. Por otro lado, en la desnutrición proteínico-calórica 9) y en adultos mal nutridos y caquécticos (27) existe un aumento relativo del tejido colágeno interfibrilar que

**Figura 2:**




**Sección transversal del muslo que establece la relación entre el músculo cuadriceps (sitio de la biopsia) y las estructuras vitales de la región.**

ocurre como consecuencia de una disminución del tejido sarcoplasmico Este tejido colágeno, presente en la biopsia, contribuye al peso de la muestra, pero contiene únicamente de 3 a 5 mEq de K por kilogramo de tejido húmedo libre de grasa Debido a esto, la concentración de este elemento en el tejido muscular se diluye, y se observan así valores bajos Estos fenómenos de dilución, constituyen lo que podríamos llamar una deficiencia "falsa", y para evitar esta situación artificial la mayoría de los autores están de acuerdo en utilizar el nitrógeno no colágeno como parámetro de expresión del potasio (mEq de K/g de NNC).

En el estudio aquí descrito únicamente dos pacientes tenían una deficiencia "real" de potasio, y a pesar de la suplementación de K por vía oral, no se corrigió esta alteración durante la fase de estabilización. Sin embargo, en otra serie de 11 pacientes estudiados en el Hospital General de Guatemala y basados en el criterio antes mencionado (K/NNC), los autores encontraron que cuatro de ellos tenían verdadera depleción de potasio. Puede, pues, decirse que sí existe esta alteración pero sólo en un número limitado de pacientes, y que ni las características clínicas ni los datos del análisis sérico permiten distinguirlos. Parece ser entonces, que existe una adaptación metabólica y que la célula muscular solamente acepta cierta cantidad de potasio que varía de acuerdo a su contenido de nitrógeno no colágeno (NNC). Puede aseverarse, pues, que a excepción de los pocos casos que presentan una "deficiencia real" de potasio, en la desnutrición proteínico-calórica ocurre una disminución de la masa de proteína celular acompañada de un descenso proporcional del potasio a este mismo nivel

El grupo de investigadores de la Unidad Tropical de Investigaciones Metabólicas en Jamaica utiliza el contador de cuerpo total, y expresa el isótopo natural K40 medido en este aparato, en base al peso corporal (13), definiendo como una verdadera depleción de potasio únicamente aquellos casos en que la concentración de K es menor de 30 mEq/kg de peso Es razonable suponer que estos casos son concentraciones bajas de K por unidad de peso sean similares a los descritos por nosotros con razones de K/NNC inicialmente reducidos Ambas situaciones ocurren rara vez, casi por lo general en pacientes con desnutrición severa de tipo edematoso, y con vómitos y/o diarrea Otro hecho importante ob-

servado por Alleyne (28) es que cuando existe esta depleción aguda, la eficacia de la terapia oral con KCl es rápida y eleva a cifras normales la concentración de K total por unidad de peso en el curso de los primeros cinco dias, se desconoce, sin embargo, la distribución de K en el organismo A partir del quinto día de tratamiento, la recuperación del potasio corporal total es linear a la de la masa proteínica (ICT). Los hallazgos de Alleyne (28) y los nuestros sugieren que, aparentemente, con pocas excepciones, en el niño desnutrido hay un descenso del contenido total de potasio, pero paralelo a la disminución de la proteína sarcoplásmica. La cantidad total de este catión sólo podrá elevarse a medida que aumenta la masa tisular activa de todo el organismo, y de la cual el músculo forma parte importante. En los casos en que se suscitan pérdidas agudas de K ocurre una verdadera depleción, quedando sin saturarse lo que algunos autores llaman "capacidad para ligar potasio" (14,28). Esta capacidad está constituida posiblemente por proteínas intracelulares que actúan como sitios de fijación para el potasio En la desnutrición proteínico-calórica habria un descenso del número total de sitios de fijación, pero los que subsistieran estarían saturados al máximo, tratando de mantener en equilibrio la relación potasio a nitrógeno. Si a esta alteración se agregan las pérdidas agudas de K por vómitos y/o diarrea, algunos de los sitios de fijación de K quedarían temporalmente vacios (no saturados), los cuales al administrar potasio en forma terapéutica se saturarían La deficiencia "real" de potasio puede corregirse fácilmente utilizando de 4 a 6 mEq de K/kg/dia por vía oral o intravenosa, sin embargo, hay que recordar que la célula puede aceptar únicamente la cantidad de potasio que su capacidad de fijación le permite, y qué dosis excesiva pueden ser toxicas y hasta fatales

## *RESUMEN*

La introducción del uso de la aguja pediátrica Baylor para la obtención de biopsias musculares ha permitido el desarrollo de estudios longitudinales en pacientes desnutridos, en forma satisfactoria y con el mínimo de riesgos. Se detalla la técnica empleada para la obtención de estas muestras y se incluye un análisis descriptivo de los cambios observados. En el niño con desnutrición proteínico-calórica existe por lo general una "adaptación" a nivel muscular. Sin ningún intento de adentrarse en hipótesis de mecanismos, esto último podría explicar por qué la relación de potasio a nitrógeno no colágeno se mantiene constante, equilibrio éste que únicamente se rompe en pocos casos ocurriendo entonces una verdadera depleción

La normalización de la concentración de potasio muscular en base a peso de tejido húmedo, no se modifica con la suplementación de potasio en presencia de una dieta de mantenimiento proteínico La recuperación de este parámetro es lenta y casi paralela a la de la proteína no colágena y a la del índice de creatinina/talla, reflejo de la masa muscular total

Esta técnica permitirá en el futuro investigar los mecanismos de estas alteraciones a nivel celular en pacientes con desnutrición proteínico-calórica abriendo también las puertas a estudios de la fisiopatología de muchas otras enfermedades

## *REFERENCIAS*

1. Trowell, H C, Davies, J N P. & Dean, R F. A: Kwashiorkor Edward Arnold Ltd, London, 1954 p. 64-203

2. Frenk, S, Metcoff, J, Gómez, F. Ramos Galván, R, Cravioto, J & Antonowicz, I Intracellular composition and homeostatic mechanisms in severe chronic infantile malnutrition II Composition of tissues Pediatrics, 20 103-120. 1957.

3 Monkeberg, F, Gase, O & Artigus, R · Estudio de los electrolitos intracelulares por medio de la biopsia muscular

en deshidratación aguda del lactante desnutrido Pediatría Panam., 2: 114, 1959.

4. Vis, H., Dubois, R., Vanderborght, H. & De Maeyer, E.: Etude des troubles électrolytiques accompagnant le kwassiokor marastique. Revue Francaise d'Etudes cliniques et Biologiques, 10: 729-741, 1965.

5. Caddell, J. L. & Goddard, D. R. Studies in protein-calorie malnutrition. I. Chemical evidence for magnesium deficiency. New Engl. J. Med., 276: 533-535, 1967.

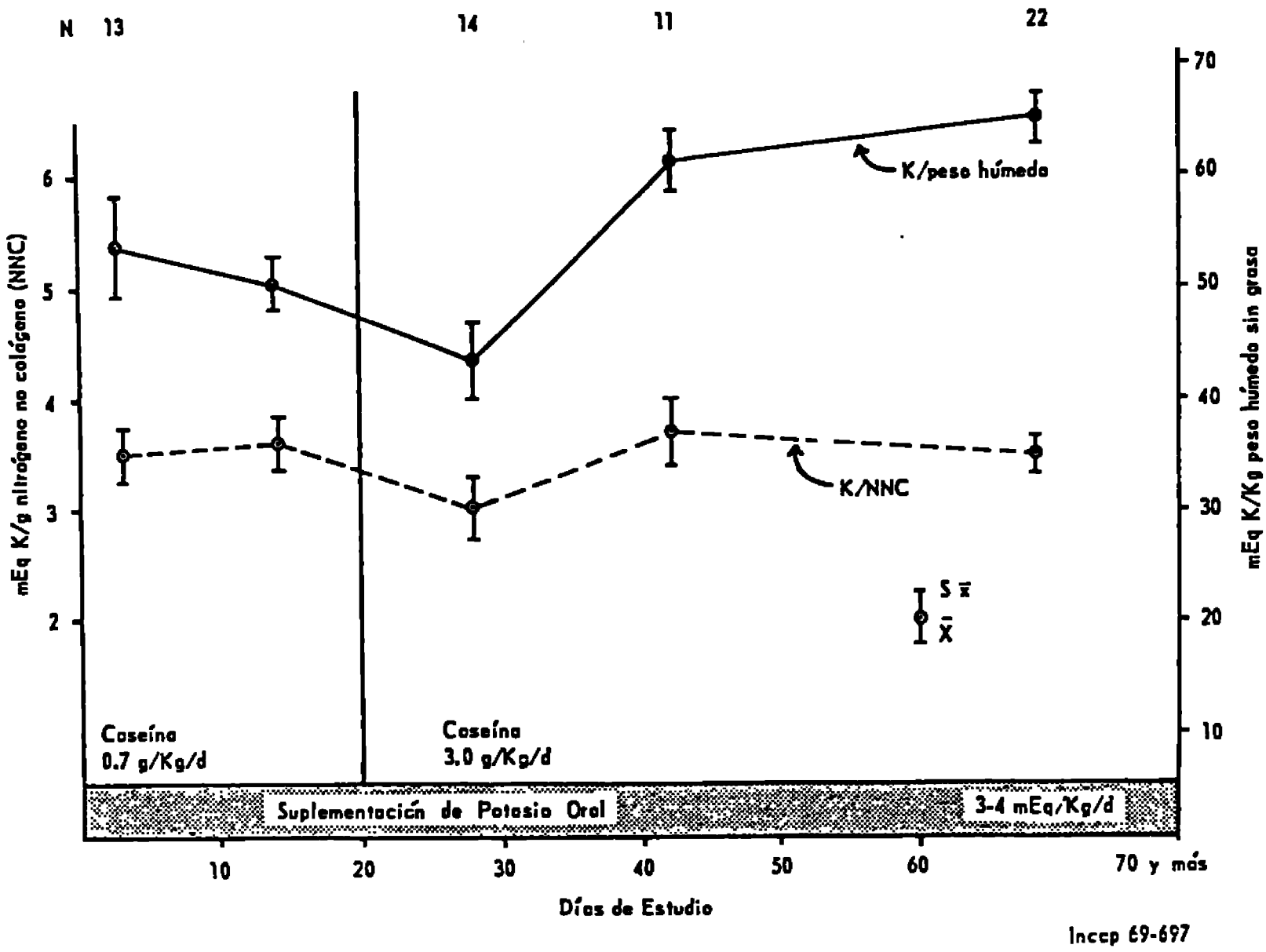
6. Metcoff, J., Frenk, S., Yoshida, T., Torres Pinedo, R., Kaiser, E. & Hansen, J. D. L.: Cell composition and metabolism in kwashiorkor (severe protein-calorie malnutrition in children). Medicine, Baltimore, 45: 365-390, 1966.

7. —Nichols, B. L., Hazlewood, C. F. & Barnes, D. J.: Percutaneous needle biopsy of cuadriceps muscle: potassium analysis in normal children. J. Pediat., 72: 840-852, 1968.

8. Tejeda V., C.: Informe preliminar sobre hallazgos patológicos en el síndrome pluricarencial de la infancia en Guatemala. Rev. Colegio Med. Guatemala, 6: 1-10, 1955.

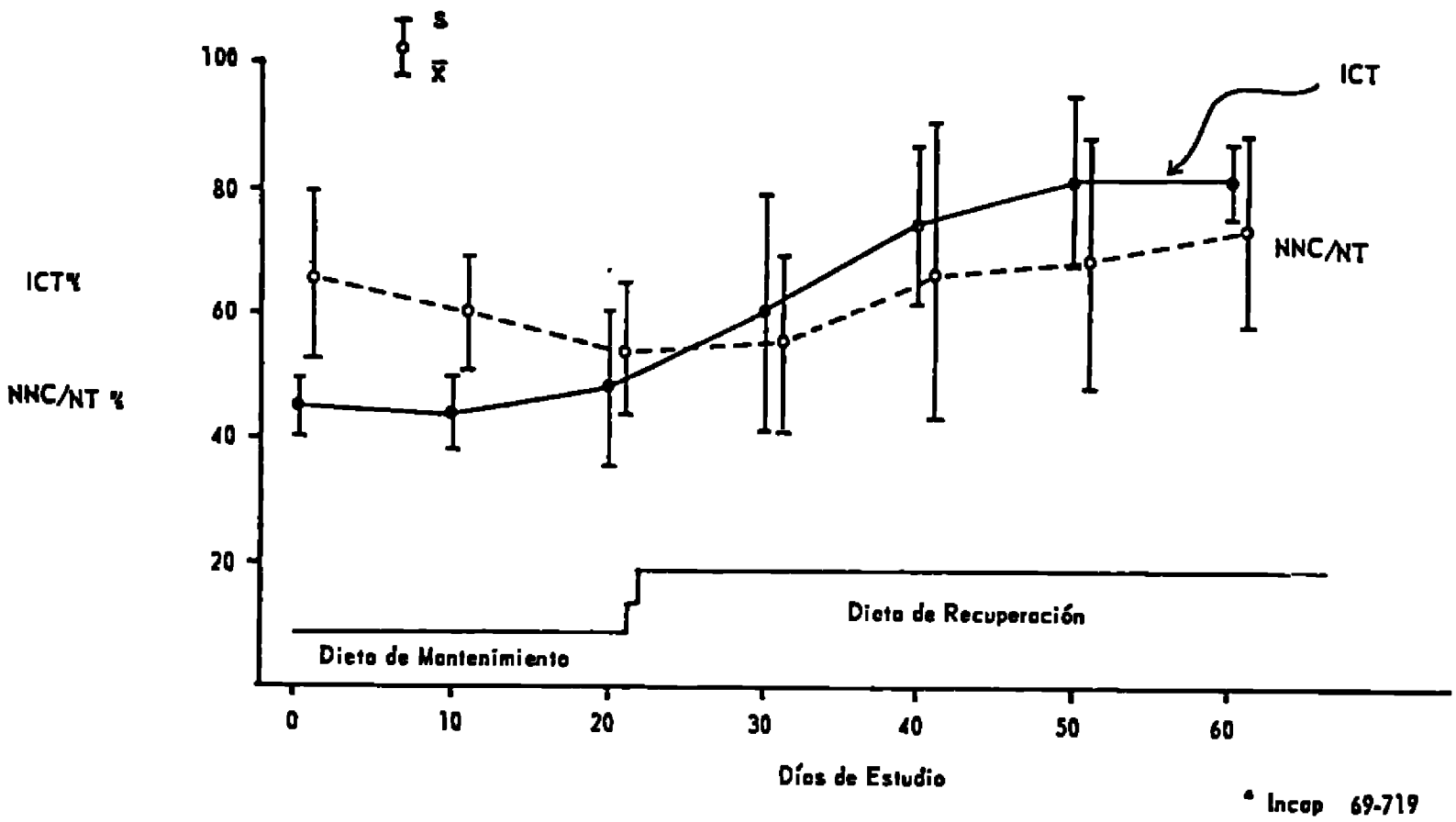
9. Montgomery, R. D.: Muscle morphology in infantile malnutrition. J. Clin Pathol., 15: 511-521, 1962.

**Gráfica 2:**




**Estudio longitudinal del potasio muscular en trece niños desnutridos, expresado en base a Nitrógeno no colágeno y en base a peso de tejido húmedo.**

**Gráfica 8:**




**Recuperación de la masa muscular (ICT) y de la razón Nitrógeno no colágeno a Nitrógeno total (NNC/NT) en tres niños desnutridos.**

10. Ramírez Lassepas, M.: Histopatología muscular en la desnutrición infantil. Tesis de graduación. Facultad de Ciencias Médicas de la Universidad de San Carlos de Guatemala, Guatemala, 1963.

11. Waterlow, J. C. & Mendes, C. B.: Composition of muscle in malnourished human infants. Nature, 180: 1361-1362, 1957.

12. Garrow, J. S.: Total body-potassium in kwashiorkor and marasmus. Lancet, 2: 455-458, 1965.

133. Alleyne, G. A. O.:: Studies on total body potassium in infantile malnutrition: the relation to body fluid space and urinary creatinine. Clin., 34: 199-204, 1968.

14. Nichols, B. L., Alleyne, G. A. O., Barnes, D. J. & Hazlewood, C. F.: Relationship between muscle potassium and total body potassium in infants with malnutrition. J. Pediat., 74: 49-57, 1969.

15. Instituto de Nutrición de Centro América y Panamá. Desnutrición severa en la infancia. Rev. Col. Méd. Guatemala, 7: 221-278, 1956. (Monografía No. 3).

16. Viteri, F., Alvarado, J., Luthringer, D. G. & Wood, R. P. II: Hematological changes in protein-calorie malnutrition Vitamins Hormones, 26: 573-615, 1968.

17. Viteri, F. & Alvarado, J.: The creatinine height index its use in the guantification of protein depletion and repletion in protein calorie malnourished children. Enviado a Pediatrics, para consideración.

18. Garrow, J. S., Fletcher, K. & Halliday, D.: Body composition in severe infantile malnutrition. J. Clin. Invest., 44: 417-425, 1965.

19. Hansen, J. D. L. & Brock, J. F. Potassium deficiency in the pathogenesis of nutritional oedema in infants. Lancet, 2: 477, 1954.

20. Hansen, J. D. L. & Jenkinson, V.: Electrolyte and nitrogen metabolism in kwashiokor. S. Afr. J. Lab. Clin. Med. 2: 206-231, 1956.

21. González Chirinos, P. & Ramos Galván, R.: El patrón electrolítico en niños con desnutrición de tercer grado. Bol. med. Hosp. Inf. (Méx.), 21: 89-98, 1964.

22. Hansen, J. D. L. Comunicación personal.

23. Wayburne, S.: Malnutrition in Johannesburg. En: Calorie deficiencies and protein deficiencies; Proc. of a Colloquium held in Cambridge, 1967. R. A. McCance & E. M. Widdowson (Eds.). J. & A. Churchill Ltd., London, 1968. p. 7-21.

24. Back, E. H., Montgomery, R. D. & Ward, E. E.: Neurological manifestations of magnesium deficiency in infantile gastroenteritis and malnutrition. Arch. Dis. Child., 37: 196-109, 1962.

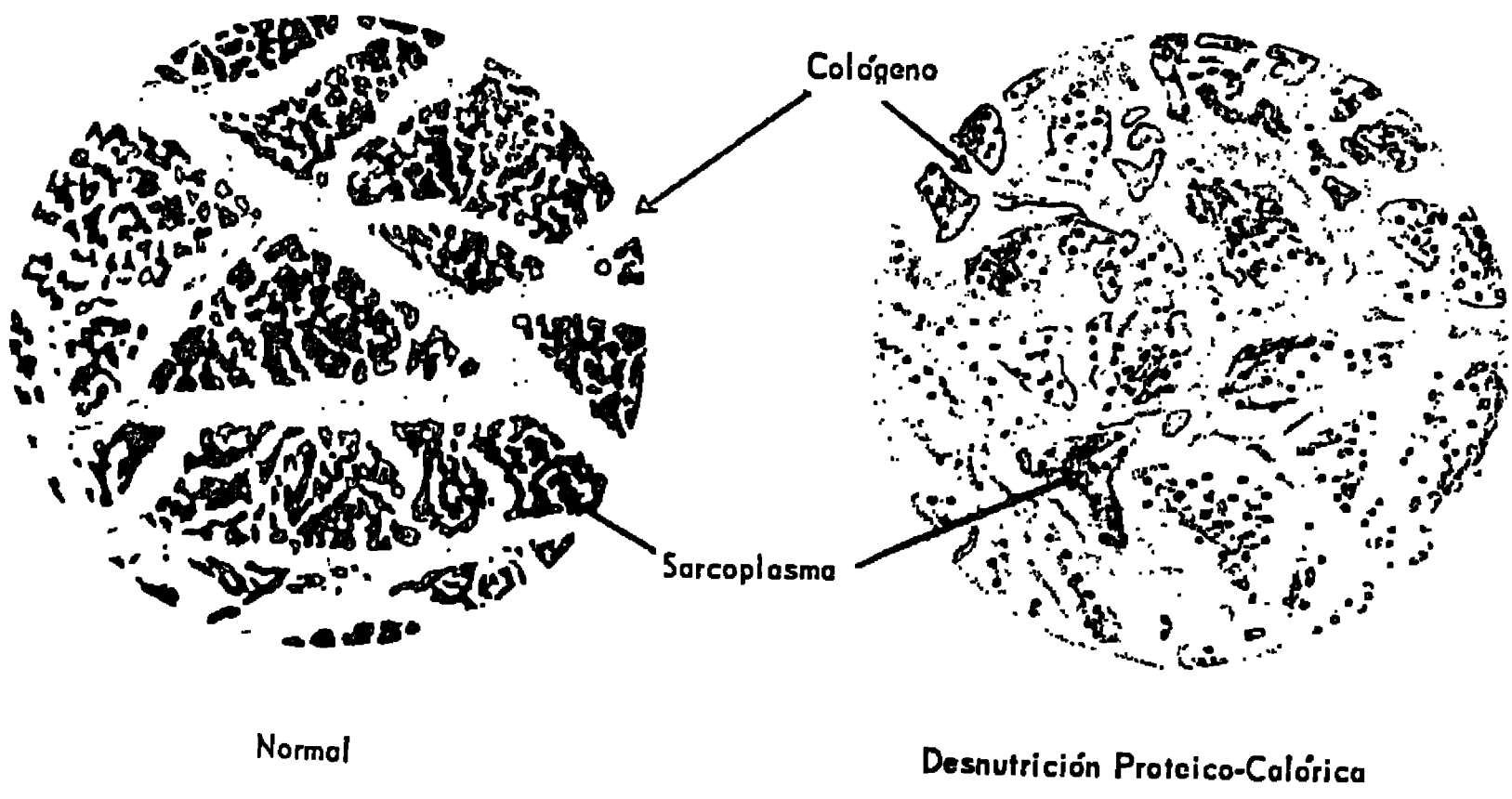
25. Montgomery, R. D. Magnesium balance studies in marasmic kwashiorkor. J. Pediat., 59: 119-123, 1961.

26. Linder, G. C., Hansen, J. D. L. & Karabus, C. D. The metabolism of magnesium and other inorganic cations and of nitrogen in acute kwashiorkor. Pediatrics, 31: 552-568, 1963.

27. Mason, K. E. Effect of nutritional deficiencies upon muscle. En: The structure and function of muscle. G. H. Bourne (Ed.), v. III, Pharmacology and disease. Academic Press, New York, 1960. p. 171-207.

28. Alleyne, G. A. O. Brit. J. Med. (en prensa).

**Figura 3:**




**Sección transversal (esquema) de músculo normal y músculo de niños desnutridos.**

**CUADRO No. 1**

**COMPARACION ENTRE LA CONCENTRACION DE POTASIO EN EL SUERO Y EN BIOPSIAS MUSCULARES DE NIÑOS CON DESNUTRICION PROTEINICO-CALORICA**

| Clave | Edad en meses | mEq de K/l de suero | mEq de K/kg de tejido húmedo libre de grasa | Depleción de masa muscular en base al ICT* |
|---|---|---|---|---|
| PC-180 | 29 | 3 6 | 47.4 | 0.79 |
| PC-182 | 16 | 4 6 | 28 9 | 0 40 |
| PC-185 | 55 | 4 6 | 63 2 | 0.45 |
| PC-186 | 21 | 2.8 | 70 2 | 0 45 |
| PC-187 | 58 | 4.1 | 35.2 | 0.37 |
| PC-188 | 65 | 4 4 | 67.6 | 0.55 |
| PC-189 | 67 | 3 8 | 46 3 | 0 24 |
| PC-194 | 15 | 5.3 | 59 4 | 0.37 |
| PC-196 | 34 | 4.3 | 26.7 | 0.51 |
| PC-197 | 35 | 3 1 | 42 2 | 0 43 |
| Valores normales | | 4.3 | 61 1 | 1 0 |
| D.E.** | | 0 7 | 10 1 | 0.08 |

* Indice de Creatinina/Talla.
** Desviación estándar

# Métodos de evaluación del estado nutricional proteínico-calórico en pre-escolares de condiciones socio-económicas diferentes.

# Repercusión nutricional del sarampión en niños crónicamente sub-alimentados.[1]

FERNANDO E. VITERI[2], LEONARDO J. MATA[3] y MOISES BÉHAR[4]
Instituto de Nutrición de Centro América y Panamá (INCAP), Guatemala, C. A.

## RESUMEN

Se estudiaron 194 niños de edad preescolar de cinco grupos correspondientes a tres categorías socioeconómicas diferentes; uno, de condición alta, otra mediana, y tres de situación socioeconómica baja. Dentro de los niños incluídos en esta última categoría, se estudió un grupo de 23 niños un mes después de haber sufrido sarampión. En todos los preescolares estudiados se midió el peso, la talla, la circunferencia del brazo y el panículo adiposo tricipital, así como la concentración sérica de proteínas totales y albúmina, la razón de urea/creatinina urinaria, y el índice de creatinina/talla (ICT) en muestras de orina colectadas en un período de 3 horas o más. Se encontró que con excepción del grupo de niños de alto nivel socioeconómico todos presentaban un franco retardo de peso así como de talla para su edad cronológica. Sin embargo, el promedio del resto de las medidas antropométricas fue predominantemente normal para todos los grupos aunque hubo un mayor número de niños con mediciones por debajo de lo normal en los grupos de condición socioecenómica baja. Además, el grupo post-sarampión presentó con mayor frecuencia grosor de panículo adiposo tricipital por debajo del 10 percentile, tanto para su edad cronológica como para su edad/talla. Desde el punto de vista bioquímico, la relación de

---

1. Este trabajo se llevó a cabo con ayuda financiera de los Institutos Nacionales de Salud de los Estados Unidos de América (NIH) con sede en Bethesda, Maryland (Subvención Nº 5-RO1 AM-00981), y con fondos provistos por el Instituto Tecnológico de Massachusetts, Cambridge, Mass, E. U. A. (Contrato Nº 5059-1).
2. Jefe de la División Biomédica del INCAP.
3. Jefe de la División de Microbiología de la misma Institución.
4. Director del Instituto de Nutrición de Centro América y Panamá.
Publicación INCAP E-660.
Recibido: 10-12-1971.

**urea/creatinina urinaria acusó francas diferencias entre los grupos de situación socioeconómica alta y los niños de condición socioeconómica subóptima. El ICT mostró un franco descenso en el grupo post-infeccioso con respecto al resto de los grupos, los que —de acuerdo a este indicador— presentaban una masa muscular adecuada para su talla. Se comenta la interpretación de cada uno de estos indicadores y sus principales limitaciones, y se demuestra, a nivel de campo, la repercusión nutricional de una infección severa en niños crónicamente subalimentados, la cual parece afectar más profundamente y de manera más prolongada el estado de nutrición proteínica que la calórica.**

## INTRODUCCION

En la mayoría de los países en vías de desarrollo, la deficiencia proteínico-calórica crónica de tipo leve o moderado constituye uno de los problemas más serios de salud pública (1). A nivel de poblaciones, esta situación ha sido puesta de manifiesto por estudios de consumo de alimentos (2), de crecimiento y desarrollo de preescolares (3) y de estadísticas vitales (4), así como por la incidencia de niños con signos y síntomas de deficiencia proteínico-calórica severa.

Sin embargo, poco se sabe del estado nutricional calórico y proteínico de la gran mayoría de niños que sólo manifiestan retardo en el crecimiento y desarrollo. Asimismo, se dispone de conocimientos limitados acerca de las repercusiones específicas de los procesos infecciosos sobre el estado de nutrición calórico y proteínico de la población general, aun cuando en estudios metabólicos se ha encontrado que las enfermedades infecciosas inducen pérdidas apreciables de nitrógeno (5, 6), afectando así el estado nutricional proteínico del niño (7). Tan sólo en años recientes ha surgido evidencia de las repercusiones nutricionales que las enfermedades infecciosas repetidas tienen en niños que viven en un estado de subnutrición crónica (8, 9).

Con el fin de conocer más a fondo la magnitud del problema, y evaluar así de manera más precisa el efecto de acciones tendientes a mejorar la nutrición del niño, se han propuesto diversas mediciones antropométricas y bioquímicas que definan con mayor exactitud el estado nutricional proteínico y calórico del niño moderadamente desnutrido. Para ese propósito se ha empleado: a) el retraso ponderal del niño en función de la edad como un indicador del grado de severidad de la desnutrición (10); b) el retraso estatural *per se* y el ponderal,

para la talla del niño (11), y c) otra serie de medidas antropométricas. Entre estas últimas, el diámetro del brazo menos la adiposidad a nivel tricipital se ha considerado como representativo del estado de nutrición proteínica (12, 13), mientras que la medición del panículo adiposo refleja el estado de reservas calóricas del niño.

Además se han propues indicadores bioquímicos que reflejan aspectos específicos del metabolismo proteínico del niño en el momento de someterse a examen (14). Dentro de éstos, se ha utilizado la concentración de albúmina sérica (15) y la relación de aminoácidos no esenciales a esenciales en el suero (16). Por otro lado, en orina se ha empleado la relación de urea a creatinina (17) y la excreción de hidroxiprolina (18). Recientemente, y con la idea de que la eliminación de creatinina refleja la masa muscular, la cual disminuye progresivamente con la deficiencia proteínica (19), se ha sugerido el empleo del "índice de creatinina/talla" (ICT) (20). Concretamente, éste consiste en la razón entre la eliminación de creatinina urinaria por unidad de tiempo del niño bajo estudio, sobre la eliminación de creatinina que es de esperar para un niño bien nutrido de igual talla que la del niño investigado. Según se ha podido comprobar, bajo condiciones de estudios metabólicos este índice es de suma utilidad (21, 22), y como lo demuestra su íntima correlación con el potasio corporal total, refleja la masa magra del niño (23). Es importante señalar que la medida de la masa magra relativa por medio del ICT está corregida para la talla del niño, independiente de su edad.

El presente trabajo se llevó a cabo con el objeto de evaluar varios de estos indicadores como métodos de diagnóstico del estado de nutrición proteínica y calórica del preescolar con retardo pondoestatural. Un segundo propósito fue valorar las repercusiones nutricionales de una infección severa (sarampión) valiéndose de esos indicadores.

## MATERIAL Y METODOS

### *Población*

El estudio incluyó un total de 193 niños preescolares de cuatro poblaciones distintas de la República de Guatemala, las cuales se escogieron en base a una apreciación gruesa de su estado socioeconómico. Ciertas características de los grupos

investigados se detallan en el Cuadro No. 1. En la ciudad de Guatemala se estudiaron 50 preescolares que asistían a un jardín de niños. Todos eran hijos de profesionales o comerciantes pertenecientes a un estrato socioeconómico alto o dentro de la categoría mediana-alta, con historia de buena o excelente nutrición y ambiente higiénico adecuado. Los niños eran de ascendencia caucásica o mestiza.

En San Lucas Sacatepéquez, los niños se catalogaron como de situación socioeconómica media-baja. Esta comunidad se encuentra situada a 25 minutos de viaje en automóvil de la ciudad capital; muchos de sus habitantes conmutan diariamente a la ciudad y la población —de extracción racial indígena o mestiza— tiene características de cierta prosperidad económica. Sin embargo, las condiciones de vivienda y saneamiento ambiental son todavía deficientes.

Los niños procedentes de Santiago Sacatepéquez y de Santa María Cauqué eran predominantemente de ascendencia indígena, Maya, y de nivel económico bajo, siendo los hábitos alimenticios e higiénicos así como las características de vivienda de estas comunidades, muy deficientes. Se tomaron dos grupos de niños de Santa María Cauqué: el primero, de 49 niños aparentemente sanos, y el otro de 23 niños que habían tenido sarampión el mes previo al estudio.

En el mismo Cuadro se observa la edad cronológica y la edad correspondiente a la talla de los niños, si se asume que la talla del niño representa el 50 percentilo de los patrones de Stuart y Stevenson (24). Desde el punto de vista de la edad cronológica, los niños procedentes de la ciudad de Guatemala, San Lucas Sacatepéquez y Santiago Sacatepéquez, eran menores que los de Santa María Cauqué. Sin embargo, exceptuando los de la ciudad capital, todos presentaban un franco retraso estatural para su edad. Como consecuencia de este hecho, la edad/talla de los niños de alto nivel socioeconómico resultó ser significativamente superior a la edad/talla de los niños sanos de las otras tres comunidades ($p < 0.05$).

*Antropometría*

Se tomaron las siguientes medidas: 1) Peso obtenido con el niño descalzo y vistiendo únicamente un mínimo de ropa. El peso promedio de la ropa fue de 300 gramos. 2) Talla de pie (descalzo), colocando al niño contra una superficie per-

**CUADRO Nº 1**
**CARACTERISTICAS DE LOS NIÑOS ESTUDIADOS**

| Grupo | Población | Estado socioeconómico | Condición clínica | Sexo M | Sexo F | Edad cronológia (meses) | Edad/ talla (meses) |
|---|---|---|---|---|---|---|---|
| I | Ciudad de Guatemala | Alto | Sanos | 28 | 22 | 46.8 ± 2.2[a] | 45.6 ± 2.3[b] |
| II | San Lucas Sacatepéquez | Mediano - bajo | Sanos | 10 | 10 | 46.2 ± 4.7[a] | 31.2 ± 3.3 |
| III | Santiago Sacatepéquez | Bajo | Sanos | 21 | 31 | 51.5 ± 2.1[a] | 31.4 ± 2.0 |
| IV | Santa María Ca· | Bajo | Sanos | 22 | 27 | 62.8 ± 2.5 | 37.1 ± 2.4 |
| V | Santa María Cauqué | Bajo | Post-sarampión | 11 | 12 | 67.4 ± 2.3 | 37.0 ± 3.7 |

[a] $p < 0.05$ con grupos IV y V.

[b] $p < 0.05$ con todos los grupos, salvo el grupo V.

pendicular a la plataforma en que estaba parado para la toma de esta medición, empleando una escuadra sobre la cabeza y una cinta exacta. 3) El perímetro del brazo se midió con una cinta metálica a nivel del punto medio entre el acromión y el olécranon, evitando presionar los tejidos blandos. El grosor del panículo adiposo tricipital se obtuvo con el calibrador de Lange y Brózek (25), midiéndolo en la cara posterior del brazo, a la misma altura en que se determinó el perímetro. Con estas medidas se calculó el diámetro del brazo corregido para panículo adiposo asumiendo que el perímetro es circular y que el panículo adiposo representa el promedio de la adiposidad total.

Se tomó una muestra de sangre y se recolectó orina por un período de tres horas o más, medida exactamente y después de haber descartado la orina de la primera micción. Cabe mencionar que los niños habían consumido un desayuno ligero.

En el suero sanguíneo se determinaron proteínas totales por refractometría (26) y albúmina por electroforesis en acetato de celulosa (27). En la muestra de orina se midió nitrógeno de urea por el método de Barker (28) y creatinina por la técnica de Clark y Thompson (29). De esta manera se obtuvo la razón de nitrógeno de urea/creatinina. La excreción de creatinina urinaria se calculó en términos de mg por minuto y se proyectó a 24 horas (30).

## RESULTADOS

Los resultados obtenidos se detallan en el Cuadro No. 2. Los datos relativos a peso para talla demuestran que en el grupo de alto nivel socioeconómico los niños acusaron un peso promedio superior al de las normas de Stuart y Stevenson (24) para niños de igual talla. En San Lucas Sacatepéquez y Santiago Sacatepéquez, los promedios de peso para talla también excedieron ligeramente los establecidos por los mismos investigadores (24) para niños de igual talla, mientras que en Santa María Cauqué, los niños, ya fuesen sanos o después de afectados por sarampión, acusaron promedios de 100 y 99% de peso para talla de los mencionados patrones. Vale la pena subrayar que no se constataron diferencias significativas entre el peso para talla en los dos grupos de niños estudiados en Santa María Cauqué.

## CUADRO Nº 2

## RESULTADOS ANTROPOMETRICOS Y BIOQUIMICOS EN LOS NIÑOS INCLUIDOS EN EL ESTUDIO

| Grupo | Comunidad y condición clínica | Peso para talla (%) | Perímetro de brazo (cm) | Panículo adiposo tricipital (mm) | Diámetro corregido de brazo (mm) | Suero | | Orina | TCT |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Proteínas (g/100 ml) | Albúmina (g/100 ml) | N de urea / creatinina | |
| I | Ciudad de Guatemala Sanos | 110 ± 1.6[a] | 17.2 ± 0.2[a] | 10.5 ± 0.3[b] | 44.3 ± 0.6[a] | 6.90 ± 0.06[c] (N = 33)[g] | 4.21 ± 0.05 (N = 33) | 10.5 ± 0.8[b] | 0.96 ± 0.04 |
| II | San Lucas Sacatepéquez Sanos | 105 ± 2.0[d] | 15.5 ± 0.3[e] | 10.1 ± 0.4[f] | 39.4 ± 1.2 | 7.43 ± 0.07 (N = 19) | 4.26 ± 0.05 (N = 19) | 9.3 ± 1.3[f] | 0.90 ± 0.07 |
| III | Santiago Sacatepéquez Sanos | 104 ± 1.1[e] | 14.4 ± 0.3 | 9.5 ± 0.3 | 37.7 ± 0.9 | 7.65 ± 0.08[d] (N = 33) | 4.40 ± 0.04 (N = 33) | 4.0 ± 0.5 | 1.09 ± 0.05 (N = 29) |
| IV | Santa María Cauqué Sanos | 100 ± 0.8 | 15.1 ± 0.2 | 9.4 ± 0.3 | 38.8 ± 0.6 | 7.30 ± 0.06 (N = 37) | 4.23 ± 0.04 (N = 37) | 4.2 ± 0.6 | 0.94 ± 0.06 |
| V | Santa María Cauqué Post-sarampión | 99 ± 1.8 | 14.9 ± 0.2 | 9.0 ± 0.5 | 38.5 ± 0.7 | 7.08 ± 0.08[f] (N = 22) | 4.08 ± 0.07 (N = 22) | 3.3 ± 0.8 | 0.72 ± 0.05[a] |

[a] $p < 0.05$ con todos los otros grupos.

[b] $p < 0.05$ con todos los otros grupos excepto el II.

[c] $p < 0.05$ con todos los otros grupos excepto el V.

[d] $p < 0.05$ con grupos IV y V.

[e] $p < 0.05$ con grupo III.

[f] $p < 0.05$ con todos los otros grupos excepto el I.

[g] $p < 0.05$ Datos sobre determinaciones efectuadas cuando el número fue inferior al total de niños estudiados.

Con respecto a la circunferencia del brazo, el grupo de la ciudad de Guatemala presentó valores significativamente superiores a los demás, mientras que el de Santiago Sacatepéquez tuvo los niveles más bajos. De nuevo pudo verificarse la ausencia de diferencias significativas entre los dos grupos investigados en Santa María Cauqué, así como entre éstos y los niños de San Lucas y de Santiago Sacatepéquez.

Las mediciones de panículo adiposo tricipital revelaron que los niños de nivel socioeconómico alto y mediano tenían un panículo adiposo mayor que los niños de nivel socioeconómico bajo. Sin embargo, entre éstos no se determinaron diferencias significativas. El diámetro del brazo, corregido para adiposidad (índice de muscularidad), mostró ser significativamente superior en el grupo de la ciudad de Guatemala con respecto a todos los otros grupos estudiados. No hubo diferencias de significancia estadística entre ninguno de los otros grupos incluídos en la investigación.

El grupo de niños de la ciudad de Guatemala acusó valores de proteínas séricas totales inferiores a los de todos los otros grupos, salvo el de Santa María Cauqué que, según se dijo, fue estudiado un mes después de sufrir sarampión. El grupo de Santiago Sacatepéquez, de situación socioeconómica baja, tuvo niveles más altos de proteínas séricas totales que los otros dos grupos del mismo nivel socioeconómico, en Santa María Cauqué, representados por niños aparentemente sanos o después de haber tenido sarampión. La concentración de albúmina sérica fue más alta en el grupo estudiado en Santiago, que en los otros, con excepción de San Lucas. El promedio más bajo de albúmina sérica se encontró en el grupo post-sarampión de Santa María Cauqué. aun cuando las diferencias no fueron estadísticamente significativas.

La relación de nitrógeno de urea a creatinina urinaria mostró niveles francamente superiores en los niños de la ciudad de Guatemala y de San Lucas Sacatépequez, mientras que los grupos de baja situación socioeconómica mostraron niveles bajos. De nuevo, el grupo post-sarampión de Santa María acusó el nivel mínimo, aunque las diferencias entre éste y los otros grupos de bajo nivel socioeconómico no alcanzaron significado estadístico.

Con respecto al índice de creatinina/talla (ICT). en todas las poblaciones de niños estudiadas se obtuvieron valores pro-

medio normales (0.9 o más), con excepción del grupo post-sarampión de Santa María, el cual mostró valores francamente inferiores y significativamente diferentes a los de todos los otros grupos.

Los Cuadros Nos. 3 y 4 muestran la distribución porcentual de los niños dentro de cada grupo estudiado, de acuerdo a los siguientes límites: a) *peso para edad* - según los límites establecidos en la clasificación de Gómez (10); b) *peso para talla* - tres valores: 90, 92 y 95% del que era de esperar; c) *circunferencia del brazo, panículo adiposo y diámetro del brazo corregido por adiposidad* - el décimo percentilo de los valores de McCammon (31) calculados tanto para la edad/talla como para la edad cronológica; d) *diámetro del brazo corregido por adiposidad* - restando el 50 percentilo de panículo adiposo tricipital al décimo percentilo del perímetro; e) *proteínas y albúmina séricas* - 6 5 y 3.4 g por 100 mililitros, respectivamente; f) *ICT* - considerando como valores límites 70% y 85% de los valores esperados (20, 21, 32).

Según los resultados de la distribución de las medidas antropométricas (Cuadro No. 3), el grupo de alto nivel socioeconómico presenta características idénticas a las de niños norteamericanos; además, el porcentaje de casos por debajo de los límites escogidos aumenta a medida que baja la situación socioeconómica de las poblaciones estudiadas. En todos los casos, el grupo de Santa María Cauqué —investigado después de haber sufrido sarampión— presentó un mayor porcentaje de niños por debajo de los límites establecidos. Sin embargo, únicamente el perímetro de brazo para la edad cronológica de los niños y el grosor de panículo adiposo, tanto para la edad cronológica como para la edad/talla, muestran diferencias significativas con el grupo control de la misma población, constituído por niños aparentemente sanos.

Desde el punto de vista bioquímico (Cuadro No. 4) se observa esencialmente la misma tendencia, salvo que los valores obtenidos para el ICT en el grupo post-sarampión de Santa María Cauqué. fueron significativamente diferentes de los del grupo de niños aparentemente sanos de las misma comunidad.

## CUADRO Nº 3

## DISTRIBUCION DE VALORES ANTROPOMETRICOS EN LAS POBLACIONES INVESTIGADAS (% DE NIÑOS)

| Grupo | Población | Peso para edad % | | Peso para talla % | | | < 10 percentilo del perímetro de brazo, para | | <10 percentilo de panículo adiposo tricipital, para | | <10 percentilo de diámetro de brazo menos adiposidad para | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | <60 | <75 | <90 | <92 | <95 | Talla | Edad | Talla | Edad | Talla | Edad |
| I | Ciudad de Guatemala Sanos | 0 | 0 | 2 | 4 | 4 | 2 | 2 | 2 | 2 | 6 | 6 |
| II | San Lucas Sacatepéquez Sanos | 0 | 20 | 5 | 10 | 10 | 24 | 29 | 5 | 5 | 24 | 33 |
| III | Santiago Sacatepéquez Sanos | 4 | 41 | 2 | 6 | 12 | 48 | 58 | 19 | 8 | 43 | 59 |
| IV | Santa María Cauqué Sanos | 2 | 61 | 4 | 12 | 21 | 33 | 49 | 8 | 8 | 48 | 54 |
| V | Santa María Cauqué Post-sarampión | 5 | 62 | 9 | 17 | 30 | 43 | 74[a] | 22[a] | 22[a] | 52 | 56 |

[a] $p < 0.05$ con niños sanos de la misma comunidad (Grupo IV).

CUADRO Nº 4

DISTRIBUCION DE VALORES BIOQUIMICOS EN LAS POBLACIONES INVESTIGADAS (% DE NIÑOS)

| Grupo | Población | Proteínas séricas (g/100 ml) < 6.5 | Albúmina sérica (g/100 ml) <3.4 | Indice de creatinina/talla <0.70 | <0.85 |
|---|---|---|---|---|---|
| I | Ciudad de Guatemala Sanos | 6 | 0 | 13 | 35 |
| II | San Lucas Sacatepéquez Sanos | 12 | 5 | 18 | 40 |
| III | Santiago Sacatepéquez Sanos | 9 | 3 | 3 | 14 |
| IV | Santa María Cauqué Sanos | 8 | 4 | 18 | 38 |
| V | Santa María Cauqué Post-sarampión | 15 | 7 | 45[a] | 64[a] |

[a] $p < 0.05$ con todos los otros grupos.

## DISCUSION

Los resultados del presente estudio ponen de manifiesto varios hechos de importancia: 1) Que el estado socioeconómico de las poblaciones estudiadas se asocia a las características de crecimiento y al estado nutricional de niños de edad preescolar. 2) Que el niño moderadamente desnutrido por lo general está adaptado, o compensado, ya que mantiene niveles de peso, adiposidad y masa muscular o magra, normales para su talla. 3) Que una infección severa en grupos de población de bajo nivel socioeconómico y cuya nutrición es deficiente, afecta de manera prolongada tanto su nutrición calórica como la proteínica. En base a los resultados de peso para talla y a los promedios de panículo adiposo, parece ser, sin embargo, que la nutrición calórica se recupera más rápidamente que la proteínica.

El grupo de alto nivel socioeconómico contrasta con los demás por tener todos los valores antropométricos dentro de los límites establecidos como normales para poblaciones estadounidenses de edad y sexo similares. Es de interés destacar que aún el grupo de población considerado como de situación socioeconómica mediana, presenta una clara disminución en talla que, para una edad promedio de tres años diez meses, representa ya un retraso promedio de un año tres meses. Este retardo estatural es aún más evidente en los grupos de bajo nivel socioeconómico. Asimismo, es importante considerar que las edades de los niños en los diversos grupos de población estudiados no son iguales, ya que los de Santa María Cauqué eran mayores que los de las otras poblaciones. Es posible que por este motivo, en ellos el retardo estatural relativo es mayor que en el grupo de Santiago Sacatepéquez.

Exista o no un retardo ponderal para la edad, en todos los grupos estudiados, el peso para talla resulta ser adecuado. Este hecho sugiere que en los niños de las edades estudiadas, el retardo ponderal para la edad es fundamentalmente una consecuencia del retardo estatural.

El hecho de que en el grupo post-sarampión el promedio de peso haya sido adecuado para su talla podría sugerir que los niños se habían recuperado totalmente del efecto de la infección severa. Los promedios del grosor del panículo adiposo tricipital y del diámetro del brazo corregido por adiposidad

parecerían confirmar parcialmente esta sugerencia. Sin embargo, el análisis de la distribución porcentual de casos por debajo de los límites considerados como normales, revela un panorama distinto, que tiende a reflejar más fielmente tanto las características relacionadas a diversos niveles socioeconómicos, como las consecutivas a una infección severa previa: 1º) Es evidente que el número de niños con valores antropométricos sub-normales tiende a aumentar conforme la condición socioeconómica disminuye. 2º) El efecto del sarampión se traduce en un mayor número de niños con panículo adiposo por debajo del 10 percentilo, tanto para la edad cronológica como para la edad/talla; además, el perímetro del brazo es inferior cuando éste se compara al 10 percentilo de niños de igual edad, aunque no así en relación con el 10º percentilo de niños de igual talla.

Desde el punto de vista bioquímico, las proteínas séricas totales en promedio, fueron inferiores en el grupo de alto nivel socioeconómico que en los restantes, mientras que los valores de albúmina sérica fueron fundamentalmente iguales en todos los grupos, salvo el de Santiago Sacatepéquez que acusó niveles más altos. Esta discrepancia entre los niveles de proteínas séricas totales y los de albúmina, refleja los valores más altos de globulina previamente determinados en grupos de población de bajo nivel socioeconómico que viven bajo condiciones deficientes de higiene personal y ambiental (33, 34). En efecto, la fracción $\gamma$ globulina fue la responsable de la mayor parte del alza de las proteínas séricas totales en los grupos de nivel socioeconómico mediano y bajo ($p<0.05$ con el grupo de nivel socioeconómico alto). Los niveles promedio de proteínas y de albúmina no reflejaron el efecto de una infección severa previa sobre el estado de nutrición proteínica. La distribución porcentual de valores bajos de estas determinaciones bioquímicas de nuevo destacan la normalidad del grupo de alto nivel socioeconómico Al mismo tiempo, en los grupos de menor nivel socioeconómico, el número de valores inferiores a lo normal es elevado y tiende a aumentar aún más en el grupo postsarampión, a pesar de que las diferencias no son estadísticamente significativas.

La relación de nitrógeno de urea a creatinina indica que tanto el grupo de nivel socioeconómico alto como el de nivel socioeconómico mediano, consumían significativamente más

proteínas que los grupos de status socioeconómico bajo. Es de interés especular en cuanto a un probable efecto predominante del factor higiénico sobre el retardo estatural y la elevación de $\gamma$ globulinas séricas, ya que ambos ocurren en el grupo de nivel socioeconómico mediano, a pesar de que en base a la razón urea/creatinina, la ingesta proteínica parece adecuada. Parte del retardo estatural podría también deberse a efectos ambientales desfavorables tempranos, fundamentalmente de carácter nutricional o infeccioso (prenatales o con anterioridad a los dos años de edad).

En contraste con todas las mediciones previas, el índice de creatinina/talla reveló no sólo diferencias significativas en cuanto a distribución de valores bajos, sino también en términos de promedio, entre el grupo estudiado después de un episodio infeccioso severo y los restantes, incluyendo los niños de la misma comunidad que no habían sufrido recientemente de sarampión. Sin embargo, este índice no llega a 0.85 en 35% de los sujetos de alto nivel socioeconómico. Este hallazgo podría explicarse: primero, por el hecho de que la excreción urinaria de creatinina no es constante en el curso de 24 horas; segundo, debido a que en varios de estos niños el flujo urinario fue escaso, y por último, a causa de un vaciamiento incompleto de la vejiga durante la colección de orina. Cualesquiera de estas causas da origen a valores bajos cuya magnitud se magnifica ocho veces al extrapolarlos a 24 horas.

A pesar de las limitaciones impuestas por la metodología empleada, el ICT es un método sensitivo para detectar la depauperación proteínica, y permite demostrar el impacto del sarampión —y probablemente de otras infecciones severas— sobre el estado nutricional proteínico de la población general. Estos estudios parecen indicar igualmente que después de una infección severa, tanto el estado de nutrición calórica —reflejada por el grosor del panículo adiposo tricipital— como el peso para la talla, se recuperan más rápidamente que el estado de nutrición proteínica. Esta disparidad en la velocidad de recuperación nutricional calórica y proteínica es semejante a la que se observa en niños con desnutrición proteínico-calórica severa bajo condiciones de tratamiento hospitalario, en quienes la mayoría de las veces se aprecia una recuperación más rápida del peso que de la masa proteínica (20, 35). De la misma manera, estos resultados explican cómo un proceso infec-

cioso severo puede constituir un factor precipitante de desnutrición proteínica severa en poblaciones de bajo nivel socioeconómico (36).

El análisis de los distintos indicadores utilizados en el estudio aquí descrito ilustra claramente que cada uno de ellos está midiendo un fenómeno diferente: así, el peso para la edad y el perímetro del brazo constituyen una especie de resumen de toda la historia nutricional del niño, incluyendo, en parte, su situación nutricional global del momento. La talla para la edad refleja únicamente la historia del niño, y está menos sujeta a fluctuaciones bruscas. El peso para la talla es un indicador más específico del estado nutricional calórico actual y lo mismo aplica a la medición del panículo adiposo, siendo más sensible esta última. El diámetro del brazo corregido para el panículo adiposo refleja un estado crónico de subnutrición fundamentalmente proteínica, ya que indirectamente mide el grado de muscularidad. Sin embargo, cabe subrayar que este indicador no fue lo suficientemente sensible como para establecer diferencias significativas entre los grupos de bajo nivel socioeconómico incluídos en este estudio, ni en términos de valores promedio, ni al expresar los datos en función de prevalencia de niveles bajos. Esta falta de sensibilidad se encuentra también en niños estudiados bajo condiciones metabólicas, ya que su coeficiente de correlación con el ICT en esas condiciones es menor de 0.5.

La relación nitrógeno de urea/creatinina refleja principalmente la ingesta proteínica en los días previos al estudio. En poblaciones subalimentadas, los valores de proteínas séricas totales tienen la enorme limitación de que mecanismos no siempre claramente definidos pueden elevarlos; entre ellos cabe indicar el alza de los niveles de $\gamma$ globulinas como consecuencia de estímulos antigénicos.

Según se sabe, los valores séricos de albúmina sólo son afectados significativamente en estadíos avanzados de deficiencia proteínica, aun cuando en casos individuales los niveles de albúmina desciendan lenta y no significativamente, conforme avanza un proceso de desnutrición proteínica. Por otro lado, el ICT parece ser un indicador sensible de descompensación nutricional proteínica, que refleja la masa muscular en relación a la talla. Este índice. por lo tanto, no está afectado por situaciones alimentarias de corta duración, y es normal

mientras el niño no esté sufriendo de un déficit proteínico-calórico prolongado que demande un catabolismo muscular acelerado. Una de las limitaciones del ICT es su gran variabilidad cuando la colección de orina se hace por períodos cortos (± 3 horas), en contraste con períodos de 24 horas o más (20-23, 32). El ICT obtenido en períodos cortos de colección de orina obviamente sólo puede servir para la clasificación de grupos de población, y no para definir la masa proteínica de casos individuales. Esta última puede definirse de manera individual únicamente por medio de colecciones de orina por períodos largos y preferiblemente repetidos (20-23).

La presente investigación subraya la importancia de ciertas mediciones antropométricas y bioquímicas en la definición del estado nutricional de grupos de niños de edad preescolar. Con base en los resultados obtenidos, se sugiere que el peso para la talla y el grosor del panículo adiposo tricipital son mediciones útiles para determinar el estado actual de nutrición calórica, y que el índice de creatinina/talla es la medición más sensible para determinar el estado de nutrición proteínica del momento, en estudios transversales.

## AGRADECIMIENTOS

Los autores agradecen sinceramente la valiosa colaboración de los Doctores Edmundo Avalos, Carlos Beteta y Juan José Urrutia, en lo que respecta a la obtención de muchos de los datos de que se da cuenta en este trabajo.

## SUMMARY

**Methods for evaluating protein-calorie nutritional s atus in preschool children from different socio-economic levels. Nutritional repercussions of measles in chronically undernourished children**

A total of 194 preschool age Guatemalan children from three socio-economic categories, were studied. These were divided into five groups: one group of children had high socio-economic standards; the second came from an intermediate status, and the last three corresponded to the low socioeconomic level. In the low socio-economic groups, 23 children were studied one month after an episode of measles. The following measurements were obtained: weight, height, arm circumference, tricipital skinfold, total serum protein and albumin concentration, urea/creatinine ratio and creatinine/height index in urine samples collected in a 3-hour timed period. With the exception of those children belonging to the high socio-economic

group, all others had frank retardation in weight and height for-age. However, all the other antropometric measurements, including weight-for-height, were predominantly normal in all groups, even though the number of children with subnormal values was higher in the low socio-economic groups. The post-measles group had a higher prevalence of subnormal skinfold values.

Biochemically, the urea/creatinine ratio was significantly higher in children from the high and medium socio-economic groups, than in those belonging to the low socio-economic level. Total serum proteins and albumin concentrations did not show differences suggestive of protein deficiency, either in the various socio-economic groups or in the post-measles group. Gamma globulins were found elevated in the children not pertaining to the high socio-economic group, and probably reflected poor environmental conditions. The creatinine/height index was clearly diminished in the post-infectious group, as compared to all the others, wich, according to this indicator, had normal muscle mass-for-height. The interpretation of each one of the indicators of nutritional condition tested in the study, is discussed.

The deleterious effects of a severe infection in suboptimally nourished children is also demonstrated at the field level. It appears that the nutritional impact of measles is more pronounced and longer-lasting in terms of protein nutrition than in terms of calorie nutrition.

## BIBLIOGRAFIA

(1) Scrimshaw, N. S. & M. Béhar. World wide occurrence of protein malnutrition. Fed. Proc., 18 (Suppl. 3): 82-88, 1959.

(2) Flores, M. & B. García. The nutritional status of children of preschool age in the Guatemalan community of Amatitlán. 1. Comparison of family and child diets. Brit. J. Nutr., 14: 207-215, 1960.

(3) Jelliffe, D. B. The Assessment of the Nutritional Status of the Community (With Special Reference to Field Surveys in Developing Regions of the World). Geneva, World Health Organization, 1966, 271 p. (WHO Monograph Series Nº 53).

(4) Gordon, J. E., J. B. Wyon & W. Ascoli. The second year death rate in less developed countries. Am. J. Med. Sci., 254: 357-380, 1967.

(5) Beisel, W. R., W. D. Sawyer, E. D. Ryll & D. Croxier. Metabolic effects of intracellular infections in man. Ann. Intern. Med., 67: 744-779, 1967.

(6) Scrimshaw, N. S., C. E. Taylor & J. E. Gordon. Interactions of Nutrition and Infection. Geneva, World Health Organization, 1968, 329 p. (WHO Monograph Series Nº 57).

(7) Viteri, F. Datos no publicados.

(8) Mata, L. J., J. J. Urrutia & A. Lechtig. Infection and nutrition of children of a low socioeconomic rural community. Am. J. Clin. Nutr., 24: 249-259, 1971.

(9) Mata, L. J., J. J. Urrutia & B. García. Effect of infection and diet on child growth: experience in a Guatemalan village. En: Nutrition and Infection. G. E. W. Wolstenholme and Maeve O'Connor, eds. London, Great Britain, J. & A. Churchill, Ltd., 1967, p. 112-126. (Ciba Foundation Study Group Nº 31).

(10) Gómez, F. Desnutrición. Bol. méd. Hosp. Infant. (México), 3: 543-551, 1946.

(11) Ramos Galván, R. Desnutrición y crecimiento físico. Bol. méd. Hosp. Infant. (México), 21 (Supl. Nº 1): 11-36, 1964.

(12) Jellife, D. B. & E. P. P. Jellife. Prevalence of protein-calorie malnutrition in Haitian preschool children. Am. J. Pub. Health, 50: 1355-1366, 1960.

(13) McFie, J. & H. F. Welbourn. Effect of malnutrition in infancy on the development of bone, muscle and fat. J. Nutrition, 76: 97-105, 1962.

(14) Arroyave, G. Proposed methodology for the biochemical evaluation of protein malnutrition in children. En: Protein-Calorie Malnutrition. A Nestlé Foundation Symposium. A. von Muralt, ed. Berlín, Springer-Verlag, 1969, p. 48-56.

(15) Hansen, J. D. L. Features and treatment of kwashiorkor at the Cape. Proceedings of a Colloquium held in Cambridge, April 1967. En: Calorie Deficiencies and Protein Deficiencies. R. A. McCance and and E. M. Widdowson, eds. London, J. & A. Churchill Ltd., 1968, p. 33-47.

(16) Whitehead, R. G. Rapid determination of some plasma aminoacids in subclinical kwashiorkor. Lancet, 1: 250-252, 1964.

(17) Arroyave, G., A. A. J. Jansen & M. Torrico. Razón nitrógeno ureico/creatinina como indicador del nivel de ingesta proteica. I. Efecto de la ingesta de agua sobre la excreción "basal" de urea y creatinina de niños con estados nutricionales diferentes. Arch. Latinoamer. Nutr., 16: 203-212, 1966.

(18) Whitehead, R. G. Hydroxyproline creatinine ratio as an index of nutritional status and rate of growth. Lancet, 2: 567-570, 1965.

(19) Mendes, C. B. & J. C. Waterlow. The effect of a low-protein diet, and of refeeding, on the composition of liver and muscle in the weanling rat. Brit. J. Nutr., 12: 74-88, 1958.

(20) Viteri, F. E. & J. Alvarado. The creatinine height index: its use in the estimation of the degree of protein depletion and repletion in protein calorie malnourished children. Pediatrics, 46: 696-706, 1970.

(21) Viteri, F. E. & J. Alvarado. Aspectos fisiopatológicos y respuestas adaptativas en la desnutrición calórica y en la desnutrición proteínica. Rev. Col. Méd. (Guatemala), 21: 175-230, 1970. Publicado también en: El Problema de la Desnutrición Proteínico-Calórica en el Istmo Centroamericano. Guatemala, INCAP, 1971, 245 p. (Monografía Nº 7 del Instituto de Nutrición de Centro América y Panamá).

(22) Viteri, F. E., J. Alvarado, D. G. Luthringer & R. P. Wood, II. Hematological changes in protein calorie malnutrition. Vitamins Hormones, 26: 573-615, 1968.

(23) Alleyne, G. A O, F. E. Viteri & J. Alvarado. Indices of body composition in infantile malnutrition: total body potassium and urinary creatinine. Am. J. Clin. Nutr., 23: 875-878, 1970.

(24) Stuart, H. C. & S S. Stevenson. Care and evaluation of well children Prysical growth and development. En: Textbook of Pediatrics. W. E Nelson, ed. 6th ed Philadelphia, W. B. Saunders Co., 1954, p. 10-66.

(25) Lange, K O. & J. Brózek. A new model of skinfold caliper. Am. J. Phys. Anthropol., 19: 98-99, 1961 (Abstract Nº 28).

(26) Interdepartmental Committee on Nutrition for National Defense. Manuel for Nutrition Surveys. 2nd. ed. Bethesda, Md, ICNND, 1963.

(27) Beckman Manual RM-1M-2. Model R-101. Microzone electrophoresis cell (Preliminary Instruction Manual). Palo Alto, California, Spinco Division, Beckman Instruments, Inc, Stanford Industrial Park, August, 1963, 38 p.

(28) Barker, S B The direct colorimetric determination of urea in blood and urine J. Biol. Chem, 152: 453-463, 1944. (c. f. Nutr. Abst. Revs., 14. 21, 1944-45 (Abst. 19).

(29) Clark, L. C Jr & H. L Thompson. Determination of creatine and creatinine in urine. Anal. Chem., 21: 1218-1221, 1949.

(30) Arroyave, G. & C. M de Arroyave. El uso de períodos cortos de recolección de orina en la estimación de la excreción diaria de creatinina. Arch Venez. Nutr., 12: 259-266, 1962.

(31) Mc Cammon, R W. with a foreword by A. Damon. Human Growth and Development. Sprinfield, Illinois, Charles C. Thomas, 1970, 295 p.

(32) Viteri, F. E, J. Alvarado & G. A. O. Alleyne. Reply to Drs. Méndez and Burkirk. Am. J. Clin. Nutr., 24: 386-387, 1971.

(33) Holmes, E. G, M. W. Stanier & M. D. Thompson. The serum protein pattern of Africans in Uganda: relation to diet and malaria. Trans. Roy. Soc. Trop. Med. Hyg., 49: 376-384, 1955.

(34) Arroyave, G, N S Scrimshaw, O. Pineda & M. A. Guzmán. Electrophoretic pattern of hyperproteinemic sera in a population group of rural Panama Am. J. Trop. Med. Hyg., 9: 81-84, 1960.

(35) Alvarado, J, F. E. Viteri & M. Béhar. Tratamiento hospitalario de la desnutrición proteínico-calórica severa. Rev. Col. Méd. (Guatemala), 21: 231-245, 1970. Publicado también en: El Problema de la Desnutrición Proteínico-Calórica en el Istmo Centroamericano. Guetemala, INCAP, 1971, 245 p. (Monografía Nº 7 del Instituto de Nutrición de Centro América y Panamá).

(36) Scrimshaw, N. S, M. Behar, F. Viteri, G. Arroyave & C. Tejada. Epidemiology and prevention of severe protein malnutrition (kwashiorkor) in Central America. Am. J. Pub. Health., 47: 53-62, 1957.

# Clasificación funcional de poblaciones desnutridas en la República de El Salvador [1].

## Desarrollo metodológico

**V. Valverde, F. Trowbridge, I. Beghin, B. Pillet, Isabel Nieves, Nancy Sloan, T. Farrell, P.R. Payne, J.L. Joy y R.E. Klein**

*Generalmente, los típicos programas gubernamentales sobre nutrición carecen de datos precisos sobre la magnitud de los grupos que podrían beneficiarse de las intervenciones, sobre los diferentes grupos de posibles beneficiarios, así como su ubicación; y acerca de las características socioeconómicas y culturales que pueden servir para identificarlos fácilmente. En el sistema de clasificación funcional para la determinación de problemas nutricionales, se recogen datos detallados sobre el comportamiento humano y las limitaciones sociales a nivel familiar y comunitario. Los datos se interpretan luego en términos generales con el fin de deducir cómo contribuyen estos factores a crear situaciones de insuficiencia nutricional en grupos más amplios. Mediante este nuevo sistema, se podrían proponer a los planificadores y responsables medidas más eficaces para reducir el número de los que viven en condiciones de penuria.*

### El concepto

Se ha recogido una gran cantidad de datos sobre malnutrición infantil en América Central y Panamá desde los años treinta. Esta labor ha culminado con los estudios recientes realizados por el Instituto de Nutrición de Centroamérica y Panamá y la Oficina de Investigación Internacional, Institutos Nacionales de Sanidad (1972), en Honduras, por el Sistema de Análisis y Planificacion de la Alimentación y Nutrición (1976), y por el Instituto de Nutrición de Centroamérica y Panamá Unidad de Análisis del Sector Salud (1976), en Nicaragua.

Dichos datos y estudios son, sin embargo, de utilidad limitada tanto para los planificadores como para los administradores, especialmente si se trata de establecer prioridades por regiones en un país, de escoger programas adecuados para resolver problemas nutricionales, y de elaborar proyectos específicos para determinadas regiones y subgrupos de familias en las mismas.

Por ejemplo, no es posible poder responder a las preguntas « qué intervención » y « para quién » con los datos agrupados a nivel nacional. Este hecho acentúa la necesidad de definir los problemas nutricionales de los países en desarrollo en particular, no sólo de forma práctica, sino también para que puedan ser de una utilidad inmediata.

Tal sistema permitiría, además, una mejor comprensión de la relación mutua entre los factores que causan los problemas nutricionales. Un aspecto fundamental es el carácter heterogéneo de la población de un país, así como la presencia

[1] El estudio sobre el terreno en que se basa este documento fue patrocinado, en parte, por el Gobierno de El Salvador, la Fundación Ford, la Fundación W.K. Kellogg, la Misión del USAID en El Salvador y ROCAP/AID.

Los autores, en octubre de 1977, formaban parte del personal del Instituto de Nutrición de Centroamérica y Panamá (INCAP), Guatemala, C.A., excepto F. Trowbridge, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland; P.R. Payne, Department of Human Nutrition, London School of Hygiene and Tropical Medicine; y J.L. Joy, Department of Nutritional Sciences, University of California, Berkeley, California.

de grupos profesionales, sociales y culturales característicos. Estas diferencias afectan tanto al proceso por el que la malnutrición llega a constituir un problema, como al proceso por el que se puede mejorarla o erradicarla.

Percatándose de esto, Joy (1973) introdujo el concepto de « clasificación funcional de poblaciones desnutridas », es decir, la agrupación de las poblaciones en categorías que pueden utilizarse en la planificación de la nutrición y del desarrollo. Cada categoría funcional posee una serie de características comunes, a saber, el mismo problema nutricional, o la pertenencia a un grupo identificable (geográfico, socioeconómico, etc.). La tercera característica[2] es que los miembros de un determinado grupo tienen una elevada probabilidad de responder del mismo modo a determinadas intervenciones. En este documento se añade una cuarta categoría: la posibilidad de llegar al grupo a través de las actuales divisiones y estructuras administrativas.

Hakim y Solimano (1976) y Payne (1976) examinaron las hipótesis en las que se basaban los anteriores esfuerzos para integrar componentes nutricionales en planes nacionales en los países en desarrollo. La necesidad de definir los problemas nutricionales y alimentarios en el marco de una clasificación funcional ha sido subrayada por Joy y Payne (1975) y la FAO (1975).

Abercrombie (1975) señaló también la importancia de identificar diversas categorías de personas pobres como medida inicial para resolver la situación de penuria. En un trabajo recientemente realizado en cuatro comunidades agrícolas rurales de economía de subsistencia en Guatemala oriental, Valverde *et al.* (1977) cuantificaron y clasificaron las familias según el tamaño de las explotaciones, la ocupación y el estado nutricional de sus hijos.

La « clasificación funcional » de poblaciones malnutridas, por tanto, es esencialmente un nuevo método de recoger y presentar datos. Difiere de los sistemas anteriores en que: (1) comienza por la identificación de distintas clases de personas afectadas en una determinada región; (2) estudia más profundamente sus problemas, comportamiento y expectativas particulares; (3) calcula la magnitud de cada grupo; y (4) trata de evaluar sus respectivos problemas por regiones o subregiones. El hecho de presentar en forma desglosada los datos recogidos permite reunificarlos después según las necesidades del planificador: a nivel nacional, por regiones, por direcciones administrativas, por estratos socioeconómicos o por tipo de empleo. Ello, a su vez, facilitará la identificación de los programas pertinentes, que tienen por objeto reducir la malnutrición de grupos de familias o de individuos en una determinada región.

Un primer intento por aplicar este tipo de metodología se ha realizado en El Salvador, donde el Gobierno había expresado su interés en este asunto y ofrecido su colaboración.

[2] Esencial para el método de Joy, aunque no aparece específicamente identificado en la referencia citada.

## Establecimiento de la metodología

### Objetivos y organización del proyecto

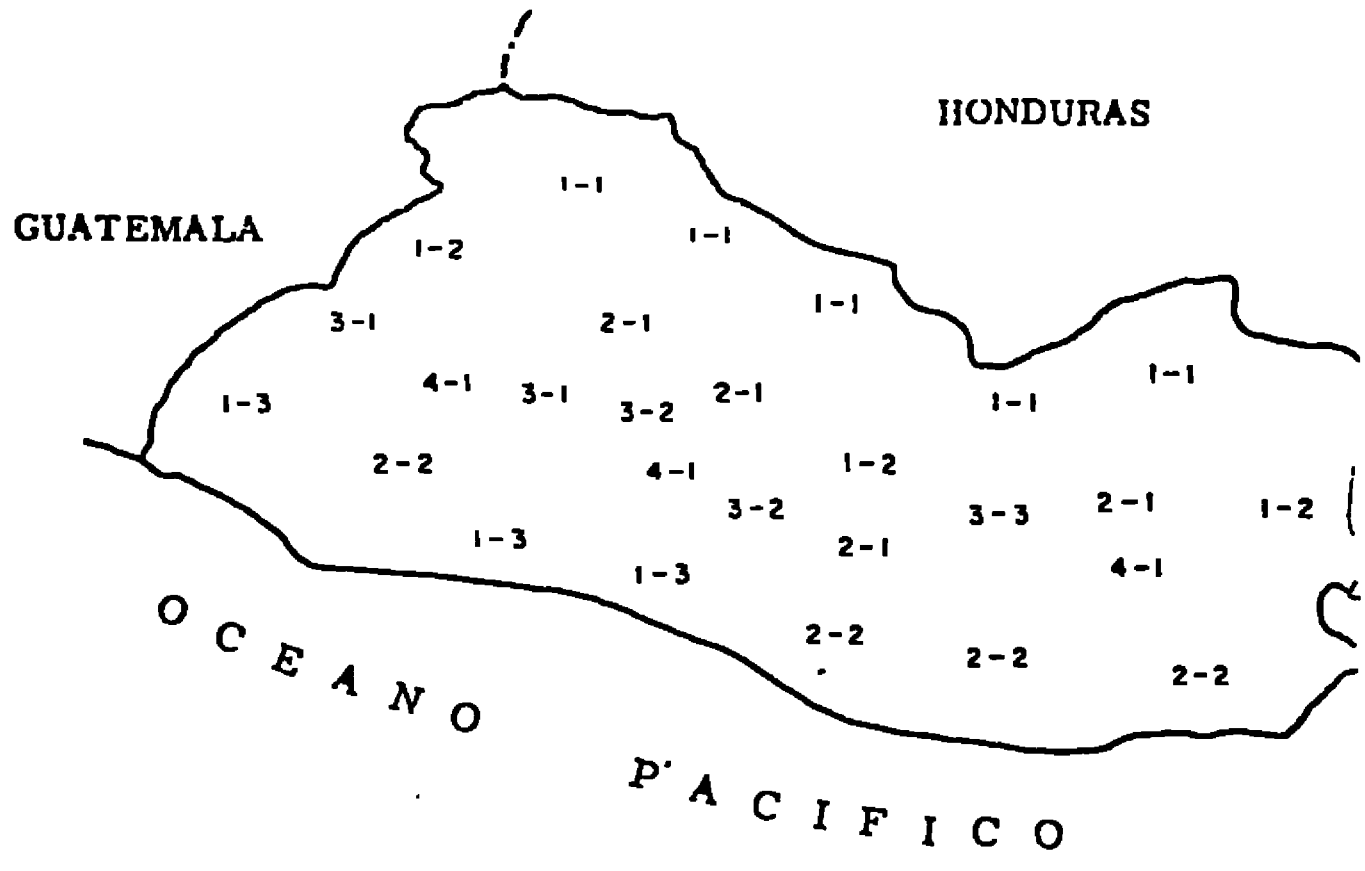
Los objetivos del proyecto realizado en El Salvador fueron, primero, actualizar e integrar los datos sobre nutrición y socioeconómicos, con el fin de orientar los programas existentes, y los que se habían de determinar en la futura política nacional en materia de alimentación y nutrición; y, segundo, establecer una metodología para elaborar una clasificación funcional.

Se creó un comité asesor multidisciplinario para definir la naturaleza y el tipo de datos que habían de recogerse, establecer los métodos de recopilación, preparar un plan analítico provisional y dirigir las actividades generales de campo. Se redactó una lista de nueve preguntas que se detallan en el Cuadro 1.

### Fuentes de información en el país

Investigando en las publicaciones de datos generales sobre las condiciones políticas, económicas, demográficas y agrícolas de El Salvador, se pudo obtener material bastante útil, por ejemplo, censos de la vivienda, demográficos y agrícolas de 1971, la lista de límites administrativos de cada municipio[3] del país, y el censo demográfico de 1976, en el que se determina el número de casas y la

[3] El Salvador se ha dividido, desde el punto de vista administrativo, en 14 departamentos, 261 municipios y 2 057 cantones.




1-1 Subregion septentrional de explotacion agricola marginal
1-2 Subregion central de explotacion agricola marginal
1-3. Subregion costera de explotacion agricola marginal
2 1 Subregion central de explotacion agricola intensiva
2 2 Subregion costera de explotacion agricola intensiva
3 1 Subregion occidental de explotacion cafetalera
3-2 Subregion central de explotacion cafetalera
3 3 Subregion oriental de explotacion cafetalera
4-1. Grupos urbanos

**Figura 1.** Ubicación de las subregiones agrícolas y grupos urbanos de El Salvador

CUADRO 1 PREGUNTAS A LAS QUE HA DE RESPONDER EL PROYECTO DE CLASIFICACIÓN FUNCIONAL

1. ¿Qué regiones de El Salvador, descritas en términos geográficos y administrativos, tienen más problemas de malnutrición?
2. ¿Cuáles son las características sociales, económicas y sanitarias generales de los grupos de población que viven en diferentes regiones?
3. ¿Cual es la población total de cada región y subregión? ¿Cuántos niños en edad preescolar malnutridos hay en cada region? ¿Cuál es el número aproximado de mujeres lactantes y gestantes?
4. ¿Cual es la importancia relativa de los diferentes factores sociales, economicos y culturales como causas de malnutrición en cada region, y en las distintas categorias de poblacion?
5. ¿Cual es la magnitud de estas categorías de población y cual su estado nutricional?
6. ¿Qué tipo de programas son más importantes para las diferentes regiones y categorias de poblacion? ¿Cuantas personas responden a los programas identificados?
7. ¿Cuál es la viabilidad politica, economica y operativa de los programas propuestos? ¿Quienes seran en definitiva los beneficiarios de los mismos?
8. ¿Qué cantidad de recursos economicos se necesita para reducir sensiblemente el numero de familias que padecen malnutricion en las diferentes regiones?
9. ¿Como pueden detectarse los cambios del estado nutricional, utilizando los actuales sistemas de informacion?

poblacion total de cada canton. Se descartó otro material, cuando los datos no podian desglosarse por regiones o subregiones, o los métodos utilizados al recogerlos no se consideraron suficientemente fidedignos.

## DEFINICIÓN DE REGIONES Y LÍMITES ADMINISTRATIVOS

Cuando se elaboró el proyecto, se estaban revisando las divisiones regionales del país. Fue necesario, por tanto, delimitar las regiones especificamente para el proyecto. Esto se realizó teniendo en cuenta los diferentes sistemas de utilización de tierras suponiendo que cada uno de ellos correspondería a: (1) ambientes socioculturales más o menos homogéneos, y (2) tipos análogos de problemas nutricionales.

Con el fin de facilitar datos útiles para los planificadores y administradores, se eligió el municipio como la unidad administrativa más conveniente, ya que no se disponía de datos sobre los límites cantonales. Los mapas de utilización de tierras y otras encuestas y datos censales existentes permitieron dividir el país en tres regiones agrícolas: (1) *explotación agrícola marginal o de subsistencia*; (2) *explotación agrícola intensiva* (cultivos comerciales para la exportación); y (3) *producción de café*. En una cuarta categoría « urbana » se incluyeron todos los cantones y capitales de municipios que tenían 10 000 habitantes o más en 1976. Se establecieron subregiones, tomando como base criterios geográficos hasta formar un total de nueve grupos ubicados como aparecen en la Figura 1. Las cuatro regiones se utilizaron como marcos de muestra para todas las actividades de campo posteriores. En el Cuadro 2 se resumen los resultados de la clasificación inicial por regiones y subregiones, en términos de la superficie de las tierras y de la población.

## USO DE LOS DATOS CENSALES

Los datos demográficos y los censos de la vivienda iniciales se agregaron y resumieron a nivel familiar para cada uno de los 2 057 cantones de El Salvador. Las variables incluían, sexo y distribución por edades, estado civil, número de miembros de la familia, ocupación, grado de alfabetización, asistencia escolar, grado de enseñanza alcanzado, mortalidad de recién nacidos y niños, fertilidad, situación en cuanto a propiedad de la casa, tipo de casa, fuentes de agua, evacuación de desechos e industrias domésticas. Estas variables se subdividieron ulteriormente; por ejemplo, el agua por fuentes, etc. Se calcularon totales y porcentajes para cada variable y sus respectivas subdivisiones. Estos datos, reunidos y resumidos por cantones, se exponen ahora en forma flexible y pueden utilizarse fácilmente para preparar ficheros secundarios a nivel municipal, o pueden reunirse según varios otros criterios, como las superficies abarcadas por los centros sanitarios, servicios de extensión agrícola, o pueden interpretarse como promedios o porcentajes nacionales. Pueden utilizarse también para señalar las características especiales de los cantones, como por ejemplo, elevado índice de desempleo, bajo nivel de escolaridad, etc.

## ENCUESTAS DE CAMPO

Todos los datos considerados esenciales para el proyecto, pero no obtenidos de estudios en curso, se reunieron mediante encuestas sobre el terreno. Se realizaron tres tipos de estudio, evaluación del estado nutricional, evaluación socioeconómica de las familias, y estudios etnográficos descriptivos de la vida comunal.

CUADRO 2 CLASIFICACIÓN SEGÚN LA POBLACIÓN Y LA REGIÓN

| Región | Subregión | Superficie | Poblacion | Densidad |
|---|---|---|---|---|
| | | *km²* | | *pob./km²* |
| Urbana | Urbana [1] | — | 1 154 590 | — |
| Café | Occidental | 1 437 | 304 679 | 212 |
| | Central | 681 | 124 065 | 182 |
| | Oriental | 408 | 104 975 | 257 |
| TOTAL | | 2 526 | 533 719 | 211 |
| Explotacion agricola intensiva | Central | 337 | 137 903 | 409 |
| | Costera | 3 605 | 497 905 | 138 |
| TOTAL | | 3 942 | 635 808 | 161 |
| Explotación agricola marginal | Septentrional | 6 560 | 745 388 | 114 |
| | Central | 4 474 | 719 077 | 161 |
| | Costera | 3 531 | 436 798 | 124 |
| TOTAL | | 14 565 | 1 901 263 | 130 |

[1] Todos los cantones y capitales municipales con una poblacion de 10 000 habitantes o más

*Evaluación del estado nutricional*

Este aspecto de la investigación tenía por objeto evaluar el estado nutricional de los lactantes y los niños en edad pre-escolar Dicha evaluación fue necesaria para identificar y establecer las diferencias potencialmente importantes que existían en cuanto al estado nutricional entre las regiones, y entre las categorías demográficas en cada región. Se tomó una muestra de 6 409 niños de ambos sexos, comprendidos entre los 6 y los 59 meses de edad, procedentes de 148 comunidades distribuidas en todas las regiones. Se realizaron mediciones de peso, altura y circunferencia del brazo mediante visitas a domicilio

La muestra urbana solamente incluyó a niños que vivían en las chabolas de San Salvador Se recogieron también medidas antropométricas de una muestra nacional de 787 niños de 6 meses a 59 meses de edad, comprendidos en una encuesta nacional sobre el estado en cuanto al consumo de vitamina A realizada por otro grupo del INCAP en 1976 Estos datos se compararon luego con los valores establecidos para niños normales en los países desarrollados, y los resultados se analizaron a nivel nacional, regional, y, en algunos casos, subregional

*Evaluación socioeconómica de las unidades familiares*

Sobre la composición, ocupación, instrucción, migración diferentes indicadores de salud e ingresos, y producción agrícola de la familia se reunieron datos socioeconómicos de dos grupos de la población para identificar las características familiares asociadas con la malnutrición en cada región, y los factores conexos comunes a todas las regiones, y para respaldar con datos cuantitativos los estudios descriptivos de la comunidad Realizada la encuesta sobre el estado nutricional en las cuatro regiones, se seleccionó, con fines comparativos, un total de 625 familias « de bajo índice de riesgo/bien nutridas » y 625 « de elevado índice de riesgo/malnutridas » (que tuvieran por lo menos un niño con un 75% de suficiencia de peso por edad)

*Estudios descriptivos sobre la vida comunitaria*

La razón fundamental por la que se realizan estos estudios fue la de conocer mejor la relación compleja existente entre los factores sociales, culturales y económicos, y los procesos relacionados con la nutrición y la salud

Se seleccionaron cuatro comunidades, consideradas representativas de las regiones respectivas, según criterios demográficos, geográficos y económicos Los etnógrafos vivieron en cada comunidad durante seis a ocho semanas, aplicando los metodos clásicos de investigación antropológica: observación mediante la participación y entrevistas Los datos se recogieron de acuerdo con una guía práctica, especialmente elaborada para este estudio, después de consultar con el comité asesor Posteriormente, se visitaron otras comunidades de la misma región, con objeto de evaluar la aplicabilidad de las observaciones Por ultimo, las notas obtenidas sobre el terreno se organizaron, analizaron, interpretaron y presentaron en informes separados, de conformidad con los subtitulos indicados en el Cuadro 3.

CUADRO 3 INFORMES SOBRE LA VIDA COMUNITARIA

MODELOS DE SUBSISTENCIA
- — Actividades economicas y sistemas de tenencia de tierras
- – Economia y servicios comunitarios
- — Economía domestica

ALIMENTACIÓN Y NUTRICION
- – Elaboracion y almacenamiento de alimentos
- — Regimen y habitos alimentarios
- — Alimentacion de lactantes y niños
- — Creencias y actitudes con relacion a los alimentos

SANIDAD E HIGIENE
- — Saneamiento del medio
- — Higiene personal de las madres los niños
- — Uso de servicios medicos disponibles
- – Creencias y costumbres asociadas

PARTO Y CRIANZA
- — Relaciones hombre mujer y reproduccion
- – Embarazo y parto
- – Crianza

COMUNICACION, ENSEÑANZA, CAMBIO E INNOVACION
- — Comunicacion
- – Cambio e innovacion
- – Enseñanza y oportunidades analogas

## ANÁLISIS DE DATOS

El proceso de análisis de datos tenía por objeto principalmente responder a las preguntas enumeradas en el Cuadro 1 Para las dos primeras, sobre las diferencias regionales y el estado nutricional, se utilizaron los datos antropométricos y del censo de la población de 1976 La caracterización de las regiones en términos socioeconómicos se basó principalmente en los datos disponibles a nivel cantonal y/o municipal, mediante un nuevo análisis de los censos de la población, la vivienda y la agricultura realizados en 1971.

Los datos sobre el estado nutricional de los niños procedentes de diferentes categorías de familias (preguntas 3, 4 y 5) se basaron en los datos obtenidos de las encuestas socioeconómicas y antropométricas. Se estimó el estado nutricional de los niños procedentes de diferentes tipos de familias, y se cuantificó la magnitud total de estas categorias utilizando los censos de población realizados en 1971 y 1976

Para los temas referentes al tipo, importancia y costo de las intervenciones nutricionales (preguntas 6, 7 y 8) hubo que agrupar todos los datos recogidos Por ejemplo los datos permitieron analizar el efecto y la respuesta de subgrupos o poblaciones específicas en una determinada región, es décir, la respuesta de los agricultores sin tierras en regiones de economía de subsistencia a los programas de aumento de la disponibilidad de tierras y de la producción, de salarios mínimos, servicios higiénicos, enseñanza, y organización de la comunidad

Por ultimo, con respecto a la inspección nutricional (pregunta 9), los estudios antropometricos regionales necesarios para la ejecución de este proyecto sirvieron para corroborar los datos sobre nutrición de la infancia, recopilados por funcionarios gubernamentales en centros sanitarios Esta información, reunida habitualmente por los servicios sanitarios, se compone de un diagnóstico médico básico de la malnutrición y de datos sobre el peso de todos los niños menores de cinco años, que se registra en algunas zonas en la primera visita anual a la clínica Como consecuencia de esta convalidación

de los datos recogidos en centros sanitarios. En 1977, el Gobierno, utilizando su sistema de datos, comenzó a aplicar un programa de vigilancia de la nutrición. En años futuros se incorporarán otros indicadores, una vez refinados y convalidados

## Mejoras futuras

Aunque se recogieron muchas enseñanzas útiles de este primer ensayo de establecimiento de una metodología un examen crítico de la labor llevada a cabo en El Salvador pone de manifiesto ciertos aspectos que conviene mejorar en la futura elaboración de la clasificacion funcional Se analizan a continuación tres de dichos aspectos

### Procedimientos organizativos

El estudio reveló que los estudios sobre la comunidad y los perfiles familiares podrían realizarse en un periodo de unas 10 a 12 semanas, segun la importancia y diversidad de la comunidad y la naturaleza especifica del problema Lo ideal sería encargar esta labor a antropólogos capacitados No obstante si se dispone de una apropiada y detallada guía practica, será suficiente un personal bien preparado y competente en ciencias sociales Además, este tipo de actividad de campo debe ser precedido de un examen exhaustivo de la documentación relativa tanto a los problemas nutricionales como al medio ambiente cultural del lugar en que se ha de realizar la investigacion

Asimismo, importa especialmente que ese tipo de investigación sea supervisado cuidadosamente por profesionales competentes en antropologia social o cultural, y que todos los miembros del equipo conozcan bien la finalidad del sistema de clasificación funcional.

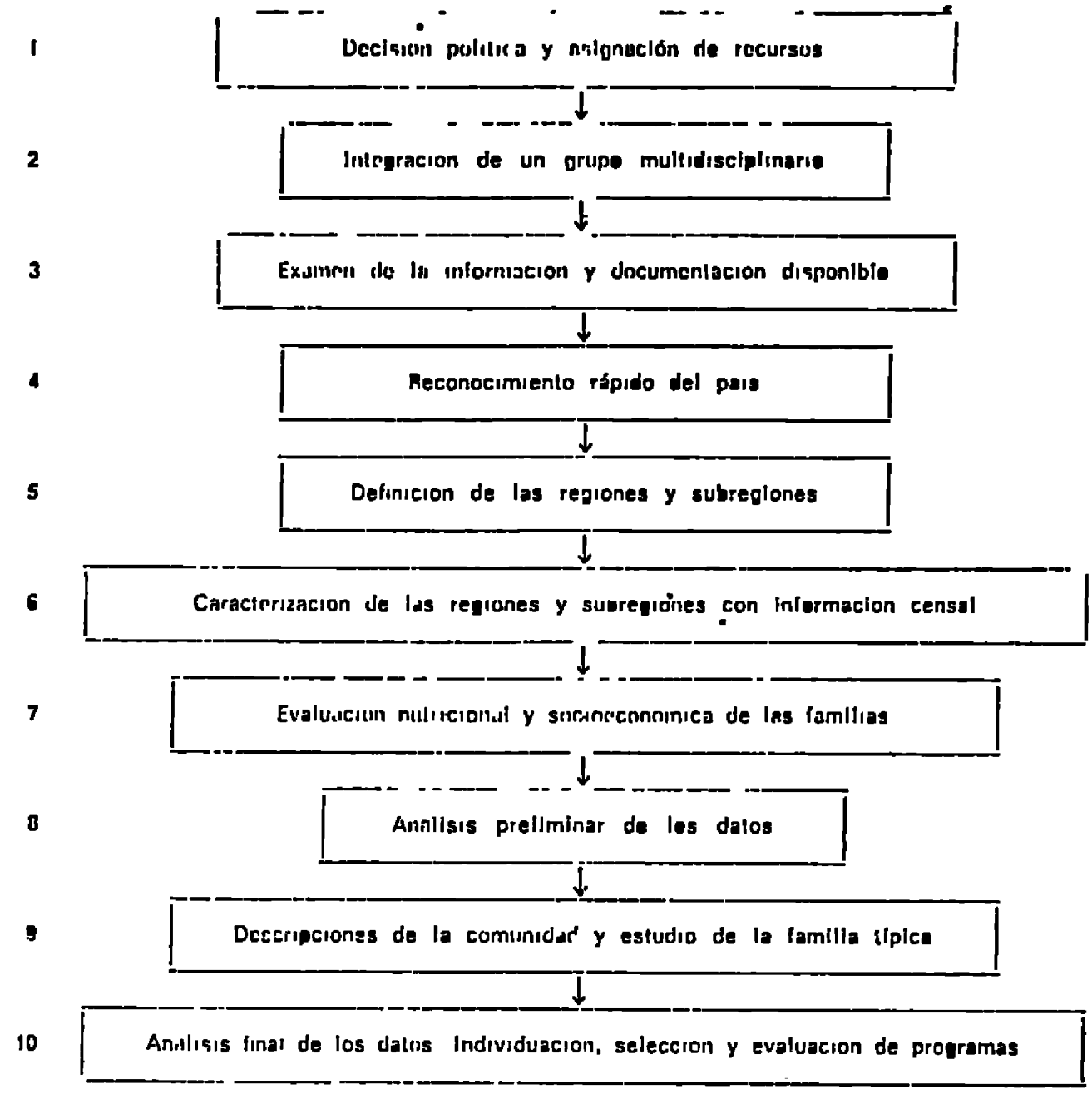


Figura 2. Orden sugerido de etapas para elaboracion de la clasificacion funcional

### Tipos de datos que han de reunirse

La estrategia más eficaz que debe utilizarse en ese tipo de proyectos es, sin duda, la de los estudios antropometricos Sin embargo, el tamaño utilizado en las regiones fue mayor del necesario Los datos sobre 700 a 800 niños, tomados de 20 a 25 lugares escogidos al azar en cada región, habrían proporcionado información fidedigna sobre la extensión de la malnutrición infantil en las distintas regiones Aunque hubiesen sido convenientes, los datos referentes a otras deficiencias nutricionales en grupos de adultos (por ejemplo, anemia por falta de hierro en mujeres embarazadas), habrian aumentado notablemente los costos

La encuesta socioeconomica a nivel familiar tiene una importancia decisiva para todo el estudio, por lo que se deberia decidir y establecer oportunamente una definicion detallada de las variables que habrán de utilizarse en el proyecto En la encuesta socioeconómica habría que incluir a todas las familias escogidas para los estudios antropométricos, ya que, en los países en que o no existen datos censuales o éstos no son fidedignos, el carácter representativo de los datos socioeconómicos es de importancia vital para el proyecto

Los estudios descriptivos de la vida comunitaria han demostrado ser instrumentos utiles para distinguir los problemas de diferentes regiones e identificar las medidas pertinentes para resolverlos

Los estudios de observación de los perfiles de familias típicas que no están incluidos en este estudio, junto con las descripciones de la comunidad, habrían aclarado la naturaleza del problema Estos estudios y descripciones serán útiles en el futuro para mejorar el proceso de identificación de las medidas pertinentes para tipos especificos de familias, así como la respuesta potencial a las intervenciones De hecho, los perfiles familiares son esenciales en un sistema de clasificación funcional, ya que, junto con las descripciones de las comunidades, ayudan a dar una respuesta a preguntas como las siguientes:

- ¿Cómo ha de cambiar la situación financiera familiar para que disminuya sensiblemente su riesgo de malnutrición?
- ¿De qué manera podrían cambiarse los hábitos alimentarios de una familia con objeto de reducir su riesgo de malnutrición?
- ¿Mediante qué formas de actividad social se podrían obtener los cambios necesarios?
- ¿Hasta qué punto podría resolverse de este modo el problema general?

En una serie tan complicada de estudios, es necesario planificar y dividir cuidadosamente cada fase, y evaluar periódicamente las actividades. En la Figura 2 se resume gráficamente un orden teórico de etapas para elaborar la clasificación funcional.

Una clasificación funcional puede utilizarse para inducir a los poderes públicos a asignar recursos, pero debe ir precedida de decisiones políticas y de la asignación de recursos para combatir la malnutrición. De no ser así, la elaboración de la clasificación puede resultar simplemente un ejercicio académico.

La segunda etapa consiste en establecer un grupo que se encargue directamente del proyecto. Lo ideal sería que en el grupo participaran quienes hubieran de utilizar los datos recogidos.

La tercera etapa consiste en un examen amplio de los datos relativos a preparación de planes de alimentación y nutrición, así como los datos sobre las características geográficas, demográficas, sociales, económicas, agrícolas y de otro tipo del país. Las decisiones sobre el grado de desglose deseable de los datos para fines prácticos deberían adoptarse en las fases iniciales del proyecto. Los ficheros de datos, al nivel escogido, se establecerían inmediatamente.

La cuarta etapa, decisiva para todo el proceso, consiste en una serie de visitas en todo el país para obtener un conocimiento intuitivo de la naturaleza del problema. Tales visitas deberían incluir conversaciones oficiosas con un grupo representativo de residentes locales sobre los problemas que ellos consideran como más apremiantes. Es también valiosa la observación directa de las actividades relacionadas con la producción, elaboración y preparación de los alimentos.

Estas visitas sirven de gran ayuda para preparar el equipo mecánico de recogida de datos y prever posibles problemas en los procedimientos de toma de muestras.

La quinta etapa consiste en dividir el país en regiones convenientes. El grupo deberá examinar los pros y contras de utilizar las divisiones regionales actuales del país o de establecer otras nuevas. Cualquiera que sea la decisión final, deberán definirse explícitamente los límites administrativos de cada región.

La sexta etapa consiste en la caracterización de regiones y subregiones en término socioeconómicos generales. Esto debe realizarse teniendo en cuenta todos los datos recogidos hasta el momento por el proyecto, más el material censal. En este punto, es esencial examinar los ficheros de datos censales con expertos en estadística y programadores de computadoras, ya que, por ejemplo, la reunificación o desglose de los datos, y su transferencia a un sistema de computadora diferente, y el análisis subsiguiente pueden resultar muy caros. Tales exámenes deberán asegurar que la serie de datos que se obtenga sea lo suficientemente flexible para los distintos análisis subsiguientes. La comprobación de la homogeneidad de las regiones por variables socioeconómicas puede dar lugar a una nueva valorización del desglose regional inicial. El levantamiento gráfico de estas variables indicará si la división regional inicial es o no adecuada.

Así, las regiones que disponen de datos demográficos servirán de bases de muestreo para la evaluación del estado nutricional, las características socioeconómicas de las familias, los estudios descriptivos y cualquier otra información pertinente que haya de reunirse.

Una vez que se hayan establecido las bases del muestreo, se puede proceder a la séptima etapa, la de las evaluaciones nutricionales y socioeconómicas. Los etnógrafos deberían participar en los equipos de estudios antropométricos de campo que visitan a las comunidades, para comenzar a identificar, en regiones y subregiones, las comunidades que satisfacen los requisitos para los estudios descriptivos sobre la vida comunitaria y los perfiles familiares. Estas visitas proporcionarán también a los etnógrafos una excelente oportunidad para obtener información general sobre las regiones en su conjunto, y empezar a recoger notas sobre la vida comunitaria.

Como octava etapa, y antes de comenzar los estudios descriptivos a nivel comunitario, conviene hacer un primer intento de análisis de datos. Habría que comenzar identificando categorías de población por región, su tamaño, frecuencia de los problemas de malnutrición y sanitarios, factores asociados, etc. Ello servirá de orientación para seleccionar los tipos de familias que han de incluirse en los estudios de perfiles, y centrará estos últimos en problemas específicos de categorías diversas de población. No obstante, el etnógrafo deberá tener cuidado de que estos análisis preliminares no influyan en sus observaciones:

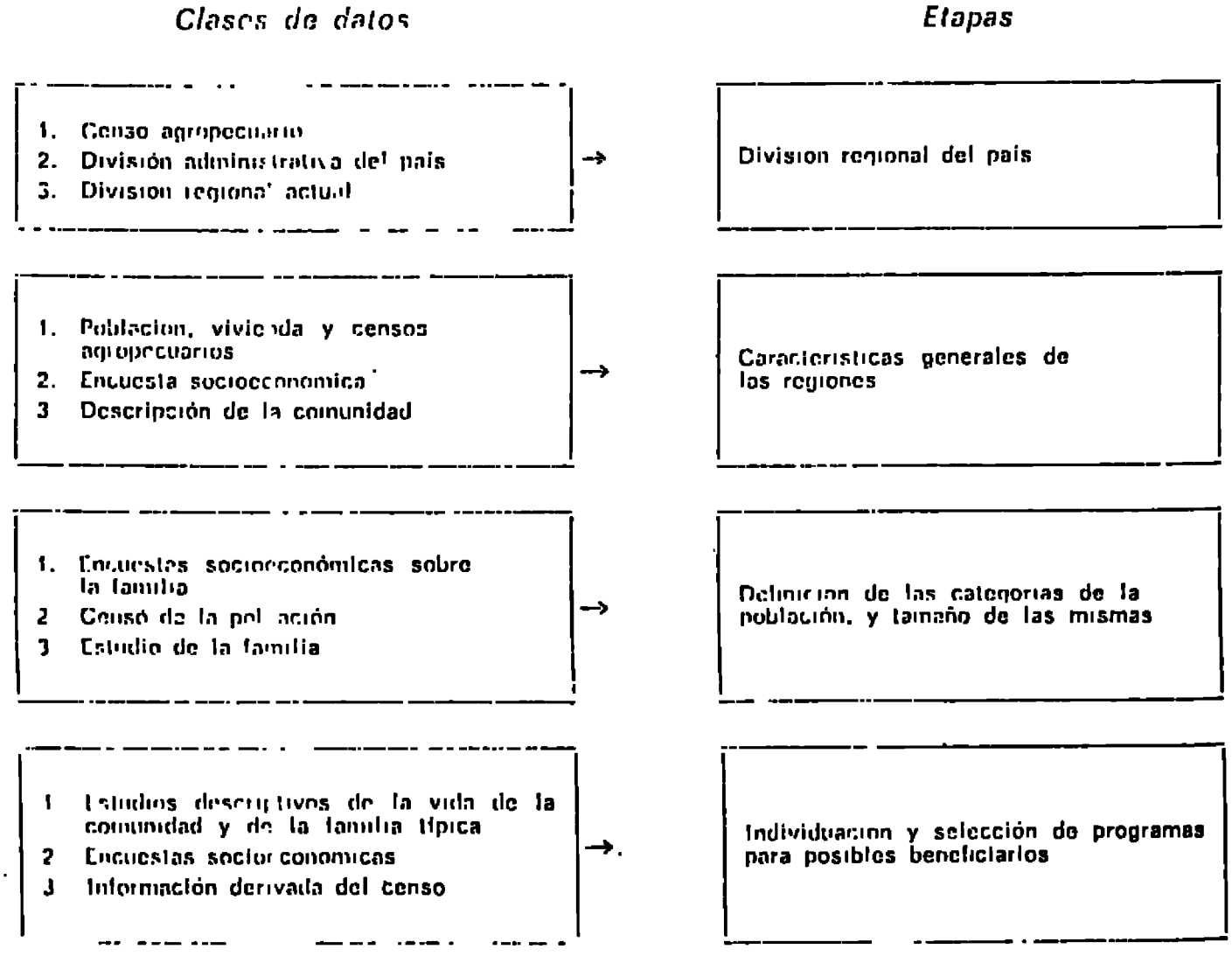



Figura 3. Clases de datos para diversas etapas de los métodos de clasificación funcional

Como novena etapa seguirán las descripciones de comunidades y los perfiles familiares.

La décima etapa consiste en clasificar las categorías de población por regiones y subregiones, calculando su magnitud e identificando y evaluando posibles programas.

Por último, las intervenciones seleccionadas pueden integrarse en un plan general de « alimentación y nutrición », « reducción de la pobreza », « mejoramiento de las condiciones de vida », « desarrollo rural » o cualquier otro título conveniente.

La aportación potencial de las diferentes fuentes de datos en la labor de integración se ilustra, a modo de ejemplo, en la Figura 3.

## Conclusión

El estudio realizado en El Salvador confirma la preocupación inicial de los autores sobre las dificultades que plantea la identificación de categorías de grupos que viven en situación de penuria en una población. No obstante, basándose en este estudio y otros trabajos de Valverde *et al.* (1977) y Rawson y Valverde (1976), los autores estiman que pueden superarse los problemas metodológicos que plantea la adopción de un sistema de clasificación funcional.

La elaboración de una clasificación funcional permite comprender mejor las causas de la malnutrición, pero no sirve necesariamente para concebir programas nuevos y milagrosos, materialmente distintos de los que suelen realizarse en los países en desarrollo. Por consiguiente, no hay que esperar que con este método van a obtenerse nuevas respuestas definitivas o globales. Siempre será necesario examinar constantemente los resultados y análisis con los planificadores y administradores.

Un sistema de clasificación funcional no sólo es valioso por el tipo de información que proporciona para la elaboración de programas, sino también porque, durante su elaboración, plantea cuestiones fundamentales sobre el proceso de desarrollo del país, y muestra cómo ciertos programas no concebidos principalmente para reducir la malnutrición pueden contribuir considerablemente al mejoramiento de la nutrición. Un examen de los programas a la luz de la clasificación funcional, y la selección final de las mejores alternativas probablemente darán lugar a la evaluación de la viabilidad política y económica de las medidas propuestas.

Las actividades llevadas a cabo en El Salvador cuestan, aproximadamente, 100 000 dólares EE.UU. En otro país de extensión parecida, los costos de una labor de este tipo podrían reducirse notablemente. Actualmente no es posible estimar el costo de las actividades posteriores a esta primera fase. Es evidente que, para determinar el valor real de la clasificación funcional, habrá que comparar los costos y los resultados con los métodos tradicionales de evaluación del estado nutricional.

Se construyen carreteras en El Salvador
Intervención en el desarrollo rural

La experiencia adquirida en El Salvador demuestra también que los gastos marginales extraordinarios que supone estudiar la estructura completa en vez de centrar las actividades de reunión de datos en categorías críticas de familias malnutridas, son pequeños si se comparan con el costo total necesario para establecer el mecanismo de evaluación. Además, si se intenta seriamente definir con claridad el problema de la nutrición, el proceso de elaboración de una clasificación funcional deberá considerarse, entonces, como un proceso constante y no como una actividad que se realiza una vez para siempre.

Las principales conclusiones de este proyecto se han comunicado al Gobierno de El Salvador. Actualmente, el Ministerio de Planificación está determinando la política alimentaria y nutricional nacional del país. Teniendo en cuenta la política nacional, se determinarán las actividades específicas que habrán de incluirse en el Plan de Alimentación y Nutrición. El Ministerio de Planificación está utilizando los datos del proyecto para determinar el tipo y la situación geográfica de los programas que habrán de emprenderse.

## Bibliografía


ABERCROMBIE, K.C. ¿Quiénes son y dónde 1975 están? *Ceres*, 45: 49-51.

FAO. *Food and nutrition planning*. Ro- 1975 me. 105 p. Nutrition Consultants Report Series Nº 35.

HAKIM, P. & SOLIMANO, G. Nutrition and 1976 national development — establishing the connection. *Food Policy*, 1(3): 249-259.

INSTITUTO DE NUTRICIÓN DE CENTROAMÉ- 1972 RICA Y PANAMÁ Y OFICINA DE INVESTIGACIÓN INTERNACIONAL. INSTITUTOS NACIONALES DE SANIDAD. *Nutritional evaluation of the population of Central America and Panama. Regional summary.* Washington, D.C., U.S. Department of Health, Education and Welfare. 165 p. DHEW Publication Nº (HSM) 72-8120.

INSTITUTO DE NUTRICIÓN DE CENTROAMÉ- 1976 RICA Y PANAMÁ/UNIDAD DE ANÁLISIS DEL SECTOR SALUD. *Análisis preliminar de la situación de salud en Nicaragua. Evaluación del componente nutricional dentro del sector salud*. Guatemala. 70 p.

JOY, J.L. Food and nutrition planning. 1973 *J. agric. Econ.*, 24: 165-192.

JOY, J.L. & PAYNE, P.R. La nutrición y 1975 la planificación del desarrollo nacional. *Aliment. Nutr. (FAO)*, 1(4): 2-10.

PAYNE, P.R. Nutrition planning and food 1976 policy. *Food Policy*, 1(2): 107-115.

RAWSON, I.G. & VALVERDE, V. The eti- 1976 ology of malnutrition among preschool children in rural Costa Rica. *J. trop. Pediat.*, 22: 12-17.

SISTEMA DE ANÁLISIS Y PLANIFICACIÓN DE 1976. LA ALIMENTACIÓN Y NUTRICIÓN. *Evaluación de las áreas prioritarias del problema nutricional de Honduras y sus posibles soluciones.* Tegucigalpa, D.C., Honduras, C.A. 495 p.

VALVERDE, V. *et al.* Relationship between 1977 family land availability and nutritional status. *Ecol. Fd Nutr.*, 6: 1-16.

THE AMERICAN JOURNAL OF CLINICAL NUTRITION
Vol. 20, No. 7, July, 1967, pp. 723-733
*Printed in U.S.A.*

# Blood-Selenium Levels and In Vitro Red Blood Cell Uptake of $^{75}Se$ in Kwashiorkor[1,2]

R. F. BURK, JR., B.A.,[3] W. N. PEARSON, PH.D.,[4]
RAYMOND P. WOOD II, M.D.,[5] AND F. VITERI, M.D., D.SC.[6]

THE STUDIES OF SCHWARZ and his coworkers (1, 2) have shown that selenium effectively prevents the development of dietary liver necrosis in the rat while Scott et al. (3) have shown it to be effective in preventing exudative diathesis in the chick. In addition, white muscle disease in sheep (4) and hepatosis dietetica in the pig have been reported to respond to treatment with selenium (5, 6).

Against this background of demonstrated essentiality in animals little is known about selenium metabolism in the human. The only report dealing with the possible involvement of selenium in human nutrition is that of Schwarz (7, 8). He reported that two children with kwashiorkor, who had failed to respond to the usual dietary treatment, were stimulated to grow by the administration of selenium. The validity of his interpretation of the data has been questioned (9), however, and further reports have not appeared. With this controversial report in mind and because Majaj et al. (10, 11) have reported a vitamin E-responsive macrocytic anemia to occur in some children with kwashiorkor, it was considered relevant to attempt to measure selenium nutriture in children with this disease. Accordingly, this report records selenium blood levels and the in vitro uptake by red blood cells of $^{75}Se$ in well-nourished children and in children with kwashiorkor.

## MATERIALS AND METHODS

These studies were performed both at the Institute of Nutrition of Central America and Panama (Guatemala City) and in the Division of Nutrition of Vanderbilt University School of Medicine, Nashville, Tennessee. The adaptation to blood of the fluorometric procedure for selenium determination and the development of the $^{75}Se$-uptake techniques were carried out in Nashville. The patients with kwashiorkor were studied at INCAP during the summers of 1965 and 1966. In 1965, all analyses were performed at INCAP, but in 1966 blood samples were transported to Nashville for selenium determination and for determination of radioactivity.

[1] From the Division of Nutrition, Vanderbilt University Medical School, Nashville, Tennessee and the Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Central America. INCAP Publication no. 1421.

[2] These studies were supported by grants from the Selenium-Tellurium Foundation, the Nutrition Foundation and the Advanced Research Projects Agency (Project AGILE). The latter contract was monitored by the Nutrition Section, Office of International Research, National Institutes of Health under ARPA Order 580, Program Plan 298. The studies in Guatemala were supported partly by Public Health Service Grant 5 ROI AM00981.

[3] Third-year Medical Student (Justin Potter Scholar). Vanderbilt University Medical School. [4] Associate Professor of Biochemistry, Vanderbilt University Medical School. [5] Surgeon, U.S. Public Health Service, Institute of Nutrition of Central America and Panama (INCAP) Guatemala. Present address: Resident in Surgery, University of Colorado Medical Center, Denver, Colorado. [6] Chief, Biomedical Division, Institute of Nutrition of Central America and Panama (INCAP), Guatemala.

*Determination of Blood-Selenium Levels*

Because the measurement of selenium in blood is not a common laboratory determination, it seemed desirable to record here our procedure in detail. The method, except for some minor details, is similar to that described by Cummins et al. (12) except that measurement of the final product is fluorometric rather than colorimetric.

*Principle*

The samples are digested in a mixture of sulfuric acid, perchloric acid, and sodium molybdate. After pH adjustment and other manipulations designed to reduce excess oxidative reagent and mask interfering ions, the selenium is complexed with diaminobenzidine (DAB). The resulting piazselenol is then extracted into toluene and measured fluorometrically.

*Apparatus*

Both an Aminco-Bowman spectrophotofluorometer (1 cm light path, square cuvette) and a Turner model 11 photofluorometer (7.5 x 100 mm round cuvettes) were used. The former is to be preferred because of its somewhat greater sensitivity. An electric micro-Kjeldahl rack and 100 ml Kjeldahl flasks were used for the digestions. A Corning model 7 pH meter was employed where required. Separatory funnels (125 ml) with ungreased glass stopcocks were used for the toluene-extraction step.

*Reagents*

*1*) Selenium standard: $SeO_2$ was dried overnight at 102 C. It was dissolved in water to give a concentration of 0.2 $\mu$g Se/ml solution.
*2*) Digestion mixture: 10 g of sodium molybdate were dissolved in 150 ml of water and 150 ml of sulfuric acid were carefully added. Then 200 ml of perchloric acid (70–72%) were added.
*3*) Glass beads: boiled in digestion mixture previously to remove contaminants.
*4*) Metacresol purple: 0.1% in 0.0026 N NaOH.
*5*) Saturated NaOH.
*6*) Formic acid (90%).
*7*) Hydroxylamine hydrochloride (40% solution).
*8*) EDTA (disodium salt) 0.2 M.
*9*) DAB: 0.5% aqueous solution of 3,3'diaminobenzidene tetrahydrochloride. The commercial compound should be recrystallized twice by dissolving it in the least amount of water possible and adding 4 N HCl. A 0.5% solution was prepared fresh daily using the recrystallized compound about 10 min before use.
*10*) Concentrated ammonium hydroxide.
*11*) Toluene (spectrograde).
*12*) Distilled deionized water (laboratory distilled water was passed through a Crystalab Deeminizer (Col-Parmer Instrument and Equipment Co., Chicago, Illinois)).

*Procedure*

One milliliter of whole blood, plasma, or red blood cells is placed into a 100-ml Kjeldahl flask containing two glass beads. Five milliliters of the digestion mixture are added and the solution is digested until it becomes clear and there are no traces of solid material upon close examination. Digestion is usually completed in 15 min. The flask and its contents are allowed to cool and the contents are then transferred to a 250-ml beaker. Quantitative transfer is insured by rinsing the flask with 10 ml of water. Two drops of metacresol purple are added to the solution and it is neutralized with saturated NaOH (yellow to blue or purple color). Approximately 4 ml of NaOH are required. The pH of the solution is then adjusted to 2.2 with 90% formic acid (Corning pH meter). Approximately 1.0 ml of formic acid is required. Four milliliters of hydroxylamine hydrochloride are added to reduce excess oxidative reagent, and 4.0 ml of 0.2 M EDTA are added to mask interfering ions such as iron, copper and vanadium which also complex with DAB. The pH is readjusted, if necessary, to 2–2.4 with $NH_4OH$ or formic acid.

Two milliliters of 0.5% DAB are added. The solution is mixed gently and the reaction is permitted to proceed in the dark at room temperature for 45 min. The reaction is stopped by adjusting the pH to 7.0 with $NH_4OH$, using the pH meter.

The solution is made up to 40 ml and poured into a 125-ml separatory funnel. Three milliliters of toluene are added and the mixture is shaken vigorously for 1 min. The layers are

permitted to separate and the aqueous (lower) layer is discarded. The toluene extract is then transferred to an appropriate cuvette for reading. Samples were activated at 450 mμ and read at 580 mμ in the Aminco-Bowman spectrophotofluorometer. When the Turner photofluorometer was used, filters 47B and 23A were employed as primary and secondary filters respectively. Blanks and standards (preferably in duplicate) should be included in each run. Under our conditions the standard curve was linear up to at least 0.5 μg of selenium.

*Comments*

Samples were analyzed in duplicate. Duplicate normal blood samples from one of us (RB) were included in every batch of samples run in Nashville as a check on the validity of the run. It was found that extreme care was necessary to prevent contamination. Because early erratic results were traced to dirty glassware, a cleaning procedure was initiated involving detergent washing followed by soaking in 50% nitric acid. The glassware was then rinsed with deionized water and dried in an oven. Different batches of commercial DAB showed variable blanks. These could be reduced by recrystallizing the DAB twice with 4 N HCl. One batch contained a yellow fluorescent material which appeared to be similar to that obtained in the selenium DAB coupling reaction. This batch was not improved by recrystallization. Similar problems with this reagent have been reported by Christian et al. (13).

*Procedure for Determination of Red Cell $^{75}Se$ Uptake Principle*

This procedure is patterned after that described by Wright and Bell (14). Whole blood anticoagulated with heparin is shaken with $^{75}SeO_3$ at 37 C under an atmosphere of $O_2$-$CO_2$ (95%–5%). The red blood cells are then separated by centrifugation, washed with 0.85% NaCl solution and their radioactivity determined. The $^{75}Se$ uptake is expressed as the percentage of the total counts found in the red blood cells.

*Apparatus*

A Dubnoff-type reciprocating shaker and 10-ml Erlenmeyer flasks with one-holed rubber stoppers provided with a short glass rod to plug the hole are used for the incubation. The $O_2$-$CO_2$ mixture is introduced into these flasks through an 18-gauge needle.

*Reagents*

1) 0.85% NaCl solution.
2) 0.16 μg Se/ml in 0.85% NaCl solution:

The Se is in the form of $Na_2{}^{75}SeO_3$. The samples used in our laboratory had, on arrival, a specific activity of about 0.5 μc/μg.

*Procedure*

Two milliliters of heparin-anticoagulated whole blood are pipetted into a 10-ml Erlenmeyer flask. One-tenth milliliter of the $^{75}Se$ solution is added, the $O_2$-$CO_2$ atmosphere is introduced through the hole in the rubber stopper (≐15 sec) and the glass rod is quickly inserted to seal the flask. The flask is placed in the metabolic shaker (37 C) and the slow shaking is commenced. More $O_2$-$CO_2$ mixture is introduced at 1 and 2 hr. At the end of 3 hr the flask is placed in an ice-water mixture to inhibit further uptake, and a small sample of blood is removed for a microhematocrit determination. A measured amount of the remaining blood is removed, placed into a counting tube, and centrifuged. An aliquot of plasma is removed to another counting tube for the measurement of its radioactivity. The red cells are washed twice with 0.85% saline and counted. The radioactivity of the plasma and red blood cells is corrected to a hematocrit of 40, and the red blood cell $^{75}Se$ uptake is expressed as the percentage of the total number of counts in the red cell counting tube.

$$^{75}\text{Se uptake } (\%) = \frac{\text{cpm red cell counting tube (corrected)} \times 100}{\text{cpm plasma counting tube (corrected)} + \text{cpm red cell counting tube (corrected)}}$$

Variations in this procedure will be discussed under RESULTS.

*Other Procedures*

Serum-vitamin E levels were determined by the micromethod of Quaife et al. (15). Hemoglobin values were measured by the cyanmethemoglobin procedure (16) and hematocrits were obtained by a microprocedure. Total-serum proteins were determined using a re-

fractometric procedure (17). Red cells were separated into old and young cells as follows: Washed cells suspended in 0.85% saline solution to a hematocrit of about 80% were centrifuged in a Wintrobe tube for 45 min at 10,000 × gravity. Equal volumes of red cells were taken from the top and bottom of the cell column in the Wintrobe tube. These were washed into counting tubes and counted.

## RESULTS

Blood-selenium levels were obtained on 10 well-nourished healthy adults and in two children. These values, which we consider to define the "normal" range, are seen in Table I. The mean value was 0.22 μg/ml with a standard deviation of 0.02. Samples from persons who resided in four geographical areas were analyzed but no location effect was detected. A whole blood sample from one of us (RB) was included in each group of samples analyzed in Nashville. The mean of 12 such determinations was 0.20 μg/ml with a standard deviation of 0.015.

TABLE I

Blood-Selenium Content in Healthy Adults

| Subject | Age | Sex | Location[a] | Whole Blood Se Content, μg/ml |
|---|---|---|---|---|
| RB[b] | 23 | M | Guatemala-Tennessee | 0.20 |
| MAB | 18 | F | Mississippi | 0.21 |
| ADB | 50 | F | Mississippi | 0.25 |
| JDB | 16 | M | Mississippi | 0.23 |
| JO | ±35 | M | Virginia | 0.21 |
| DO | ±11 | M | Virginia | 0.22 |
| BB | 23 | M | Guatemala | 0.28 |
| RW | 29 | M | Guatemala | 0.22 |
| JA | 23 | M | Tennessee | 0.19 |
| Mc | ±50 | M | Tennessee | 0.20 |
| RM | 9 | M | Guatemala | 0.22 |
| JB | 22 | M | Tennessee | 0.20 |
| | | | | Mean = 0.22 μg/ml |
| | | | | SD = 0.02 |

[a] Principal residence at time of blood collection.

[b] Many analyses were performed on the subject both in Guatemala and in Nashville. There was no discernible difference in the levels obtained.

About two-thirds of the selenium in blood is contained in the red blood cells and one-third appears in the plasma. When the contents of these two blood fractions are adjusted for hematocrit and added together they usually approximate closely the value obtained on an equivalent quantity of whole blood.

The initial studies of kwashiorkor patients were carried out in Guatemala during the summer of 1965. The 12 children who were selected for the study fell into two groups. Six had recovered clinically from kwashiorkor and had been residents of the ward for up to 1 year at the time of the study. These were considered to be "controls." In the other group, there were four newly admitted kwashiorkor cases who had received no treatment prior to the time the blood sample was drawn and two patients with kwashiorkor who had been under treatment (special diets, electrolytes, antibiotics, etc.) for 2½–3 months prior to the blood sampling.

The blood-selenium levels in the two groups of children with kwashiorkor are seen in Table II. The children with untreated kwashiorkor (*PC-162, PC-163, Se-10,* and *PC-165*) had whole blood-selenium levels that were less than half of those observed in the adult controls (Table I) and lower than those observed in the children who had recovered from kwashiorkor. Although the latter group had blood-selenium levels that were higher than those observed in the untreated infants, they were lower than those recorded in the adult controls. The two children (*PC-159, PC-160*) who had been under short-term treatment for kwashiorkor (2½–3 months) had whole blood-selenium levels similar to those found in the untreated patients. Of the 12 children studied, two were clearly anemic (*Se-10, PC-165*) but no attempt was made to characterize the anemia. All of the patients with untreated kwashiorkor were hypoproteinemic.

Serum-tocopherol levels in the untreated

TABLE II

Blood-Selenium Levels in Guatemalan Children (1965)[a]

| Subject | Age, years | Hb, g/100 ml | % Hct | TPP, g/100 ml | Vitamin E, mg/100 ml | Se Content | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Whole blood, μg/ml | Plasma, μg/ml | Red blood cells, μg/ml | Plasma protein, μg/g | Hemoglobin, μg/g |
| *Recovered From Kwashiorkor ("controls")* | | | | | | | | | | |
| PC-147 | 3 | 13.6 | 39 | 6.9 | 0.71 | 0.12 | 0.07 | 0.16 | 1.08 | 0.43 |
| PC-148 | 3 | 13.1 | 36 | 7.3 | 0.58 | 0.15 | 0.12 | 0.31 | 1.59 | 0.52 |
| PC-152 | 3 | 13.2 | 37 | 7.5 | 1.10 | 0.14 | 0.10 | 0.21 | 1.31 | 0.53 |
| PC-151 | 3 | 15.7 | 42 | 6.4 | 0.46 | 0.13 | 0.07 | 0.18 | 1.41 | 0.44 |
| PC-154 | 3 | 13.3 | 39 | 7.1 | 0.83 | 0.15 | 0.12 | 0.26 | 1.69 | 0.53 |
| PC-156 | 5 | 13.2 | 38 | 7.0 | 1.43 | 0.14 | 0.13 | 0.23 | 1.89 | 0.61 |
| | Mean | 13.7 | | 7.0 | | 0.14[b] | 0.10 | 0.23 | 1.50 | 0.51 |
| | SD | 1.0 | | 0.4 | | 0.01 | 0.02 | 0.05 | 0.29 | 0.07 |
| *Kwashiorkor Untreated or under Treatment* | | | | | | | | | | |
| PC-162 | 2 | 12.0 | 34 | 7.0 | 0.21 | 0.07 | 0.06 | 0.09 | 1.47 | 0.24 |
| PC-163 | 2 | 12.2 | 37 | 3.7 | 0.37 | 0.09 | | | | |
| Se-10 | 1 | 8.7 | 27 | 4.2 | 0.80 | 0.09 | 0.05 | 0.08 | 1.24 | 0.22 |
| PC-165 | 5 | 10.5 | 32 | 4.2 | 0.32 | 0.06 | | | | |
| PC-159 | 2 | 12.3 | 36 | 6.8 | 2.64 | 0.06 | | 0.10 | | 0.28 |
| PC-160 | 8 | 12.3 | 34 | 8.1 | 0.78 | 0.10 | | | | |
| | Mean | 11.3 | | 5.2 | | 0.08[b] | | | | |
| | SD | 1.5 | | 1.8 | | 0.02 | | | | |

TPP = total plasma protein.

[a] Each selenium value represents the mean of a single duplicate determination.

[b] *b* versus *b* significantly different $P < 0.001$.

patients were similar to those reported by Scrimshaw et al. (18) and Majaj et al. (10). *Patient PC-159* had received vitamin E supplementation which accounts for his elevated serum-vitamin E level.

In a few instances, more than one blood value was obtained on the same child. Two values were recorded for three children and three values were recorded for two children. These data are presented in Fig. 1. In every instance, the blood-selenium levels, initially low, were found to reach normal levels after treatment. It should be emphasized that no selenium supplements were given in any of these studies. *Subject PC-159,* for example, had a blood-Se level of only 0.06 μg/ml after treatment for 93 days. Fifty days later, his blood-selenium level had risen to 0.13 μg/ml and a "normal" level (0.19 μg) was recorded on the 446th day of his residency on the ward. This child consumed a casein-based, methionine-supplemented diet during his first 60 days on the metabolic ward, which contained by analysis only 0.05 μg Se/g. After

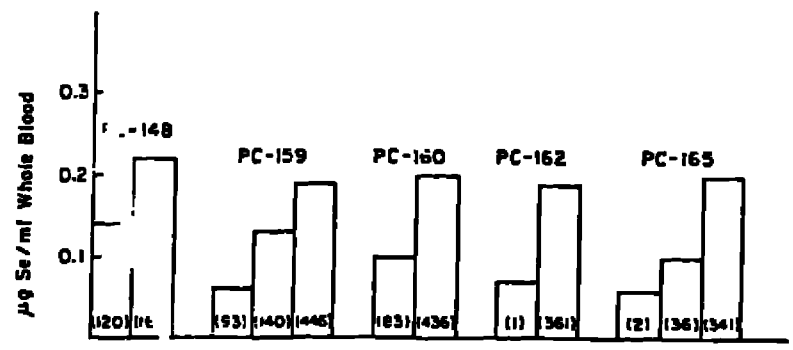



FIG. 1. Whole blood-selenium levels in five kwashiorkor patients at various times after admission to the metabolic ward. Numbers in parentheses indicate day of residence on ward when blood sample was taken.

this dietary regime, various diets of other composition were fed, but the approximate selenium content of only one of these is known. This was the so-called "soya" diet which was found to contain 0.25–0.32 μg/g. This diet was fed for a 3-day period about 10 days prior to obtaining the second blood specimen (day 143) and for almost 2 months thereafter.

*Subject PC-160*, whose blood selenium level was 0.10 μg/ml after 83 days on the ward, consumed the casein-methionine diet for 10 days of this period and a whole milk diet thereafter. A variety of diets was consumed thereafter and he was found to have a "normal" blood level of selenium 12 months later.

*Subject PC-148* received a variety of diets during his first 120 days on the ward but the casein diet was not included. After determination of the first blood-selenium level, he received the "soya" diet during 12 of the next 60 days including the 9 days just prior to his second blood analysis.

*Subject PC-162* received a variety of diets during his 1st year of residency on the ward and prior to his second blood

TABLE III

Blood-Selenium Levels in Guatemalan Children (1966)

| Subject | Age, years | Hb, g/100 ml | % Hct | TPP, g/100 ml | Se Content | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Whole blood, μg/ml | Plasma, μg/ml | Red blood cells, μg/ml | Plasma protein, μg/g | Hemoglobin, μg/g |
| *Controls* | | | | | | | | | |
| K-10 | 7 (18)[a] | 12.4 | 38 | 5.8 | 0.33 | 0.23 | 0.56 | 3.97 | 1.53 |
| K-11 | 7 (45) | 9.7 | 34 | 8.4 | 0.28 | 0.20 | 0.46 | 2.86 | 1.44 |
| K-12 | 3 (21) | 9.8 | 32 | 8.6 | 0.21 | 0.22 | 0.41 | 2.56 | 1.22 |
| K-13 | 8 (49) | 10.4 | 33 | 7.0 | 0.14 | 0.09 | 0.13 | 1.27 | 0.26 |
| K-16 | 9 (104) | 14.0 | 44 | 7.8 | 0.24 | 0.13 | 0.37 | 1.62 | 1.07 |
| PC-159 | 3 (446) | 14.0 | 38 | 7.8 | 0.24 | 0.13 | 0.37 | 1.25 | 0.98 |
| PC-160 | 6 (436) | 12.8 | 36 | 7.7 | 0.20 | 0.11 | 0.30 | 1.43 | 0.78 |
| PC-162 | 3 (361) | 13.5 | 38 | 7.3 | 0.19 | 0.12 | 0.31 | 1.64 | 0.82 |
| PC-165 | 6 (341) | 14.8 | 42 | 7.6 | 0.20 | 0.12 | 0.32 | 1.58 | 0.81 |
| | Mean | 12.3 | | 7.5 | 0.23 | 0.15 | 0.36 | 2.02 | 0.99 |
| | SD | 1.9 | | 0.8 | 0.05 | 0.05 | 0.12 | 0.92 | 0.39 |
| *Kwashiorkor Patients* | | | | | | | | | |
| K-1 | 3 (3) | 9.0 | 29 | 3.6 | 0.07 | 0.06 | 0.19 | 1.67 | 0.56 |
| K-2 | 9 (7) | 7.0 | 22 | 5.0 | 0.09 | 0.05 | 0.20 | 1.00 | 0.57 |
| K-3 | 6 (9) | 9.7 | 28 | 5.9 | 0.08 | 0.08 | 0.19 | 1.36 | 0.52 |
| K-4 | 4 (4) | 11.7 | 36 | 4.2 | 0.12 | 0.05 | 0.21 | 1.19 | 0.59 |
| K-6 | 7 (1) | 11.9 | 35 | 3.1 | 0.12 | 0.06 | 0.22 | 1.93 | 0.59 |
| K-8 | 12 (1) | 5.6 | 21 | 3.4 | 0.08 | 0.06 | 0.26 | 1.77 | 0.89 |
| K-9 | 6 (9) | 10.3 | 33 | 5.9 | 0.24 | 0.15 | 0.57 | 2.54 | 1.62 |
| K-14 | 8 (2) | 11.6 | 35 | 4.5 | 0.09 | 0.04 | 0.13 | 0.89 | 0.26 |
| K-15 | 3 (2) | 9.0 | 29 | 5.0 | 0.13 | 0.06 | 0.35 | 1.20 | 1.00 |
| | Mean | 9.5 | | 4.5 | 0.11 | 0.07 | 0.26 | 1.51 | 0.74 |
| | SD | 2.2 | | 1.0 | 0.05 | 0.03 | 0.13 | 0.52 | 0.40 |
| | | | | | $P < 0.01$ | $P < 0.01$ | NS | NS | NS |

[a] Numbers in parentheses indicate the number of days in the hospital prior to drawing blood for this analysis.

analysis. *Subject PC-165* was fed the casein-methionine diet from admission until a second blood-selenium level was determined 36 days later. He consumed a variety of diets thereafter.

In the summer of 1966, nine children with kwashiorkor were studied at Guatemala General Hospital. These children gave a clinical impression of that disease and all had decreased plasma-protein levels as seen in Table III. The controls were children from the same hospital who, although hospital patients, were not considered to be malnourished. A few subjects from the metabolic ward at INCAP who had completely recovered from kwashiorkor were also included in the control group. These children had been residents of the metabolic unit for periods of time ranging from 11 to 15 months.

The blood-selenium levels in the kwashiorkor and control groups are shown in Table III. One patient with kwashiorkor (*K-9*) had a normal blood-selenium value and one control patient (*K-13*) had a low blood-selenium value. In spite of this, all mean selenium parameters were lower in the children with kwashiorkor than in the controls but only the differences in the selenium content of whole blood and of plasma reached statistical significance. The levels obtained in the kwashiorkor patients resembled those found in untreated patients in the first study. The blood-selenium levels in the control children were similar to those found in healthy adults (Table I).

Measurements of red blood cell $^{75}Se$ uptakes were carried out in these subjects by the technique described previously. Only 3-hr uptakes were determined. Preliminary studies using control blood revealed a curvilinear uptake of selenium with time when the incubation was carried out under an atmosphere of $O_2$-$CO_2$ (95%–5%). Considerably less uptake was observed if air or nitrogen atmospheres were used suggesting that selenium uptake is, at least in part, an active process.

TABLE IV

Red Blood Cell-$^{75}Se$ Uptakes in Kwashiorkor and Control Patients

| Subject | Age, years | Hb, g/100 ml | TPP, g/100 ml | Whole Blood-Se Level, μg/ml | Per Cent $^{75}Se$ Uptake |
|---|---|---|---|---|---|
| *Patients with Kwashiorkor* | | | | | |
| K-1 | 3 | 9.0 | 3.6 | 0.07 | 22.1 |
| K-2 | 8 | 7.0 | 5.0 | 0.09 | 24.6 |
| K-3 | 6 | 9.7 | 5.9 | 0.08 | 19.4 |
| K-4 | 3 | 11.7 | 4.2 | 0.12 | 27.8 |
| K-6 | 7 | 11.9 | 3.1 | 0.12 | 21.4 |
| K-8 | 12 | 5.6 | 3.4 | 0.08 | 23.2 |
| K-9 | 6 | 10.3 | 5.9 | 0.24 | 15.8 |
| K-14 | 8 | 11.6 | 4.5 | 0.09 | 18.2 |
| K-15 | 3 | 9.0 | 5.0 | 0.13 | 18.9 |
| | Mean | 9.5 | 4.5 | 0.11 | 21.3 |
| | SD | 2.2 | 1.0 | 0.05 | 3.7 |
| *Controls* | | | | | |
| K-10 | 7 | 12.4 | 5.8 | 0.33 | 14.6 |
| K-11 | 7 | 9.7 | 8.4 | 0.28 | 13.0 |
| K-12 | 3 | 9.8 | 8.6 | 0.21 | 17.0 |
| K-13 | 8 | 10.4 | 7.0 | 0.14 | 13.0 |
| K-16 | 8 | 14.0 | 7.8 | 0.24 | 13.2 |
| PC-159 | 3 | 13.3 | 7.2 | 0.19 | 7.6 |
| PC-160 | 6 | 12.8 | 7.7 | 0.20 | 11.8 |
| PC-162 | 3 | 13.5 | 7.3 | 0.19 | 12.4 |
| PC-165 | 6 | 14.8 | 7.6 | 0.20 | 11.6 |
| | Mean | 12.3 | 7.5 | 0.23 | 12.7 |
| | SD | 1.9 | 0.8 | 0.05 | 2.5 |

These uptakes in nine subjects with kwashiorkor and in the nine control subjects are seen in Table IV. In 3 hr, the red blood cells from control subjects took up 12.7% of the total added radioactivity while the red blood cells from the kwashiorkor patients took up 21.3%. The difference is highly significant ($P < 0.01$).

Because the kwashiorkor patients had low plasma-protein levels (4.5 g/100 ml) when compared to the controls, it seemed possible that some of the $^{75}Se$ might complex with plasma proteins in the whole blood incubation system. This might reduce the amount of selenite ion available

for uptake by the red cells and explain why kwashiorkor patients would show higher red blood cell $^{75}Se$ uptakes.

This possibility was examined in the following fashion. Radioactive selenite (3.4 m$\mu$c) was added to 4 ml of normal plasma and the mixture was incubated under an $O_2$-$CO_2$ atmosphere for 3 hr. Then, a 2-ml aliquot of the sample was placed on a column of Sephadex G-200 (1.6 cm x 30 cm) and the plasma proteins were eluted with tris buffer (pH-7.7). The Sephadex G-200 was employed because it best separates proteins of different size, and it was of interest to determine whether $^{75}SeO_3^{2-}$ was associated with plasma globulin which is usually normal in kwashiorkor or with plasma albumin which is decreased in kwashiorkor. The protein content of the 2-ml fractions collected was determined by measuring their optical densities at 280 m$\mu$ in a Beckman DB spectrophotometer and the location of the $^{75}Se$ was determined by measuring the radioactivity of each tube in well-type scintillation counter. The elution pattern obtained is seen in Fig. 2. It is readily apparent that the bulk of the protein comes off the column before the largest $^{75}Se$ peak. Only very small $^{75}Se$ peaks are associated with the large OD peak. The small OD peak occurring at *fraction 24* (which coincides with the large $^{75}Se$ peak) was examined for the presence of protein by the Folin-Ciocalteu method and by paper electrophoresis. Neither revealed the presence of protein. Thus *fractions 21–32* containing 84% of the $^{75}Se$, contained no detectable protein. The location of albumin and globulin in the large OD peak was determined by paper electrophoresis with the following results: *fraction 10,* globulin (mostly gamma); *fraction 14,* albumin with a faint trace of globulin; and *fraction 18,* albumin. Thus it is seen that only a small portion of the total radioactivity becomes associated with the plasma protein during the incubation procedure and that which does is found in both the globulin and the albumin. The possibility that the main peak of radioactivity is selenite was examined by a separate experiment in which $^{75}Se$ (as selenite ion) was placed on a Sephadex column and eluted with tris buffer. The experiment depicted in Fig. 2 was then repeated on this same column. The main peak of radioactivity was found in the same position in both experiments suggesting that it is either inorganic selenite or selenite associated with some small molecule.

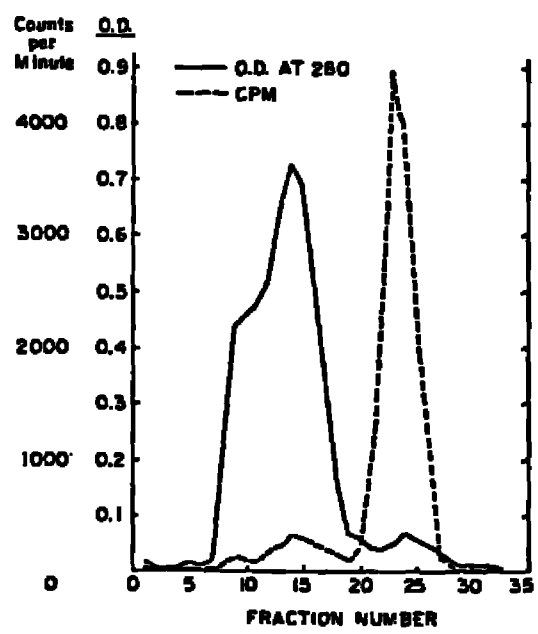



FIG. 2. Fractionation of plasma + $^{75}SeO_3^{2-}$ on a Sephadex G-200 column. Fraction size = 2.0 ml.

The nature of the association of selenium with the red blood cell was also examined. Red cells recovered from a $^{75}Se$-uptake experiment were lysed and the stroma removed by centrifugation. The supernatant solution, the protein component of which is almost exclusively hemoglobin, was then subjected to Sephadex chromatography under the identical conditions used in the studies of plasma proteins. The hemoglobin content of each fraction was measured by absorption at 280 m$\mu$ in a Beckman DB spectrophotometer and the radioactivity of each fraction was determined in a well-type scintillation counter. The results are seen in Fig. 3. The hemoglobin peak and radioactivity peak coincide suggesting that the bulk of the absorbed $^{75}Se$ becomes at-

tached to the hemoglobin molecule. The second small peak of radioactivity was not associated with a protein peak and its elution position suggests that it may be either inorganic selenium or selenium associated with a small molecule such as glutathione or an amino acid.

Because of the marked association of selenium with the hemoglobin molecule it occurred to us that reticulocytes might be preferentially absorbing selenium and subsequently incorporating it into the hemoglobin molecule. This possibility was examined by incubating red cells with $^{75}Se$ followed by the separation of the cells into different age groups by centrifugation (see METHODS). The radioactivity of the very young cells was found to be similar to that of older cells so it appeared that reticulocytes do not preferentially absorb selenite ion.

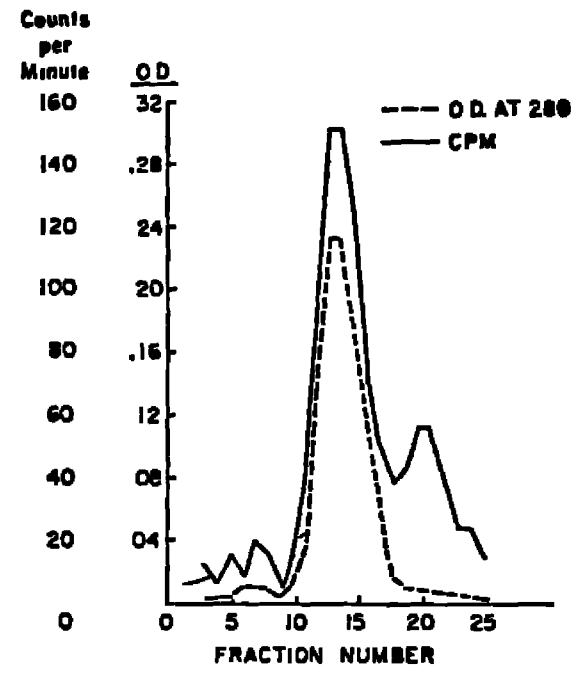



FIG. 3. Fractionation of radioactivity and protein (hemoglobin) in red blood cell hemolysates after incubation in vitro with $^{75}SeO_3^{2-}$. Fraction size = 2.0 ml.

## DISCUSSION

Only limited data are available on selenium distribution in blood. Taussky et al. (19) using a DAB fluorometric method in chicks, found serum levels of 0.1–0.2 μg/g and red blood cell levels of 0.25–0.30 μg/g. These values do not differ markedly from the values reported here. Bowen and Cawse (20) analyzed eight samples of human blood by the neutron activation technique and reported a range of values of 0.26–0.37 μg/milliliter with a mean of 0.32. It is not reported from whom the samples were collected. Brune et al. (21) used neutron-activation analysis and recorded a mean blood-selenium level of 0.12 μg/milliliter in six "normal" adult subjects. Our "normal" figure of 0.22 μg/milliliter lies precisely between these two means. Whether these differences represent a function of methodology or geographic variability (England; Sweden; United States) is not known. Neither can one draw inferences from these data on the comparability of the fluorometric and neutron-activation methods other than that the reported values all lie in the same range.

The blood-selenium levels in healthy adults recorded in this study fell within a fairly narrow range even though the determinations were carried out both in Guatemala and Nashville and the subjects lived in different locations. The difference between the selenium-blood levels in the 1965 controls and the 1966 controls is probably a real one. Consistent laboratory error may be excluded because the blood of RB gave similar values each year whether analyzed in Nashville or Guatemala. It is possible that different selenium intakes are responsible because the 1965 controls all received semipurified diets of one type or another during most of their tenure in the metabolic ward. Four of the five 1966 control children from the Guatemala General Hospital had blood-selenium levels that were considerably higher than those of the four controls who had recovered from kwashiorkor and were still residents of the metabolic ward in INCAP. The limited dietary information available suggests that the blood-selenium levels were increased more rapidly in children who were fed the selenium-rich "soya" diet than in children fed the selenium-poor

casein diet. In any event, it is evident that blood-selenium levels are but slowly restored during the recovery of children from kwashiorkor (Fig. 1).

Children with kwashiorkor have reduced blood-selenium levels for two reasons. In the first place, they are both anemic and hypoproteinemic and accordingly circulate reduced levels of selenium-containing proteins. Secondly, their serum proteins and hemoglobin contain less selenium although this difference did not reach statistical significance (see Table III). Although it would seem reasonable that reduced blood-Se content is compatible with reduced whole body stores, it is not known whether blood selenium is truly in equilibrium with total body stores and it would be difficult to establish this in the human being.

The enhanced uptake of $^{75}Se$ by the red blood cells of children with kwashiorkor may reflect their reduced selenium content. That this occurs in experimental animals is known from the studies of Wright and Bell (14) who showed that blood samples from sheep fed a ration low in selenium took up more and more selenium as the experiment progressed. Uptakes of other ions by the red cells were not changed. The selenium content of the red blood cells was not determined but it may be presumed that it was reduced. They suggested that this technique might be used as an index of selenium status and our results tend to confirm their conclusion.

The failure of $^{75}Se$ clearly to associate with serum protein in vitro is of interest. McConnell et al. (22) have demonstrated that 10 min after the subcutaneous injection of $^{75}SeO_3^{2-}$ into a dog, 50–70% of the total serum $^{75}Se$ is protein "bound" as determined by dialysis. This increases to 95% after 48 hr. This binding probably indicates the actual incorporation of the selenite into these proteins. The trichloroacetic acid precipitation of the serum proteins yielded consistently higher "protein bound" selenium values than did dialysis. In our laboratory the addition of trichloroacetic acid to plasma incubated in vitro with $^{75}SeO_3^{2-}$ was found to precipitate most of the radioactivity. This does not necessarily indicate Se-protein binding because it may be the result of combination of the selenite with the plasma protein in salt form when trichloroacetic acid is added. Under our conditions of incubation, little association of selenite with plasma protein was observed if the proteins were fractionated on Sephadex columns.

The results of this study suggest that children with kwashiorkor have reduced stores of selenium. Whether this contributes significantly to the clinical syndrome of this complex disease is not known and it is apparent that further investigations are required to determine whether a primary dietary lack or a secondary metabolic derangement is involved. The known dietary requirement for this element by a variety of species would imply that it is indeed a required nutrient for man.

We are grateful to Miss Cristina Contreras and Mrs. Elvira de Orozco, who carried out many of the determinations included in *Other Procedures*. The enthusiastic cooperation of Dr. V. Arguetavon Kaenel, Professor of Pediatrics at San Carlos University and head of the Department of Pediatrics at the Guatemala General Hospital, made the studies in the summer of 1966 possible. The skillful assistance of Miss Martha Anne Burk with the selenium analyses shown in Table III is gratefully acknowledged.

## ADDENDA

*1*) After this manuscript had been submitted for publication, two publications which bear on this subject came to our attention. The first (*Lancet* ii: 592, 1966) reports a growth response to selenium supplementation in three malnourished children. In addition, three of five malnourished children with anemia (macrocytic?) showed reticulocyte response on treatment with selenium. The second publication (*Clin. Chim. Acta* 16: 311, 1967) records the selenium content of blood and human tissues as measured by radioactivation analysis. The mean

values for whole blood reported are similar to those recorded here.

2) Although the selenium method described here has performed satisfactorily, problems have arisen occasionally presumably related to the use of different batches of diaminobenzidine. Accordingly, another method based on that of J. H. Watkinson (*Anal Chem.* 38: 92, 1966), which uses diaminonaphthalene as the fluorogen, is now in use.

## REFERENCES


1. SCHWARZ, K, AND C. M FOLTZ Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J Am. Chem. Soc.* 79: 3292, 1957.
2. SCHWARZ, K Vitamin E, trace elements and sulfhydryl groups in respiratory decline in vitro (rat): mode of action of tocopherol and related compounds. *Vitamins Hormones* 20: 463, 1962.
3. SCOTT, M. L., H. E BUTTERS AND M. C. NESHEIM. Vitamin E-like activity of dried brewer's yeast and other foodstuffs. *Poultry Sci.* 36: 1155, 1957.
4. DRAKE, C, A. B. GRANT AND N. J. HARTLEY. Selenium and animal health. I. The effect of alpha-tocopherol and selenium in the control of field outbreaks of white muscle disease in lambs II The effect of selenium on unthrifty weaned lambs *N. Zealand Vet. J.* 8: 4, 1960
5. EGGERT, G R, E PATTERSON, W. T. AKERS AND E. L. R. STOKSTAD. Vitamin E and selenium in the nutrition of the pig *J. Animal Sci.* 16 1037, 1957.
6. GRANT, C. A, AND B THAFVELIN. Selenium and nutritional necrosis of the liver in the pig. Preliminary note on the prophylactic action of selenium under experimental conditions. *Nord. Veterinarmed.* 10· 657, 1958.
7. SCHWARZ, K. Development and status of experimental work on factor-3-selenium. *Federation Proc* 20. 666, 1961.
8. SCHWARZ, K Selenium and kwashiorkor *Lancet* i: 1335, 1965
9. WATERLOW, J C. Selenium content of fishflour and kwashiorkor. *Lancet* i. 869, 1965.
10. MAJAJ, A. S, J. S. DINNING, S A. AZZAM AND W. J. DARBY. Vitamin E-responsive megaloblastic anemia in infants with protein calorie malnutrition *Am. J Clin. Nutr.* 12: 374, 1963.
11. MAJAJ, A. S. Vitamin E-responsive macrocytic anemia in protein-calorie malnutrition. *Am. J. Clin. Nutr.* 18: 362, 1966.
12. CUMMINS, L. M., J. L. MARTIN AND D. D. MAAG. An improved method for determination of selenium in biological materials. *Anal. Chem.* 37: 430, 1965.
13. CHRISTIAN, G. D., E. C. KNOBLOCK AND W. C. PURDY. Polarographic determination of selenium in biological materials. *J. Assoc. Offic. Agr. Chemists* 48. 877, 1965.
14. WRIGHT, P. L. AND M. C. BELL. Selenium and vitamin E influence upon the *in vitro* uptake of $Se^{75}$ by ovine blood cells. *Proc. Soc. Exptl. Biol. Med.* 114: 379, 1963.
15. QUAIFE, M. L, N. S. SCRIMSHAW AND O. H. LOWRY. A micromethod for assay of total tocopherols in blood serum. *J. Biol. Chem* 180: 1229, 1949.
16. HAINLINE, A. Hemoglobin. In: *Standard Methods of Clinical Chemistry.* New York: Academic, Vol II, 1958, p. 49.
17. WOLF, A. V., J. B. FULLER, E. J. GOLDMAN AND T. D. MURRAY. New refractometric methods for the determination of total proteins in serum and in urine. *Clin. Chem.* 8: 158, 1962.
18. SCRIMSHAW, N. S., M. BEHAR, G. ARROYAVE, C. TEJADA AND F. VITERI. Kwashiorkor in children and its response to protein therapy. *J. Am. Med. Assoc.* 164: 555, 1957.
19. TAUSSKY, H. H., A. WASHINGTON, E. ZUBILLAGA AND A. T. MILHORAT. Distribution of selenium in tissues of normal and dystrophic chickens *Nature* 206: 509, 1965.
20. BOWEN, H. J. M., AND P. A. CAWSE. The determination of selenium in biological material by radioactivation. *Analyst* 88: 721, 1963.
21. BRUNE, D., K. SAMSAHL AND P. O. WESTER. A comparison between the amounts of As, Au, Br, Cu, Fe, Mo, Se, and Zn in normal and uraemic human whole blood by means of neutron activation analysis. *Clin. Chim. Acta* 13: 285, 1966.
22. MCCONNELL, K. P., C. H. WARNITZ AND D. M ROTH. Time distribution studies of selenium-75 in dog serum proteins. *Texas Rept. Biol. Med.* 18: 438, 1960.

**Serum levels of IgG, IgA and IgM were found to be either normal or elevated in PCM patients, with no relation to the degree of protein–calorie depletion, the percentage of white cells and plasmocytoid lymphocytes in peripheral blood, or the prognosis. The IgG rose in a child developing mumps even though his diet was only sufficient for nitrogen equilibrium. These results still do not elucidate the discrepancy that exists between elevated immunoglobulin fractions and the high mortality rate associated with the presence of infection in protein–calorie malnutrition.**

# Serum Immunoglobulins in Edematous Protein–Calorie Malnourished Children*

## *Studies in Guatemalan Children at INCAP*

JORGE ALVARADO, M.D.,** DAVID G. LUTHRINGER, M.D.***

INCREASED susceptibility to many infections in protein–calorie malnourished children is a well-known phenomenon.[1,2] In turn, these infections are important, not only as precipitants of kwashiorkor,[3,4] but also as contributors to the high fatality rate in this disease.[5,6]

The abundance of assessments of the capacity of children with protein–calorie malnutrition (PCM) to form humoral antibodies, as tested by challenge with attenuated viral and bacterial vaccines, has given variable results. For example, Brown and Katz[7] found no antibody response following yellow fever vaccine. Impaired responses to mumps vaccine and to diphtheria toxoid have been reported,[8,9] but to typhoid and oral poliomyelitis vaccines the serologic responses have been normal.[7,10,11] It should be noted that these studies were done in coincidence with the giving of adequate protein therapy, and hence the responses observed may reflect the antibody-forming capacity of rapidly changing organisms during nutritional recovery rather than in static malnutrition. In PCM the rate of synthesis of gamma globulin does not seem to be impaired.[12] In fact, its synthesis is greatly increased when a superimposed infection is present, in contrast to a simultaneous decrease in the synthesis of albumin.[12] Little information is available concerning the pattern of the other serum immunoglobulins in PCM.

The investigations here described deal with the serum immunoglobulins in protein–calorie malnourished children before protein repletion was initiated. The basic question was whether immunoglobulin deficiency might be implicated in the high morbidity and mortality rates associated with infection in PCM.

### Subjects

The investigations were carried out with 25 children suffering from PCM ("malnourished group") as determined by accepted clinical characteristics—poor dietary history, wasting, edema, skin and hair changes, apathy.[13]

The children were patients either in INCAP's Clinical Center or in the Pediatrics Ward of the Guatemala General Hospital. They were studied within 72 hours of ad-

* This work was sponsored in part by the National Institutes of Health of the U. S. Public Health Service (Grant No. AM-00081 12 and NIH Agreement No. 71X3003X9008-15), and by the World Health Organization.

** Assistant Chief, Biomedical Division, Institute of Nutrition of Central America and Panama (INCAP) Guatemala C. A.

*** Formerly, Medical Officer of the Biomedical Division, INCAP. Present Address: 1801 Eye Street, N.W., Washington, D. C. 20006.

INCAP Publication I-527.

mission, before an adequate protein intake was started. In age they ranged from 1 year and 6 months to 5 years and 11 months. Height and weight features of these children are shown in Table 1.

The percentage of expected weight for height has been selected as an index of caloric depletion, 100 per cent corresponding to the Boston 50th percentile. The mean value of all cases on admission was 89% ± 23 6 (1 S.D.), but if minimum weight is used (after clinical edema has disappeared), the mean value changes to 76% ± 3 9 (1 S.D ). This last figure was accurately obtained only in 13 children studied at INCAP's Clinical Center.

Twelve out of these 13 children were studied again by strict clinical and biochemical criteria once their nutritional recovery had been achieved. These children constitute the "recovered group."

## Controls

Fourteen children served as "control group." These children came from families of low socioeconomic status and had the same age distribution as the patients. These controls, regular attenders at a day-care nursery, were selected according to the following criteria: normal growth pattern during the previous three months, absence of known clinical infection, no recent immunizations.

## Methods

Venous blood samples were used for hematologic studies, including peripheral blood smears. Standard biochemical methods were used to assess nutritional status. Total serum protein was determined by refractometry.[14] Serum-protein electrophoresis was performed on cellulose acetate strips. Serum for immunoglobulin determinations was rapidly separated and kept frozen until assayed by the radial immunodiffusion technique.[15] Hyland's Immuno-Plates and immunoglobulin standards were utilized in this study (Hyland Laboratories, Los Angeles, California).

Single determinations were done on most of the samples. Random duplicates of the standards and samples gave a coefficient of variation from 5 to 15 per cent.

The patient's 24-hour urinary creatinine excretion, compared with that excreted by a normal child of the same height, independent of age, served as an index of protein depletion. This index indirectly reflects muscle mass which in itself represents approximately 43 per cent of total protein mass, and is referred to as the creatinine/height index (CHI).[16, 17]

Children suffering from PCM were treated according to our standard therapeutic regimens,[18] utilizing casein supplemented with methionine as the source of protein. A level of 0 7 Gm. of protein/Kg./day was provided for a period of 7 to 10 days, and then increased gradually to 3 or 4 Gm./Kg /day. Clinical histories were kept of recent previous infections, and detailed progress records of those that occurred during their hospitalization. Bacteriologic studies and serum immunoglobulin determinations were performed simultaneously in a few patients. Parasitologic examinations for ova and parasites were

TABLE 1. *Clinical and Biochemical Characteristics of the Malnourished, Recovered, and Control Children Studied*

| Group | Age in Months | % Expected Weight for Height | % Height for Age | Total Serum Proteins | Creatinine Height Index |
|---|---|---|---|---|---|
| Malnourished | 36 ± 15 3* (25)** | 89 ± 23 6 (25) | 86 ± 6 3 (25) | 4 07 ± 0 53* (25)** | 0 54 ± 0 15 (19) |
| Recovered | 36 ± 12 5 (12) | 104 ± 7 6 (12) | 88 ± 5 1 (12) | 7 40 ± 0 56 (12) | 0 96 ± 0 08 (12) |
| Controls | 45 ± 11 8 (14) | 106 ± 6 5 (14) | 92 ± 2.3 (14) | 7 18 ± 0 64 (13) | 0 96 ± 0 04 (13) |

* Average ± 1 S D.
** Number of cases

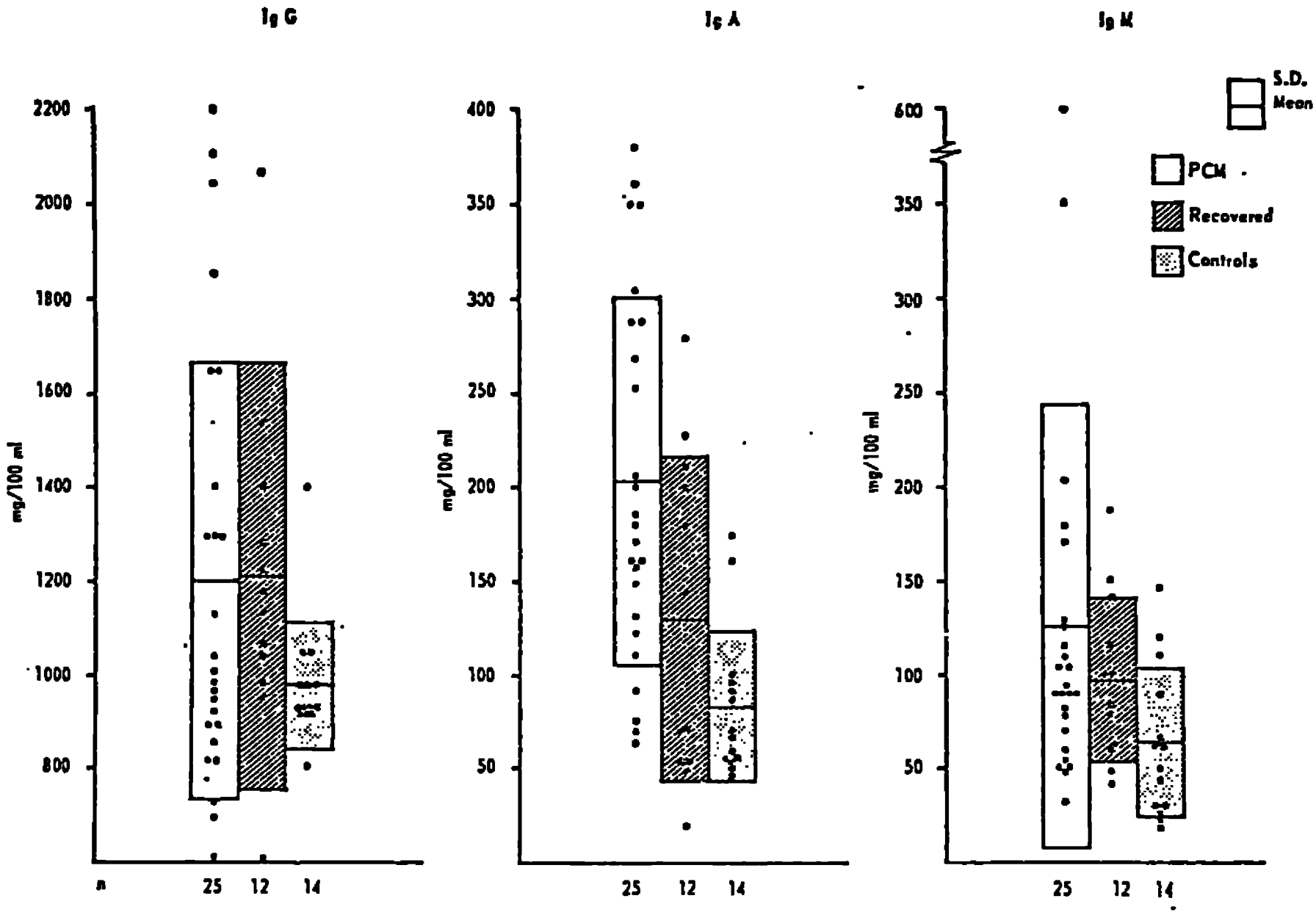



FIG. 1. Levels of serum immunoglobulin fractions in malnourished, recovered and control children.

made in stools collected from all patients. Chest x-rays were taken upon admission. After the clinical edema had disappeared (usually 10–18 days after initiation of an adequate diet) a tuberculin test was applied to all children. None of the patients showed evidence of granulomatous disease.

## Results

Although indices of adequate protein nutrition are scarce and difficult to assess, two parameters were selected for this purpose (Table 2). The first parameter was the level of total serum proteins. This was found to be definitely depressed in the "malnourished group" and normal in the other two groups. The average serum albumin levels were 1.78, 4.21 and 4.06 Gm./100 ml., respectively, for the malnourished, recovered and control groups.

The second parameter was the creatinine/height index (CHI).[16, 17] Normal values in this study, as in others carried out at INCAP,[17] are always above 0.85; they usually reach 1.0 when full nutritional recovery is attained, and from then onwards remain close to this figure. The CHI in every one of our malnourished children was below 0.70 with an average value of 0.54.

No direct relation was found between the CHI (creatinine/height index) and the serum levels of immunoglobulin fractions IgG, IgA, and IgM.

No significant differences were found among the three groups—malnourished, recovered, control—with respect to IgG and IgM values (Fig. 1). Five of the 25 malnourished patients studied had IgM levels above 150 mg./100 ml., including one child (PC-210) who had 600 mg./100 ml. and was in adequate hydration when tested.

Patients with severe diarrhea had the highest values of both IgM and IgA serum immunoglobulins.

Between the malnourished group and the normal controls a significant statistical differ-

TABLE 2. *Serum Immunoglobulins in the Groups of Children Studied*

| Group | Immunoglobulins | | |
|---|---|---|---|
| | IgG | IgA | IgM |
| Malnourished | 1,201 ± 465 (25) | 203 ± 98 (25)*† | 126 ± 118 (25) |
| Recovered | 1,210 ± 456 (12) | 130 ± 87 (12)* | 97 ± 44 (12) |
| Controls | 977 ± 136 (14) | 83 ± 40 (14)† | 64 ± 30 (14) |

* $p < 0.05$ within groups.
† $p < 0.001$ within groups.

ence in regard to IgA levels ($p < 0.001$) was observed. The differences in levels between malnourished and recovered children was also significant ($p < 0.05$). No significant difference in levels was observed between recovered and normal children. Figure 2 compares the IgA values for these Guatemalan children with values reported for healthy Scandinavian children.[19]

When the children with PCM were divided into two groups according to their age: below or above 36 months, the mean serum immunoglobulin fractions of the two groups did not differ significantly. Age, therefore, does not explain the results obtained. The values for the 86 per cent of the normal control children in our study fall within 2 S.D. of the mean values reported for normal Scandinavian children [19] (Fig. 2).

Three cases were studied serially (Table 3). Throughout, patient 196 had a constant increase in the IgA fraction. The last determination, done one week after a lactose load test of four days duration, demonstrated lactose intolerance. Patient 198 maintained high levels of IgA throughout the first 62 days of hospitalization and had severe diarrhea during the first week after admission even when given a lactose- and gluten-free diet. Child 216 was studied at more frequent intervals during the first two weeks of hospitalization; parotitis was obvious on the fifth day, and the IgG level rose rapidly even while dietary protein was given in amounts which only allow nitrogen equilibrium. Clinically, this patient behaved as an otherwise normal child, had high fever four days after the onset of parotitis and developed no complications.

Three of our malnourished patients had a fatal course. Patient 183 died as a consequence of electrolyte imbalance and severe lactic acidosis eight days after admission; pa-

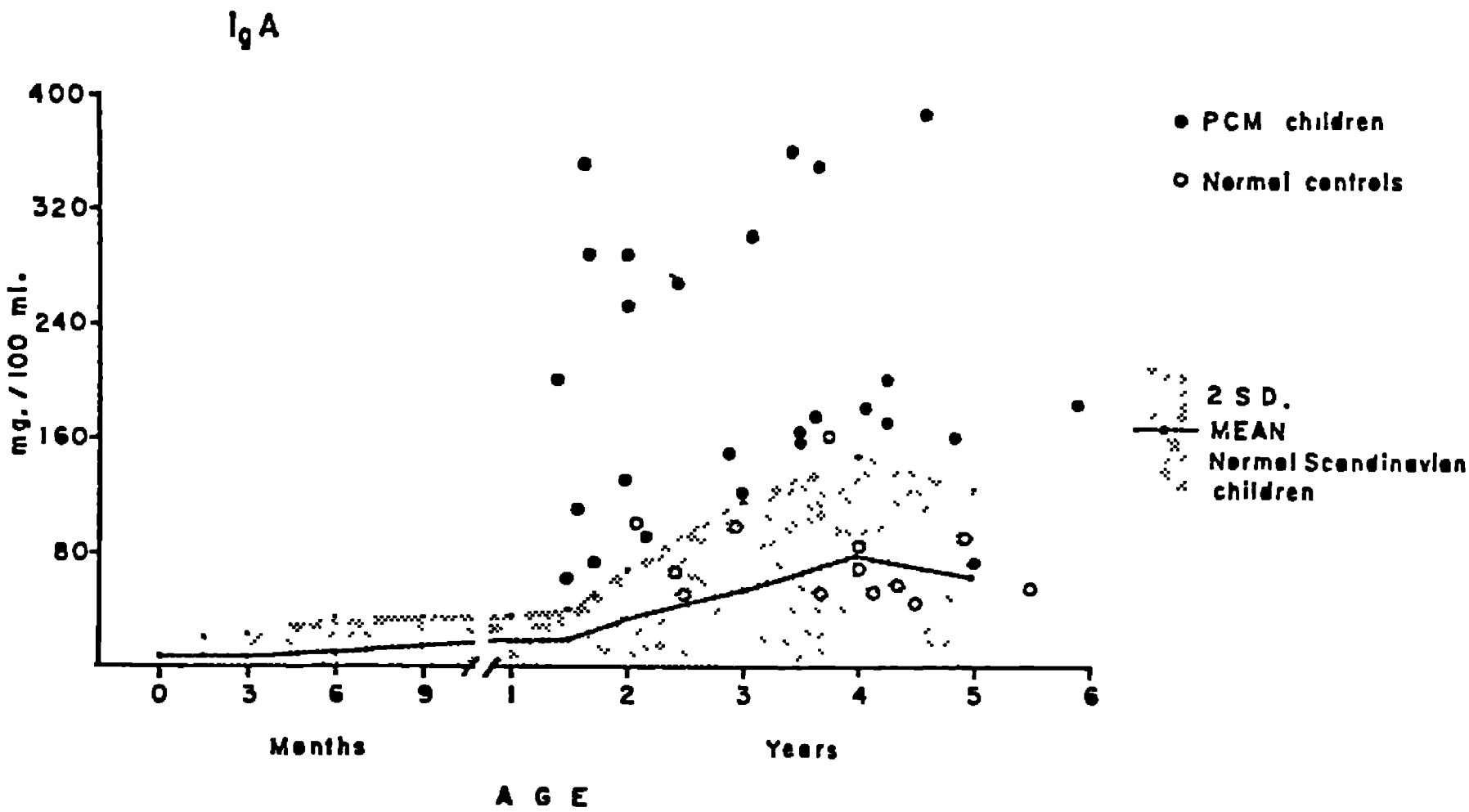



FIG. 2. Serum IgA levels in protein-calorie malnourished children and normal Guatemalan controls compared with healthy Scandinavian children (Adapted from Johansson, D. O. and Berg, T.: Acta Paed. Scand. 56. 572–579, 1967.)

TABLE 3. *Longitudinal Studies*

| Code | Days of Hospital Stay | Diarrhea | Respiratory Infection | Other Diseases | Total Serum Proteins (Gm %) | Serum Albumin (Gm %) | Immunoglobulins IgG | IgA | IgM |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | (mg %) | | |
| 196 | 1 | + | 0 | Herpes simplex | 3.3 | 1 53 | 820 | 148 | 78 |
| | 28 | 0 | 0 | 0 | 7 1 | 4 04 | 1,050 | 122 | 140 |
| | 65 | 0 | + | Fever | 5 1 | 3 04 | 1,350 | 215 | 58 |
| | 120 | Lactose intolerance | 0 | 0 | 6 9 | 4 60 | 1,175 | 200 | 41 |
| 198 | 1 | ++ | + | 0 | 3 6 | 1 73 | 2,050 | 360 | 65 |
| | 45 | 0 | 0 | 0 | 7.9 | 4 91 | 1,100 | 173 | 120 |
| | 62 | 0 | 0 | 0 | 8 1 | 5 36 | 980 | 280 | 100 |
| 216 | 1 | ++ | 0 | Conjunctivitis | 3 1 | 1 32 | 890 | 171 | 136 |
| | 3 | + | 0 | Conjunctivitis | 3 2 | 1 40 | 890 | 160 | 104 |
| | 8 | + | 0 | Mumps | 4 5 | 1 64 | 1,450 | 304 | 180 |
| | 18 | 0 | 0 | 0 | 6 4 | 3 17 | 1,530 | 163 | 166 |

tient 184 died without 48 hours from severe bronchopneumonia, and patient 213 died after 12 days of hospitalization from severe generalized septicemia caused by *Pseudomonas aeruginosa*. None of these fatal cases had subnormal levels in any of the immunoglobulin fractions that were measured.

Total white blood cell and differential counts in 14 of the malnourished children, done upon admission and simultaneously with the chemistry studies, showed no positive correlations between the percentage of any of the white cells, including the abnormal circulating plasmocytoid lymphocytes and the three immunoglobulin fractions studied.

In bacteriologic investigations of those patients with severe diarrhea or with suspected generalized sepsis, *Salmonella* was found in the stools of two with severe gastrointestinal symptoms, and *Pseudomona aeruginosa* grew in culture samples taken from the skin, blood and feces of patient 213. No relation was found between the highest rectal temperature observed during the first week of admission and the level of any of the Ig fractions. Of interest, however, is that none of the patients had severe hypothermia (a common sign in septicemia).

Intestinal parasites, when present, were usually the combination of helminths and protozoa. Neither severe infections nor a significant relation between the immunoglobulin levels and the parasitic load were observed

## Discussion

With rare exceptions, children with PCM are either incubating or harboring clinical or subclinical infections of varied etiology. This would explain the stimulation for an increased rate of synthesis of serum immunoglobulin in some PCM patients, as observed by Cohen and Hansen,[12] and ourselves. Furthermore, these rises suggest that the high incidence of infection associated with PCM is not a consequence of immunologic deficiency as measured by levels of IgG, IgA and IgM in serum.

The significantly higher-than-normal levels of serum IgA observed in this and other series of malnourished children, as recently reported,[8, 20, 21] have not yet been adequately explained. The elevations in some of the malabsorptive and the diarrheal disorders,[22] could be related to repetitive gastrointestinal insults, which are of common occurrence in children with PCM. An abnormal permeability of the gastrointestinal mucosa, if present, could facilitate the entry into the blood stream of the IgA secreted by the plasma cells in the *lamina propria*. Immunofluorescence studies of biopsies of gastrointestinal mucosa in celiac disease have revealed an increased number of plasma cells infiltrating the *lamina propria*, the majority of them staining with antisera to IgA and a few with antisera to IgG and IgM.[23] Morphologic studies of duo-

denal and rectal biopsies in PCM children have shown lymphoid and plasma cell infiltrations of the *lamina propria*, which do not entirely disappear with nutritional recovery.[24] If these plasma cells are producing IgA, as is the case in celiac disease,[25] this may help explain the rise of serum IgA levels in PCM. The persistence of the high IgA levels after nutritional recovery, could similarly reflect what is occurring in the intestinal mucosa.

In a recently reported study of serum immunoglobulin levels[21] in malnourished children, the control group consisted of children from rural communities where PCM and intermittent diarrhea are prevalent. These subjects had neither clinical nor biochemical evidence of malnutrition, but their serum IgA values were similar to those of the "recovered" patients in our present study and differ significantly from values in children with PCM.

## Acknowledgments

The authors express their sincere appreciation to Mr. José Luis Escobar, for his valuable technical assistance, to Mrs. Ilse de Melgar, for her assistance in the statistical analysis of the data, and to Dr. Victor Argueta von Kaenel, Head of the Pediatric Department of the Guatemala General Hospital, for allowing us to study patients under his care. They also gratefully acknowledge the encouragement received from Dr. Fernando Viteri, Head of the Biomedical Division of INCAP, and from Dr. Moisés Béhar, Director of the Institute.

## References

1. Gordon, J. E., Chitkara, I. D. and Wyon, J. B.: Weanling diarrhea. Amer. J. Med. Sci. 245: 345, 1963.
2. Scrimshaw, N. S., Wilson, D. and Bressani, R.: Infection and kwashiorkor. J. Trop. Pediat. 6: 37, 1960.
3. Scrimshaw, N. S., Taylor, C. E. and Gordon, J. E.: Interactions of nutrition and infection. Geneva, World Health Organization, 1968 (WHO Monograph Series No. 57), p. 26.
4. Salomon, J. B., Mata, L. J. and Gordon, J. E.: Malnutrition and the common communicable diseases of childhood in rural Guatemala. Amer. J. Pub. Health 58: 505, 1968.
5. Tejada V., C., Béhar, M. y Cofiño U., E.: Estudio clínico patológico de las bronconeumonías del niño desnutrido. Rev. Col. Méd. (Guatemala) 7: 134, 1956.
6. Lawless, J., Lawless, M. M. and Garden, A. S.: Admission and mortality in a children's ward in an urban tropical hospital. Lancet 2: 1175, 1966.
7. Brown, R. E. and Katz, M.: Antigenic stimulation in undernourished children. E. Afr. Med. J. 42: 221, 1965.
8. Kumate R., J.: Desnutrición e inmunología. *In* Desnutrición en el Niño, R. Ramos Galván, C. Mariscal Abascal, A. Viniegra Carreras and B. Pérez Ortiz, Eds. México, D. F., Impresiones Modernas, S. A., 1969, p. 121.
9. Olarte, J., Craviото, J. and Campos, B.: Inmunidad en el niño desnutrido. I. Producción de antitoxina diftérica. Bol. Méd. Hosp. Infant. (Méx.) 13: 467, 1956.
10. Pretorius, P. J. and De Villiers, L. S.: Antibody response in children with protein malnutrition. Amer. J. Clin. Nutr. 10: 379, 1962.
11. Kumate, J., Benavides, L., Hikimura, J. y Herrera Romo, L.: Respuesta inmunológica en fiebre tifoidea. Bol. Méd. Hosp. Infant. (Méx.) 19: 17, 1962.
12. Cohen, S. and Hansen, J. D. L.: Metabolism of albumin and γ-globulin in kwashiorkor. Clin. Sci. 23: 351, 1962.
13. Viteri, F., Béhar, M., Arroyave, G. and Scrimshaw, N. S.: Clinical aspects of protein malnutrition. *In* Mammalian Protein Metabolism, H. N. Munro and J. B. Allison, Eds. vol. 2, New York, Academic Press, Inc., 1964, pp. 523–568.
14. Instructions for use and care of T. S. meter and the T. C. Refractometer. A. O. Instrument Company, Buffalo, New York 14215, U. S. A.
15. Fahey, J. L. and McKelvey, E. M.: Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immun. 94: 84, 1965.
16. Viteri, F., Alvarado, J., Luthringer, D. G. and Wood, R. P. III: Hematological changes in protein-calorie malnutrition. Vitamins Hormones 26: 573, 1968.
17. Viteri, F. and Alvarado, J.: The creatinine height index, its use in the quantification of protein depletion and repletion in protein–calorie malnourished children. Pediatrics (In press).
18. Alvarado, J., Viteri, F. and Béhar, M.: Tratamiento hospitalario de la desnutrición proteínico-calórica severa. Rev. Col. Méd. (Guatemala), 1970. (In press)
19. Johansson, D. O. and Berg, T.: Immunoglobulin levels in healthy children. Acta. Paediat. Scand. 56: 572, 1967.
20. Keet, M. P. and Thom, H.: Serum immunoglobulins in kwashiorkor. Arch. Dis. Child. 44: 600, 1969.
21. Lechtig, A., Arroyave, G., Viteri, F. and Mata, L. J.: Inmunoglobulinas séricas en la desnutrición proteínico–calórica de niños preescolares. Arch. Latinoamer. Nutr., 1970. (In press)
22. Najjar, S. S., Stephan, M. and Asfour, R. Y.: Serum levels of immunoglobulins in marasmic infants. Arch. Dis. Child. 44: 120, 1969.
23. Rubin, W., Fauci, A. S., Sleisenger, M. H. and Jeffries, G. H.: Immunofluorescent studies in adult celiac disease. J. Clin. Invest. 44: 475, 1965.
24. Schneider, R., Alvarado, J. and Viteri, F.: Histopathological study of the intestinal mucosa in malnutrition: A preliminary report. *In:* Pediatrics in Latin America. Report of a Macy Conference, Santiago, Chile, March 24 to 26, 1969. Josiah Macy Jr. Foundation, p. 31.
25. Thompson, R. A., Asquith, P. and Cooke, W. T.: Secretory IgA in the serum. Lancet 2: 517, 1969.

THE AMERICAN JOURNAL OF CLINICAL NUTRITION
Vol. 23, No. 7, July, 1970, pp. 875–878
*Printed in U.S.A.*

# Indices of Body Composition in Infantile Malnutrition: Total Body Potassium and Urinary Creatinine[1]

G. A. O. ALLEYNE,[2] M.D., M.R.C.P., F. VITERI,[3] M.D., AND J. ALVARADO,[3] M.D.

HITHERTO, an increase in weight and height on treatment has been the most widely used index of recovery from malnutrition, and when the recovery process is well established an increase in weight usually reflects an increase in lean body mass. It has been shown that there are gross abnormalities of body composition in malnutrition (1–4) and, in the early stages of recovery, increase in weight may be masked by changes in body composition.

It would be ideal, therefore, to have a simple method of assessing total body composition at regular intervals. There are no such simple methods, but we feel that some measure of lean body mass or muscle mass would serve the purpose equally well. Urinary creatinine is held to be a measure of muscle mass even in malnutrition (5, 6), and Cheek (7) has proposed that total muscle mass can be estimated from the 24-hr urinary creatinine. Stearns et al. (8) have measured urinary creatinine in a large series of normal children and given values for the increments of 24-hr urinary creatinine with height. On the basis of these normal data one can determine the creatinine excretion appropriate for a healthy child of a given height. If then the 24-hr urinary creatinine and height of the malnourished child are known, the creatinine–height index may be calculated and is some measure of the degree of deficit of muscle mass in that child (9–11).

Total body potassium is also a measure of lean body mass and indirectly of muscle mass, since the major fraction of body potassium is in muscle. By the same analogy the degree of potassium and hence lean body mass deficit can be calculated if we know the total body potassium and

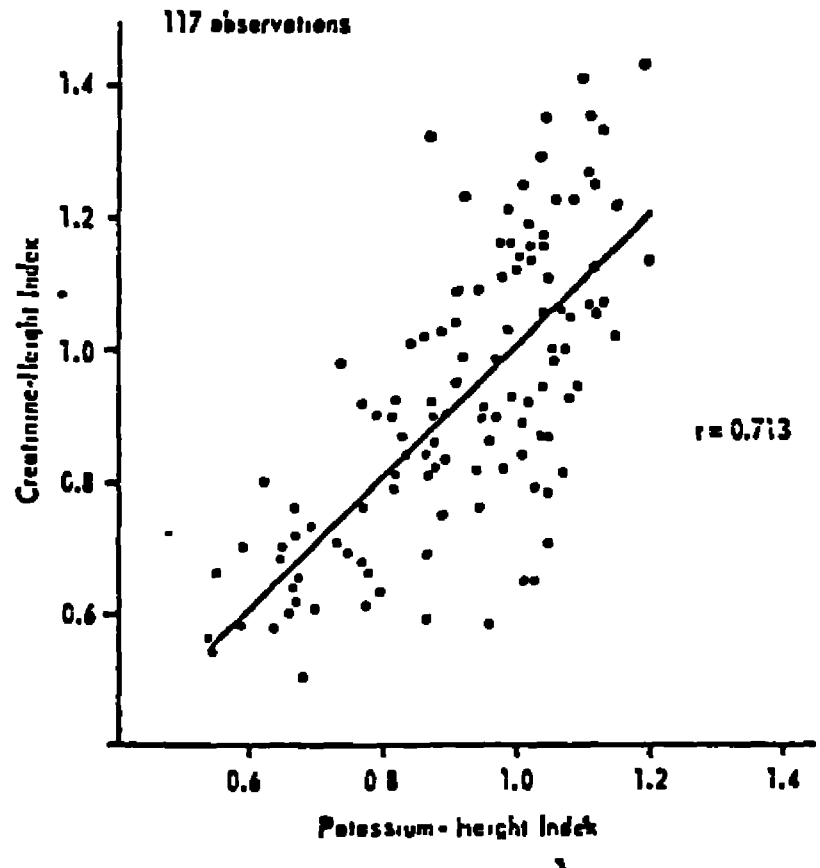



FIG. 1. Relationship between total body potassium per centimeter of height and height in infants fully recovered from malnutrition.

[1] Supported in part by a grant from the Wellcome Trust.

[2] Present address: Medical Research Council, Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston 7, Jamaica.
[3] Present address: Instituto de Nutrición de Centro América y Panamá, Carretera Roosevelt Zona 11, Guatemala, Centro-América.

height of the child and the total body potassium appropriate for a normal child of the same height. In this study we show that there is a good degree of correlation between the muscle mass deficit as measured by the creatinine–height index and the lean body mass deficit as measured by the potassium–height index.

TABLE I

Creatinine–height index (CHI) and potassium–height index (KHI) in infants recovering from malnutrition

| Days after admission | Number of observations | CHI | KHI |
|---|---|---|---|
| | | *Mean* | |
| 0–5 | 8 | 0.61 (0.016)* | 0.67 (0.038) |
| 6–10 | 11 | 0.72 (0.033) | 0.67 (0.021) |
| 11–20 | 14 | 0.77 (0.048) | 0.83 (0.031) |
| 21–30 | 22 | 0.96 (0 041) | 0 90 (0.021) |
| 31–40 | 19 | 1.04 (0.046) | 1.01 (0.019) |
| 41–50 | 18 | 1.07 (0 039) | 1.00 (0.021) |
| 51–60 | 11 | 0.99 (0.063) | 1.05 (0.025) |
| More than 60 | 12 | 1.01 (0.071) | 1.07 (0 056) |

* Figures in parentheses indicate SEM.

## MATERIAL AND METHODS

The patients studied were malnourished children admitted to the Medical Research Council's Tropical Metabolism Research Unit. The typical clinical picture has been described previously (12). Timed 24-hr specimens of urine were collected from a series of 25 male infants with the use of a glass adaptor strapped to the perineum. Total body potassium was measured in a 4-II liquid scintillation whole body counter. Urinary creatinine was measured with alkaline picrate (13).

*Indices:*

$$\text{Creatinine–height index (CHI)} = \frac{\text{24-hr urine creatinine/cm height}}{\text{24-hr urine creatine/cm height for a normal child of same height.}}$$

$$\text{Potassium–height index (KHI)} = \frac{\text{total body potassium/cm height}}{\text{total body potassium/cm height for a normal child of same height.}}$$

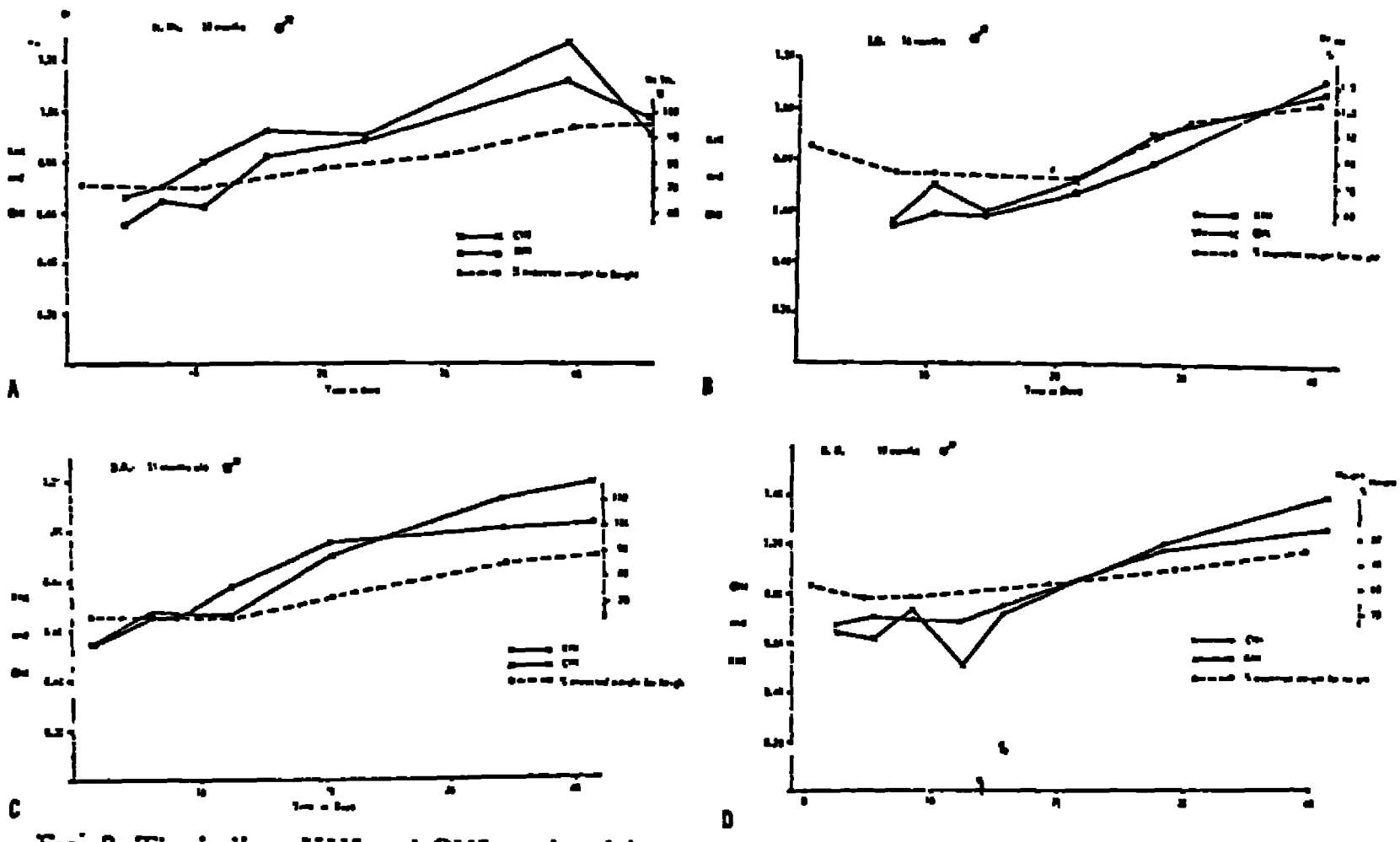



FIG. 2. The indices, KHI and CHI, and weight as a percentage of expected weight for height in four children recovering from malnutrition.

As mentioned above, the normal creatinine excretion was obtained from the data of Stearns et al. (8). The normal data for total body potassium were derived from total body potassium measurements in a series of 37 children who had been treated in this Unit and had recovered from malnutrition (Fig. 1).

## RESULTS

The CHI and KHI at different stages of recovery from malnutrition are shown in Table 1. It is clear that on admission to the hospital there was a 30–40% reduction in tissue mass as shown by both indices, which approached normal after approximately 4 weeks. Figure 2, *A–D* shows the weight chart and both indices in four children recovering from malnutrition.

## DISCUSSION

The major aim of this study was to validate the use of the creatinine–height index by comparison with an independent measure of lean body mass. The use of body potassium as an index of lean body mass in the early stages of recovery from malnutrition is quite justified, since it has been shown that a true potassium deficiency is rapidly corrected within the first few days of therapy, and, thereafter, a low body potassium represents not potassium deficiency but a reduction of lean body mass (14).

It is important to use height rather than weight in the derivation of any indices of normality of body composition, since height is not affected by the rapid changes in body composition that take place during recovery.

We would hope that CHI finds widespread use as a measure of muscle mass. It would then be possible to assess various physiological functions in terms of the CHI instead of weight, which as we have stated is a rather poor index of body composition and change.

Although in this study we have used a single 21-hr urine collection, the accuracy of the results would have been improved if the mean of three consecutive 24-hr collections had been taken, as it has been shown that the coefficient of variation in urinary creatinine excretion is reduced from 13% in 24-hr collections to 6.9% in 3-day collections (11). The wide variation in urinary creatinine found in some malnourished children soon after admission (5) may be avoided if urine is collected for 2 or 3 days.

## SUMMARY

Indices of muscle mass and lean body mass are derived from 24-hr urinary creatinine and total body potassium. These indices change in parallel in children recovering from protein–calorie malnutrition.

## REFERENCES

1. Garrow, J. S., K. Fletcher and D Halliday. Body composition in severe infantile malnutrition. *J. Clin Invest* 44 417, 1965.
2. Alleyne, G. A. O. Studies on total body potassium in infantile malnutrition: the relation to body fluid spaces and urinary creatinine *Clin. Sci.* 34. 199, 1968.
3. Halliday, D. Chemical composition of the whole body and individual tissues of two Jamaican children whose deaths resulted primarily from malnutrition *Clin. Sci.* 33. 365, 1967.
4. Alleyne, G. A O, D Halliday, B. L. Nichols and J. C. Waterlow. Chemical composition of organs of children who died from malnutrition. *Brit. J. Nutr.* 23 783, 1969.
5. Standard, K. L, V. S. Wills and J. C Waterlow. Indirect indicators of muscle mass in malnourished infants *Am. J. Clin. Nutr.* 7: 271, 1959
6. Arroyave, G, D. Wilson, M. Béhar and N S. Scrimshaw. Serum and urinary creatinine in children with severe protein malnutrition *Am. J. Clin Nutr* 9: 176, 1961.
7. Cheek, D. B *Human Growth* Philadelphia: Lea & Febiger, 1968.
8. Stearns, G., K J Newman, J B McKinley and P C. Jeans *Ann N.Y. Acad Sci.* 69 835, 1958.
9. Viteri, F., G. Arroyave and M. Béhar. *Abstract 7th Intern. Congr. Nutr.* p. 46, 1966.
10. Viteri, F., J. Alvarado, D. Luthringer and R. P. Wood III. Hematological changes in protein-caloric malnutrition. *Vitamins Hormones* 66: 587, 1968.

11. VITERI, F., AND J. ALVARADO. *Paediatrics.* Submitted for publication.
12. WATERLOW, J. C., J. CRAVIOTO AND J. M. L. STEPHEN. Protein malnutrition in man. *Advan. Protein Chem.* 15: 131, 1960.
13. BOSNES, R. W., AND H. H. TAUSSKY. On the colorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.* 158: 581, 1945.
14. ALLEYNE, G. A. O. Studies on total body potassium in malnourished infants. Factors affecting potassium repletion. *Brit. J. Nutr.* 24: 205, 1970.

# 14 The Importance of Accurate Measures of Malnutrition

Moisés Béhar
Director, Instituto de Nutrición de Centro America y Panama, Guatemala City

Dr. Bengoa stressed in his paper—I believe very rightly so—the need of an indicator that can be of practical value in assessing the extent of the problem of protein-calorie malnutrition (PCM) in a community.

In the preindustrialized areas of the world, evidence obtained through dietary, biochemical, clinical, and anthropometrical studies, as well as from the analysis of morbidity and mortality data, indicates that the prevalence of PCM in all of its different forms and degrees is greater than would be suspected by nonspecialized persons on the basis of the occurrence of the severe forms, kwashiorkor and marasmus. The above-mentioned studies are complicated, expensive, and time-consuming; furthermore, the information that they provide is difficult for nonspecialists to interpret. Therefore, it would be extremely useful to have indicators that are easily obtainable and that are sufficiently specific and sensitive to be used in determining the magnitude of the problem, both for diagnostic and for program evaluation purposes.

One of the reasons why malnutrition has not received sufficient attention by policy-makers at a national level in many countries is that its real magnitude is frequently unknown or underestimated. As of the last few years, great efforts are being made in the planning of health programs, but even health planners have been unable to give malnutrition its proper place among health problems, partly because of the difficulties in estimating its real magnitude.

Only under situations of acute hunger, as have recently occurred in Biafra and India, do health authorities and the entire society recognize malnutrition as a problem. It would be extremely useful to demonstrate that under "normal" conditions of life more than half the population of a country has suffered or is suffering from some degree of malnutrition. [1] For this purpose, indicators like the one suggested by Dr. Bengoa can be useful.

Responsible persons in underdeveloped countries, frequently even health personnel, have accepted some characteristics of the population like small body size, lack of initiative and ambition, and low work efficiency as genetic characteristics, although available scientific information suggests that these are more frequently the result of environmental factors, among which malnutrition is usually one of the most important. For this reason, it would also be very useful to demonstrate a correlation between anthropometric indicators—like the one suggested by Dr. Bengoa—and functional parameters of the subjects such as resistance to infection, psychological behavior, or learn-

ing capability, even if this correlation does not necessarily prove a direct causal relationship with malnutrition, but rather the influence of the total environment.

There are, of course, limitations in an indicator like body size, which were briefly analyzed by Dr. Wilson, but we should keep in mind that an indicator is not necessarily a direct measurement of the phenomenon but only a practical way of evaluating its magnitude.

In regard to the intervention programs for the control of PCM, we agree with the epidemiological approach recommended by Dr. Bengoa. Through this approach, it will also be possible to select population groups, either in terms of socioeconomic condition or in respect to age, which are more vulnerable and in greater need of attention This is particularly true for the implementation of measures within the secondary or tertiary level of prevention, such as treatment, nutrition rehabilitation, supplementary feeding, or nutrition education.

In relation to the age groups that should receive greater attention, it is interesting to observe how the situation has changed as more and better information on the effects of malnutrition has been obtained. Until about twenty years ago. supplementary feeding and nutrition education programs were organized mainly for schoolchildren. probably because this group was easier to reach. However, experimental studies revealed that the small size of school-age children cannot be modified significantly by supplementary feeding. [2] Furthermore, the severe cases of protein-calorie malnutrition, mainly kwashiorkor, occurring predominantly between the second and fourth years of life. received great international attention. [3.4] On the basis of these and other related observations, emphasis was progressively and correctly moved from the school-age to the preschool-age child. It was also observed that in the more primitive communities where prolonged breast-feeding (usually over one year of age) is still a common practice, cases of severe PCM, particularly kwashiorkor. were seldom seen before the age of 1 year For these reasons, the group of children from 1 to 4 years old was identified as the one at greater risk and was therefore granted the highest priority in applied programs Later on, however, it was observed that even in those communities where prolonged breast-feeding is a common practice, the growth of children was not satisfactory as of the fourth to sixth month of age. Morbidity and mortality statistics also indicated a great risk for children during the second half of their first year of life and during their second year, decreasing rapidly thereafter. [5] In addition, because of its dramatic quality and probably also

its exotic name, kwashiorkor initially received more attention, but later on the importance of marasmus and other severe types of PCM occurring before the first year of life was recognized in population groups undergoing cultural transition, where weaning takes place earlier, and often improperly. [6] It was then realized that all children under 5 years of age, including those under one year, would have to be considered.

More recently, interest has progressively been focused on the possibilities of permanent damage in young children's mental development caused by malnutrition both before and in the 6 months after birth. [7,8] So far, available evidence on the effect of the mother's nutrition on the newborn has been contradictory or inconclusive. [9] On a carefully controlled longitudinal study, now under way in Guatemala, information thus far obtained suggests a good association between caloric intake of mothers during pregnancy (estimated by dietary surveys) and the weight of their newborns. [10] Furthermore, preliminary data suggest that it is possible to correct the low birth weight, so frequent in these babies, by correcting the inadequate dietary intake of their mothers with supplementary feeding during pregnancy. [11] Babies with low birth weight are at a much greater risk of early death, as was recently confirmed by INCAP studies. [12]

All this information indicates the need for directing greater attention to pregnant and lactating mothers, and to children during their first months of life in any applied nutrition program. It seems, therefore, that high priority should be given to the development of a very strong program on maternal and child health within the health plans of the developing countries; not only family planning but also nutrition should be among its fundamental components.

Recent observations in Central America support Dr. Bengoa's contention that activities at the level of secondary and tertiary prevention, particularly those actions which are under the direct responsibility of health agencies, can reduce the incidence of severe and advanced cases without modifying substantially the prevalence of mild or moderate cases. These last forms, although clinically not very dramatic, can be of greater public health significance because they are more prevalent, and especially if their association with functional damage is further documented. The control of these forms requires a strong coordinated program at the level of primary prevention, which should correct the basic and interrelated problems of insufficient and inadequate supply of foods, low purchasing power, and low educational level. This can be done only through coordinated multisectoral programs, properly oriented by

a national food and nutrition policy and constituting an important component of the national development plan.

In order to convince the planners and the policy-makers of the need and feasibility of this last approach, efforts are being made to sell the idea on an economic basis, that is, to demonstrate that the expenses involved in measures of direct nutritional benefits are a good investment. Dr. Wilson indicated the complexity of this approach and the difficulty in obtaining the basic information required. Still, I believe that it is possible to obtain a reasonably good estimate of the immediate economic losses due to malnutrition in a given community by calculating, among other items, the expenses incurred in the treatment of cases, and the losses due to absenteeism of workers because of diseases related to malnutrition, as well as those resulting from reduced work performance. However, these immediate losses are probably much lower than those stemming from early malnutrition. The effects of malnutrition during the intrauterine period and in the course of the first few years of life, in terms of lower learning capacity and inefficient integration of the labor force in a technological society, are much more difficult to estimate in economic terms; furthermore, they appear after a time lag of at least 10 to 20 years. This is, of course, a serious limitation to planners or to politicians interested in investments with a more immediate return. Therefore, the improvement of these fundamental programs is postponed, and the gap between the developed and the underdeveloped societies increases.

I agree that, for effectiveness, any intervention program to control malnutrition has to be an ambitious one, which may even require significant changes in the socioeconomic and political structure of the country. Therefore, very careful planning is fundamental

Some countries and international agencies interested in the field are already working in this direction. In my opinion, the efforts for socioeconomic development in the last decade have demonstrated that greater attention to human resources and improvement of the quality of life of the total population of the country is needed. This is not only the final objective of development but also a mechanism for achieving a real and harmonious socioeconomic development, not just economic growth.

I fully appreciate, therefore, the interest in and efforts toward utilizing a more strict planning methodology, for which I am sure this conference will be of great value. Still, I think that in planning the control of malnutrition we should not completely forget the humanitarian aspects. Unfortunately for us who are interested in nutrition, there is very little probability that a highly

influential or policy-making citizen will go to bed hungry or will have a child dying from kwashiorkor or marasmus, as is happening every day to thousands of people in large areas of the world. If that were the case I am sure that, in addition to the "rational" economic approach to the prevention of malnutrition, a higher social and humanitarian consciousness would be awakened.

## References

1. Béhar, M. Prevalence of malnutrition among preschool children. In *Malnutrition, Learning, and Behavior.* N. S. Scrimshaw and J. E. Gordon, eds., Cambridge, Mass.: MIT Press, 168, p. 30.

2. Scrimshaw, N. S., and M. A. Guzmán. The effect of dietary supplementation and the administration of vitamin $B_{12}$ and aureomycin on the growth of school children. In *Current Research on Vitamins in Trophology.* Nutrition Symposium Series No. 7. New York: National Vitamin Foundation, 1953, p. 101.

3. Brock, J. F., and M. Autret. *Kwashiorkor in Africa.* FAO Nutritional Studies No. 8. Rome. FAO, 1952.

4. Autret, M., and M. Béhar. *Síndrome Policarencial Infantil (Kwashiorkor) and its Prevention in Central America.* FAO Nutritional Studies No. 13. Rome. FAO, 1954.

5. Gordon, J. E., J. B. Wyon, and W. Ascoli. The second year death rate in less developed countries. *Amer. J. Med. Sci.* 254.357, 1967.

6. McLaren, D. S. A fresh look at protein-calorie malnutrition. *Lancet* 2.485, 1966.

7. Scrimshaw, N. S., and J. E. Gordon. *Malnutrition, Learning, and Behavior.* Cambridge, Mass. MIT Press, 1968.

8. Winick, M. Cellular growth in intrauterine malnutrition. *Ped. Clin. North America* 17.69, 1970.

9. Bergner, L. and M. W. Susser. Low birth weight and prenatal nutrition, An interpretative review. *Pediatrics* 46:946, 1970.

10. Lechtig, A., J. P. Habicht, E. de León, G. Guzmán, and M. Flores. Influencia de la nutrición materna sobre el crecimiento fetal en poblaciones rurales de Guatemala. I. Aspectos dietéticos. *Arch. Latinoamer. Nutr.* In press.

11. Lechtig, A., J. P. Habicht, E. de León, and G. Guzmán. Influencia de la nutrición materna sobre el crecimiento fetal en poblaciones rurales de Guatemala. II. Suplementación alimentaria. *Arch. Latinoamer. Nutr.* 22:117, 1972.

12. Mata, L. J. *Nutrition and Infection.* Protein Advisory Group Bulletin No. 11. Geneva, 1971.

**Nutrition**
Editors: A. CHÁVEZ, H. BOURGES, S. BASTA, Mexico
Publishers: S. KARGER, Basel SEPARATUM (Printed in Switzerland)

Proc. 9th int Congr. Nutrition, Mexico 1972, vol 2, pp 276–279 (Karger, Basel 1975)

# Bone Maturation in Children Recovered from Malnutrition

M. A. GUZMÁN

Institute of Nutrition of Central America and Panamá (INCAP), Guatemala

Since growth is conditioned by the adequacy of nutrition, it is not surprising that skeletal development may be affected detrimentally by limited nutrition.

In the literature, one finds early reports to this effect based on the gross assessment of 'bone age', established by comparison of radiographs of selected sites, with corresponding age-sex specific norms [7] Although this approach results in estimates which may be considerably affected by measurement error, the results obtained through its application have, nevertheless, suggested that retardation in bone development begins early in life, manifesting itself in a pattern that is quite uniform among populations of widely different ethnic extraction but with a similar background of deficient nutrition [2, 8, 11]. Furthermore, the retardation in bone age reported for these populations in terms of net time deviations from the norm closely parallel similar estimates of time lags established on the basis of either height or weight [8].

The problem of skeletal growth retardation has been studied using different alternative methods, all of which attempt to reduce the large measurement errors commonly associated with the comparison technique described previously [3, 5]. In our case, we have evaluated bone development on the basis of age-sex specific counts of ossification centers, determined from left hand-wrist radiographs, and following the methodology described by GARN and ROHMANN [5]. The counting of ossification centers present is complemented with radiogrammetric measurements of the external and internal diameters at the midshaft of the second metacarpal bone, which permits estimation of cortical thickness [3]. By using this approach, we have been able to assess bone development, with sufficient sensitivity and a good

reliability, from birth to 5 years of age for different population groups in the rural highlands of Guatemala [9, 10, 12].

Results from a longitudinal study of 5 years' duration [10], evaluated in terms of bone development using the methods described, suggest that improvement of the usual diet of a village through the daily administration of a high quality protein supplement (Incaparina) resulted in better age-sex specific ossification status (greater number of centers present) and improved bone growth (more centers appearing during fixed time intervals) for the children of the supplemented village in comparison with children from similar villages without food supplements [9, 10].

Parallel studies conducted with 96 children hospitalized for protein-calorie malnutrition at the Roosevelt Hospital in Guatemala City revealed that the ossification status of these children does not differ from that of ambulatory children from control villages in the Guatemalan highlands [6]. On the other hand, radiogrammetric measurements of second metacarpal cortical thickness in village and hospitalized Guatemalan children revealed no systematic reduction in outer bone diameter at midshaft, but a significantly larger inner diameter was found in the hospitalized children. This indicates a reduction of cortical bone at the endosteal surface [4]. The difference in cortical bone between the hospitalized and the village children becomes more apparent if the bone diameter measurements are used to calculate midshaft cortical area, and the results expressed in terms of percent cortical area [4].

Another series of studies on the bone development of children, also conducted in Guatemala, evaluated radiogrammetric results for 84 children with protein-calorie malnutrition who died and were autopsied at the Roosevelt Hospital during the period from August 1964 to November 1965.

Preliminary analyses of the results from these studies indicate that there is no significant difference between the number of ossification centers present in these children and the number of centers present in either their village, or hospitalized, age-sex specific counterparts. The cortical thickness of the second metacarpal of the autopsied cases was, however, less than the cortical thickness of the second metacarpal of both the village and the hospitalized children. In this case, the percent cortical area of the autopsied cases was substantially less than that of comparable living children. In the necropsy series, it was also possible to carry out direct measurements of midshaft diameters on the second metacarpal bone removed at the time of autopsy; the results from such measurements closely parallel the previously described radiogrammetric results.

*Table I.* Percent ash content of the second metacarpal bone in children dying from protein-calorie malnutrition at the Roosevelt Hospital in Guatemala during August 1964–November 1965

| Age, years | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | n | $\bar{x}$ | s | n | $\bar{x}$ | s |
| <1 | 11 | 42.3 | 13 3 | 3 | 51.7 | 12 7 |
| 1 | 9 | 48 8 | 10 1 | 16 | 52 8 | 9 4 |
| 2 | 7 | 51 3 | 4.1 | 6 | 53 3 | 7 0 |
| 3 | 2 | 58 5 | 1 0 | 2 | 59.0 | 5 6 |
| 4 | 4 | 59 8 | 10 8 | – | – | – |
| 5 and over | 6 | 53 5 | 3.3 | 3 | 56.0 | 2 0 |
| Total | 39 | 49.7 | 10.7 | 30 | 53.5 | 8 4 |

In 69 of the 84 cases included in the necropsy series, it was also possible to carry out ash determinations; the results are presented in table I. These determinations suggest that the children dying from protein-calorie malnutrition have a dried, defatted bone ash content that does not differ from reported normal values [1, 13]. In other words, the bone loss evidenced by reduced cortical thickness really represents a loss of total bone; in this event, the detrimental effect of early malnutrition may well produce irreversible damage to the skeletal structure, such as thinning of the bone and changes in the epiphyses [6]. A sequence of these events may be permanently recorded in the bone as lines of arrest (Harris' lines), which are commonly visible in hand-wrist radiographs of children from areas where malnutrition is common. The real biological significance of these lines, however, still remains unclear. On the other hand, the thinning of the bone results in decreased breaking strength and a generally reduced compression strength [6]; the changes in the epiphyses may produce a reduction in the length of bones which may well be irreversible, thus contributing to the small body size which uniformly characterizes the populations of regions where malnutrition is prevalent [8]. In any case, the available evidence suggests that conditions of poor nutrition result in skeletal changes which may permanently affect the normal functions of the individual.

## *References*


1 Bodansky, M. and Bodansky, O.: Biochemistry of disease; 2nd ed , p. 792 (The McMillan Co., New York 1952)

2 Chan, S T.; Chang, K S.F., and Hsu, F K : Growth and skeletal maturation of Chinese children, Amer. J. phys. Anthropol. *19:* 289–300 (1961).

3 Garn, S. M ; Feutz, E ; Colbert, C., and Wagner, B : Comparison of cortical thickness and radiographic microdensitometry in the measurement of bone loss; in Progress in development of methods in bone densitometry, pp. 65–77 (National Aeronautics and Space Administration, Washington D.C. 1966).

4 Garn, S M , Guzmán, M A , and Wagner, B.: Subperiosteal gain and endosteal loss in protein-calorie malnutrition: Amer. J. phys. Anthropol. *30*. 153–155 (1969)

5 Garn, S. M and Rohmann, C : The number of hand-wrist centers. Amer. J phys Anthropol *18:* 293–299 (1960).

6 Garn, S M.; Rohmann, C , and Guzmán, M. A : Malnutrition and skeletal development in the pre-school child; in Pre-school child malnutrition, pp. 43–62 (Nat. Acad. Sci.National Research Council, Washington, D C. 1966).

7 Greulich, W. W. and Pyle, S. I.: Radiographic atlas of skeletal development of the hand and wrist; 2nd ed. (Stanford University Press, Palo Alto California 1959).

8 Guzmán, M A · Impaired physical growth and maturation in malnourished populations, in Scrimshaw and Gordon Malnutrition, learning and behaviour, pp 42–54 (MIT Press, Cambridge 1968)

9 Guzmán, M A ; Rohmann, C., Flores, M ; Garn, S. M , and Scrimshaw, N S : Osseous growth of Guatemalan children fed a protein-calorie supplement. Fed Proc *23*. 338 (1964)

10 Guzmán, M. A ; Scrimshaw, N. S ; Bruch, H A , and Gordon, J. E : Nutrition and infection field study in Guatemalan villages, 1959–1964 VII Physical growth and development of preschool children Arch. environm Hlth *17* 107–118 (1968).

11 Jones, P. R. M. and Dean, R. F. A.: The effects of kwashiorkor on the development of the bones of the hand. J. trop Pediat *2*. 51–68 (1956)

12 Salomon, J B ; Klein, R E ; Guzmán, M. A , y Canosa, C.: Efectos de la nutrición e infecciones sobre desarrollo óseo de niños en un área rural en Guatemala. Arch. lat -amer. Nutr. *22*. 417–449 (1972).

13 White, A., Handler, P., and Smith, E L : Principles of biochemistry, p 4 (McGraw Hill Book Co , New York 1964)


Author's address: Dr. Miguel A. Guzman, Institute of Nutrition of Central America and Panama, Apartado 11–88, *Guatemala* (Guatemala)

# Sialic Acid Content of Red Blood Cells from Protein-Calorie Malnourished Children and During Recovery, and from Normal Children and Adults[1]

C. CONTRERAS AND F. E. VITERI

*Biomedical Division, Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala*

Received February 26, 1973

CONTRERAS, C., and VITERI, F. E. 1973. Sialic acid content of red blood cells from protein-calorie malnourished children and during recovery, and from normal children and adults. Can. J. Physiol Pharmacol. 51, 853–858.

Sialic acids were measured in the red cells of two groups of subjects. One group consisted of 12 children with severe protein-calorie malnutrition (P C.M ), six of them were followed longitudinally throughout the recovery period The control group included 28 normal children and 11 normal adults. All subjects were studied hematologically and the sialic acid content of the red cells was determined in three layers of erythrocytes, separated according to their density by ultracentrifugation. The results indicate that there are no alterations in the content of sialic acids in the red cells of children with severe P.C.M. Furthermore, they show that the sialic acid content of the red cell is not influenced by various levels of red cell folates nor by differences in the concentration of serum proteins, serum iron, percentage saturation of transferrin, serum folates, or serum vitamin $B_{12}$.

CONTRERAS, C., et VITERI, F. E 1973. Sialic acid content of red blood cells from protein-calorie malnourished children and during recovery, and from normal children and adults Can J. Physiol Pharmacol 51, 853–858.

Chez deux groupes de sujets, nous avons mesuré le contenu en acides sialiques des globules rouges Un groupe comprenait 12 enfants sujets à une malnutrition protéo-calorique (P C.M ) grave, six d'entre eux ont été également observés durant le période de rétablissement Le groupe témoin comprenait 28 enfants normaux et 11 adultes Tous les sujets ont été soumis a des tests hématologiques Nous avons déterminé le contenu en acides sialiques au niveau des trois couches de globules rouges telles qu'obtenues par ultracentrifugation Les résultats indiquent que le contenu en acides sialiques des globules rouges n'est pas modifié chez l'enfant atteint de P.C M. Ils indiquent également que le contenu des globules rouges en acides sialiques n'est pas modifié par le contenu globulaire en folates ni par le contenu sérique en proteines, fer folates et vitamine $B_{12}$, ni par le taux de saturation de la transferrine

[Traduit par le journal]

## Introduction

There is, to our knowledge, no information on the effect of nutrition on the sialic acid contents of the red blood cells, even though they are important components of membranes (Eylar *et al.* 1962; Cook *et al.* 1961) and of erythrocuprein (Kimmel *et al.* 1959), which partially determines the red cell life span (Markowitz *et al.* 1959).

In children suffering from kwashiorkor and marasmickwashiorkor, several authors (Shehata *et al.* 1965; Fayad *et al.* 1969; Patwardhan *et al.* 1971) have shown a marked increase in the total protein-bound hexose in serum. On the other hand, Patwardhan *et al.* (1971) observed altered concentrations of serum glycoproteins, a finding that the authors interpreted as the result of infection on protein-calorie deficiency.

In uncomplicated protein-calorie malnutrition (P C M.), changes such as low serum copper and ceruloplasmin (Lahey *et al* 1958), a mild decrease in red cell survival (Lanzkowsky *et al.* 1967), and a reduced number of red cells (Viteri *et al.* 1968*a*, 1968*b*) are known to occur. Viteri and colleagues (1968*a*, 1968*b*) have suggested that this finding is the result of adaptation, which involves reduction of the total circulating hemoglobin as a consequence of diminution in active tissue mass. If hemolysis plays an important role in the hematological alterations observed in P C M, an increase in the sialic acid content of erythro-

[1]This work was partially supported by Advanced Research Projects Agency (Project AGILE), under ARPA Order No 580, Program Plan No. 298, and monitored by the Nutrition Program, National Center for Chronic Disease Control, Bureau of Disease Prevention and Environmental Control, U S Public Health Service, and NIH Grant AM-0981

cytes in patients with severe P.C.M. would be expected since young red blood cells have a higher content of these acids, based on the results of Yachnin and Gardner (1961). On the other hand, if erythropoiesis were markedly impaired in severe P.C.M, the red blood cell content of these acids would be reduced, since older erythrocytes have a smaller content of sialic acids.

The concentration of sialic acids was measured in red blood cells of children with severe uncomplicated P.C.M. and during nutritional recovery in order to estimate the age composition of the circulating erythrocytes. Thereafter, relationships were investigated between the concentration of sialic acids in red blood cells and the serum and erythrocyte contents of important nutrients (proteins, iron, folates, and vitamin $B_{12}$) known to be deficient in cases of uncomplicated P.C.M., and during nutritional recovery on certain experimental diets (Viteri *et al.* 1964).

## Materials and Methods

Two groups of subjects were studied: (1) Normal subjects, 11 well-nourished adults, and 28 children 43 to 96 months old, (2) malnourished subjects, 12 children with severe P.C.M. of the edematous type and without infectious complications, between 22 and 84 months of age, admitted to the pediatric section of the General Hospital of Guatemala and to the Clinical Center of the Institute of Nutrition of Central America and Panama (INCAP). Six of these patients were studied periodically during their nutritional recovery.

All children were treated following a standard protocol used at INCAP's Clinical Center (Alvarado *et al.* 1970): upon admission water and electrolyte imbalances were corrected while the children were maintained for approximately 4 days on an adaptation diet that provided 0.7 g of protein (casein + 0.2% methionine) and 70 cal/kg body weight per day. Twenty to thirty percent of the calories were given as vegetable fat. From then on, and depending on tolerance, the concentration of the diet was increased progressively to reach 3–4 g of protein and 120–150 cal/kg body weight per day. This level of intake was usually attained within 10 days of admission. Later on, casein was progressively substituted by milk. Throughout hospitalization a multivitamin and mineral supplement was administered. It included iron, folic acid, and vitamin $B_{12}$. Protein depletion and repletion were estimated by the creatinine–height index (Viteri and Alvarado 1970).

Red cells were obtained from heparinized venous blood, which was placed at 4 °C immediately after withdrawal. Packed red cell volume was measured by the method of McGovern *et al.* (1955). Blood samples were centrifuged at 2000 × *g* and 4 °C for 10 min. Plasma and the buffy coat containing leukocytes were removed by suction. The packed erythrocytes were transferred to polyallomer tubes and centrifuged at 67 000 × *g* and 4 °C for 2 h in a preparative ultracentrifuge (Spinco model L2-65), using a swinging bucket rotor (Type SW-39) and following the method of Rigas *et al.* (1961). The resulting packed erythrocyte column was arbitrarily divided into three equal layers by cutting the tubes to isolate erythrocytes of different densities. They were identified as fractions 1, 2, and 3 from top to bottom. Fraction 1 should contain most of the reticulocytes; fraction 2, mature red cells; and fraction 3, older red cells (Rigas *et al.* 1961; Borun *et al.* 1957). Red blood cell counts were routinely carried out on each layer using a Coulter counter model B (Coulter 1956). Reticulocyte counts were performed according to the method of Brecher and Schneiderman (1950) to check the success of blood cell fractionation. Chemical determination of sialic acids was performed on each of the three erythrocyte layers by the thiobarbituric acid method of Warren (1959), using a Beckman DB spectrophotometer. The absorption spectrum of the acid chromophore from each sample was always obtained in the wave length range of 480–600 (mμ) nm. A representative spectrum is illustrated in Fig. 1. The equation and constants proposed by Tishkoff (1966) were applied to calculate the sialic acid concentration. Sialic acid content was expressed as *N*-acetylneuraminic acid (NANA).

Two lots of crude neuraminidase-receptor-destroying enzyme from *Vibrio cholerae* filtrates were used.[2] In paired determinations ($n = 18$) enzyme lot No. 2 gave consistently lower values (81.6%) than enzyme lot No. 1. Therefore, values obtained with the enzyme lot No. 2 were corrected to express all results as if obtained with the enzyme lot No. 1. The purity of the *N*-glycolyl- and *N*-acetylneuraminic acids[3] used as reference standards was checked by descending paper chromatography, using as a solvent system a mixture of butanol, *n*-propanol, and 0.1 *N* HCl in the ratio of 1:2:1 (Mårtensson *et al.* 1958). All other chemicals used were reagent grade.

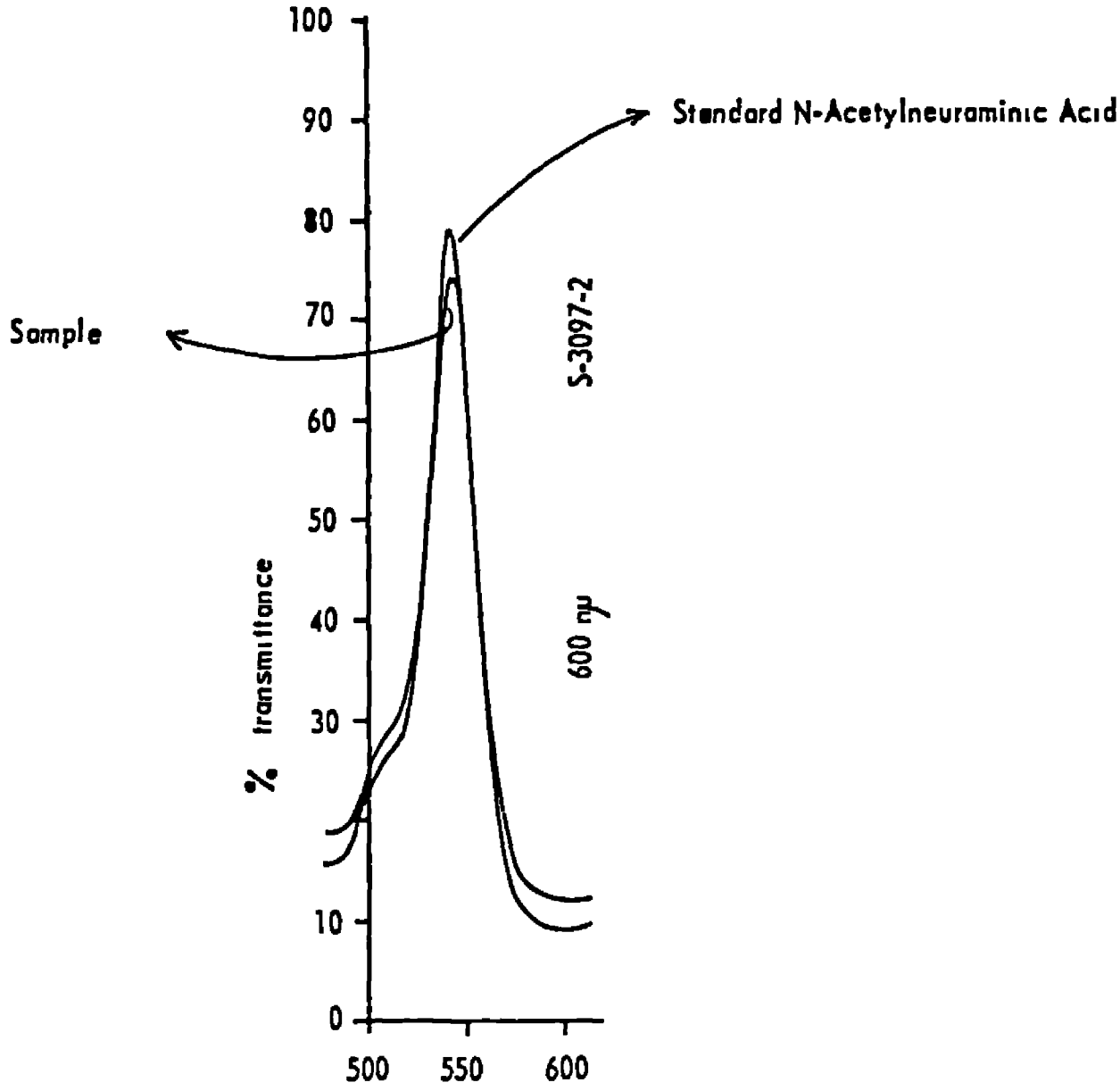
Plasma proteins were measured by refractometry, and hemoglobin content by the method of Crosby *et al.* (1954), while serum iron levels and total iron binding capacity were estimated by Ramsay's technique (Ramsay 1958). Serum folates were quantitatively determined using *Lactobacillus casei* as the assay microorganism (Herbert 1961). Estimation of serum $B_{12}$ levels was made by the *Euglena gracilis* method of Anderson (1964).

## Results

Data for P.C.M. children and for children fully recovered from P.C.M. are presented in

[2]Enzyme lot No. 1 was supplied by Sigma Chemical Company and enzyme lot No. 2 by Calbiochem.

[3]Supplied by Sigma Chemical Company.




Wavelength Range (mμ)

FIG 1. Absorption spectrum of the thiobarbituric acid chromophore

TABLE 1. Reticulocyte counts and sialic acids content of red blood cells from children with P C.M. and fully recovered

| | Red cell layers | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Reticulocytes (% of total) | 70.2 ± 3 3 ($n$ = 15) | 20 4 ± 2 2* ($n$ = 15) | 9.4 ± 1.7* ($n$ = 15) |
| NANA (μg/$10^{10}$ RBC) | 146 0 ± 6 0 ($n$ = 16) | 124 0 ± 4 0* ($n$ = 16) | 117 0 ± 5 0* ($n$ = 14) |
| NANA (μg/ml packed RBC) | 161 0 ± 6 0 ($n$ = 16) | 145 0 ± 6 0 ($n$ = 16) | 145 0 ± 7 0 ($n$ = 14) |

*Significantly different ($p$ < 0 005) from layer 1
NOTE The red cells are separated into three layers by centrifugation Data are mean values ± S E

Table 1. This table shows the reticulocyte counts on each layer and confirms that, as expected, the reticulocyte content decreased from the top to the bottom layer. The levels of NANA also tended to diminish from top to bottom. When expressing the sialic acid content as concentration per $10^{10}$ red cells, a significant difference was observed between layer 1 and the other two layers. However, when NANA was expressed as micrograms per milliliter of packed red blood cells, no significant differences were observed between layers

TABLE 2. Biochemical and hematological data and red blood cell sialic acid content in P.C.M. children, according to hospitalization period

| Hospitalization period (days) | Serum proteins (g/100 ml) | Packed RBC volume (%) | Serum iron (μg/100 ml) | Saturation of TIBC* (%) | RBC folates (ng/ml) | Serum $B_{12}$ (pg/ml) |
|---|---|---|---|---|---|---|
| 0–3 | 4.4 ± 0.2 ($n$ = 12) | 32.0 ± 1.0 ($n$ = 12) | 51.0 ± 4.0 ($n$ = 12) | 52.0 ± 7.0 ($n$ = 12) | 169.0 ± 22.0 ($n$ = 11) | 442.0 ± 85.0 ($n$ = 12) |
| 4–20 | 6.6 ± 0.4 ($n$ = 10) | 29.0 ± 1.0 ($n$ = 10) | 186.0 ± 74.0 ($n$ = 10) | 46.0 ± 8.0 ($n$ = 10) | 136.0 ± 12.0 ($n$ = 8) | 395.0 ± 55.0 ($n$ = 9) |
| 21–40 | 7.5 ± 0.3 ($n$ = 10) | 30.0 ± 0.8 ($n$ = 10) | 87.0 ± 31.0 ($n$ = 10) | 25.0 ± 7.0 ($n$ = 10) | 150.0 ± 15.0 ($n$ = 10) | 334.0 ± 57.0 ($n$ = 10) |
| 41+ | 7.6 ± 0.1 ($n$ = 16) | 34.0 ± 0.6 ($n$ = 16) | 55.0 ± 7.5 ($n$ = 10) | 15.0 ± 2.0 ($n$ = 10) | 178.0 ± 26.0 ($n$ = 10) | 302.0 ± 34.0 ($n$ = 7) |

| | RBC sialic acid content in red cell layer (NANA μg/ml packed RBC) | | | RBC sialic acid content in red cell layer (NANA μg/$10^{10}$ RBC) | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| 0–3 | 140.0 ± 7.0 ($n$ = 11) | 144.0 ± 8.0 ($n$ = 12) | 147.0 ± 10.0 ($n$ = 12) | 143.0 ± 10.0 ($n$ = 5) | 119.0 ± 8.0 ($n$ = 6) | 118.0 ± 10.0 ($n$ = 6) |
| 4–20 | 146.0 ± 10.0 ($n$ = 10) | 119.0 ± 12.0 ($n$ = 9) | 138.0 ± 9.0 ($n$ = 9) | 156.0 ± 20.0 ($n$ = 4) | 123.0 ± 16.0 ($n$ = 3) | 101.0 ± 10.0 ($n$ = 2) |
| 21–40 | 176.0 ± 30.0 ($n$ = 10) | 155.0 ± 9.0 ($n$ = 10) | 150.0 ± 8.0 ($n$ = 9) | 161.0 ± 0 ($n$ = 1) | 130.0 ± 0 ($n$ = 1) | — — |
| 41+ | 149.0 ± 8.0 ($n$ = 16) | 144.0 ± 9.0 ($n$ = 16) | 147.0 ± 8.0 ($n$ = 16) | 139.0 ± 5.0 ($n$ = 6) | 129.0 ± 5.0 ($n$ = 6) | 121.0 ± 6.0 ($n$ = 6) |

*TIBS is the total iron binding capacity.
NOTE: Data are mean values ± S.E.

TABLE 3. Total sialic acids of the three red blood cell layers in the four groups of subjects studied

| Group | Packed cell volume (%) | Sialic acid content expressed as NANA μg/ml packed RBC in red cell layers | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| P.C.M. children | 32.0 ± 1.0 ($n$ = 12) | 140.0 ± 7.0 ($n$ = 11) | 141.0 ± 8.0 ($n$ = 12) | 147.0 ± 10.0 ($n$ = 12) |
| Children fully recovered from P.C.M. | 34.0 ± 0.6 ($n$ = 16) | 149.0 ± 8.0 ($n$ = 16) | 144.0 ± 9.0 ($n$ = 16) | 147.0 ± 8.0 ($n$ = 16) |
| Control children | 37.0 ± 0.6 ($n$ = 29) | 148.3 ± 6.0 ($n$ = 28) | 146.0 ± 6.0 ($n$ = 28) | 143.0 ± 5.0 ($n$ = 28) |
| Control adults | 48.0 ± 1.0 ($n$ = 16) | 155.0 ± 3.0 ($n$ = 15) | 157.0 ± 4.0 ($n$ = 15) | 159.0 ± 4.0 ($n$ = 16) |

NOTE: Data are mean values ± S.E.

Table 2 shows the results obtained in the malnourished children, grouped by days of hospitalization in four categories: acute phase (0–3 days), early recovery stage (4–20 days), late recovery (21–40 days), and full recovery (41+ days). Differences in sialic acid content between layers generally persist when expressed by micrograms per $10^{10}$ red cells, although no differences were detected between the various stages of recovery. Furthermore, the NANA values obtained from severely malnourished children, fully recovered children, and normal children and adults (controls) are similar (Table 3). The values in the blood from normal adults tend to be higher in the three red cell layers.

An attempt was also made to correlate the sialic acid content of red blood cells from P.C.M. and recovered children with some of the biochemical and hematological parameters in-

vestigated. There exists no relationship between the sialic acid content and any other nutritional or hematological variable measured, either by groups of children or individually. These included packed cell volume, hemoglobin concentration, serum iron, percentage saturation of transferrin, red cell folates, and serum vitamin $B_{12}$. Tables 4 and 5 are presented as illustrations of this fact. Red cell folate level was chosen, in this instance, as the independent variable; based on the distribution of these levels the children were grouped in quartiles

## Discussion

The results obtained provide evidence that in contrast to alterations in serum glycoproteins, red cell sialic acid content remains normal in P.C.M. Findings in the three red blood cell (RBC) layers also indicate that old cells contain less sialic acid than young cells. This suggests that even though during the process of malnutrition bone marrow production may have decreased or even stopped (Viteri *et al* 1968*a*, Kho and Tumbelaka 1960, Ghitis *et al* 1963; Adams *et al.* 1967), when the malnourished children were studied their red cell production was probably keeping up with its demands, mostly determined by the children's active tissue mass. This interpretation is in agreement with the blood reticulocyte content of P C.M. children (Viteri *et al* 1968*a*).

The fact that the red cell content of sialic acid does not appear altered in severe uncomplicated P.C.M. also agrees with our unpublished observations (Viteri, F. E., and Alvarado, J.: unpublished observations) that the red cell half-life is essentially normal in children with severe protein-calorie malnutrition and without infectious complications. Also it may be concluded that protein, iron, folate, and vitamin $B_{12}$ deficiencies diagnosed on the bases of serum and/or red cell contents of these nutrients do not influence the total sialic acid content of red cells.

TABLE 4. Hematological and nutritional biochemical data of children with P C.M and during recovery Results are grouped based on red cell folates

| | | Group No. 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| RBC folates (ng/ml) | $\bar{X}$ | 74 0 | 127 0 | 168 0 | 250 0 |
| | S E. | 9 0 | 4 0 | 3 0 | 15 0 |
| | (*n*) | 6 | 12 | 12 | 9 |
| Packed RBC volume (%) | $\bar{X}$ | 30 0 | 31 0 | 31.0 | 33 0 |
| | S E. | 2 0 | 1 0 | 1.0 | 0.8 |
| | (*n*) | 6 | 12 | 12 | 18 |
| MCV ($\mu m^3$) | $\bar{X}$ | 86 0 | 82.0 | 84 0 | 84.0 |
| | S E. | 2 0 | 2 0 | 3 0 | 2.0 |
| | (*n*) | 5 | 11 | 10 | 13 |
| MCHC (g/100 ml RBC) | $\bar{X}$ | 31 0 | 32 0 | 32 0 | 32 0 |
| | S E. | 0 8 | 0.6 | 0 7 | 0 4 |
| | (*n*) | 5 | 11 | 10 | 13 |
| Serum iron (µg/100 ml) | $\bar{X}$ | 77 0 | 92 0 | 123 0 | 72 0 |
| | S E | 27 0 | 26 0 | 65 0 | 11 0 |
| | (*n*) | 6 | 12 | 12 | 12 |
| Saturation of TIBC (%) | $\bar{X}$ | 36 0 | 33 0 | 30.0 | 43 0 |
| | S E. | 13 0 | 6 0 | 8 0 | 8 0 |
| | (*n*) | 6 | 12 | 12 | 12 |
| Serum vitamin $B_{12}$ (pg/ml) | $\bar{X}$ | 251 0 | 488 0 | 305 0 | 420 0 |
| | S E | 82 0 | 70.0 | 35 0 | 76 0 |
| | (*n*) | 6 | 11 | 12 | 9 |

NOTE MCV is the mean corpuscular volume and MCHC the mean corpuscular hemoglobin concentration

TABLE 5. Sialic acid content of the three red blood cell layers in blood obtained from P C.M. children and during recovery. Results are grouped based on red blood cell folates

| Group No. | | RBC folates ng/ml | Sialic acid (NANA µg/ml RBC) in red cell layers 1 | 2 | 3 |
|---|---|---|---|---|---|
| 1 | $\bar{X}$ | 74.0 | 142.0 | 147 0 | 143.0 |
| | S E. | 9.0 | 10.0 | 9.0 | 7 0 |
| | (*n*) | 6 | 6 | 6 | 6 |
| 2 | $\bar{X}$ | 127.0 | 159 0 | 136 0 | 150.0 |
| | S E. | 4.0 | 22.0 | 15.0 | 12.0 |
| | (*n*) | 12 | 12 | 11 | 11 |
| 3 | $\bar{X}$ | 168.0 | 150 0 | 137.0 | 145.0 |
| | S.E. | 3.0 | 16.0 | 9.0 | 8.0 |
| | (*n*) | 12 | 11 | 12 | 11 |
| 4 | $\bar{X}$ | 250.0 | 151 0 | 144 0 | 145 0 |
| | S.E. | 15.0 | 7.0 | 6 0 | 7 0 |
| | (*n*) | 9 | 18 | 18 | 18 |
| | | | Sialic acid (NANA µg/$10^{10}$ RBC) in red cell layers 1 | 2 | 3 |
| 1 | $\bar{X}$ | 74.0 | 127.0 | 128 0 | 112.0 |
| | S E. | 9.0 | 0 | 9.0 | 4 0 |
| | (*n*) | 6 | 2 | 2 | 2 |
| 2 | $\bar{X}$ | 127.0 | 172.0 | 123 0 | 128 0 |
| | S E. | 4.0 | 0 | 0. | 0 |
| | (*n*) | 12 | 1 | 1 | 1 |
| 3 | $\bar{X}$ | 168 0 | 143 0 | 121 0 | 103 0 |
| | S E. | 3.0 | 7 0 | 6 0 | 17.0 |
| | (*n*) | 12 | 4 | 4 | 3 |
| 4 | $\bar{X}$ | 250 0 | 149.0 | 125 0 | 122 0 |
| | S.E. | 15 0 | 10.0 | 7 0 | 6 0 |
| | (*n*) | 9 | 9 | 9 | 8 |

The authors express their appreciation to Doctor Victor Argueta Von Kaenel, Head of the Pediatric Section and Director of the San Juan de Dios de Guatemala Hospital, for his valuable cooperation in allowing them to study some of his patients. They are indebted to Doctor Jorge Alvarado and Doctor Humberto Mansylla for their assistance in the care of the patients. They also wish to thank Mrs. Sara de Castañeda for her assistance in the preparation of the manuscript.

ADAMS, E. B., SCRAGG, J. N., NAIDOO, B. T., LILJESTRAND, S. K., and COCKRAM, V. I. 1967. Observations on the aetiology and treatment of anaemia in kwashiorkor. Br. Med. J. 3, 451–454.

ALVARADO, J., VITERI, F., and BEHAR, M. 1970. Tratamiento hospitalario de la desnutrición proteínico-calórica severa. Rev. Col. Med. (Guatemala), 21, 231–245.

ANDERSON, B. B. 1964. Investigations into the Euglena method for the assay of the vitamin $B_{12}$ in serum. J. Clin. Pathol. 17, 14–26.

BORUN, E. R., FIGUEROA, W. G., and PERRY, S. M. 1957. The distribution of $Fe^{59}$ tagged human erythrocytes in centrifuged specimens as a function of cell age. J. Clin. Invest. 36, 676–679.

BRECHER, G., and SCHNEIDERMAN, M. 1950. A time-saving device for the counting of reticulocytes. Am. J. Clin. Pathol. 20, 1079–1083.

COOK, G. M. W., HEARD, D. H., and SEAMAN, G. V. F. 1961. Sialic acids and the electrokinetic charge of the human erythrocyte. Nature, 191, 44–47.

COULTER, W. 1956. Proc. Natl. Electron. Conf. Hialeah, Florida.

CROSBY, W. H., MUNN, J. I., and FURTH, F. W. 1954. U.S. Armed Forces Med. J. 5, 693.

EYLAR, E. H., MADOFF, M. A., BRODY, O. V., and ONCLEY, J. L. 1962. The contribution of sialic acid to the surface charge of the erythrocyte. J. Biol. Chem. 237, 1992–2000.

FAYAD, I. M., METWALLI, O. M., SHUKRY, A. S., and ISMAIL, S. M. 1969. Serum mucoproteins in protein-calorie deficiency. Gaz. Egypt. Pediatr. Assoc. 17, 199.

GHITIS, J., VELEZ, H., LINARES, F., SINISTERRA, L., and VITALE, J. J. 1963. Cali-Harvard nutrition project. II. The erythroid atrophy of kwashiorkor and marasmus. Am. J. Clin. Nutr. 12, 445–451.

HERBERT, V., FISHER, R., and KOONTZ, B. J. 1961. The assay and nature of folic acid activity in human serum. J. Clin. Invest. 40, 81–91.

KHO, L. K., and TUMBELAKA, W. A. 1960. The pathogenesis of anemia in kwashiorkor. Ann. Paediatr. 194, 257–272.

KIMMEL, J. R., MARKOWITZ, H., and BROWN, D. M. 1959. Some chemical and physical properties of erythrocuprein. J. Biol. Chem. 234, 46–50.

LAHEY, M. E., BEHAR, M., VITERI, F., and SCRIMSHAW, N. S. 1958. Values for copper, iron and iron-binding capacity in the serum in kwashiorkor. Pediatrics, 22, 72–79.

LANZKOWSKY, P., MCKENZIE, D., KATZ, S., HOFFENBERG, R., FRIEDMAN, R., and BLACK, E. 1967. Erythrocyte abnormality induced by protein malnutrition. II. 51-Chromium labeled erythrocyte studies. Br. J. Haematol. 13, 639–649.

MARKOWITZ, H., CARTWRIGHT, G. E., and WINTROBE, M. M. 1959. Studies on copper metabolism. XXVII. The isolation and properties of an erythrocyte cuproprotein (erythrocuprein). J. Biol. Chem. 234, 40–45.

MÅRTENSSON, E., RAAL, A., and SVENNERHOLM, L. 1958. Sialic acids in blood serum. Biochim. Biophys. Acta, 30, 124–129.

MCGOVERN, J. J., RICHARDSON JONES, A., and STEINBERG, A. G. 1955. The hematocrit of capillary blood. New Engl. J. Med. 253, 308–312.

PATWARDHAN, V. N., MAGHRABI, R. H., MOUSA, W., GABR, M. K., and EL MARAGHY, S. 1971. Serum glycoproteins in protein-calorie deficiency disease. Am. J. Clin. Nutr. 24, 906–912.

RAMSAY, W. N. M. 1958. Plasma iron. *In* Advances in clinical chemistry. Vol. 1. *Edited by* H. Sobotka and C. P. Stewart. Academic Press Inc., New York. pp. 1–39.

RIGAS, D. A., KOLER, R. D., and SWISHER, K. 1961. Ultracentrifugal fractionation of human erythrocytes on the basis of cell age. J. Lab. Clin. Med. 58, 242–246.

SHEHATA, A. H., ABDEL HAY, A., KAMEL, G., FAYAD, I., and TALAAT, M. 1965. Biochemical studies in kwashiorkor. IV. Protein-bound polysaccharides. J. Endocrinol. Metab. (Egypt), 11, 33.

TISHKOFF, G. H. 1966. Erythrocyte mucoids in acquired autoimmune hemolytic anemia. Blood, 28, 229–240.

VITERI, F. E., and ALVARADO, J. 1970. The creatinine–height index: its use in the estimation of the degree of protein depletion and repletion in protein calorie malnourished children. Pediatrics, 46, 696–706.

VITERI, F. E., ALVARADO, J., LUTHRINGER, D. G., and WOOD, R. P., II. 1968*a*. Hematological changes in protein-calorie malnutrition. Vitam. Horm. 26, 573–615.

———. 1968*b*. Adaptation of the erythropoietic system to protein calorie deficiency. Fed. Proc. 27, 485 (Abstr. 1509).

VITERI, F., BEHAR, M., ARROYAVE, G., and SCRIMSHAW, N. S. 1964. Clinical aspects of protein malnutrition. *In* Mammalian protein metabolism. Vol. II. *Edited by* H. N. Munro and J. B. Allison. Academic Press Inc., New York and London. p. 523.

WARREN, L. 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234, 1971–1975.

YACHNIN, S., and GARDNER, F. H. 1961. Measurement of human erythrocyte neuraminic acid: relationship to haemolysis and red blood cell virus interaction. Br. J. Haematol. 7, 464–475.

# The Head Circumference / Chest Circumference Ratio in Mild-to-Moderate Protein-Calorie Malnutrition

*by* REYNALDO MARTORELL, CHARLES YARBROUGH, ROBERT M. MALINA, JEAN-PIERRE HABICHT, AARON LECHTIG AND ROBERT E. KLEIN

*Division of Human Development, Institute of Nutrition of Central America and Panama (INCAP), Calzada Roosevelt, Zone 11, Guatemala City, Guatemala, C.A.*

The objective of this report is to evaluate the utility of the ratio of head to chest circumference (head circumference/chest circumference × 100: H/C ratio) as an index of the nutritional status of individuals and populations.

The use of the ratio has been recommended for the evaluation of young children from 6 months to 5 years, specifically, it has been suggested that the percentage of children at different ages who have larger head than chest circumference should be calculated and reported[1] Technically, the H/C ratio appears to be appropriate for field conditions for it does not require expensive equipment, not even a tape measure is necessary as a piece of string will do[1].

The rationale behind the H/C ratio is as follows at birth, head circumference is generally larger than chest circumference After birth, the circumference of the head does not grow as rapidly as that of the chest In well-nourished children the circumference of the chest becomes larger than that of the head between 6 and 12 months of age, producing a mean H/C ratio of less than 100%[1-3]. On the other hand, in severe protein-calorie malnutrition there may be poor growth of the pectoral muscles or even wasting such that the head remains larger than the chest circumference at later ages, yielding a mean H/C ratio greater than 100%[1]. Thus, in some malnourished populations the mean circumference of the chest does not become larger than that of the head until as late as the third and fourth year of life[1,4-6].

Though many studies have reported mean H/C ratios, little is known about the variability in the ratio both in well-nourished and poorly-nourished populations This results from the fact that in most instances the H/C ratio is derived from the population means or medians of head ($\overline{H}$) and chest circumference ($\overline{C}$) as $\overline{H}/\overline{C}$ rather than from individual ratios as $(\Sigma H/C)/n$, where n=number of individual ratios Variability can be estimated from the latter but not from the former. Lacking estimates of the variability in the H/C ratio, it is impossible to know what percentage of children have unacceptable H/C ratios after 1 year of life in well-nourished populations Without this knowledge, it is difficult to evaluate what is normal to expect at the individual or population level.

As opinion regarding the value of the H/C ratio in the evaluation of early growth and development varies[1-7], we examined the H/C ratio in a moderately malnourished population of Guatemalan children, birth to seven years of age, to make inferences as to the utility of the ratio to discriminate well-nourished and moderately-malnourished populations and individuals

## Methods

The data presented here are mixed longitudinal observations on 1,119 clinically normal rural Guatemalan Ladino children under study by the Division of Human Development of the Institute of Nutrition of Central America and Panama (INCAP). Although the nutritional and health standards of these villages are better than the average for rural Guatemala, mild-to-moderate protein-calorie malnutrition is prevalent in this population[8]. From the basic sample, 5,012 head and 4,982 chest circumference measurements are available. The sample comprises 84% of all possible examinations on children who were 0 to 84 months of age within the period of January 1969 through May 1, 1972 in four villages in the Department of El Progreso, northeast of Guatemala City. The villages are on the Atlantic slopes of the Guatemalan highlands at altitudes between 300 and 1,100 metres.

Head and chest circumferences were taken by trained and standardised anthropometrists. The two measurements are included in an extensive anthropometric battery taken on all children Both circumferences were measured with a flexible steel tape to the nearest millimeter, and with the child in a seated position Head (fronto-occipital) circumference was measured as the maximum circumference of the head with the tape passing above (but not including) the supra orbital ridges and over the maximum occipital prominence Care was taken to ensure that the tape passed

TABLE I. Means and standard deviations for the head-chest circumference ratios (H/C ratio) and the ratio of mean head and chest circumferences (H/C ratio) in Guatemalan and Denver children.

| | Boys | | | | Girls | | | |
|---|---|---|---|---|---|---|---|---|
| | Guatemala | | | Denver | Guatemala | | | Denver |
| | H/C ratio | | H/C ratio | H/C ratio | H/C ratio | | $\overline{H}/\overline{C}$ ratio | H/C ratio |
| Age group (months) | X | S.D. | X | X | X | S.D. | X | X |
| Birth | 108.6 | 6.1 | 108.1 | 106.5 | 108.5 | 3.4 | 108.5 | 104.6 |
| 0.5 | 105.1 | 4.8 | 104.8 | —* | 105.1 | 5.7 | 104.9 | —* |
| 3 | 101.4 | 4.7 | 101.3 | 101.3 | 102.3 | 4.5 | 101.9 | 102.1 |
| 6 | 101.9 | 4.5 | 101.7 | 100.2 | 100.8 | 4.5 | 100.5 | 99.5 |
| 9 | 103.0 | 4.4 | 102.6 | 99.6 | 101.7 | 4.3 | 101.7 | 99.8 |
| 12 | 101.7 | 4.5 | 101.6 | 99.4 | 101.7 | 4.6 | 101.6 | 99.8 |
| 15 | 101.1 | 4.2 | 100.9 | —* | 100.9 | 4.8 | 100.7 | —* |
| 18 | 100.6 | 4.3 | 100.4 | 99.4 | 100.2 | 4.5 | 100.0 | 99.8 |
| 21 | 99.3 | 4.1 | 99.1 | —* | 99.7 | 4.3 | 99.5 | —* |
| 24 | 98.4 | 4.2 | 98.3 | 99.8 | 98.3 | 4.5 | 98.3 | 100.2 |
| 30 | 96.5 | 4.1 | 96.3 | 99.6 | 96.6 | 4.5 | 96.4 | 100.4 |
| 36 | 95.0 | 3.9 | 94.8 | 99.0 | 95.0 | 4.7 | 94.9 | 100.0 |
| 42 | 93.6 | 4.0 | 93.4 | 98.3 | 95.0 | 3.9 | 94.8 | 99.0 |
| 48 | 92.6 | 4.1 | 92.4 | 97.7 | 93.5 | 4.2 | 93.3 | 98.6 |
| 60 | 90.7 | 4.4 | 90.6 | 95.7 | 92.1 | 3.9 | 92.0 | 96.6 |
| 72 | 89.3 | 4.2 | 89.3 | 94.3 | 90.1 | 3.9 | 89.8 | 94.3 |
| 84 | 87.1 | 3.8 | 87.0 | 90.7 | 89.0 | 3.6 | 88.8 | 91.8 |

*Information not available at these ages.

around the head at the same level on each side. Firm pressure was applied to the tape to compress underlying hair. Chest circumference was measured at mid-respiration with the tape placed at the level of the xiphoid process and below the inferior angles of the scapulae. The tape was applied in such a manner as to permit skin contact without compression of underlying tissues.

Measurements were made at birth and at 16 specific age intervals through 84 months. The specific age intervals and sample size per age group are indicated later in the report. The permitted variation around the measurement intervals was ±3 days at 15 days of age, ±5 days from 3 through 24 months of age, and ±7 days from 30 through 84 months of age. Prior to analysis, head and chest measurements which seemed unusual for the age of the child were checked and discarded if not verified by re-measurement. The rate of discard was approximately 0·4%.

Measurement variability estimates for the H/C ratio were obtained from the following routine procedure. Each week a random sample of 10% of all subjects examined the previous week is re-measured following the standard field procedure. The measurement standard deviation of the H/C ratio was obtained from a repeated measure analysis of variance which effectively controlled for short-term growth. The results were that the measurement standard deviation of the ratio was 2·6% (n = 144 replicates).

For comparison with the moderately-malnourished Guatemalan sample, the reported data on head and chest circumference of Denver children[11] are used as an example of a well-nourished population. The H/C ratio was derived from the reported means of head and chest circumferences of the Denver data.

## Results

Means and standard deviations for the H/C ratio derived from individual Guatemalan children, along with $\overline{H}/\overline{C}$ ratios derived from Guatemalan and American age-specific mean head and chest circumferences are presented in Table I. As expected, the H/C ratio decreases with age. The mean H/C ratios approximate 100% in Guatemalan children of both sexes at 18 months and are lower than 100% at 21 months of age, indicating a cross-over of the two measurements approximately one year later than that reported for well-nourished children[1–3]. Variation around the mean H/C ratio values, as expected, is considerable so that at 3 months of age some children already have chest circumferences larger than head circumferences. Conversely, at the older ages studied some children have larger head than chest circumferences.

The age-sex specific variability in the H/C ratio falls slightly with age, the median standard deviation being 4·2%. This variability is of similar magnitude in middle-class Japanese children[3]. As mentioned previously the measurement standard deviation of the H/C ratio is 2·6%. Therefore, the ratio of the measurement variance (6·76) to the median population variance (17·64) is 38%. That is, 38% of the population variance of the

H/C ratio is due to variability in measurement and cannot serve to explain or be explained by other variables such as nutrition. The corresponding statistics for length and weight, two commonly used indicators of nutritional status, are 1 and 6% respectively in our sample[10]. Therefore, the H/C ratio, from measurement variability criteria alone, seems to be a poor index of nutritional status.

It is apparent in Table I that age-specific mean H/C ratios derived from individual Guatemalan children are essentially identical with $\overline{H}/\overline{C}$ ratios derived from age-specific mean head and mean chest circumferences. Similar observations are evident in the data reported by TERADA and HOSHI[3] on Japanese children 1 through 36 months We take this to indicate that the ratio of the means is statistically adequate for population estimates of the H/C ratio. However, variability cannot be estimated for the ratio of the means.

As stated earlier, the H/C ratio crosses 100% between 18 and 21 months for both Guatemalan boys and girls In the Denver sample however, the situation is more complex In Denver boys, the $\overline{H}/\overline{C}$ ratio crosses 100% between 6 and 9 months while in girls the $\overline{H}/\overline{C}$ ratio does not finally cross 100% until after 36 months. While Denver boys and girls may differ in terms of the age at which head circumference becomes less than chest circumference, in fact, if we look at the ratio as a continuum, both sexes are quite similar in terms of the $\overline{H}/\overline{C}$ ratio. For both Denver boys and girls, the $\overline{H}/\overline{C}$ ratio fluctuates between 99 0% and 100 4%, quite a narrow range, from 6 through 36 months of age. This indicates that Denver children over these ages have mean head and chest circumferences that are approximately equal in size

## Discussion

Between 3 and 24 months, Guatemalan and Denver children have H/C ratios that are fairly similar In other words, there is no apparent substantial difference between malnourished Guatemalan and better-nourished Denver children in the ratio of mean head and chest circumference. Consequently, the H/C ratio does not seem to be a good discriminator between these two populations of varying nutritional status. Furthermore, the adequacy of the H/C ratio as an index of nutritional status is questioned by the fact that after 24 months the H/C ratio is actually smaller in Guatemalan children than in Denver children. That is, after 24 months, Guatemalan children have larger chest relative to head circumference when compared to Denver children This reflects a greater stunting in head circumference relative to chest circumference in Guatemalan children[11].

Since the H/C ratio cannot discriminate populations, it is certain that the ratio would also lack the power to distinguish individuals from the two populations. The variability in the individual head and chest circumference measures are similar in the Guatemalan and Denver data[9,11]. Further, the variability in the H/C ratio is similar in Guatemalan and middle-class Japanese children[3] Consequently, it is reasonable to assume that the variability in the H/C ratio noted in our population is quite similar to that which would be observed in the Denver data. Judging from the fact that the H/C ratio is quite close to 100% up to about 4 years of age in the Denver sample, and assuming a standard deviation of around 4% as in the Guatemalan sample, a quarter or more of Denver children from one to four years of age would have ratios greater than 100% and consequently be classified as malnourished This suggests poor specificity of the H/C r asatio a discriminator of nutritional status.

In contrast, after 2 years of age, the H/C ratio would not classify as many children as being malnourished in the Guatemalan sample as it apparently does in the Denver sample, suggesting low sensitivity for the ratio. The variation in H/C ratios among Guatemalan children is more apparent in the percentiles presented in Table II At two years of age, for example, 25% of the children have H/C ratios of 100% or higher. At three years of age, approximately 10% of the children have H/C ratios of 100% or higher. It is not until four years of age in the rural Guatemalan sample of boys, and five years of age in the sample of girls that only 3% of the children have H/C ratios of 100% or higher.

One can ask whether the small percentage of children at 4 who have a ratio of 100 or higher are in fact children with markedly retarded growth. The result of this analysis is presented in Table III where the length, weight, head and chest circumference of 22 such 4-year-old children are expressed in terms of the sex-specific percentile distribution of the study sample[11,12]. Included in this analysis are children measured subsequent to the preparation of Tables I and II so as to increase sample size. It is clear that in comparison to the study sample, these children are normal with respect to length and only slightly lighter in terms of weight. They are not therefore, more retarded than the population from which they were drawn It should be pointed out that none are below the third percentile for length or weight. Further, of the 22 children, 18 had normal weight for length and 4 fell within 90 to 80 percent of the standard[1]. In terms of head circumference, they are somewhat larger than the general population while in chest circumference they tend to be small Thus, it appears that the H/C ratio identifies children who, with respect to the study sample, have large heads, small chests and typical length and weights.

TABLE II Percentiles for the head-chest circumference ratios in rural Guatemalan Ladino children

| Age group (months) | Boys | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | n | Percentiles | | | | | | |
| | | 3 | 10 | 25 | 50 | 75 | 90 | 97 |
| Birth | 25 | | | 104 | 109 | 111 | | |
| 0 5 | 118 | 97 | 100 | 102 | 105 | 107 | 110 | 112 |
| 3 | 202 | 93 | 96 | 98 | 102 | 104 | 107 | 110 |
| 6 | 194 | 94 | 97 | 98 | 102 | 104 | 107 | 108 |
| 9 | 191 | 95 | 97 | 100 | 103 | 105 | 108 | 111 |
| 12 | 186 | 93 | 96 | 98 | 101 | 104 | 107 | 108 |
| 15 | 185 | 94 | 96 | 98 | 101 | 103 | 105 | 108 |
| 18 | 174 | 93 | 95 | 98 | 100 | 103 | 105 | 108 |
| 21 | 162 | 91 | 94 | 96 | 99 | 101 | 104 | 106 |
| 24 | 180 | 90 | 93 | 96 | 98 | 100 | 103 | 105 |
| 30 | 163 | 90 | 92 | 93 | 96 | 98 | 101 | 104 |
| 36 | 164 | 88 | 90 | 93 | 94 | 97 | 99 | 101 |
| 42 | 166 | 87 | 89 | 91 | 93 | 95 | 98 | 100 |
| 48 | 157 | 86 | 87 | 90 | 93 | 94 | 97 | 99 |
| 60 | 142 | 81 | 85 | 88 | 91 | 93 | 95 | 97 |
| 72 | 127 | 80 | 84 | 87 | 89 | 91 | 94 | 96 |
| 84 | 115 | 81 | 82 | 84 | 87 | 89 | 91 | 93 |

| Age group (months) | Girls | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | n | Percentiles | | | | | | |
| | | 3 | 10 | 25 | 50 | 75 | 90 | 97 |
| Birth | 12 | | | 105 | 108 | 111 | | |
| 0 5 | 107 | 95 | 99 | 102 | 105 | 108 | 111 | 114 |
| 3 | 160 | 94 | 97 | 99 | 102 | 104 | 106 | 111 |
| 6 | 159 | 91 | 95 | 98 | 101 | 103 | 105 | 108 |
| 9 | 158 | 93 | 96 | 99 | 101 | 104 | 106 | 109 |
| 12 | 154 | 94 | 97 | 99 | 102 | 103 | 106 | 111 |
| 15 | 142 | 93 | 95 | 98 | 100 | 103 | 106 | 110 |
| 18 | 153 | 92 | 95 | 97 | 100 | 102 | 105 | 108 |
| 21 | 136 | 92 | 95 | 97 | 100 | 101 | 104 | 106 |
| 24 | 141 | 91 | 93 | 95 | 98 | 100 | 103 | 106 |
| 30 | 150 | 89 | 91 | 93 | 96 | 99 | 102 | 104 |
| 36 | 143 | 86 | 90 | 92 | 95 | 97 | 100 | 103 |
| 42 | 146 | 88 | 90 | 92 | 95 | 97 | 99 | 101 |
| 48 | 148 | 86 | 88 | 91 | 93 | 95 | 98 | 101 |
| 60 | 149 | 85 | 87 | 90 | 92 | 94 | 96 | 98 |
| 72 | 133 | 83 | 85 | 87 | 90 | 92 | 95 | 97 |
| 84 | 118 | 83 | 84 | 86 | 89 | 91 | 93 | 95 |

In conclusion, while the ratio of head to chest circumference may be useful to identify severely malnourished children, the evidence examined suggests that the ratio has no power to discriminate either populations or individuals who are well nourished from those moderately malnourished

## Summary

This report compares moderately-malnourished Guatemalan children, birth to seven years of age, and well-nourished Denver children of similar age in terms of the ratio of head to chest circumference (H/C ratio)

The mean H/C ratios were very similar in both populations from 3 to 24 months while after 24 months the mean H/C ratios were actually smaller in the Guatemalan sample The ratio therefore, was not useful in detecting differences in nutritional status between the populations studied It was estimated that after two years of age, the H/C ratio would, contrary to expectations, classify a greater percentage of Denver than Guatemalan children as having H/C ratios greater than 100%, and hence, as malnourished Furthermore, four-year-old Guatemalan children with H/C ratios of 100% or more had typical lengths and weights when compared to the general population In conclusion, the evidence presented suggests that the H/C ratio has no power to discriminate either populations or individuals who are well nourished or moderately malnourished

## References

(1) Jelliffe, D. B. (1966). The Assessment of the Nutritional Status of the Community. World Health Organization, Geneva. (WHO Monograph Series No. 53).

(2) Nelson, W. E. (Ed). (1964). Textbook of Pediatrics. Saunders Co., Philadelphia.

(3) Terada, H., Hoshi, H. (1965). *Acta Anatomica Nipponica*, 40, 368.

(4) Dean, R. F. A. (1965). *Courrier*, 15, 73.

(5) Datta Banik, N. D., Krishna, R., Mane, S. I. S., Raj, L. (1970). *Indian J. Pediat.*, 37, 438.

(6) Neumann, C. G., Shanker, H., Uberoi, I. S. (1969). *Indian J. Med. Res.*, 57, 1,122.

(7) Silver, H. K., Kempe, C. H., Bruyn, H. B. (1971). Handbook of Pediatrics. Los Altos California.

(8) Habicht, J-P., Schwedes, J. A., Arroyave, G., Klein, R. E. (1973). *Am. J. Clin. Nutr.*, 26, 1,046.

(9) Hansman, C. (1970). *in* Human Growth and Development. R. W. McCammon (Ed). Charles C. Thomas, Springfield, Illinois, p. 101.

(10) Martorell, R., Habicht, J-P., Yarbrough, C., Guzmán, G., Klein, R. E. *Am. J. Phys. Anthrop.* in press.

(11) Malina, R. M., Habicht, J-P., Martorell, R., Lechtig, A., Yarbrough, C., Klein, R. E. *Am. J. Clin. Nutr.* in press.

(12) Yarbrough, C., Habicht, J. P., Malina, R. M., Lechtig, A., Klein, R. E. (1975). *Am. J. Phys. Anthrop.*, 42, 439.


TABLE III. Percentile distribution of anthropometry of 22 four-year-old Guatemalan children with H/C ratios of 100% or more.

| Percentile grouping | Length | Weight | Head circumference | Chest circumference |
|---|---|---|---|---|
| > 97 | 1 | 0 | 4 | 0 |
| ⩾ 75 < 97 | 3 | 2 | 7 | 0 |
| ⩾ 50 < 75 | 6 | 6 | 5 | 1 |
| ⩾ 25 < 50 | 3 | 6 | 3 | 4 |
| ⩾ 3 < 25 | 9 | 8 | 3 | 13 |
| < 3 | 0 | 0 | 0 | 4 |

# Patterns of Cortical Bone Growth in Moderately Malnourished Preschool Children

By J. H. Himes,[1] R. Martorell,[2] J-P. Habicht,[2]
C. Yarbrough,[2] R. M. Malina,[1] and R. E. Klein[2]

## ABSTRACT

The growth patterns of five measures of second metacarpal cortical bone are described for a mixed-longitudinal sample of 710 rural Guatemalan preschool children with mild to moderate protein-calorie malnutrition, and are compared to well-nourished children. The Guatemalan children are severely retarded in metacarpal growth compared to the well-nourished children of the same age and sex. Further, when compared to well-nourished children of the same stature and weight, the Guatemalan children have less metacarpal cortex for a given body size. Retardation in metacarpal cortical bone in the moderately malnourished children is not only a reflection of an overall smaller body size, but suggests a differential skeletal response to nutritional stress.

Severe protein-calorie malnutrition in laboratory animals is associated with characteristic alterations in bone growth including thinned cortices, osteoporosis, medullary expansion, coarse trabeculation, lines of arrested growth and overall growth retardation (Pratt and McCance, 1960; Dickerson and McCance, 1961; Adams, 1969). Osseous changes similar to those produced in experimental protein-calorie malnutrition have also been reported for children with frank protein-calorie malnutrition (El Nawaby et al. 1962; Garn et al. 1964, 1969; Adams and Berridge, 1969). However, there is little information concerning bone growth in children who suffer from mild to moderate or subclinical protein-calorie malnutrition, even though such malnutrition is widespread in many parts of the world (Jelliffe, 1966; Béhar, 1968).

One finding perhaps related to the retarded bone growth in malnourished children is that protein-calorie malnutrition, even in its

[1] Department of Anthropology, University of Texas at Austin, Austin, Texas 78712.

[2] Division of Human Development, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala, C.A.

*Human Biology, September 1975, Vol. 47, No. 3, pp. 337-350.*

milder forms is also associated with an overall growth deficiency in body size (Dreizen and Stone, 1962; Guzmán, 1968). With a deficiency in the growth of bone and body size accompanying protein-calorie malnutrition, it may be that both bone dimensions and body size are uniformly retarded, thus reflecting an overall retardation in body growth. Preliminary analyses of our data (unpublished) have shown that in mild to moderately malnourished children second metacarpal cortical bone and body size are positively correlated.

The purpose of this report is to describe the growth patterns of selected measures of second metacarpal cortical bone in a mixed-longitudinal sample of mild to moderately malnourished Guatemalan preschool children in reference to well-nourished children, and to determine if the diminished bone growth of the Guatemalan children is proportionate to the retardation in stature and weight.

## Materials and Methods

The present investigation is part of a longitudinal study investigating the effects of mild to moderate protein-calorie malnutrition on physical growth and mental development which is being carried out by the Division of Human Development at the Institute of Nutrition of Central America and Panama (INCAP) (Klein et al. 1973). Children in the study are participants in a voluntary dietary supplementation program being conducted in four rural *Ladino* villages in Guatemala. Before the institution of the project, village children suffered from various degrees of protein-calorie malnutrition, much of it severe. The supplementation program, which includes medical care, has now almost eliminated clinical or severe protein-calorie malnutrition and has greatly reduced the previously high childhood mortality. None of the children in the present sample suffer from clinical malnutrition yet there are very few children who are adequately nourished. The dietary patterns and biochemical findings of rural Guatemalan children have been well documented through a national nutrition survey conducted by INCAP (1969) which indicated that young children generally suffered from mild to moderate protein-calorie malnutrition. These national findings were substantiated in a subsample of our study population (Habicht et al. 1973). Anthropometric indices of nutritional status are consistent with the dietary and biochemical findings, indicating retarded growth in height, weight and skinfolds (Habicht et al. 1974,

Malina et al. 1974, in press; Yarbrough et al. in press). Further, when supplemented regularly with protein calories, village children demonstrated weight gains which did not differ significantly from those of well nourished children (Habicht et al. 1972).

The study sample is mixed-longitudinal and consists of 1729 annual anthropometric examinations of 710 *Ladino* children 1 to 7 years of age. The present analyses are concerned only with attained growth and treat the data cross-sectionally so that each child may be represented in the total sample more than once. The cross-sectional values are completely consistent with those obtained from the fully longitudinal portion of the sample. Specific understanding of attained growth is necessary before changes in growth may be properly evaluated. Investigations of longitudinal growth changes are part of the research in progress and will be dealt with specifically in a subsequent report.

All examinations were made within 7 days of the child's birthday. At each examination standardized radiographs of the left hand and wrist were taken at a fixed tube distance of 76 cm, centered over the head of the third metacarpal, and with Kodak no screen X-ray film. For children 1 year old the X-ray exposure was 15 milliamperes and 65 kilovolts at 48/60 of a second time, while children 2 years and older had an exposure of 15 milliamperes, 65 kilovolts and 1 second time. Standard procedure included appropriate screening and quality safety control (Division de Desarrollo Humano, INCAP, 1971).

The periosteal (PD) and medullary (MD) diameters of the second metacarpal were measured at the midshaft point (excluding the epiphysis) by a single observer (JHH). Metacarpal diameters were measured with a graduated loupe to the nearest 0.1 mm. Cortical thickness (CT) was obtained by subtraction of the medullary diameter from the periosteal diameter. An estimate of the cross-sectional cortical area (CA) was calculated assuming a cylindrical model: $CA = 0.785\ (PD^2 - MD^2)$ (Garn, 1970). The relative amount of the periosteal area occupied by cortex (percent cortical area) was estimated: $PCA = \left(\frac{PD^2 - MD^2}{PD^2}\right) \cdot 100$ (Garn, 1970).

The observer measurement variability for the bone variables was estimated from 25 replicate measurings across all ages. Table 1 presents the measurement standard deviations and the measurement variabilities relative to the population variabilities at a single age for each of the bone variables. There was no apparent change in measurement

Table 1

*Measurement Variability for Cortical Bone Variables*

| Variable | $S_m$ | R |
|---|---|---|
| Periosteal Diameter (mm) | 0 06 | 0.021 |
| Medullary Diameter (mm) | 0.10 | 0.042 |
| Cortical Thickness (mm) | 0.11 | 0.118 |
| Cortical Area ($mm^2$) | 0.52 | 0.071 |
| Percent Cortical Area (%) | 3.21 | 0.105 |

$S_m$ = standard deviation of the measurement

$= \sqrt{\frac{\Sigma d^2}{2n}}$ d = difference between replicate measurings

n = number of pairs

R = Ratio of variance in measurement ($S_m^2$) to population variance at a single age (Habicht, 1974).

variability with age. Although there are few directly comparable data, these variability values are well within published figures for similar studies of bone growth (Adams et al. 1969; Mazess et al. 1970).

## Results

The age-specific means and standard deviations of attained growth in the bone variables are presented for boys and girls in Table 2. In general, boys have greater mean periosteal diameter, medullary diameter and cortical area than girls, while girls have greater cortical thickness and percent cortical area.

Figures 1 and 2 present the age-specific means of the cortical bone dimensions for the Guatemalan children compared to those for better nourished U.S. white children from the recent 10-State Nutrition Survey (Garn et al. n.d.). In both populations, periosteal diameter, cortical thickness, cortical area and percent cortical area generally increase from 1 to 7 years in an approximately linear fashion, while there is little

Table 2

*Means and Standard Deviations of Attained Second Metacarpal Growth*

| | | Periosteal Diameter (mm) | | Medullary Diameter (mm) | | Cortical Thickness (mm) | | Cortical Area ($mm^2$) | | Percent Cortical Area (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | n | Mean | S D | Mean | S.D. | Mean | S D | Mean | S D. | Mean | S.D. |
| BOYS | | | | | | | | | | | |
| 1 | 185 | 3 99 | 0 35 | 3 14 | 0.38 | 0.85 | 0 24 | 4.74 | 1.39 | 37.69 | 8 83 |
| 2 | 176 | 4 28 | 0 39 | 3 24 | 0.46 | 1 04 | 0 30 | 6.09 | 1.74 | 42.36 | 10.11 |
| 3 | 167 | 4.62 | 0 40 | 3 35 | 0 52 | 1.27 | 0.31 | 7.88 | 1.74 | 47 32 | 10 34 |
| 4 | 144 | 4.90 | 0 41 | 3.36 | 0.52 | 1.54 | 0 32 | 9 92 | 1 92 | 52.79 | 9 73 |
| 5 | 125 | 5 10 | 0.41 | 3 37 | 0.55 | 1.72 | 0 34 | 11.37 | 2.12 | 56 00 | 9.73 |
| 6 | 76 | 5.26 | 0 41 | 3.38 | 0 53 | 1.87 | 0 32 | 12 59 | 2.06 | 58 37 | 8 76 |
| 7 | 48 | 5 42 | 0 46 | 3 29 | 0.54 | 2.13 | 0.30 | 14 52 | 2 28 | 63 07 | 7.66 |
| Total | 921 | | | | | | | | | | |
| GIRLS | | | | | | | | | | | |
| 1 | 182 | 3 71 | 0.34 | 2.86 | 0 37 | 0 84 | 0.22 | 4 34 | 1.14 | 40 14 | 9 12 |
| 2 | 154 | 3 98 | 0.35 | 2 93 | 0 43 | 1 05 | 0.31 | 5 64 | 1 58 | 45 36 | 10 90 |
| 3 | 151 | 4 25 | 0 40 | 2.93 | 0 47 | 1 32 | 0 32 | 7 38 | 1.80 | 52.12 | 10 27 |
| 4 | 129 | 4 51 | 0 38 | 2 92 | 0 49 | 1 59 | 0 36 | 9 22 | 1 99 | 57.70 | 10.40 |
| 5 | 100 | 4.72 | 0.41 | 2.92 | 0.53 | 1.79 | 0 37 | 10 67 | 2.18 | 61 20 | 10 09 |
| 6 | 56 | 4 93 | 0 42 | 2.87 | 0 49 | 2 05 | 0 40 | 12.47 | 2.48 | 65 50 | 8 94 |
| 7 | 36 | 5 21 | 0 49 | 2.81 | 0 49 | 2 35 | 0.34 | 14.65 | 2 16 | 70 12 | 7.49 |
| Total | 808 | | | | | | | | | | |

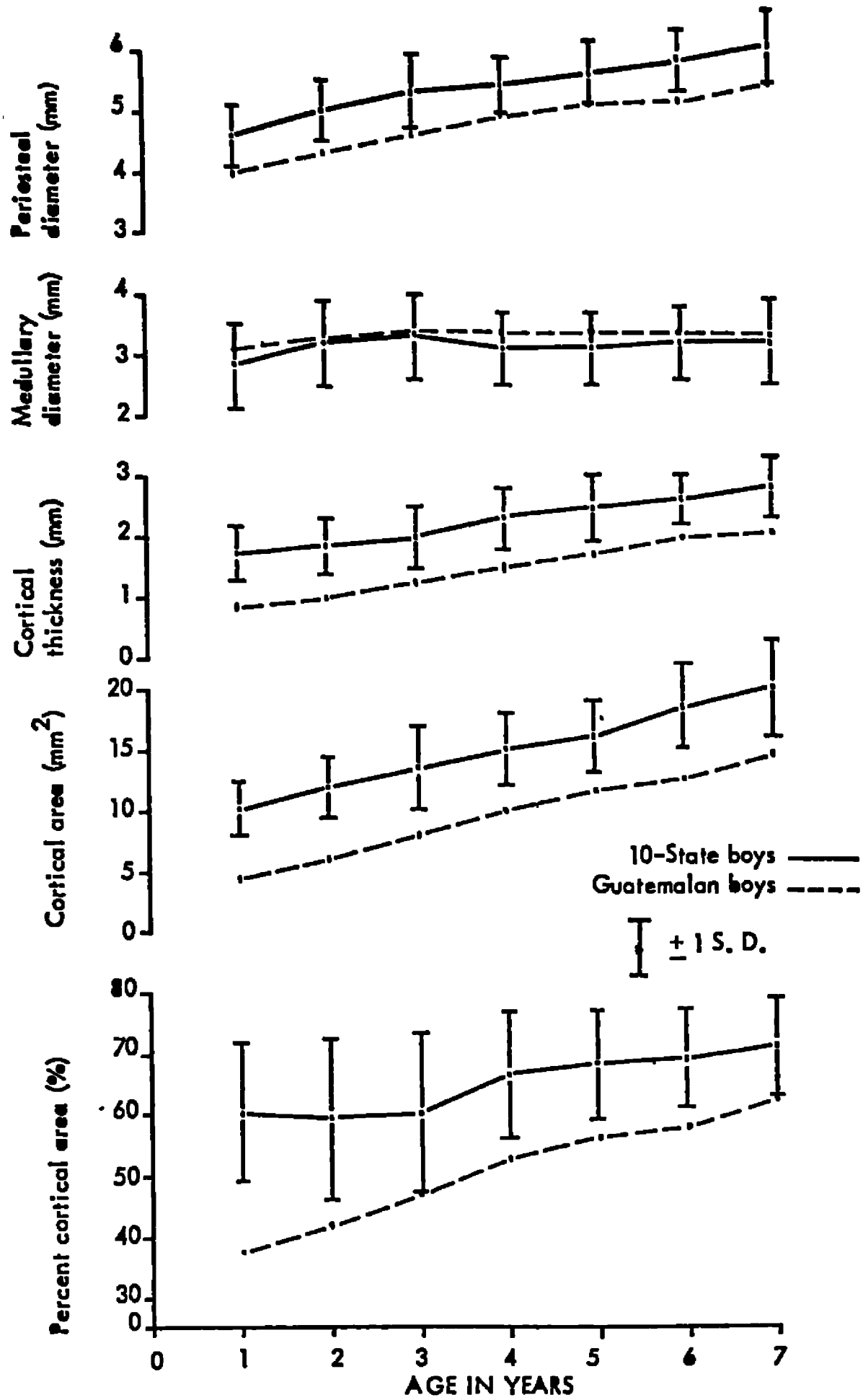



FIG. 1. Growth in cortical bone of Guatemalan boys compared to U S. children.

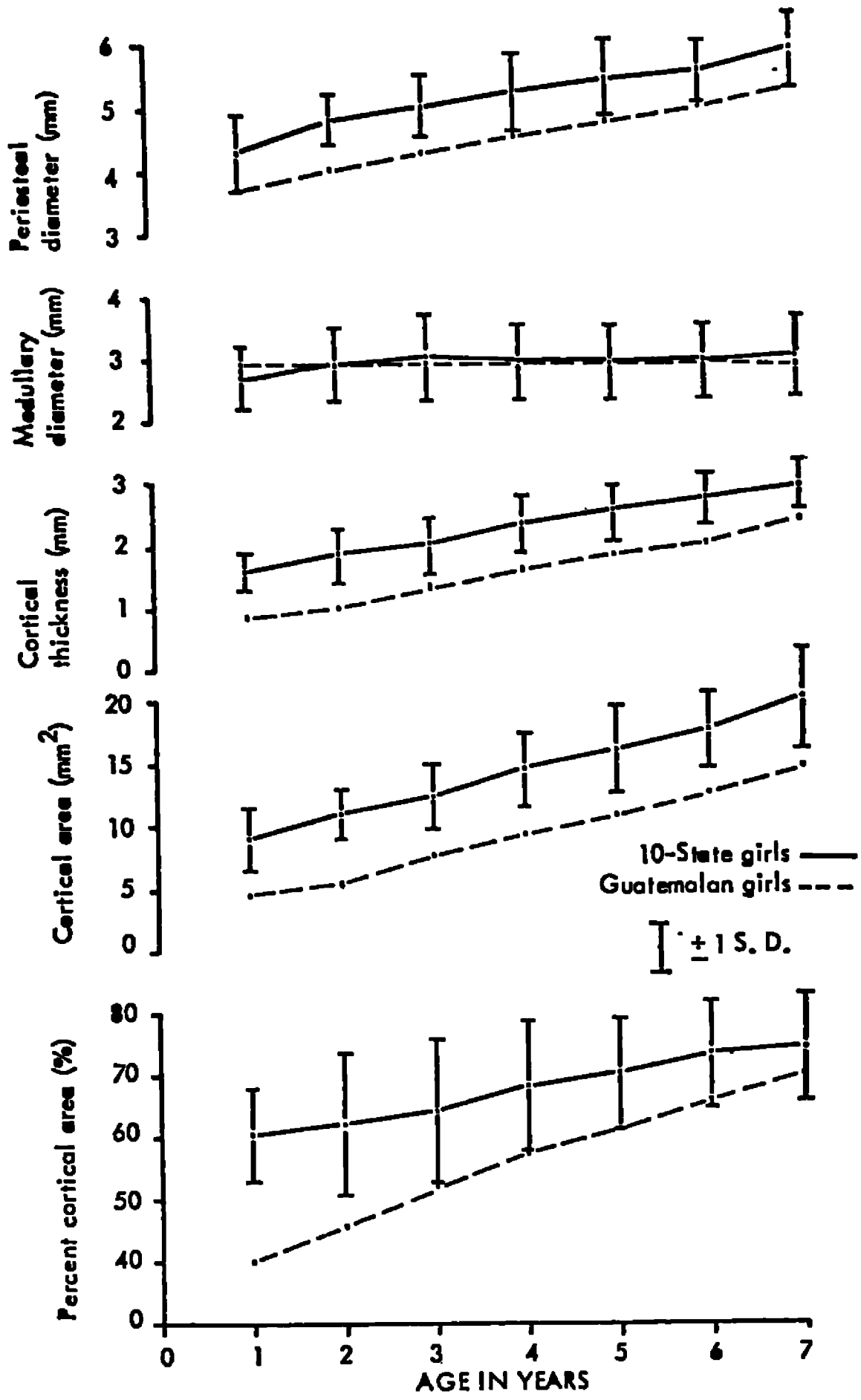



FIG 2 Growth in cortical bone of Guatemalan girls compared to U.S. children.

change in medullary diameter. The Guatemalan children are significantly retarded ($p < .001$) in periosteal diameter, cortical thickness, cortical area and percent cortical area compared to better nourished children. On the other hand, the Guatemalan boys have consistently greater medullary diameters than the U.S. boys, while the mean medullary diameter of the Guatemalan girls approximates that of the U.S. girls throughout the age range.

The absolute deficiencies in metacarpal dimensions of the Guatemalan children generally fall at least 1 standard deviation below the U.S. means. These deficiencies are rather constant throughout the age range. However, Guatemalan children show an apparent catch-up in percent cortical area relative to the U.S. children, because cortical area is increasing while medullary diameter is constant.

If the smaller cortical dimensions of the Guatemalan children only reflect overall retardation in body growth, then the well-nourished children and the Guatemalan children should have similar bone dimensions for a given body size, even though the Guatemalan children are significantly shorter and lighter than well-nourished U.S. children (Yarbrough, et al. in press). In Figures 3 and 4 age-specific means for cortical bone variables are presented relative to age-specific means for stature and weight of the U.S. and Guatemalan children. The Guatemalan body size data presented here are representative (no age and sex-specific mean differences greater than 1.0 cm or 0.4 kg) of that given in Yarbrough et al. (in press).[1]

Guatemalan children at age 1 start with less periosteal diameter, cortical thickness, cortical area and percent cortical area, for a given stature or weight than the well-nourished children. For most of the bone variables this deficiency decreases slightly throughout the age range, and for percent cortical area for body size it decreases markedly from 1 to 7 years. As medullary diameter changes little from 1 to 7 years of age (Figs. 1 and 2) there is little overall relationship with stature or weight. Thus, medullary diameter for a given body size is virtually the same for the U.S. and the Guatemalan children, although Guatemalan boys have, on the average, slightly, but consistently greater medullary diameters for body size than the U.S. boys.

[1] Stature in Guatemalan children was measured as recumbent length while stature in the U.S. sample was measured standing for children two years of age and older. Therefore, 1 cm was added to the U.S. mean stature values at those ages in order to make them more comparable to the recumbent length of the Guatemalan children.

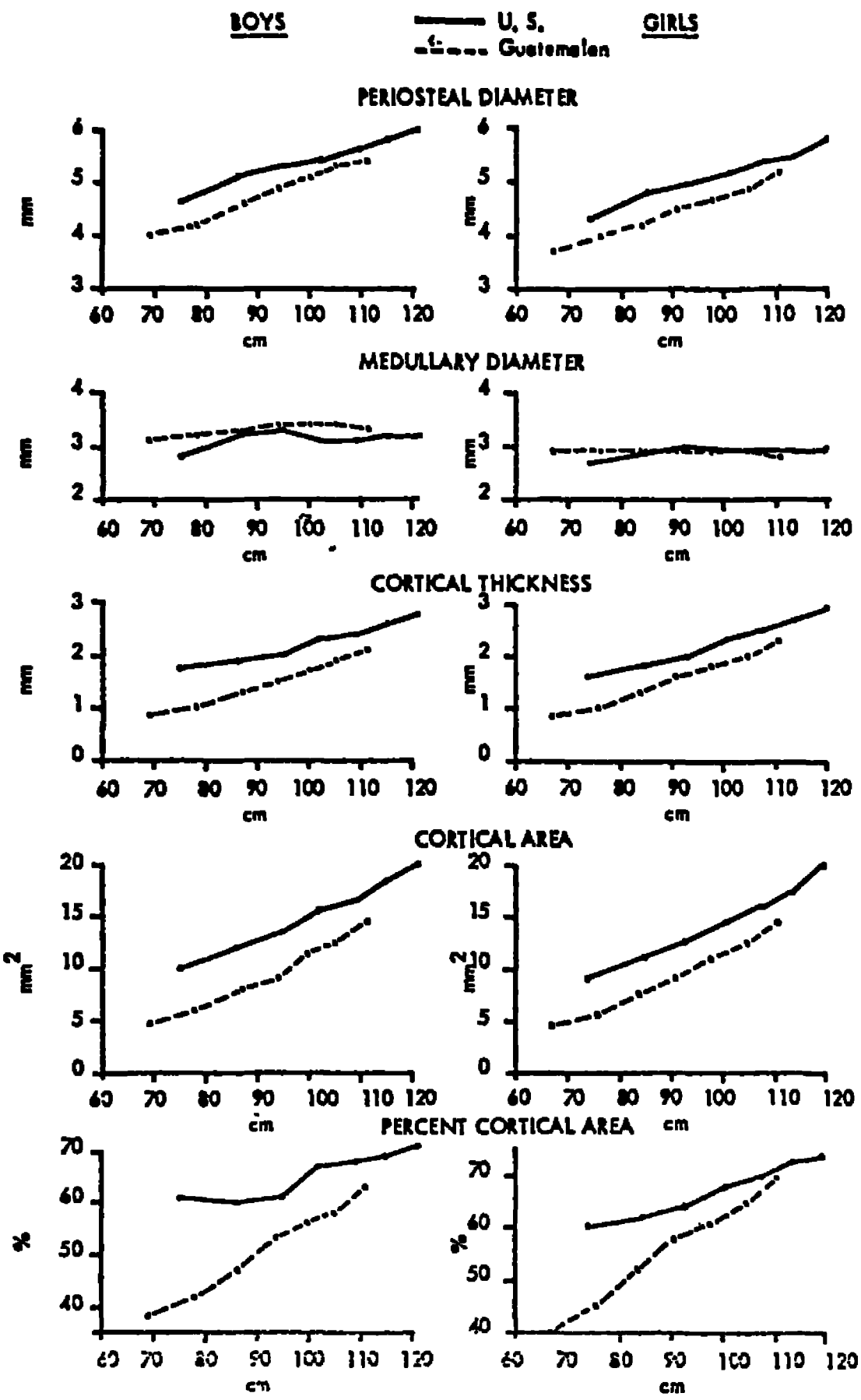



FIG. 3. Mean bone growth relative to stature.

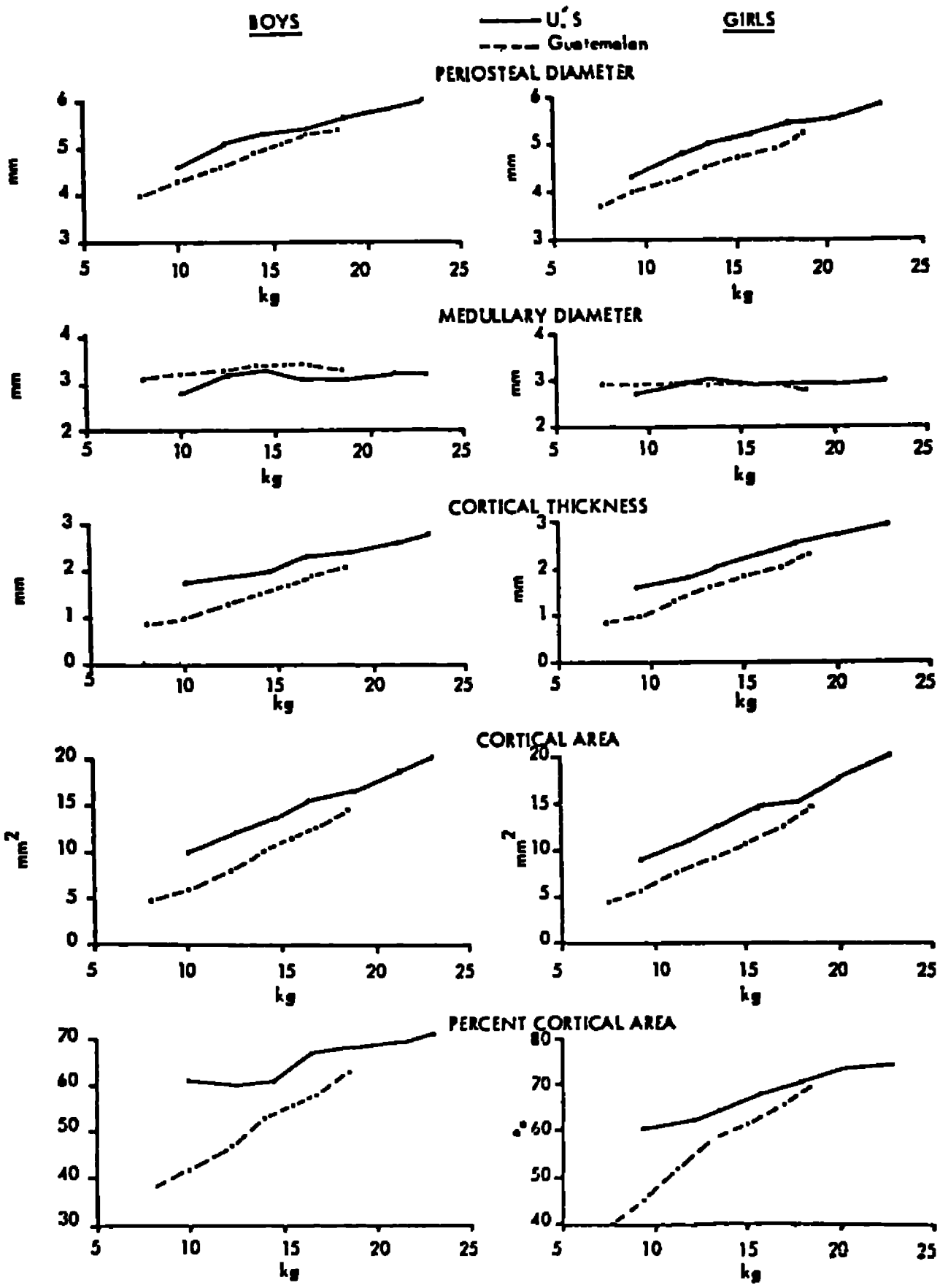



Fig. 4. Major growth relative to weight.

The deficiencies of the Guatemalan children in periosteal diameter, cortical thickness, cortical area and percent cortical area for body size are too large to be explained solely by the Guatemalan measurement variability (Table 1) and the inter-observer measurement variability for the U.S. sample (approximating 0.15 mm; Garn, personal communication). Thus, the retardation in cortical bone growth of the Guatemalan children compared to the well-nourished children is not only a reflection of stunted body size because, except for medullary diameter, the Guatemalan children have absolutely smaller cortical dimensions for a given stature or weight.

## Discussion

The moderately malnourished Guatemalan children when compared to well-nourished U.S. children have significantly smaller periosteal diameters but medullary diameters which approximate, and in the case of the boys exceed, the U.S. values. Therefore, the Guatemalan children have relatively expanded medullary diameters for their periosteal diameters, and consequently, absolutely thinner cortices and smaller cross sectional areas of cortex. Similarly, having smaller bones with less cortex, the percentage of the metacarpal cross-section occupied by cortex or percent cortical area is severely reduced.

These differences in metacarpal cortical bone between the Guatemalan and U.S. children are qualitatively similar to those reported for children with severe protein-calorie malnutrition compared to local controls (Adams and Berridge, 1969; Garn et al. 1969), with the exception that periosteal diameter was either not different or slightly larger in hospitalized children with kwashiorkor compared to village controls who suffered from mild to moderate malnutrition. Thus, in the previous studies the comparisons made were between severely malnourished and moderately malnourished children, rather than between well-nourished and severely malnourished children. This suggests that chronic protein-calorie deficiency may be a more important factor in retarding periosteal diameter growth than the more acute often rapidly precipitated bout with kwashiorkor. A similar retardation in periosteal diameter growth is also seen in children malnourished due to celiac disease (Barr et al. 1972).

The growth deficiency in bone of the Guatemalan children is of added interest in light of the children's small body size. The stunting in body size seen in Guatemala is primarily due to environmental factors

(Habicht et al. 1974). Thus, it is of interest to inquire whether bone growth and body size respond uniformly to environmental stress. One would then expect smaller children to have proportionately smaller bone dimensions, which would account for some of the absolute differences in bone between the Guatemalan and U.S. children. However, when compared to the U.S. children, the Guatemalan children have not only absolutely less metacarpal cortical bone but also smaller cortical dimensions relative to their stature and weight. This is to say that the Guatemalan children are retarded in bone and body size but the deficiency in bone is greater than that which would be expected if the diminished bone dimensions only reflected overall stunting in body size. Similarly, when Garn et al. (1966) compared tibial cortical measurements of frankly malnourished Jamaican children with those of well-nourished Ohio children of comparable tibial length, thus partially correcting for height, the malnourished children had greatly reduced cortical thickness, cortical area and percent cortical area compared to the well-nourished children. Thus, these findings and those of the present study concur in that the deficiencies in bone growth seen in moderately malnourished children show a skeletal response to malnutrition above and beyond a retardation associated solely with body size, although measures of cortical bone are related to body size.

## Acknowledgment

This research was supported by Contract (PH43-65-640) from the National Institute of Child Health and Human Development, Bethesda, Maryland and a faculty sponsored research grant from the Institute of Latin American Studies, University of Texas at Austin, Austin, Texas.

*Received: 22 October 1974.*

## Literature Cited

Adams, P. 1969 The effect of experimental malnutrition on the development of long bones. Biblio. Nutrit. Dieta 13: 69-73.

Adams, P. and F. R. Berridge 1969 Effects of kwashiorkor on cortical and trabecular bone. Arch. Dis. Childh. 44: 705-709.

Adams, P., G. T. Davis and P. M. Sweetnam 1969 Observer error and measurements of the metacarpal. Brit. J. Radiol. 42: 192-197.

Barr, D. G. D., D. H. Shmerling and A. Prader 1972 Catch-up growth in malnutrition, studied in celiac disease after institution of gluten-free diet. Pediat. Res. 6: 521-527.

BÉHAR, M. 1968 Prevalence of malnutrition among preschool children of developing countries. *In:* Malnutrition, Learning and Behavior N. S. Scrimshaw and J. E. Gordon (eds.). M.I.T. Press, Cambridge, Mass, pp. 30-41.

DICKERSON, W. T. AND R A MCCANCE 1961 Severe undernutrition in growing and adult animals. 8 The dimensions and chemistry of the long bones. Brit. J. Nutrit. 15: 567-576.

DIVISIÓN DE DESARROLLO HUMANO, INCAP 1971 Manual de Operaciones: Estudio longitudinal de desnutrición, crecimiento físico y desarrollo mental. INCAP-DDH, Guatemala, Guatemala.

DREIZEN, S AND R. E. STONE 1962 Human nutritive and growth failure. Postgrad. Med. 32: 381-386.

EL NAWABY, M., A. S. SHUKRY, A. EL HEFNY AND M. RAGAB 1962 A radiological study of the heart and bones in kwashiorkor in Egyptian children. J. Ind. Pediat. Soc. 1: 203-208.

GARN, S. M. 1970 The Earlier Gain and the Later Loss of Cortical Bone C. C Thomas, Springfield, Illinois

GARN, S. M., M. A. GUZMÁN AND B. WAGNER 1969 Subperiosteal gain and endosteal loss in protein-calorie malnutrition. Amer. J. Phys. Anthrop 30: 153-156.

GARN, S M., R L. MILLER AND K E. LARSON *n d.* Metacarpal lengths, cortical diameters and areas from the 10-State Nutrition Survey including estimated skeletal weights, weight and stature for Whites, Blacks and Mexican-Americans. Center for Human Growth and Development. University of Michigan, Ann Arbor.

GARN, S M, C. G ROHMANN, M. BÉHAR, F. VITERI AND M. A. GUZMÁN 1964 Compact bone deficiency in protein-calorie malnutrition. Science 145: 1444-1445.

GARN, S. M., C. G ROHMANN AND M. A GUZMÁN 1966 Malnutrition and skeletal development in the preschool child. *In.* Pre-School Malnutrition, Primary Deterrent to Human Progress National Academy of Science—National Research Council Pub. 1282, Washington, D.C, pp 43-73

GUZMÁN, M. A. 1968 Impaired physical growth and maturation in malnourished populations. *In·* Malnutrition, Learning and Behavior. N S. Scrimshaw and J E. Gordon (eds ). M I T. Press, Cambridge, Mass, pp 42-54.

HABICHT, J-P. 1974 Estandarización de métodos epidemiológicos cuantitativos sobre el terreno. Boletín de la Oficina Sanitaria Panamericana 76: 375-384

HABICHT, J-P, A. LECHTIG, C YARBROUGH AND R E. KLEIN 1972 The timing of the effect of supplementation feeding on the growth of rural preschool children. Paper presented at the IX International Congress of Nutrition, Mexico, Mexico, September 3-9, 1972

HABICHT, J-P., R. MARTORELL, C YARBROUGH, R. M. MALINA AND R. E KLEIN 1974 Height and weight standards for preschool children. how relevant are ethnic differences in growth potential? Lancet 1: 611-615

HABICHT, J-P., J A. SCHWEDES, G. ARROYAVE AND R. E. KLEIN 1973 Biochemical indices of nutrition reflecting ingestion of a high protein supplement in rural Guatemalan children Amer J. Clin. Nutrit. 26: 1046-1052.

INCAP 1969 Evaluación Nutricional de la Población de Centro America y Panamá. Guatemala. Institute of Nutrition of Central America and Panama. Guatemala, Guatemala.

JELLIFFE, D. B. 1966 The Assessment of the Nutritional Status of the Community. WHO Monograph No 53, WHO, Geneva.

KLEIN, R. E., J-P. HABICHT AND C. YARBROUGH 1973 Some methodological problems in field studies of nutrition and intelligence *In:* D. J. Kallen (ed.), Nutrition, Development and Social Behavior. Department of Health, Education and Welfare Pub. No (NIH) 73-242, U.S. Govt. Print. Off., Washington, D.C., pp. 61-75.

MALINA, R. M., J-P. HABICHT, R. MARTORELL, A. LECHTIG, C. YARBROUGH AND R. E. KLEIN *in press* Head and chest circumferences in rural Guatemalan Ladino children birth to seven years of age. Amer. J. Clin. Nutrit.

MALINA, R. M., J-P. HABICHT, C. YARBROUGH, R. MARTORELL AND R. E. KLEIN 1974 Skinfold thickness at seven sites in rural Guatemalan Ladino children birth through seven years of age. Human Biol. 46: 453-469

MAZESS, R. B., J R. CAMERON AND J. A. SORENSON 1970 A comparison of radiological methods for determining bone mineral content. *In:* Progress in Methods of Bone Mineral Measurement. G. P. Whedon and J R Cameron (eds ) U.S. Dept. Health, Education and Welfare, Washington, D C, pp. 455-479

PRATT, C. W. M. AND R. A. MCCANCE 1960 Severe undernutrition in growing and adult animals. 2. Changes in long bones of growing cockrels held at fixed weights by undernutrition Brit. J. Nutrit. 14: 75-84

YARBROUGH, C., J-P. HABICHT, R. M. MALINA, A. LECHTIG AND R. E KLEIN *in press* Length and weight in rural Guatemalan Ladino children birth to seven years of age. Amer. J. Phys. Anthrop.

# Sibling Similarities in Number of Ossification Centers of the Hand and Wrist in a Malnourished Population

By Renaldo Martorell,[1] Charles Yarbrough,[2] John H. Himes[3] and Robert E. Klein[2]

## ABSTRACT

Sibling correlations in the number of ossification centers of chronically malnourished Guatemalan children, 1 to 7 years old, are presented. Sister-sister correlations were found to be similar to brother-brother and brother-sister correlations. Sibling correlations for the Guatemalan sample were similar to those reported for U.S.A. children in the case of brother-brother and brother-sister correlations. Sister-sister correlations were, however, lower in the Guatemalan sample. These findings are not in general agreement with predictions made to the effect that sibling correlations should be lower in malnourished children.

Garn, Rohmann and Davis (1963) reported that in well-nourished, Ohio-born white children, genetics appeared to account for a major proportion of the variance in the number of ossification centers present at a given age, and in the time of appearance of specific hand-wrist centers. These conclusions were based on interpretations of patterns of parent-child, sibling and twin correlations. These authors hypothesized that "at lower levels of nutrition" the relative weight of genetics and nutrition could well reverse. Indeed, it follows from the theory underlying the notion of estimated heritability that as the environmental variance increases, the estimated heritability decreases.

In the present study we examine sibling correlations in the number of ossification centers of chronically malnourished Guatemalan children 1 to 7 years old, and compare them to those published for well-nourished children at the same ages (Garn et al. 1963).

## METHODS

The study took place in four rural Ladino communities of Guatemala. Protein-calorie malnutrition (Lechtig et al. 1975) and high morbidity

[1]Food Research Institute, Stanford University, Stanford, California 94305.

[2]The [illegible] of Human Development, Institute of Nutrition of Central America and Panama (INCAP), P.O. Box 1188, Guatemala City, Guatemala, C.A.

[3]The Fels Research Institute, Yellow Springs, Ohio 45387.

*Human Biology, February 1978, Vol. 50, No. 1, pp. 73–81.*

(Martorell et al. 1975) are endemic in these communities, and physical growth of children is stunted (Yarbrough et al. 1975).

Radiographs of the left hand and wrists were obtained at 3 and 6 months of age and then serially every 6 months until 4 years, after which time X-rays were taken annually until 7 years of age. The age tolerances were ± 7 days during the first two years, and ± 15 days thereafter. With the exceptions of the pisiform and sesamoids, each radiopaque center of ossification (carpals and epiphyses) was identified on the radiographs and counted to obtain the number of ossified centers present (Yarbrough et al 1973). All information utilized was collected between January 1969 and July 1975.

## Results

The mean number of ossified hand-wrist centers at ages 0 25 through 7 years is shown in Table 1 for Guatemalan boys and girls. As is true of other populations in both developed (Reynolds and Asakawa, 1951, Garn and Rohmann, 1960) and developing nations (Massé and Hunt, 1963, Malcolm, 1970), girls are more advanced in skeletal maturity than boys. The greatest differences between sexes, ranging from 5.7 to 6 7 centers, occur in the age range of 1.5 to 3.0 years.

Table 1

*Mean Number of Ossified Centers in Guatemalan Boys and Girls*

| | Boys | | | Girls | | |
|---|---|---|---|---|---|---|
| Age (years) | n | $\bar{x}$ | SD | n | $\bar{x}$ | SD |
| 1 0 | 256 | 2 71 | 1 52 | 235 | 5 26 | 3 83 |
| 1 5 | 282 | 4 22 | 2 93 | 226 | 9 87 | 5 47 |
| 2 0 | 302 | 7 15 | 4 49 | 261 | 13 89 | 5 70 |
| 2 5 | 313 | 10 82 | 4 96 | 273 | 17 52 | 5 23 |
| 3 0 | 330 | 14 42 | 4 90 | 287 | 20 06 | 4 18 |
| 3 5 | 312 | 17 26 | 4 42 | 282 | 21 72 | 3 14 |
| 4 0 | 307 | 19 50 | 3 40 | 276 | 22 89 | 2 18 |
| 5 0 | 286 | 22 18 | 1 99 | 267 | 24 43 | 1 83 |
| 6 0 | 262 | 23 53 | 1 70 | 248 | 26.04 | 1 70 |
| 7 0 | 253 | 25 05 | 1 82 | 233 | 27 13 | 1 24 |

Among well-nourished children, the number of centers tend to be more variable for girls than boys at a fixed chronological age (Garn and Rohmann, 1960). For the Guatemalan children, this is true throughout the first 2½ years of age. Thereafter, until they are 7 years old, variances are equal or slightly higher for boys (Table 1). However, a closer look at the data reveals that some of the differences in variability largely disappear when boys and girls are compared, not at a fixed chronological age, but at comparable levels of skeletal maturity, or number of centers Figure 1 shows that in regard to skeletal maturity girls are always more variable than boys, except at the extremes of the mean number of centers These differences, however, are neither large nor statistically significant (i e at point of greatest differences, $F = 1\ 33$, $f_1/f_2 = 261/313$, $p>0\ 05$)

Previous studies have shown that compared to U.S A children, rural Guatemalan children are retarded in the number of ossification centers present (Blanco et al. 1972, Yarbrough et al 1973) In our study sample, Guatemalan girls are more retarded than U.S A. girls at 2 years of age

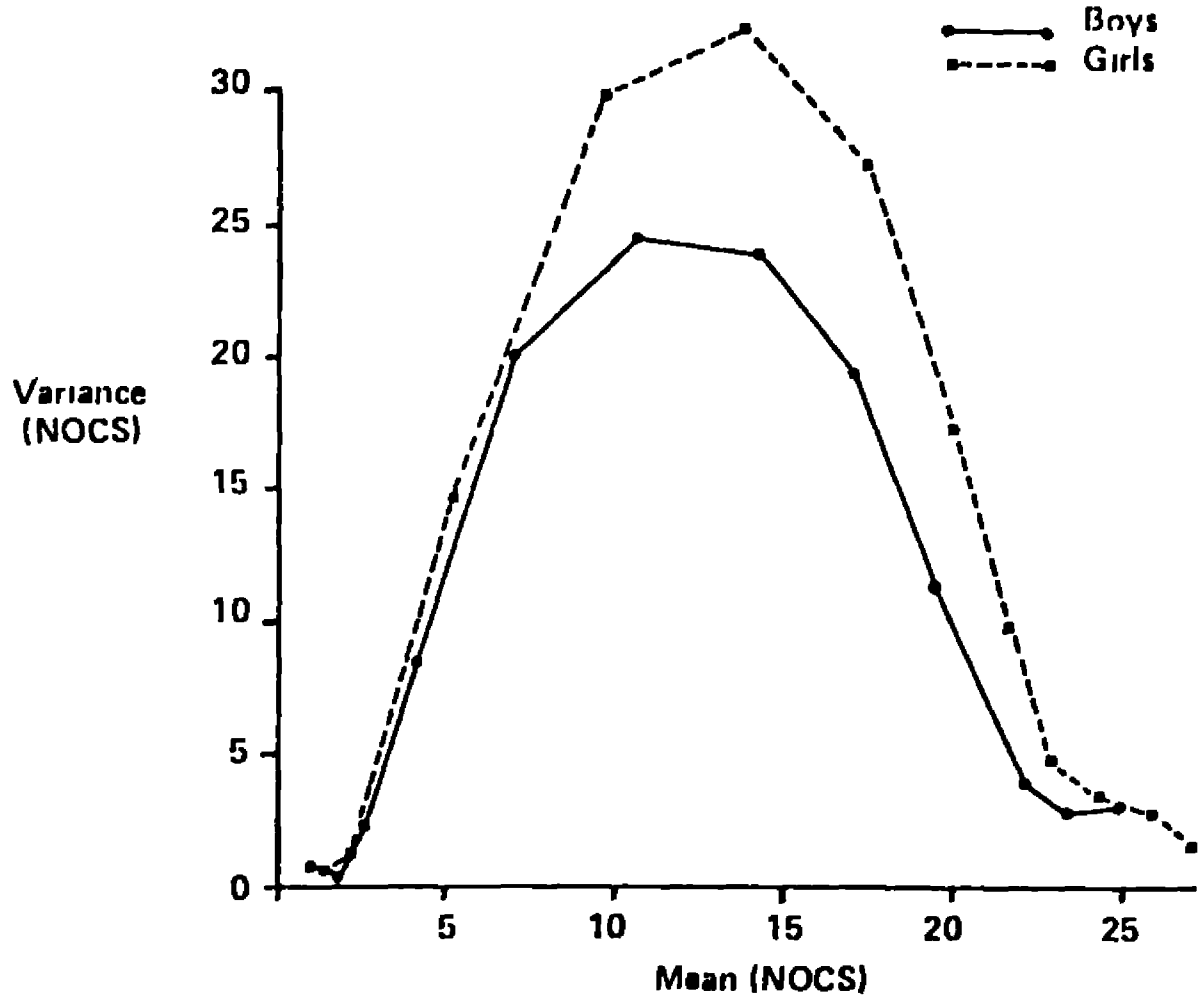
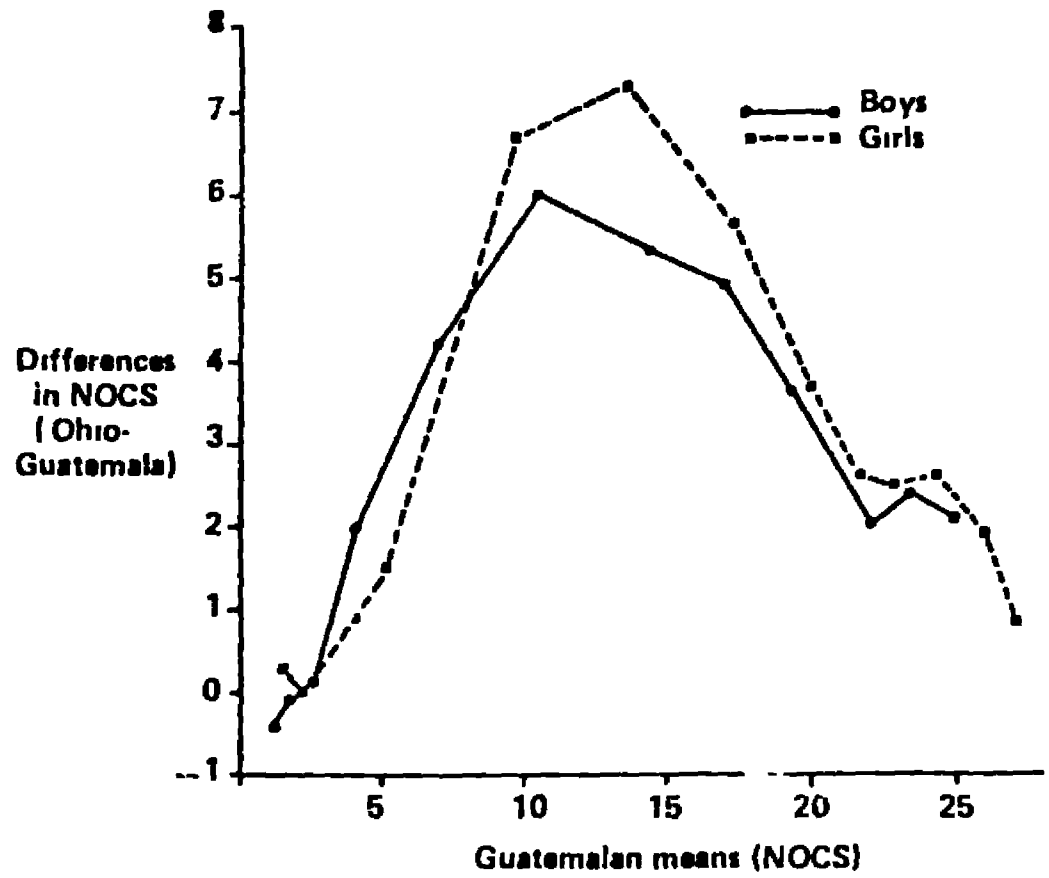



FIG 1 Relationship between age-sex-specific means and variances of number of ossification centers (NOCS) of Guatemalan children




a Garn and Rohmann (1960) report 50th percentiles A comparison of these values and mean values reported for the same Ohio boys (Yarbrough et al , 1973) reveals small differences which would not alter the conclusions drawn from this Figure

FIG 2 Difference between Ohio[a] and Guatemalan children in number of ossification centers (NOCS) present plotted as a function of the Guatemalan NOCS mean

(Garn and Rohmann, 1960), while in Guatemalan boys maximum retardation occurs at 2½ years of age. As in the case of variances, sex differences in the pattern of retardation seem to be a function of differences in the rate of maturation rather than in chronological age. The sex-specific differences in number of ossification centers between Guatemalan and U S.A. children, relative not to age but to the number of centers present, are shown in Figure 2. The periods of greatest retardation occur when Guatemalan children have 10 to 15 centers The fact that girls are more behind at the points of peak retardation,[1] reflects perhaps that girls reached 10 to 15 centers before two years of age, at a time when nutritional and health status is likely to be poorest.

Sibling correlation coefficients for the number of ossification centers are presented in Table 2 for Guatemalan and U.S. children Sibling correlations are similar in brother-brother, brother-sister and sister-sister comparisons in the Guatemalan sample. This is contrary to the findings of

Table 2

*Sibling Correlations in the Number of Ossification Centers in Guatemalan and U.S.A. Children*

| Age (years) | Guatemala | | | | | | U S A [1] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Brother-Brother | | Brother-Sister | | Sister-Sister | | Brother-Brother | | Brother-Sister | | Sister-Sister | |
| | n | r | n | r | n | r | n | r | n | r | n | r |
| 1.0 | 128 | 0 19 | 81 | 0 31 | 100 | 0 20 | 52 | 0 15 | 82 | 0 19 | 33 | 0 65 |
| 1 5 | 144 | 0 45 | 99 | 0 34 | 94 | 0 46 | 53 | 0 32 | 106 | 0 46 | 45 | 0 66 |
| 2 0 | 162 | 0 38 | 124 | 0 49 | 122 | 0 52 | 57 | 0 47 | 104 | 0.42 | 37 | 0 43 |
| 2.5 | 188 | 0 44 | 142 | 0 49 | 152 | 0 57 | 54 | 0 34 | 101 | 0 26 | 51 | 0 46 |
| 3 0 | 176 | 0 51 | 159 | 0 47 | 148 | 0 41 | 57 | 0 43 | 107 | 0 30 | 49 | 0 53 |
| 3.5 | 170 | 0 50 | 152 | 0 48 | 138 | 0 41 | 58 | 0.50 | 103 | 0 38 | 43 | 0 42 |
| 4 0 | 160 | 0 44 | 165 | 0 37 | 124 | 0 19 | 54 | 0 36 | 101 | 0 33 | 44 | 0 65 |
| 5 0 | 134 | 0 44 | 138 | 0 31 | 144 | 0 40 | 54 | 0 34 | 97 | 0 47 | 39 | 0 75 |
| 6.0 | 106 | 0 49 | 124 | 0 29 | 146 | 0 49 | 51 | 0.38 | 84 | 0 48 | 30 | 0 67 |
| 7 0 | 112 | 0 48 | 115 | 0 17 | 146 | 0 24 | 47 | 0 63 | 79 | 0 65 | 22 | 0.84 |
| Average[2] | 1480 | 0 44 | 1299 | 0 39 | 1314 | 0 40 | 537 | 0 40 | 964 | 0 40 | 393 | 0 60 |

[1]From Garn et al. (1963)

[2]Summary correlations based on all possible comparisons within a family, weighed z-scores following Snedecor and Cochran (1967) Because in both the U S A and the Guatemalan sample, children are included at more than one age group, and because children may appear as often as they have sibs, standard tests of significance are not appropriate

Garn and colleagues (1963, 1969) which show sister-sister correlations to be the highest in Fels data, suggesting strong X chromosome involvement in ossification parameters. Sibling correlations for brother-brother and for brother-sister are of similar magnitude for Guatemalan and U.S. children. Sister-sister correlations, however, tend to be higher in U.S.A. data.

## Discussion

With the exception of sister-sister correlations, sibling correlations in the number of ossification centers are similar in well-nourished U.S. children and poorly-nourished children from rural Guatemala. A similar finding was observed in comparisons of parent-child and sibling correlations for height between Guatemalan and European children (Martorell et al. 1977). Moreover, Mueller (1976) who reviewed 24 studies of parent-child correlations in stature and weight, found considerable overlap in the magnitude of correlations from well-nourished and malnourished populations. Because malnourished children are substantially retarded, not only in physical growth (Habicht et al. 1974), but in skeletal age as well (Dreizen et al. 1961), the environmental component of the phenotypic variability should increase, reducing the resemblance estimate as seen in the correlation between relatives in well-nourished populations. However, the present data are not generally supportive of this hypothesis.

Sibling-sibling correlations in growth are always greater than those for parent-child (Garn and Rohmann, 1966). This is probably due to greater environmental similarities between siblings than between parent and child. Furusho (1963) has shown that siblings closer together in age, have higher correlations for stature than siblings farther apart in age, but only when there are considerable environmental differences between the latter sibs. It may be that in rural Guatemala sibling correlations are unexpectedly high because siblings share a common environment to a greater degree than in the United States. This hypothesis, however, does not adequately explain the higher than expected parent-child correlations for height, unless one posits strong transgenerational environmental correlates.

It may be that in the four apparently homogeneously poor villages covered by our study, environmental conditions, though not optimal for growth and development, are not that different across families. In other words, children would be stunted in growth more or less uniformly, in such a way that genetic correlations would be undisturbed. This

hypothesis, however, cannot satisfactorily explain the increased height variances observed in rural Guatemala. For instance, variances in height from birth to 7 years of age for rural Guatemalan children (Yarbrough et al. 1975) are typically over 50% larger than those for Denver children of the same age (Hansman, 1970).

Strong assortative mating for traits, reflecting growth and maturity parameters, may raise sib correlations. However, we think this hypothesis is unlikely, since the husband-wife height correlation in rural Guatemala is 0.09 (n = 260, N.S.) a value much lower than 0.3, the correlation usually reported for well-nourished populations (Spuhler, 1968; Tanner et al. 1970).

In the four rural Guatemalan villages studied, mates are generally found within, or close to the community. Hence, we suspect that inbreeding may be higher than in well-nourished populations from the U.S.A. Therefore, genetic correlations would be higher, to the extent that Guatemalan siblings are more related to one another than expected. Therefore, one would have to correct for inbreeding, before comparing correlations in the two populations of interest to this study.

Concerning sex differences in sib correlations for number of centers, we should note that the original sample sizes reported by Garn et al. (1963) are quite small. The greatest number of brother-brother pairs reported is 58, and the greatest number of sister-sister pairs, 51. Using those as estimates of the appropriate sample sizes for a test of the difference in correlation sizes utilizing the standard Fisher transformations of the average correlations, we get $t = 1.36$ ($0.10 < p > 0.05$). Thus, we conclude that it is not overwhelmingly certain that sister-sister correlations are in fact higher than those between brothers.

We entertain the possibility that in both the Fels and the Guatemalan samples, the true sib-sib correlation for number of hand-wrist centers is approximately 0.4, regardless of sex. We are not suggesting, however, that sex differences do not exist in the magnitude of sib correlations for other growth and maturational variables (Palmer, 1934, Hewitt, 1957).

Sibling correlations for the number of hand-wrist ossification centers are as high in rural Guatemala as in the U.S,A. Similar findings have been reported for height and weight for various poorly-nourished populations (Mueller, 1976, Martorell et al. 1977). Whether the unexpectedly high correlations result primarily from genetic and/or environmental factors is difficult to determine. Sibling and parent-child correlations in well-nourished populations have been traditionally interpreted as largely reflecting the influence of genetic factors (Garn et al. 1976). Analyses of

resemblances in height, weight and fatfolds between adoptive parents and their adopted children raise the possibility that nongenetic familial characteristics play a greater than previously assumed role in explaining parent-child and sibling correlations in well-nourished populations (Garn et al. 1976). These findings in turn make interpretation of such correlations in poorly-nourished populations a difficult task. Research should be oriented, as suggested by Garn et al. (1976), to elucidate the relative importance of genetic and nongenetic factors in causing variability in anthropometric variables. The implications of this research for nutritional anthropometry are clear because variability in indicators such as height and weight is assumed by public health personnel in developing nations to be almost exclusively caused by environmental factors. Moreover, parental size is almost never taken into account in evaluating the nutritional status of children. Just as it is clear that genetics plays a substantial role in explaining variability in well-nourished populations, it is also evident that factors such as nutrition and disease account for much of the variability in anthropometric variables in deprived populations. What is needed, therefore, is to go further towards the quantification of the relative importance of genetic and environmental factors as causes of variability in populations living in environments that either allow or restrict attainment of genetic potential.

## Acknowledgements

This research was supported by Contract No. NO1-HD-5-0640 from the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, U.S.A.

*Received: 6 April 1977.*
*Revision Received: 18 August 1977.*

## Literature Cited

Blanco, R. A., R. M. Acheson, C. Canosa and J. B. Salomon 1972 Retardation in appearance of ossification centers in deprived Guatemalan children. Human Biol. 44: 525-536.

Dreizen, S., R. E. Stone and T. D Spies 1961 The influence of chronic undernutrition on bone growth in children. Postgraduate Med. 29: 182-193.

Furusho, T. 1963 On the factors affecting the sib correlation of stature. Japan J. Human Genet. 8: 255-264.

Garn, S. M., S. M. Bailey and P. E. Cole 1976 Similarities between parents and their adopted children. Am. J. Phys. Anthrop. 45: 539-544.

GARN, S M. AND C. ROHMANN 1960 The number of hand-wrist centers Am J Phys Anthrop. 18: 293-299

——— 1966 Interactions of nutrition and genetics in the timing of growth and development. Pediatric Clinics of North America, 13. 353-379

GARN, S. M , C ROHMANN AND A A DAVIS 1963 Genetics of hand-wrist ossification Am J Phys Anthrop 21: 33-40

GARN, S M , C G ROHMANN AND K P HERTZOG 1969 Apparent influence of the X-chromosome on timing of 73 ossification centers Am J Phys Anthrop 30. 123-128

HABICHT, J-P , R MARTORELL, C YARBROUGH, R M MALINA AND R E KLEIN 1974 Height and weight standards for preschool children Are there really ethnic differences in growth potential? Lancet, 1: 611-615

HANSMAN, C 1970 Anthropometry and related data *In* Human growth and development, R W. McCammon (ed ), C. C Thomas, Springfield, Illinois, pp 101-154

HEWITT, D 1957 Some familial correlations in height, weight and skeletal maturity Ann Human Genet 22. 26-35.

LECHTIG, A , J-P. HABICHT, H DELGADO, R E KLEIN, C YARBROUGH AND R MARTORELL 1975 Effect of food supplementation during pregnancy on birth weight Pediatrics, 56: 508-520

MALCOLM, L A. 1970 Growth and development of the Bundi child of the New Guinea Highlands Human Biol 42. 293-328

MARTORELL, R , J-P HABICHT, C YARBROUGH, A. LECHTIG, R E KLEIN AND K A. WESTERN 1975 Acute morbidity and physical growth in rural Guatemalan children Am J. Dis Child 129. 1296-1301

MARTORELL, R , C YARBROUGH, A LECHTIG, H DELGADO AND R E KLEIN 1977 Genetic-environmental interactions in physical growth Acta Paediat Scand 66. 579-584

MASSÉ, G AND E. E HUNT 1963 Skeletal maturation of the hand and wrist in West African children Human Biol. 35. 3-25

MUELLER, W H 1976 Parent-child correlations for stature and weight among school aged children a review of 24 studies Human Biol 48 379-397

PALMER, C 1934 Age changes in physical resemblance of siblings Child Develop 5 351-360

REYNOLDS, E L AND T ASAKAWA 1951 Skeletal development in infancy Am J Roent. Radium Ther 65. 403-410

SPUHLER, J N 1968 Assortative mating with respect to physical characteristics Eugenics Quarterly, 15. 128-140.

TANNER, J M , H GOLDSTEIN AND R H WHITEHOUSE 1970 Standards for children's height at ages 2-9 years allowing for height of the parents Arch. Dis Child 45. 755-762.

YARBROUGH, C , J-P. HABICHT, R E KLEIN AND A F ROCHE 1973 Determining the biological age of the preschool child from a hand-wrist radiograph Invest Radiol 8. 233-243

YARBROUGH, C , J-P HABICHT, R MALINA, A LECHTIG AND R E. KLEIN 1975 Length and weight in rural Guatemalan Ladino children birth to seven years of age Am J. Phys Anthrop 42: 439-447

# Functional classification of undernourished populations in the Republic of El Salvador [1]

## Methodological development

**V. Valverde, F. Trowbridge, I. Beghin, B. Pillet, Isabel Nieves, Nancy Sloan, T. Farrell, P.R. Payne, J.L. Joy and R.E. Klein**

*Typical government nutrition programmes usually lack precise information on the size of groups that could benefit from interventions, on the different types of potential beneficiaries and their location, and on socio-economic and cultural characteristics through which they could readily be identified.*

*In the functional classification approach to the definition of nutritional problems, detailed information on human behaviour and social constraints is collected at family and community levels. The data are then interpreted in general terms in order to understand how these factors contribute to inadequate levels of nutrition within larger groups. This new approach should enable more effective measures for reducing the numbers of those living under conditions of deprivation to be presented to planners and decision makers.*

### The concept

A large amount of data has been collected on child malnutrition in Central America and Panama since the 1930s culminating in recent studies by the Institute of Nutrition of Central America and Panama/Office of International Research, National Institutes of Health (1972), in Honduras by the Sistema de Análisis y Planificación de la Alimentación y Nutrición (1976), and by the Instituto de Nutrición de Centro América y Panamá/Unidad de Análisis del Sector Salud (1976) in Nicaragua. However, their usefulness to planners and administrators is limited, particularly when the attempt is made to establish priorities for regions within a country, to select suitable programmes for dealing with nutritional problems, and to design specific projects for specific regions and subgroups of families within them. For example the questions "what intervention" and "for whom" cannot be answered by data aggregated at national level. This experience emphasizes the need to define the nutritional problems of developing countries in particular, not only in a practical manner but also in one that may be of immediate use.

Such an approach should also lead to a better understanding of the interrelationships between the factors believed to cause nutritional problems. Basic to it is the fact that the population of any given country is heterogeneous, and comprises distinc-

[1] The field work on which this paper is based was sponsored in part by the Government of El Salvador, the Ford Foundation, the W.K. Kellogg Foundation, the USAID Mission in El Salvador, and ROCAP/AID.

The authors were, in October 1977, on the staff of the Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C.A., except for F. Trowbridge, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland; P.R. Payne, Department of Human Nutrition, London School of Hygiene and Tropical Medicine; and J.L. Joy, Department of Nutritional Sciences, University of California, Berkeley, California.

TABLE 1 QUESTIONS TO BE ANSWERED BY THE FUNCTIONAL CLASSIFICATION PROPER

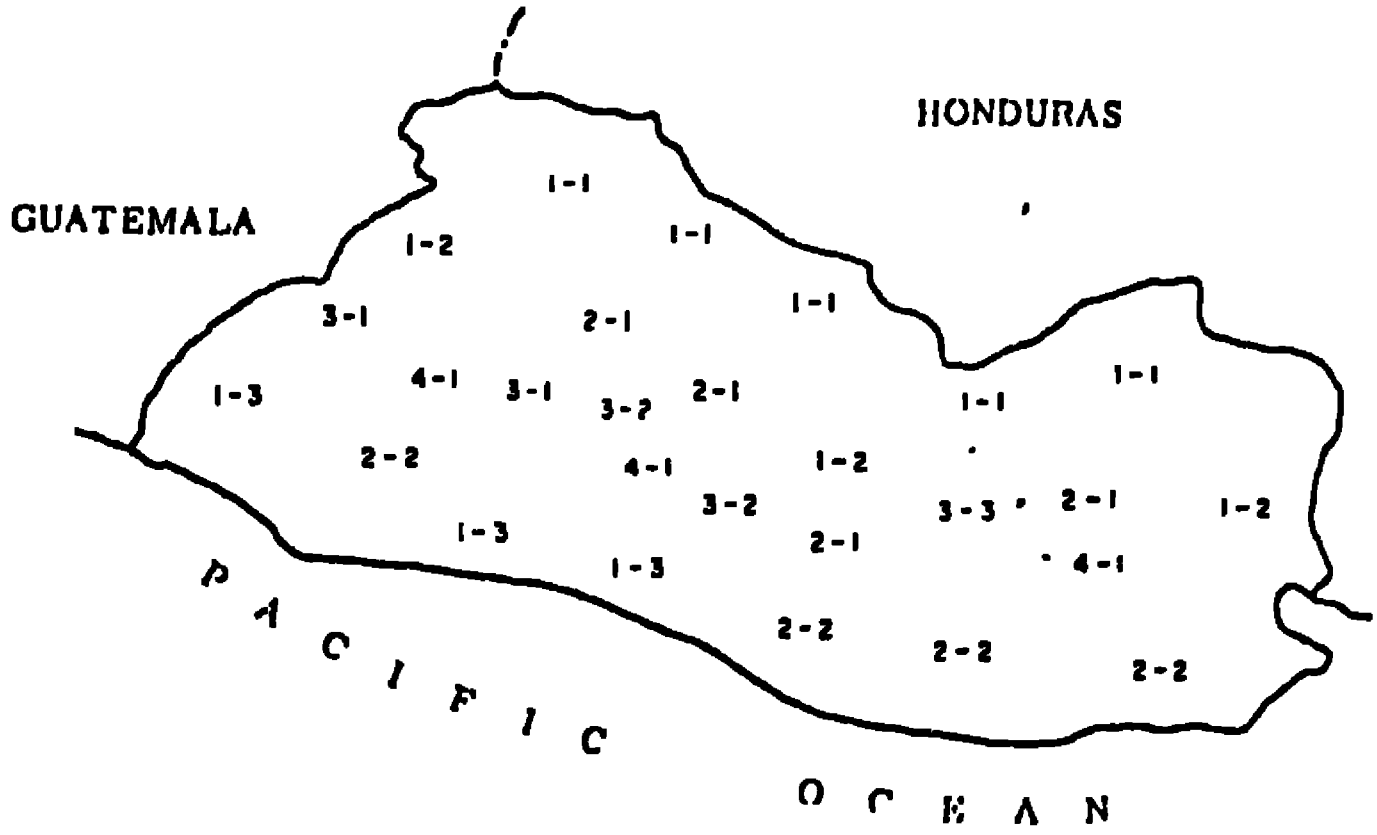
1. Which regions of El Salvador, described in geographical and administrative terms, have most malnutrition problems?
2. What are the general social, economic and health characteristics of population groups living in different regions?
3. What is the total population of each region and subregion? How many malnourished preschool children are found in each region? What is the approximate number of lactating and pregnant women?
4. What is the relative importance of the different social, economic and cultural factors as causes of malnutrition in each region, and in the distinct categories of population?
5. What is the size of these categories of population and their nutritional status?
6. What kind of programmes are most relevant for the different regions and categories of population? How many people respond to identified programmes?
7. What is the political, economic and operational feasibility of the suggested programmes? Who will finally benefit from them?
8. What amount of economic resources is needed to induce important changes in the number of families suffering from malnutrition in the different regions?
9. How can changes in nutritional status be detected, utilizing the existing information systems?

tive occupational, social and cultural groups. These differences affect both the process by which malnutrition becomes a problem, and the process through which it can be ameliorated or eradicated.

Realizing this, Joy (1973) introduced the concept of "functional classification of undernourished populations", i.e., the grouping of populations into categories that can be used in nutrition and development planning. Each functional category has a set of common characteristics, e.g., the same nutrition problem, or membership of an identifiable group (geographic, socio-economic, etc.). A third characteristic[2] is that the members of a given group have a high probability of responding in the same manner to a given intervention. A fourth category is added in this paper: the feasibility of reaching the group within the existing administrative divisions and through the existing administrative structures.

Hakim and Sollmano (1976) and Payne (1976) have reviewed the assumptions on which previous efforts to integrate nutritional components into national plans in developing countries are based. The need to define the nutritional and food problems within the framework of a functional classification has been stressed by Joy and Payne (1975) and FAO (1975). Abercrombie (1975) has also mentioned the importance of identifying sets of categories of poor people as an initial step toward the solution of deprivation. In a recent work carried out in four rural-subsistence agricultural communities of eastern Guatemala, Valverde *et al.* (1977) quantified and characterized families by size of landholdings, occupation, and nutritional status of their children.

A "functional classification" of malnourished populations, therefore, is essentially a new way of collecting and presenting information. It differs from earlier approaches in that: 1) it starts from the identification of distinct sets of affected people in a given region; 2) it studies their particular problems, behaviour and expectations in greater depth; 3) it estimates the size of each group; and 4) it attempts to assess their respective problems by region or subregion.




1-1) Northern subregion of marginal agricultural exploitation
1-2) Central subregion of marginal agricultural exploitation
1-3) Coastal subregion of marginal agricultural exploitation
2-1) Central subregion of intensive agricultural exploitation
2-2) Coastal subregion of intensive agricultural exploitation
3-1) Western subregion of coffee exploitation
3-2) Central subregion of coffee exploitation
3-3) Eastern subregion of coffee exploitation
4-1) Urban groups

*Figure 1.* Location of agricultural subregions and urban groups in El Salvador

The level of disaggregation of the data collected permits reaggregation according to the needs of the planner: at national level, by region, by administrative division, by socio-economic stratum or by type of employment. This in turn should facilitate identification of relevant programmes aimed at reducing malnutrition in groups of families or individuals in a given region.

A first attempt to develop such a type of methodology was made in

[2] Essential to Joy's approach, although not specifically identified in the quoted reference.

El Salvador, where the Government had expressed interest in this matter and offered its collaboration.

## Methodology development

### Project objectives and organization

The objectives of the project conducted in El Salvador were first, to update and integrate nutrition and socio-economic information in order to orient the existing programmes, and those to be determined by the future national food and nutrition policy; and second, to develop a methodology for elaborating a functional classification.

A multidisciplinary advisory committee was set up to define the nature and type of data to be gathered, establish the collection methods, prepare a tentative analytic plan and guide the general field operations. A nine-point list of questions, detailed in Table 1, was drawn up.

### Information sources in the country

A search of the literature for general data on the political, economic, demographic and agricultural conditions of El Salvador produced some useful material, e.g., the national housing, population, and agricultural censuses of 1971, the list of administrative boundaries for each *municipio*[3] of the country, and the 1976 population census giving the number of houses and total population in each *cantón*. Other material was discarded where data could not be disaggregated by regions or subregions, or the methods used for its collection were not considered sufficiently reliable.

### Definition of regions and administrative boundaries

At the time of the project, regional divisions of the country were being revised. It was therefore necessary to define regions specifically for the project. This was done on the basis of different patterns of land use assuming that each would correspond to: 1) more or less homogeneous socio-cultural environments, and 2) similar types of nutritional problems.

In order to provide data useful for planners and administrators, the municipio was chosen as the most convenient administrative unit since information on cantón boundaries were not available. Existing maps of land-use and other survey and census data enabled the country to be divided into three agricultural regions: 1) *marginal agricultural exploitation or of subsistence*; 2) *intensive agricultural exploitation* (cash crops for export); and 3) *coffee production*.

A fourth "urban" category included all cantones and capitals of municipios with 10 000 or more inhabitants in 1975. Subregions were designated based on geographic criteria to give a total of nine groups located as in Figure 1. The four regions were used as sample frames for all subsequent field activities. Table 2 summarizes the results of the initial classification by region and subregion in terms of land area and population.

### Use of census information

The original data of the population and housing censuses were aggregated and summarized at household level for each of the 2 057 cantones of El Salvador. The variables included: sex and age distribution, civil status, family size, occupation, literacy, school attendance, educational achievement, infant and child mortality, fertility, house ownership, type of house, sources of water, waste disposal and home industries. These variables were further subdivided; for instance, water by source, etc. Total numbers and percentages were calculated for each variable and its respective subdivisions. This information, aggregated and summarized by cantones, is now in a flexible form and can easily be used in preparing subfiles at the municipal level, or it can be aggregated according to various other criteria, such as the areas covered by health posts, agricultural extension agencies, or interpreted as national averages or percentages. It can also be used to provide characteristics of cantones with particular features such as a high level of unemployment, a low level of schooling, etc.

### Field surveys

Any information considered essential for the project, but not available

Table 2. Population/region classification

| Region | Subregion | Area | Population | Density |
|---|---|---|---|---|
| | | *Km²* | | *Pop /km²* |
| Urban | Urban [1] | | 1 154 590 | -- |
| Coffee | Western | 1 417 | 304 679 | 212 |
| | Central | 681 | 124 065 | 182 |
| | Eastern | 408 | 104 975 | 257 |
| Total | | 2 526 | 533 719 | 211 |
| Intensive agricultural exploitation | Central | 337 | 137 903 | 409 |
| | Coastal | 3 605 | 497 905 | 138 |
| Total | | 3 942 | 635 808 | 161 |
| Marginal agricultural exploitation | Northern | 6 560 | 745 388 | 114 |
| | Central | 4 474 | 719 077 | 161 |
| | Coastal | 3 531 | 436 798 | 124 |
| Total | | 14 565 | 1 901 263 | 130 |

[1] All cantones and municipal capitals with a population of 10 000 inhabitants or more.

[3] El Salvador is administratively divided into 14 departamentos, 261 municipios and 2 057 cantones

from existing studies, was gathered through field surveys. Three types of studies were conducted: evaluation of nutritional status, socio-economic evaluation of families, and ethnographic descriptive studies of community life.

*Evaluation of nutritional status*

The purpose of this part of the research was to assess the nutritional status of infants and preschool children. This was necessary to identify and establish the extent of potentially important differences in nutritional status between regions, and between categories of population within each region. A sample of 6 409 children of both sexes, aged 6 to 59 months, was taken from 148 communities distributed among all regions. Measurements of weight, height and arm circumference were obtained by means of household visits. The urban sample included only children living in the slums of San Salvador. Anthropometric measurements were also collected from a national sample of 787 children aged 6 to 59 months included in a national survey on vitamin A status conducted by another INCAP group in 1976. This information was then compared with standards for normal children in developed countries, and the results were analysed at the national, regional and, in some cases, subregional level.

*Socio-economic evaluation of family units*

Socio-economic data on family composition, occupation, education, migration, various indicators of wealth and income, and agricultural production were collected from two groups of the population to identify the family characteristics associated with malnutrition in each region, related factors common to all regions, and to provide quantitative support to the descriptive community studies. A total of 625 "low-risk/well-nourished" families and 625 "high-risk/malnourished" families (having at least one child under 75 percent adequacy of weight for age) were selected from the nutritional status survey in the four regions for comparison purposes.

*Descriptive studies on community life*

The rationale for these studies was to obtain a better understanding of the complex interrelationships between social, cultural and economic factors, and those processes related to nutrition and health.

Four communities, each considered to be representative of one of the regions, were selected based on demographic, geographic and economic criteria. The ethnographers lived in each community for six to eight weeks practising the classical methods of anthropological research - participant observation and open interviews. Data were gathered according to a field guide especially designed for this study after consultation with the advisory committee. Subsequently other communities in the same region were visited in order to assess the general applicability of the observations. Finally, field notes were organized, analysed, interpreted and presented in separate reports according to the subheading listed in Table 3.

TABLE 3. REPORTS ON COMMUNITY LIFE

| |
|---|
| SUBSISTENCE PATTERNS |
| — Economic activities and land tenure practices |
| — Community economics and services |
| — Household economics |
| FOOD AND NUTRITION |
| — Food processing and storage |
| — Diet and eating habits |
| — Infants' and children's diets |
| — Beliefs and attitudes related to food |
| HEALTH AND HYGIENE |
| — Environmental sanitation |
| — Personal hygiene of mother and children |
| — Use of available medical facilities |
| — Associated beliefs and practices |
| CHILD BEARING AND CHILD REARING |
| — Male-female relations and reproduction |
| — Pregnancy and child bearing |
| — Child-rearing practices |
| COMMUNICATION, EDUCATION, CHANGE AND INNOVATION |
| — Communication |
| — Change and innovation |
| — Education and related opportunities |

DATA ANALYSIS

The process of data analysis was aimed mainly at answering the questions listed in Table 1. The first two, on regional differences and nutritional status, utilized the anthropometric and 1976 population census data. Characterization of the regions in socio-economic terms was based mainly on the information at the cantón and/or municipal level, through the reanalysis of the population, housing and agricultural censuses of 1971.

Information on the nutritional status of children from different family categories (questions 3, 4 and 5) was based on data obtained from the socio-economic and anthropometric surveys. The nutritional status of children from different types of families was estimated, and the total size of these categories quantified using the population censuses of 1971 and 1976.

The issues dealing with the type, relevance and costs of nutritional interventions (questions 6, 7 and 8) required the integration of all data collected. For example, the data allowed an analysis of the effect and response of specific subgroups or populations in a given region, e.g., the response of landless farmers in the subsistence region to programmes of increased land availability and production, minimum salaries, health services, education, and community oganization.

Finally, in relation to nutritional surveillance (question 9), the regional anthropometric studies developed as a requirement of this project were useful in validating the nutritional data on children gathered in health posts by government officials. This information, routinely collected by the health services, consists of a basic medical diagnosis of malnutrition and information on weight, as recorded at the first annual visit to the clinic, in selected areas, of all children under five years of age. As a result of this validation of health-post data, the government, using its own informa-

tion system, began implementing a nutrition monitoring surveillance programme in 1977. Other indicators, once refined and validated, will no doubt be incorporated in future years.

## Future improvements

Although many useful lessons were learned from this first attempt to develop a methodology, a critical review of the work in El Salvador identifies aspects that need improvement in the future elaboration of functional classification. Three of these are discussed below.

### Organizational procedures

The study showed that community studies and family profiles could be carried out in a period of about 10 to 12 weeks, depending on the size and diversity of the community and the specific nature of the problem. Ideally, the human resource inputs should be trained anthropologists. However, if an appropriate and detailed field guide-line is available, mature personnel with sound foundations in the social sciences will suffice. Furthermore, field work of this nature should be preceded by an exhaustive review of the literature, both with respect to nutritional problems and to the general cultural environment where the research is to take place. It is also especially important that this type of research be carefully supervised by professionals with training in social or cultural anthropology, and that all members of the team are aware of the purpose of the functional classification approach.

### Types of data to be collected

The most efficient strategy to be used in this type of project is clearly that of anthropometric studies. However, the sample size in the regions was larger than necessary. Information on 700 to 800 children, derived from 20 to 25 sites chosen randomly in each region, would have provided reliable information on the extent of malnourished children in distinct regions. Although desirable, data regarding other nutritional deficiencies in groups other than children (e.g., iron-deficiency anaemia in pregnant women) would have increased the cost considerably.

The role of the socio-economic survey at family level is of key importance for the whole exercise, and a detailed definition of variables to be used should be decided upon and defined early in the project. All families chosen for anthropometric studies should be included in the socio-economic survey, since in countries where census data are not available or are unreliable, the representativeness of the socio-economic data is of crucial importance for the project.

Descriptive studies of community life have proved to be useful tools to differentiate problems in distinct regions and to identify relevant measures to reduce them.

Observational studies of typical family profiles, not included in this study, along with community descriptions, would have provided further insights into the nature of the problem. These will be useful in future to improve the identification process of relevant measures for specific sets of families as well as the potential response to interventions. In fact, family profiles are essential in a functional classification approach since, in conjunction with the community descriptions, they help to answer questions of the following nature

- In what ways does the family financial situation need to be changed to reduce substantially its risk of malnutrition?
- In what manner might the food habits of a family be changed in order to reduce its risk of malnutrition?
- What forms of social action might achieve the necessary changes?
- To what extent might the overall problem be solved in this way?

### Sequence of operations

Each stage must, in such a complicated series of studies, be carefully planned and phased, and activities

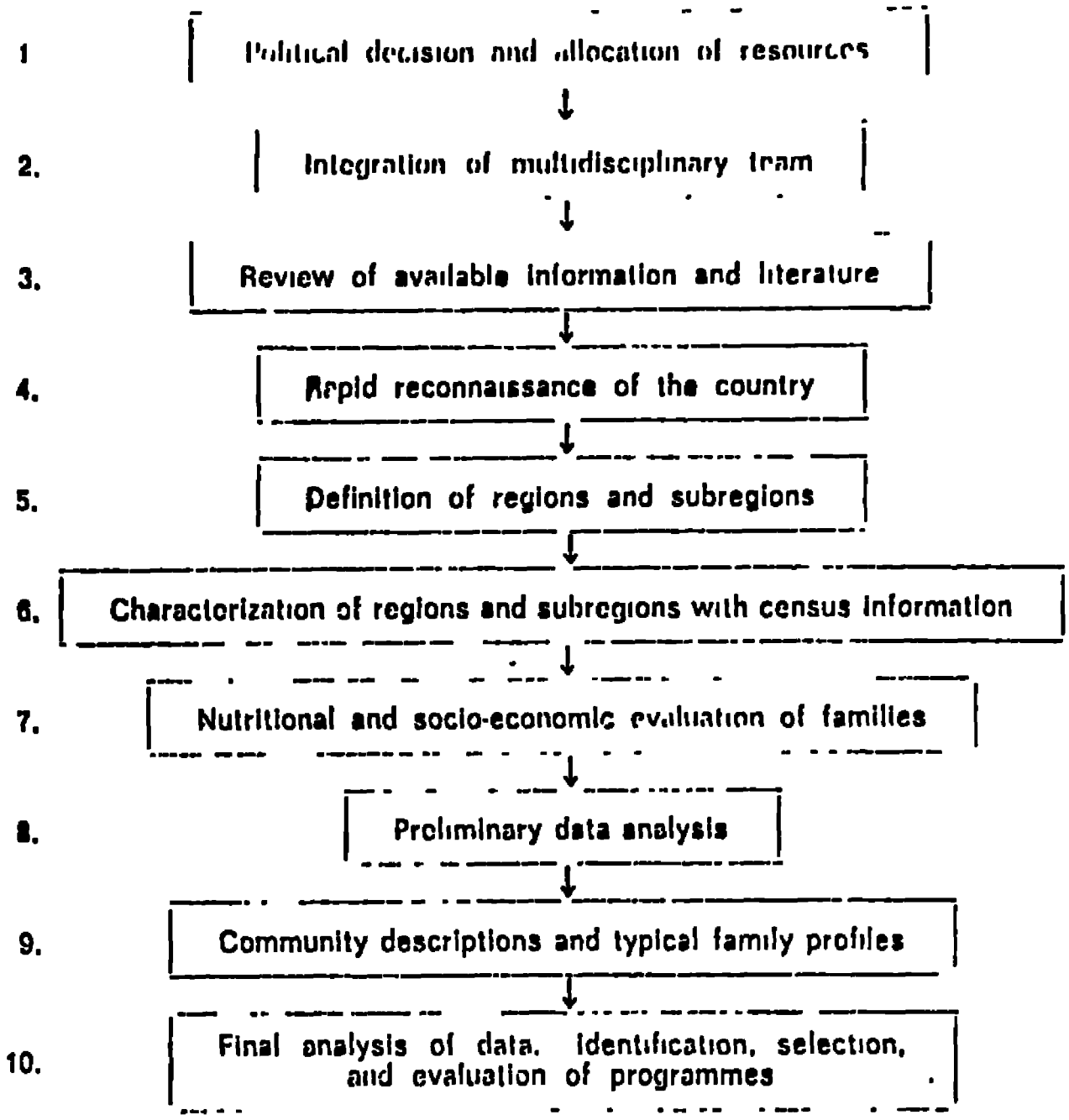



*Figure 2.* Suggested sequence of steps in the elaboration of functional classification

periodically evaluated. An idealized sequence of steps toward elaborating a functional classification is graphically summarized in Figure 2.

Although a functional classification can be used to motivate politicians to allocate resources, it should be preceded by political decisions and resource allocations to combat malnutrition. If this is not the case, elaboration of the classification may simply end up as an academic exercise.

The second step is to establish a group to be directly in charge of the project. Ideally, this should include those who will eventually make use of the information generated.

The third step consists of an extensive review of the existing data relevant to food and nutrition planning, as well as information on the geographic, demographic, social, economic, agricultural and other characteristics of the country. Decisions should be made in the early stages of the project regarding the desirable level of disaggregation of the data for operational purposes. Data files at the chosen level should be established immediately.

The fourth step, critical to the whole process, consists of a series of visits throughout the country to obtain an intuitive understanding of the nature of the problem. Such visits should include informal conversations with a cross-section of local residents regarding what problems they perceive as the most pressing. Also valuable is direct observation of activities related to food production, processing and preparation.

These visits are of great help in the preparation of data-collection hardware and in anticipating potential problems in sampling procedures.

The fifth step is to divide the country into suitable regions. The group should review the pros and cons of using existing regional divisions of the country or of defining new ones. Whatever the final decision, administrative boundaries for each region should be explicitly defined.

The sixth step is the characterization of regions and subregions in general socio-economic terms. This should be done on the basis of all the information collected by the project hitherto, plus census material. It is essential at this point to discuss the census data files with statisticians and computer programmers, since aggregating or disaggregating the data, and their transfer to a different computer system, for example, and subsequent analysis can be extremely expensive. Such discussions should ensure that the resulting data file is flexible enough for various subsequent analyses. A check on the homogeneity of the regions by socio-economic variables may lead to a reassessment of the initial regional breakdown. Mapping of these variables will indicate the adequacy of the initial regional division.

Thus, the regions with population data will serve as sample frames for the evaluation of nutritional status, socio-economic characteristics of the families, descriptive studies and any other relevant information to be collected.

Once sample frames are defined, one can proceed to the seventh step, that of nutritional and socio-economic evaluations. The ethnographers should join the anthropometry field teams visiting the communities to begin identifying, in regions and subregions, the communities that fulfil the requirements for the descriptive studies on community life and family profiles. These visits will also provide an excellent opportunity for ethnographers to obtain general information on the regions as a whole, and initiate the collection of notes on community life.

As the eighth step, it is desirable that, before starting descriptive studies at community level, a first attempt be made to analyse the data. Categories of population by region, their size, prevalence of malnutrition and health problems, associated factors, etc., should begin to be identified. This will orient the selection of types of families to be included in profile studies, and focus the latter on specific problems of distinct categories of population. However, the ethnographer should be careful not to allow these preliminary analyses to influence or bias his observations.

Community description and the family profiles follow as the ninth step.

The tenth step consists of classifying categories of population by regions and subregions, estimating their size, and identifying and evaluating possible programmes.

| *Types of data* | *Steps* |
|---|---|
| 1. Agricultural census<br>2. Administrative division of the country<br>3. Current regional division | → Regional division of the country |
| 1. Population, housing and agricultural censuses<br>2. Socio-economic survey<br>3. Community descriptions | → General characteristics of the regions |
| 1. Socio-economic family surveys<br>2. Population census<br>3. Family profiles | → Definition of categories of the population, and their size |
| 1. Descriptive studies on community life and typical profiles<br>2. Socio-economic surveys<br>3. Census information | → Identification and selection of programmes for potential beneficiaries |

*Figure 3.* Types of data for different steps of functional classification approaches

Finally, the selected interventions can be integrated in an overall plan for 'food and nutrition", "reduction of poverty", "improvement of living conditions", "rural development" or any other desirable title.

The potential contribution of the different data sources in the integration exercise is illustrated, as an example, in Figure 3.

## Conclusion

The El Salvador study confirms the authors' initial concern about the inherent difficulties involved in identifying categories of deprived groups in a population. Nevertheless, based on this study and other work by Valverde *et al.* (1977) and Rawson and Valverde (1976), they believe that methodological problems in adopting a functional classification approach can be overcome.

The elaboration of a functional classification enables the causality of malnutrition to be better understood. However, it does not necessarily identify new and miraculous programmes differing materially from those usually implemented in developing countries New definitive or packaged answers should not therefore be expected from this approach There will always be a need for continuous review of results and discussions with planners and administrators.

A functional classification approach is not only valuable because of the type of information it provides for programme design, but also because during its elaboration it raises fundamental questions on the development process of the country. It also shows how programmes not primarily conceived to reduce malnutrition can play an important role in nutrition improvement A review of programmes in the light of a functional classification and the final selection of the best alternatives can be expected to lead to the appraisal of the political and economic feasibility of proposed measures

The activities carried out in El Salvador cost approximately US$100 000 The costs of a new exercise in another country of similar size could be significantly reduced The cost of activities, beyond this first phase, cannot be estimated at present Obviously, the cost and outputs will need to be compared with traditional methods of evaluating nutrition status in order to determine the real value of functional classification.

The El Salvador experience also shows that the marginal extra cost of studying the total framework, instead of focusing the effort of data collection on critical categories of malnourished families, is small compared with the total cost of setting up the evaluation machinery. Furthermore, if a serious attempt is made to clearly define the nutrition problem, then the process of elaborating a functional classification should be considered as a permanent ongoing process and not as a once-and-for-all venture

The major findings of this Project have been communicated to the Government of El Salvador. At the present time, the Ministry of Planning is defining the National Food and Nutritional Policy for the country Based on the national policy, the specific activities to be included in the Food and Nutrition Plan will be defined The data from the Project are being utilized by the Ministry of Planning to determine the type and geographical location of programmes to be undertaken.

Village road making in El Salvador
A rural development intervention

## References


Abercrombie, K C. Who are they, where are they? *Ceres*, 45: 49-51.
1975

FAO. *Food and nutrition planning*. Rome. 105 p. Nutrition Consultants Report Series No. 35.
1975

Hakim, P. & Solimano, G. Nutrition and national development establishing the connection. *Food Policy*, 1(3): 249-259.
1976

Institute of Nutrition of Central America and Panama and Office for International Research, National Institutes of Health. *Nutritional evaluation of the population of Central America and Panama. Regional summary* Washington, D C., U S. Department of Health, Education and Welfare. 165 p. DHEW Publication No. (HSM) 72 8120
1972

Instituto de Nutrición de Centro América y Panamá/Unidad de análisis del sector salud *Análisis preliminar de la situación de salud en Nicaragua. Evaluación del componente nutricional dentro del sector salud*. Guatemala. 70 p.
1976

Joy, J L. Food and nutrition planning. *J. agric. Econ*., 24: 165-192
1973

Joy, J L., & Payne P.R. Nutrition and national development planning *Fd Nutr. (FAO)*. 1(4): 2-10.
1975

Payne, P.R Nutrition planning and food policy. *Food Policy*, 1(2): 107-115.
1976

Rawson, I G. & Valverde, V. The etiology of malnutrition among preschool children in rural Costa Rica. *J. trop. Pediat*. 22: 12-17
1976

Sistema de Análisis y Planificación de la Alimentación y Nutrición *Evaluación de las áreas prioritarias del problema nutricional de Honduras y sus posibles soluciones*. Tegucigalpa, D C, Honduras, C A. 495 p.
1976

Valverde, V *et al* Relationship between family land availability and nutritional status *Lond Fd Nutr.*, 6: 1-16.
1977

*Journal of Pediatric Gastroenterology and Nutrition*
6:387–391 © 1987 Raven Press, New York

# Effects of Nutritional Recuperation on E-Rosetting Lymphocytes and in Vitro Response to Thymosin in Malnourished Children

Jose R. Cruz, Francisco Chew, Raul A. Fernandez, Benjamin Torun, *Allan L. Goldstein, and †Gerald T. Keusch

*Division of Nutrition and Health, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala; *Department of Biochemistry, George Washington University School of Medicine and Health Sciences, Washington, D.C.; and †Division of Geographic Medicine, New England Medical Center and Tufts University School of Medicine, Boston, Massachusetts, U.S.A.*

Summary: The percentage of peripheral blood lymphocytes forming rosettes with sheep erythrocytes (E-rosettes) was determined at admission to the INCAP Clinical Center in eight acutely malnourished Guatemalan children, and again after 14 and 28–30 days of nutritional therapy. While the mean percentage of E-rosettes increased during therapy, the change (from 35.6 ± 10% to 43.3 ± 19%) did not reach statistical significance because of the variable response of different subjects. At each time period, however, in vitro incubation with the thymic factor, thymosin fraction 5, significantly increased the percentage of E-rosetting lymphocytes. The presence of thymosin responsive cells in circulation after 1 month of optimal nutritional support indicates that immature T-lymphocytes can persist in circulation in patients with severe malnutrition, even after clinical improvement. Thus, neither the percentage of E-rosettes in peripheral blood nor their response to in vitro incubation with thymosin correlated with anthropometric measures of nutritional status in individual patients. This suggests that other nutritional or nonnutritional factors may be important modulating influences on T-lymphocytes, and that prospective studies with thymic factor administration are warranted. Key Words: Peripheral blood lymphocytes—E-Rosettes—Thymosin—Malnourishment.

Defective cell-mediated immune (CMI) responses are frequently encountered in children with protein–energy malnutrition (PEM) (1–6). Because of the central role of the thymus in the development of T-lymphocytes involved in CMI responses (7) and the presence of thymic atrophy and decreased numbers and percentage of mature circulating T-cells that accompany PEM (1–6,8,9), it has been postulated that replacement with thymic factors may improve immune function in these patients (6). In the accompanying paper (10), we have shown that some but not all PEM patients of similar nutritional status, as defined by anthropometric and clinical asssessment, have subnormal proportions of mature T-lymphocytes in peripheral blood. In vitro exposure of peripheral blood lymphocytes from these subjects to a thymic peptide fraction, thymosin fraction 5 (f-5), increases the proportion of E-rosetting T-cells, suggesting that patients who demonstrate such a response in vitro have increased numbers of immature circulating pre-T-lymphocytes in vivo.

In this paper, we report the effects of nutritional therapy on the proportion of E-rosetting lymphocytes in circulation and the lymphocyte response in vitro to f-5. These data indicate that despite progressive improvement in nutritional status during 1 month of intensive nutritional support, the change in percentage of E-rosetting T-cells is variable in individual patients. More important, because cells responsive in vitro to f-5 remain in circulation, con-

Address correspondence and reprint requests to Dr. J. R. Cruz at Division of Nutrition and Health, Institute of Nutrition of Central America and Panama INCAP, P.O. Box 1188, Guatemala City, Guatemala.

tinued thymic dysfunction may be present in these patients.

## MATERIALS AND METHODS

Eight children, five boys and three girls aged 14–38 months, were admitted to INCAP's clinical center with severe edematous PEM by clinical, anthropometric, and biochemical criteria (Table 1). The research protocol was approved by the Committee on Human Rights at INCAP, and informed parental consent was obtained. Ten milliliters of peripheral blood was obtained in preservative-free heparin (20 U/ml) within 24 h of admission and on days 14 and 28–30 of hospitalization. Peripheral blood mononuclear cells were isolated and exposed to a final concentration of 20 μg/ml of the partially purified bovine thymic hormone, thymosin fraction 5 (f-5, lot number BPP-100, a gift of Hoffmann La-Roche, Nutley, NJ) in Hanks' balanced salt solution (HBSS) or HBSS alone, as described in the accompanying paper (10). Following incubation, T-lymphocytes were identified by the E-rosetting technique, and the number of nonrosetting, small-rosetting (1–2 firmly attached sheep erythrocytes), and E-rosetting lymphocytes (3 or more firmly attached sheep erythrocytes) were enumerated in a 200 cell count (10). Based on the variability in measurement of E-rosettes in our laboratory (mean, 54.62; SD, 4.2%; SD/mean, 0.077), we have arbitrarily designated a change equal to or greater than ± 1.5 SD (6.3%) as a response to either nutritional treatment or in vitro exposure to f-5.

Nutritional therapy consisted of a gradual increase in diet to 150 calories and 4 g protein/kg body weight/day over a 1-week period. Vitamin A (100,000 IU) was given orally on day 1. Daily supplementation with vitamins and trace minerals (including 60-mg elemental iron as $FeSO_4$) to meet requirements was started on day 8.

The results were examined statistically by analysis of variance.

TABLE 1. *Anthropometric and biochemical data*

| | Admission | Day 14 | Day 28–30 |
|---|---|---|---|
| Weight-for-height (% of standard)[a] | 70.0 ± 7[b] | 77.0 ± 6 | 87.0 ± 11 |
| Plasma protein (g/dl) | 4.3 ± 0.4 | 6.1 ± 1.1 | 7.3 ± 1.2 |
| Serum albumin (g/dl) | 1.8 ± 0.7 | 2.8 ± 0.7 | 3.6 ± 0.8 |
| Hemoglobin (g/dl) | 8.8 ± 1.2 | 9.0 ± 1.5 | 9.4 ± 0.9 |

[a] Based on "dry weight" calculated after disappearance of edema compared to 50th percentile of NCHS standards.
[b] Mean ± SD.

## RESULTS

### Clinical Evaluation

Table 1 summarizes the patient characteristics in the eight patients on admission. Nutritional therapy resulted in marked improvement in weight-for-height after 4 weeks in all but one child.

### E-Rosettes

The mean percentage of E-rosetting T-lymphocytes in the first blood sample was 35.6 ± 10% (Fig. 1). Although this value increased during the 4 weeks of observation, the change was not statistically significant because of variable responses of individuals ($F = 0.2868$, $p = 0.76$). By 4 weeks of nutritional support, the mean of 43.3 ± 19% was still below the value obtained in healthy children with mild growth retardation from the same population (10). The reciprocal change in the percentage of nonrosetting cells, and the minor decrease in small rosettes with time, did not reach statistical significance ($F = 0.0457$ and $0.2554$; $p = 0.96$ and $0.78$, respectively).

### Effects of f-5

In vitro treatment of lymphocytes obtained at admission with f-5 resulted in a mean increase of 8.0 ± 4.7% in the proportion of lymphocytes forming E-rosettes (Fig. 1; $F = 48.6$, $p < 0.0001$). An increase of similar magnitude was observed in the follow-up samples at 2 and 4 weeks of nutritional therapy. This increase in E-rosettes was entirely at the expense of nonrosetting cells ($F = 49.9$, $p < 0.0001$). A significant increase in response to f-5 as defined in this study (≥6.3%) was observed in 14/20 samples in which the baseline value in the absence of f-5 was less than the mean value observed in clinically well Guatemalan children [mean E-rosette values of 35% (20 samples) versus 52% (41 samples), respectively] (10). This can be compared to 1/4 responders among those with higher baseline percentage of E-rosettes (mean, 61.9%). The response to f-5 was independent of time ($F = 0.0407$, $p = 0.06$ for E-rosettes and $F = 0.0523$, $p = 0.95$ for nonrosetting lymphocytes).

### Nutritional Status and E-Rosettes

Although nutritional status and the proportion of E-rosettes increased over the 4 weeks of observa-

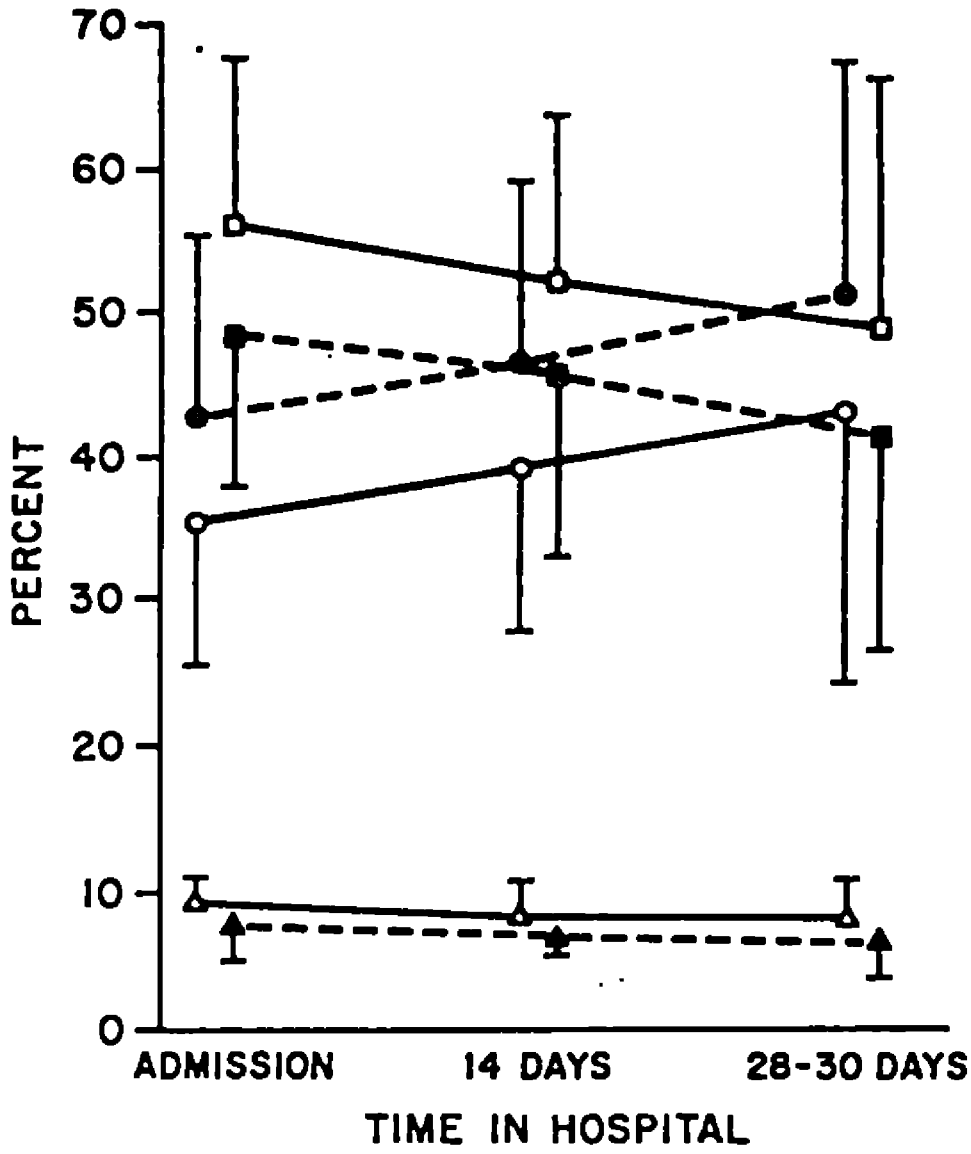



FIG. 1. Mean percentage (% 1 SD) of E-rosettes (circles), small rosettes (triangles), and nonrosetting lymphocytes (squares) among peripheral blood mononuclear cells in eight patients with protein-energy malnutrition. The open symbols represent assays in the absence of thymosin fraction 5, and the closed symbols are the results of assays following incubation in the presence of thymosin (20 μg/ml).

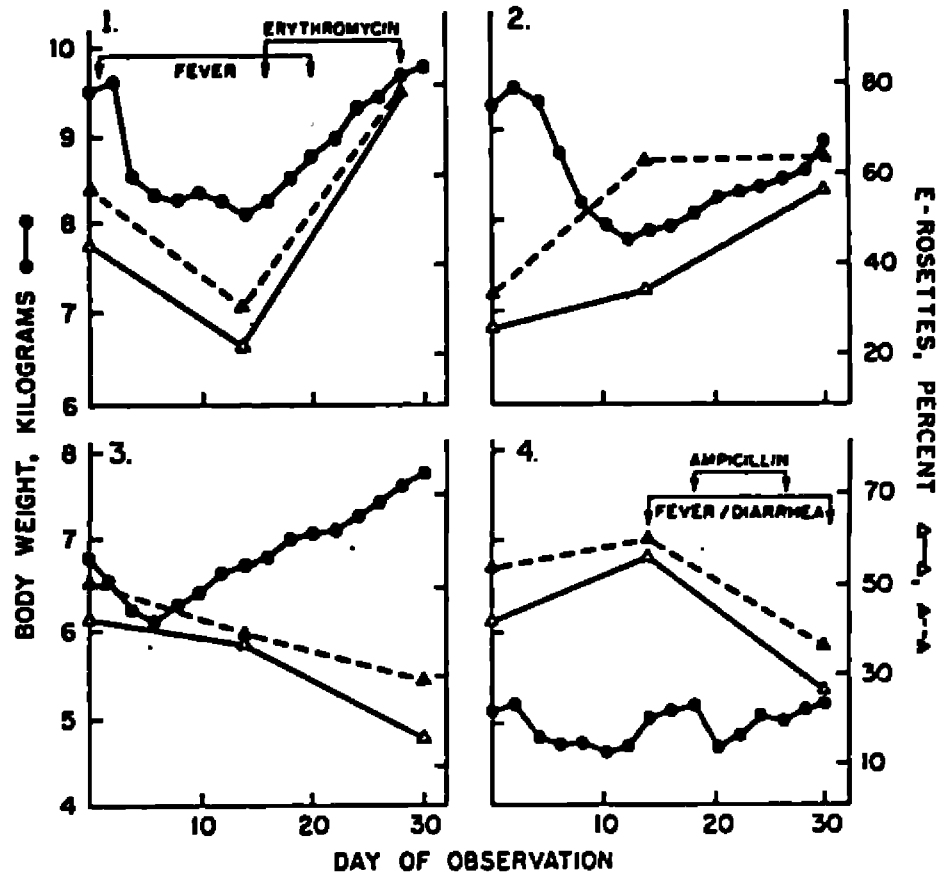



FIG. 2. Weight curve (closed circles) and the percentage of E-rosettes in the absence (open triangles) and presence (closed triangles) of 20 μg/ml of thymosin fraction 5 in four children with acute protein–energy malnutrition. Cases 563 and 567 were clinically uncomplicated and showed a good nutritional response (weight-for-height 87 and 91% of standard by 4 weeks). Percentage of E-rosettes improved in case 567 but steadily diminished in case 563. Cases 562 and 569 experienced febrile illness during the course of observation, with an associated fall in percentage of E-rosettes. In the former patient, weight-for-height failed to improve by day 30 of treatment (increase in weight-for-height from 56 to 63%) and E-rosettes dropped to 27%. In the latter child, percentage of E-rosettes improved when antibiotic treatment was initiated and was associated with improvement in nutritional status (weight-for-height, 94% by day 30).

tion in the group as a whole (Table I and Fig. 1), the response in individual patients was variable (Fig. 2). In some, clinical, nutritional, and immunological status changed in parallel; in others, there was no obvious relationship. Four cases are illustrated.

### *Case 1 (PC 569)*

A 34-month-old female had an unexplained fever for the first 3 weeks of the study and concomitant decrease in E-rosettes from 45 to 22%. When erythromycin was given, she defervesced and rapid catch-up growth began. By day 28 the percentage of E-rosettes had increased to 80%. Weight-for-height did not change from admission to day 14 (77–81% of standard), but then reached 94% of standard by day 30.

### *Case 2 (PC 567)*

A 20-month-old male had an uncomplicated clinical recovery and steady increase in percentage of E-rosettes to normal (56%) as weight-for-height reached 91% of standard. The effect of f-5 on day 14 was striking, increasing percent E-rosettes from 34 to 64%.

### *Case 3 (PC 563)*

An 18-month-old female had an uncomplicated clinical recovery, and an increase in weight-for-height from the initial value of 65–87% of standard. Nevertheless, the percentage of E-rosettes steadily decreased from 43% on admission to 36% at day 14 to 17% by day 30.

### *Case 4 (PC 562)*

A 14-month-old male had unexplained anorexia and little improvement in weight-for-height during the first 2 weeks (from 56 to only 63% of standard), but a normalization of the percentage of E-rosettes

from 42 to 57%. Fever developed on day 14. Ampicillin was begun on day 18, with defervescence, however, diarrhea began on day 20. While there was no further change in weight-for-height during this period (64% of standard at day 30), the percentage of E-rosettes decreased to 27%.

## DISCUSSION

The results of the present study show that despite clinically adequate nutritional recuperation 1 month after hospitalization, PEM patients may still have subnormal proportions of E-rosetting lymphocytes in their blood. For comparison, we have used data obtained in our laboratory from other Guatemalan children who are mildly growth retarded but clinically healthy (mean value, 52%) (10) and other data reported in the literature from Africa and Bangladesh (49.9 and 53.7%, respectively) (11,12). As it is likely that the majority of these children are at least marginally malnourished as well, these figures represent a minimum estimate of the true normal proportion of E-rosetting cells in this age group.

As we and others have noted before, in vitro incubation with thymic factors results in an increase in the proportion of E-rosetting cells in subjects with initially low values (10,13,14). Of interest in the present study, a similar magnitude of response to f-5 in vitro was found after 2 weeks and 1 month of optimal nutritional rehabilitation. This indicates that despite clinically satisfactory nutritional recuperation, f-5 responsive and presumably immature cells may still be found in the circulation. Indeed, this response to f-5 may be a useful functional indicator of recovery of the immune system with nutritional treatment for severe PEM. By 4 weeks of nutritional therapy, the mean proportion of E-rosetting cells in vitro in the presence of f-5 reached the value observed in vivo in mildly malnourished controls (10). The failure to accomplish this degree of in vitro restoration earlier in the course of treatment suggests that there may be subpopulations of cells responsive to different thymic hormones and/or a prethymic lymphocyte defect in PEM, resulting in a deficit of cells able to respond to the thymic hormone signal.

Thus, in contrast to other data (4), our results show that clinically acceptable nutritional recuperation does not guarantee concomitant reversal of the T-lymphocyte abnormalities associated with PEM. Therefore, therapeutic measures are still needed to accelerate the improvement in immunocompetence. Studies in patients with primary immunodeficiencies have led to the concept that the degree of responsiveness of T-lymphocytes to in vitro treatment with thymosin represents the level of thymic factor-associated CMI deficiency in the patient (13,14). Because of the in vitro effects of f-5 on E-rosettes in PEM patients and because in vivo administration of thymic factors to some immunosuppressed patients can improve immune function (13,14), administration of f-5 to malnourished children may represent one way to accomplish more rapid restoration of CMI functions. Because it is possible that subpopulations of T-lymphocytes could respond to different thymic factors, it may be necessary to administer various thymic factors together for maximum improvement. If successful, however, this therapy might reduce both morbidity and mortality from infection during the clinically vulnerable initial period of nutritional therapy (15,16) until full recovery of the T-lymphocyte system can occur

Acknowledgment: Supported in part by grant HD-11844 from the National Institute of Child Health and Human Development, NIH, Bethesda, MD, and a grant in geographic medicine from the Rockefeller Foundation, New York, NY.

## REFERENCES

1 Schopfer K, Douglas SD. *In vitro* studies of lymphocytes from children with kwashiorkor. *Clin Immunol Immunopathol* 1976,5 21–30
2. Chandra RK. Lymphocyte subpopulations in human malnutrition. cytotoxic and suppressor cells. *Pediatrics* 1977,59 423–7.
3 Keusch GT. Nutrition as a determinant of host response to infection and the metabolic sequellae of infectious diseases *Sem Infect Dis* 1979;2 265–303.
4. Chandra RK. Cell-mediated immunity in nutritional imbalance. *Fed Proc* 1980,39 3088–92
5. Good RA, Hanson LA, Edelman R Infections and undernutrition *Nutr Rev* 1982,40 119–28
6. Keusch GT, Wilson CS, Waksal SD Nutrition, host defenses, and the lymphoid system In: Gallin JI, Fauci AS, eds. *Advances in host defense mechanisms Vol 2.* New York. Raven Press, 1983 275–359.
7 Trainin N Thymic hormones and the immune response *Physiol Rev* 1974 54 272–315
8 Mugerwa JW The lymphoreticular system in kwashiorkor *J Pathol* 1971,105.105–9.
9. Smythe PM, Schonland M, Brereton-Stiles GG, Coovadia HM, Grace HJ, Loening WEK, Mafoyane A, Parent MA, Vos GH Thymolymphatic deficiency and depression of cell-mediated immunity in protein-calorie malnutrition *Lancet* 1971,ii 939–43
10 Keusch GT, Urrutia JJ, Torun B, Cruz JR, Smith H, Flores

R, Goldstein AL. Immature circulating lymphocytes in acutely malnourished Guatemalan infants. *J Pediatr Gastroenterol Nutr* 1987.6(2).
11. Jackson TM, Zaman SN. The *in vitro* effect of thymic factor, thymopoietin, on a subpopulation of lymphocytes from severely malnourished children. *Clin Exp Immunol* 1980;39: 717–21.
12. Olusi SO, Thurman GB, Goldstein AL. Effect of thymosin on T lymphocyte rosette formation in children with kwashiorkor. *Clin Immunol Immunopathol* 1980,15:687–91.
13. Goldstein AL, Cohen GH, Rossio JL, Thurman GR, Brown CN, Ulrich JT. Use of thymosin in the treatment of primary immunodeficiency diseases and cancer. *Med Clin N Am* 1976,60:591–606.
14. Barret DJ, Wara DW, Ammann AJ, Cowan MJ. Thymosin therapy in the DiGeorge syndrome. *J Pediatr* 1980,97.66–71.
15. Purtillo DT, Connor DJ. Fatal infections in protein-calorie malnourished children with thymolymphatic atrophy. *Arch Dis Child* 1975,50.149–52.
16. Brown KH, Gilman RH, Gaffar A, Almagir SM, Strife JL, Kapikian AZ, Sack RB. Infections associated with severe protein-calorie malnutrition in hospitalized infants and children. *Nutr Res* 1981,1:33–46.

# The effect of dietary lactose on the early recovery from protein-energy malnutrition. I. Clinical and anthropometric indices[1–5]

*Noel W Solomons,[6] MD, Benjamin Torun,[7] MD, PhD, Benjamin Caballero,[8] MD, Samuel Flores-Huerta,[8] MD, and Guido Orozco,[9] MD*

ABSTRACT To assess the advisability of using lactose-containing formulas in the rehabilitation of severely malnourished children, indices of clinical recovery, growth and restoration of body proteins and gastrointestinal function were measured longitudinally during the initial 45 days of hospitalization in 20 male, preschool children with kwashiorkor and marasmic-kwashiorkor. All patients received a diet based on cows' milk, but half were allocated to a formula pretreated with β-galactosidase to hydrolyze the lactose, while the others received the untreated, intact milk. The groups were identical with respect to clinical criteria on admission. For the final 37 days of the protocol, the subjects received 4 g of protein and 150 kcal of energy per kg per day. More diarrhea was experienced by the intact lactose group during early hospitalization. Overall, recovery was satisfactory in both cohorts, and there were no differences in rates of growth, body protein repletion, restoration of energy reserves nor intestinal functions. In conclusion, the routine reduction of lactose content from a milk-based diet for severe protein-energy malnutrition offers no advantages. *Am J Clin Nutr* 1984,40:591–600.

KEY WORDS Diarrhea, diet therapy, dietary carbohydrate, growth, lactase, lactose intolerance, milk, protein-energy malnutrition

## Introduction

Milk is a source of high quality protein, frequently used for the recuperation of children with protein-energy malnutrition (PEM). Subsidies from the World Food Program, governmental agencies, and some private voluntary organizations have facilitated the distribution of milk for famine relief and for routine programs of inpatient and outpatient treatment of endemic malnutrition. In the extensive 25-yr experience with several hundred children admitted to the Clinical Research Center of the Institute of Nutrition of Central America and Panama (INCAP), milk has been used along with a host of other protein sources as the basis of recovery diets in various forms of PEM. Based on the excellent results obtained (1), cows' milk became the main ingredient in our routine therapeutic diets. However, its suitability for malnourished patients or populations has been questioned, mainly in relation to its carbohydrate, the disaccharide, lactose. Children with severe PEM commonly have a reduced activity of intestinal lactase, the mucosal enzyme responsible for the digestion of lactose (2–5), and it has been

[1] From the Division of Human Nutrition and Biology, Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala; and the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139.

[2] Supported in part by grants-in-aid from the National Dairy Council, Rosemont, IL and the SugarLo Company, Pleasantville, NJ.

[3] Presented in part at the IV Symposium on Gastroenterology and Nutrition, Sophia Antipolis, France, November, 1981; at the Congreso Panamericano de la Leche, Buenos Aires, Argentina, April, 1982; and at the Annual Meeting of the American Society for Clinical Nutrition, Washington, DC, May, 1982.

[4] INCAP Publication I-1284.

[5] Address reprint requests to: Dr Benjamin Torun, Division of Nutrition and Health, INCAP, Apartado Postal 1188, Guatemala City, Guatemala.

[6] Associate Professor of Clinical Nutrition, MIT and Visiting Professor/Affiliated Investigator, INCAP

Received August 9, 1982.
Accepted for publication April 17, 1984.

suggested that feeding this disaccharide can retard nutritional recovery (6, 7). Low-lactose or lactose-free milk can be produced using the processes of in vitro hydrolysis (8) or ultrafiltration (9). These procedures add considerably to the cost of milk, but the increased cost might be justified if the absence of lactose in a milk-based diet were shown to accelerate recovery from PEM.

The theoretical objections to milk-feeding in PEM are generally based on two considerations: *1*) that the carbohydrate, lactose, will be poorly absorbed, with consequently decreased effective utilization of the dietary energy; and *2*) that the osmotic and fermentative effects of nonabsorbed carbohydrate in the intestine will produce or exacerbate diarrhea. In relation to the first issue, it should be considered that the appropriate formulation of the recovery diets requires supplementation of native cows' milk with additional energy sources (dextrins, sucrose, vegetable oil) to provide a food that delivers about 4 g protein and 150 to 200 kcal/kg. Lactose would represent only 11 to 16% of total energy in such diets, and recent studies in rats (10) and human volunteers (10, 11) suggest that a large portion of the energy in carbohydrates escaping digestion and absorption in the small bowel can eventually be absorbed from the colon, most probably in the form of volatile fatty acids (12). The second consideration is not easy to assess, since the criteria used in different studies to describe diarrhea are not uniform and frequently do not allow the determination of worsening of the diarrhea that often accompanies the PEM syndrome.

Thus, given an array of problems with the interpretation of previous reports, and given the newer insights into colonic carbohydrate metabolism, we undertook a systematic randomized trial of formulas based on whole cows' milk versus lactose-hydrolyzed milk during 45 days after beginning treatment of 20 Guatemalan children with severe PEM of the edematous type. This paper describes the clinical results and intestinal functions in terms of stool characteristics. An accompanying paper (13) gives detailed information about the absorption of dietary nutrients.

[7] Chief, Division of Nutrition and Health, and Director, Clinical Research Center, INCAP. [8] Fellow, World Hunger Programme, United Nations University/INCAP. [9] Resident physician, Clinical Research Center, INCAP.

## Patients and methods

### *Subjects*

The subjects were all male, Guatemalan preschool children of Maya or ladino (Mayan-Caucasian) descent, between the ages of 15 and 36 months, who were referred to INCAP for treatment of kwashiorkor or marasmic-kwashiorkor. Only 20 of 32 children originally admitted completed the experimental protocol. Of the remainder, five died shortly after hospitalization, and seven had persistent vomiting or infections that required intensive and prolonged treatment and drastically diminished spontaneous oral intake. The study protocol was approved by the Committee on Human Research of INCAP and the Committee on the Use of Humans as Experimental Subjects of MIT. Informed written consent was obtained from the patients' parents upon admission to the Clinical Research Center, after the nature and purposes of the study had been explained.

On admission, a clinical history was obtained from the child's mother, with special attention being given to antecedents of diarrheal episodes, prior administration of antibiotics and "home remedies," recent growth pattern, and concurrent infectious illnesses. All children had edema of the lower (and some of the upper) extremities, brittle and easily pluckable hair, decreased subcutaneous fat, and plasma concentrations of total proteins and albumin below 5.1 and 3.5 g/dl, respectively. The clinical severity of their edema was separately assessed by two pediatricians and classified on a scale of 1 to 3.

Routine care was provided by the full-time staff of nursing aides of the Clinical Research Center. Temperature and pulse rates were monitored every 4 h during the first 3 wk, and every 12 h thereafter. Nurses recorded and reported clinical observations at the end of the three daily shifts. Treatment for infections and other complications was started immediately upon detection after admission. The Center was attended full-time by a resident physician who examined each patient daily, and by three experienced pediatricians. Suspected infectious illnesses were confirmed by clinical examination and microbiological cultures, and antibiotic therapy and other supportive measures were instituted as indicated during the course of recovery.

### *Assignment of dietary regimen*

On the day of admission, each child was assigned to either the intact milk (IM) or the lactose-hydrolyzed milk (HM) group by a process of *stratified, binary-choice allocation*. By this process, we attempted to match the groups as closely as possible with respect to age, clinical severity, degree of edema, *estimated* weight for height deficit corrected for edema, serum protein concentrations, and history of diarrhea immediately before admission. Thus, the first child was assigned by

binary choice (a coin toss) to a given treatment. If a subsequently admitted child was closely similarly to the first child in terms of the aforementioned criteria, he would be assigned to receive the opposite dietary treatment. If a child did not substantially resemble any previously admitted patient, he was once again assigned by coin toss to receive one or the other dietary formula The final goal was to complete 10 children with each of the dietary regimens Thus, when one treatment quota had been filled, the remaining children were all assigned to the other diet This "matching" of patients was employed simply to encourage the final equivalency of the treatment groups, and not for pairing the subjects' data in the final analyses of the results.

*Diets*

Meals were prepared in the metabolic kitchen of the Clinical Research Center. They consisted of a liquid formula providing various amounts of protein and carbohydrate, advanced in a step-wise fashion during the first week of the protocol (Table 1) During the first 2 days in the hospital, the children were fed 0.7 g casein and 70 kcal/kg/day. Thereafter, the protein source was milk from a single lot of recently prepared powdered whole milk (Prolac, Guatemala City). The dry milk contained, per 100 g, 4.7 g residual humidity, 6.2 g ash, 27.4 g protein (15.7% nitrogen), 20.5 g fat, and 41.2 g carbohydrate (calculated by difference). The formulas contained milk, sucrose, soybean oil, and a mineral mixture (14) At full strength, 32% of the carbohydrate calories and 16% of total energy was derived from intact (or hydrolyzed) lactose. Table 1 shows their protein and energy delivery. Additional water was provided ad libitum, and the patients received every day vitamins, minerals, and electrolytes in adequate amounts to satisfy the needs and replenish the stores of malnourished children (14) The total daily ration was divided into five equal servings, fed at 3-h intervals between 8 AM and 8 PM. Thus, from day 8 onward, each meal would deliver 1.2 g lactose per kg of body weight. Dietary intakes were measured by differential weighing of the food containers before and after each feeding.

A commercial, food-grade, β-galactosidase from *Kluyveromyces lactis* (LactAid, SugarLo Company, Pleasantville, NJ) was added to the HM formulas at a dose of 1 drop of LactAid per 2.9 g of milk protein, equivalent to 10 drops per liter of fluid cows' milk, as recommended by the manufacturer for >90% lactose hydrolysis The liquid formula was thoroughly mixed, divided into individual meal servings, and stored in the refrigerator (4 to 6°C) for use 24 to 96 h later. The hydrolytic effect was previously assessed by comparing lactose hydrolysis after adding the enzyme to the complete HM formula, to reconstituted powdered milk alone, and to reconstituted powdered milk combined with the other ingredients of the formula After 24 h at 4 to 6°C, 93.7, 89.3, and 95.3% hydrolyses were achieved, respectively.

*Anthropometric measurements and growth assessments*

The children were weighed each morning, before breakfast, on a triple beam balance (Douglas-Homs Co, Burlingame, CA) On admission and every 7 days, the following indices were measured length; circumferences of the head, mid-arm, and calf; and subcutaneous skinfold thicknesses in the tricipital, subscapular, and paraumbilical regions (Lange calipers, Cambridge, MD) The edema-free weight on admission was calculated from the extrapolation of the lines corresponding to the initial rate of weight-loss due to diuresis of edema fluid and the initial rate of rapid catch-up growth (Fig 1). Total weight gain in 45 days was calculated from this edema-free weight and the mean weight on days 43 to 47. The daily rate of initial, rapid catch-up weight gain was calculated from the slope of the regression line of data points of body weight from the point of deflection after diuresis to the first change in velocity sited from a graphic plot. This linear segment of daily weight increments ranged from 13 to 41 days (25 ± 7, mean ± SD) in various children. Adequacy of weight for height was calculated in relation to the 50th percentile of the Harvard standards (15).

*Intestinal functions*

Each stool was evaluated in terms of physical characteristics, pH (litmus indicator paper) and the semiquantitative estimation of the concentration of reducing substances (Clinitest tablets, Ames Laboratories, Elkhart, IN) (16). Complete daily fecal output was collected and weighed on days: 2 to 5; 12 to 14; 22 to 24; 32 to 34; and 42 to 44. The children in group HM were fed the IM formula on days 46 to 50, and their stools examined to investigate changes after eating a lactose-free diet for 45 days.

Feces were classified as abnormal when they were liquid, semiliquid, or contained mucus, visible fat, or blood. Inhospital diarrhea was defined when any two of the following criteria were met in the same day: *1*)

TABLE 1
Composition of recovery diets and schedule of advancement of protein and energy content*

| Days of treatment | Protein | Energy | Fat | Lactose | Protein source |
|---|---|---|---|---|---|
| | *g/kg/day* | *kcal/kg/day* | *% kcal* | *g/kg/day* | |
| 0–1 | 0.7 | 70 | 30 | | Casein |
| 2–3 | 1 | 100 | 30 | 0.1 or 1.5 | Intact cows' milk or with >90% lactose hydrolysis |
| 4–5 | 2 | 120 | 40 | 0.3 or 3.0 | |
| 6–7 | 3 | 150 | 40 | 0.4 or 4 5 | |
| 8+ | 4 | 150 | 40 | 0.6 or 6.0 | |

* Five daily meals, each with one-fifth of the amounts described. Supplemented with minerals, vitamins, electrolytes and water.

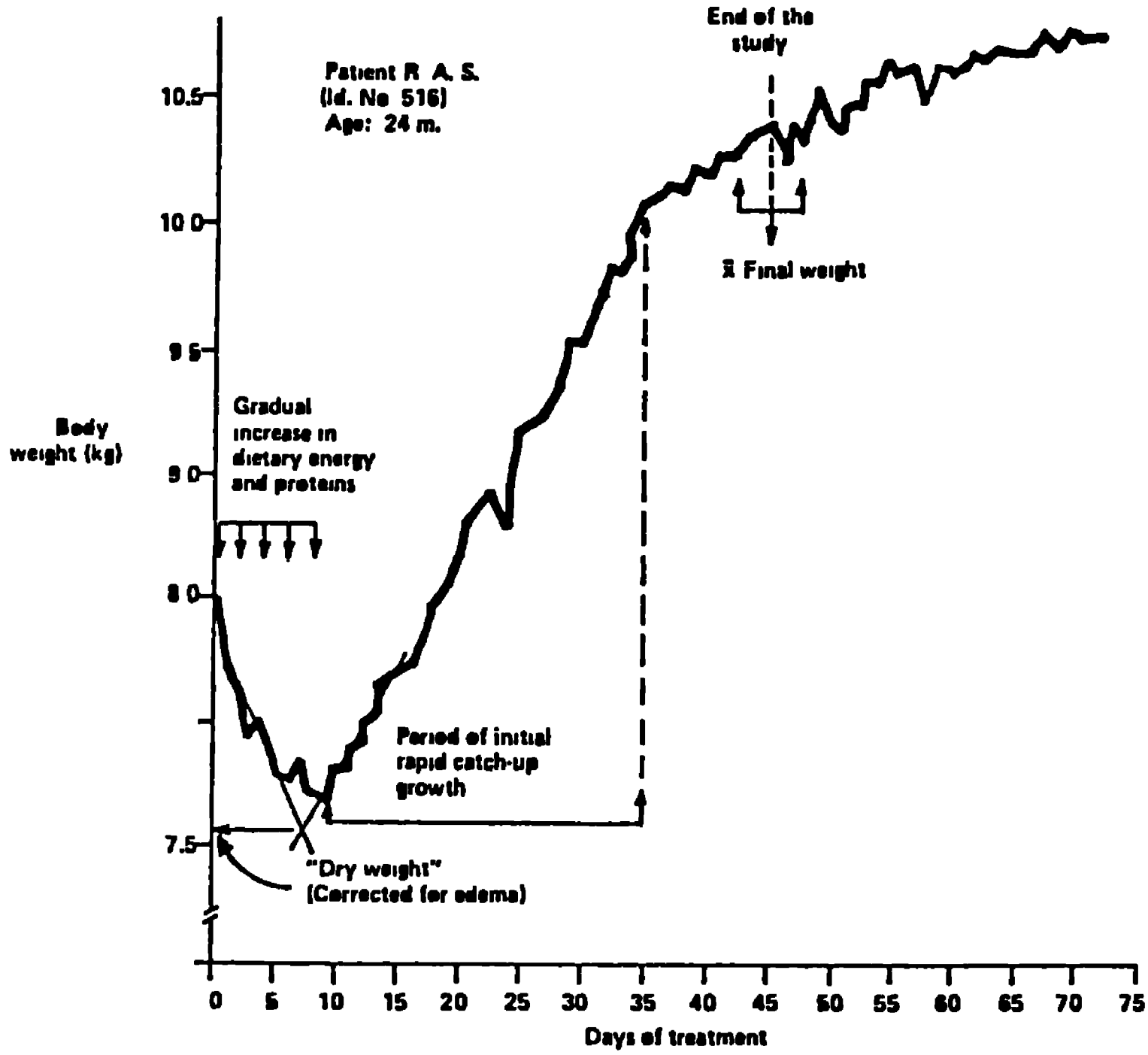



FIG 1. Daily weight chart of a patient treated for edematous protein-energy malnutrition, showing: *1*) the gradual increase in nutrient delivery from 0.7 g protein and 70 kcal to 4 g protein and 150 kcal/kg/day; *2*) the initial weight loss due to loss of edema fluid; *3*) the extrapolations to estimate the patient's weight on admission, corrected for the weight of edema ("dry weight"); *4*) the initial phase of rapid catch-up, which follows a linear function, and the subsequent moderation of weight gain; *5*) the final weight in a 45-day study.

at least half of the stools had abnormal physical characteristics; *2*) more than 150 g of feces excreted in 24 h; *3*) one or more stools had pH below 6.

*Other laboratory determinations*

A routine battery of tests, which included hematological, biochemical, and bacteriological analyses, was performed on admission, and thereafter as required by each patient's clinical evolution. The concentrations of blood hemoglobin (automated cyanomethemoglobin), plasma proteins (refractometry), and plasma albumin (bromcresol purple) were measured at weekly intervals from blood obtained by fingerprick. Serum iron and iron-binding capacity (pyridyl bisulfite) were measured initially and at the end of the study. Urinary creatinine excretion (automated picrate method) was measured in total urine collected on the same days when feces were collected and weighed. The creatinine-height index (17) was calculated as an indicator of body protein deficit and repletion. The lactose content of the diets was determined by the glucose-oxidase method before and after complete hydrolysis with β-galactosidase.

*Statistical analyses*

Weight gains were calculated by linear regression analysis. Differences between treatment groups were assessed by analysis of variance and by $\chi^2$ and "Student's" *t* test (18) as appropriate.

## Results

### *Hydrolysis of lactose in diet formula*

Analyses of 15 HM aliquots showed that 91 to 100% of lactose was hydrolyzed after treatment with LactAid.

### *Comparability of treatment groups*

Table 2 shows that on admission the groups of children assigned to either dietary

TABLE 2
Selected comparative data on admission and at the end of the study*

| | Admission to INCAP | | End of the study | |
|---|---|---|---|---|
| | Intact milk | Hydrolyzed milk | Intact milk | Hydrolyzed milk |
| Age (mo) | 23 ± 6 | 21 ± 6 | 24 ± 6 | 23 ± 6 |
| Ht (cm) | 74.6 ± 3.2 | 75.6 ± 3.5 | 76.3 ± 3.1 | 77.4 ± 3.6 |
| Ht for age (mo) | 12 ± 2 | 12 ± 3 | 13 ± 2 | 14 ± 3 |
| Wt (kg) | 6.87 ± 0.91† | 7.13 ± 1.02† | 9.14 ± 1.20 | 9.62 ± 1.34 |
| Wt for ht (%)‡ | 70 ± 8† | 72 ± 7† | 89 ± 8 | 92 ± 8 |
| Lean arm diameter (mm)§ | 28 ± 3 | 29 ± 3 | 32 ± 2 | 35 ± 3 |
| Calf circumference (cm) | 13.7 ± 1.3 | 13.9 ± 1.5 | 15.6 ± 1.4 | 16.1 ± 1.6 |
| Skinfold thickness (mm) ‖ | 3.4 ± 1.6 | 3.1 ± 1.1 | 6.4 ± 2.1 | 6 0 ± 1.6 |
| Creatinine-ht index¶ | 0 63 ± 0.16 | 0.65 ± 0.12 | 1.07 ± 0.08 | 1 06 ± 0.10 |
| Severity of edema** | 2.0 ± 0.9 | 2.1 ± 0.9 | | |
| Plasma proteins (g/dl) | 4.2 ± 0 6 | 4.4 ± 0.6 | 7.1 ± 0.3 | 7.1 ± 0.5 |
| Serum albumin (g/dl) | 2.3 ± 0.8 | 2.2 ± 0.5 | 5.4 ± 0.4 | 5.2 ± 0.3 |
| Hb (g/dl) | 9.9 ± 0 7 | 9.5 ± 2.0 | 10 6 ± 1.4 | 10 6 ± 0 8 |
| Serum iron (μg/dl) | 55 ± 19 | 58 ± 19 | 54 ± 33 | 57 ± 28 |
| Iron binding capacity (μg/dl) | 128 ± 42 | 125 ± 30 | 347 ± 21 | 349 ± 44 |

* Mean ± SD, 10 children in each group.
† Corrected for weight of edema.
‡ Relative to Harvard standards, where 100% = 50th percentile (20).
§ Corrected for skinfold thickness.
‖ Average of 3 sites: tricipital, subscapular, and parumbilical.
¶ Normal ≥0.90 (22).
** 1 = edema below knees; 2 = edema involving thighs; 3 = edema involving arms.

TABLE 3
Intestinal and absorptive function during the first three days of hospitalization

| | Intact milk | Lactose-hydrolyzed milk |
|---|---|---|
| Children with diarrhea* | 8+ | 4+ |
| Stools | | |
| No of evacuations (stools/day) | 4.0 ± 0.9‡ | 2.8 ± 1.2‡ |
| % with abnormal characteristics | 87 ± 20 | 68 ± 32 |
| Average daily wt (g/day) | 243 ± 174 | 172 ± 108 |
| Children with acid stools (pH ≤5) | 5 | 4 |
| Children with fecal reducing substances. | | |
| only traces | 2 | 4 |
| + to ++++ | 7+ | 4+ |

* With 2 or 3 of: *1*) acidic pH; *2*) > 150 g feces/day; *3*) >50% liquid stools or any stool with blood or mucus.
† Groups differ, $\chi^2$, $p < 0.05$.
‡ Means differ, "Student's" *t* test, $p < 0.05$.

treatment were similar in age, severity of PEM, anthropometric characteristics, and biochemical indices of malnutrition. Although they had similar histories of recent or current diarrhea, more children in the group fed IM than that fed HM had abnormal stools and diarrhea starting from the day of admission (Table 3).

### *Clinical evolution*

In addition to the anthropometric changes described below, both groups of patients recovered well and in a similar fashion. The clinical signs and symptoms of acute, severe PEM disappeared at about the same rate in both groups. For example, clinical (ie, "visible") edema disappeared within 3 to 15 days of treatment in group IM (10 ± 4, mean ± SD) and within 4 to 18 days (9 ± 5) in group HM. While in the Clinical Research Center, six children from each group had illnesses that did not interfere with the dietary treatment such as upper respiratory infections, otitis media, tonsilitis, diarrhea, and nonspecific fever. These were treated symptomatically, or with the appropriate antibiotics. The length of time during which the six children in each group were ill was 1 to 11 days (5.8 ± 4.2) in group IM and 2 to 15 days (7.8 ± 4.4) in group HM.

The hematological indices of all children improved, although many had not reached normal hemoglobin concentrations by the end of the study (Table 2), not an unusual finding after 45 days of treating children with severe PEM (19).

*Growth and anthropometric recovery*

The recovery of weight-for-height was parallel with the two dietary treatments, with no differences registered at any interval (Fig 2). Not all children, however, had reached a status of 91% of the standard by the end of the 45-day protocol period (Fig 3), but fully half of both cohorts had attained this landmark of recuperation. Not only were the mean final outcomes with respect to ponderal and linear growth and to recovery of anthropometric indices equivalent in both groups (Table 2, last two columns), but the distribution of the data for each parameter was also closely similar (Fig 4). Of special note is the rate of weight gain during the initial period of catch-up growth: the 8.5 ± 0.9 and 8.7 ± 1.5 g/kg/day of the IM and HM groups, respectively, are not different.

*Body proteins*

The restoration of total plasma proteins and serum albumin was rapid, and followed a parallel, identical trajectory without regard to the assignment of diet (Fig 5). After 3 wk of treatment, mean levels of both had returned to the normal range. The recovery of transferrin levels, another index of visceral protein, was adequate by the end of the study, as indicated by the increase in total iron-binding capacity (Table 2). Lean body mass, as estimated by the creatinine-height index, recovered more slowly, but, by the end of the 45 days, all patients in both cohorts had a urinary creatinine excretion appropriate to their height (Fig 3).

*Stool characteristics and diarrhea*

Table 3 shows that more children had diarrhea and fecal reducing substances dur-

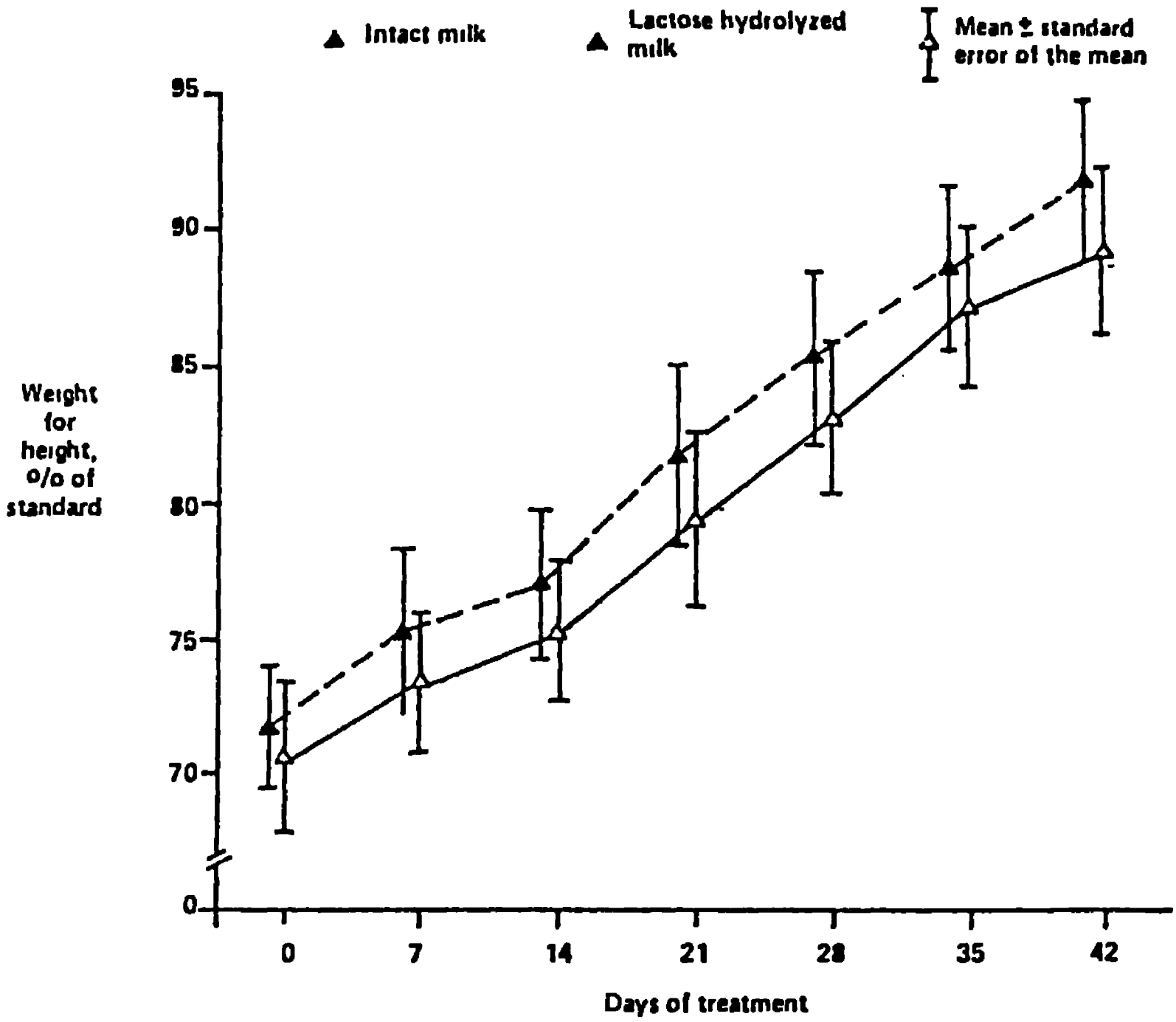



FIG 2. Weight expected for height, as percentage of the reference (median of the Harvard standards), of children treated with intact or lactose-hydrolyzed milk diets Mean ± SEM of weekly measurements; 10 children in each group.

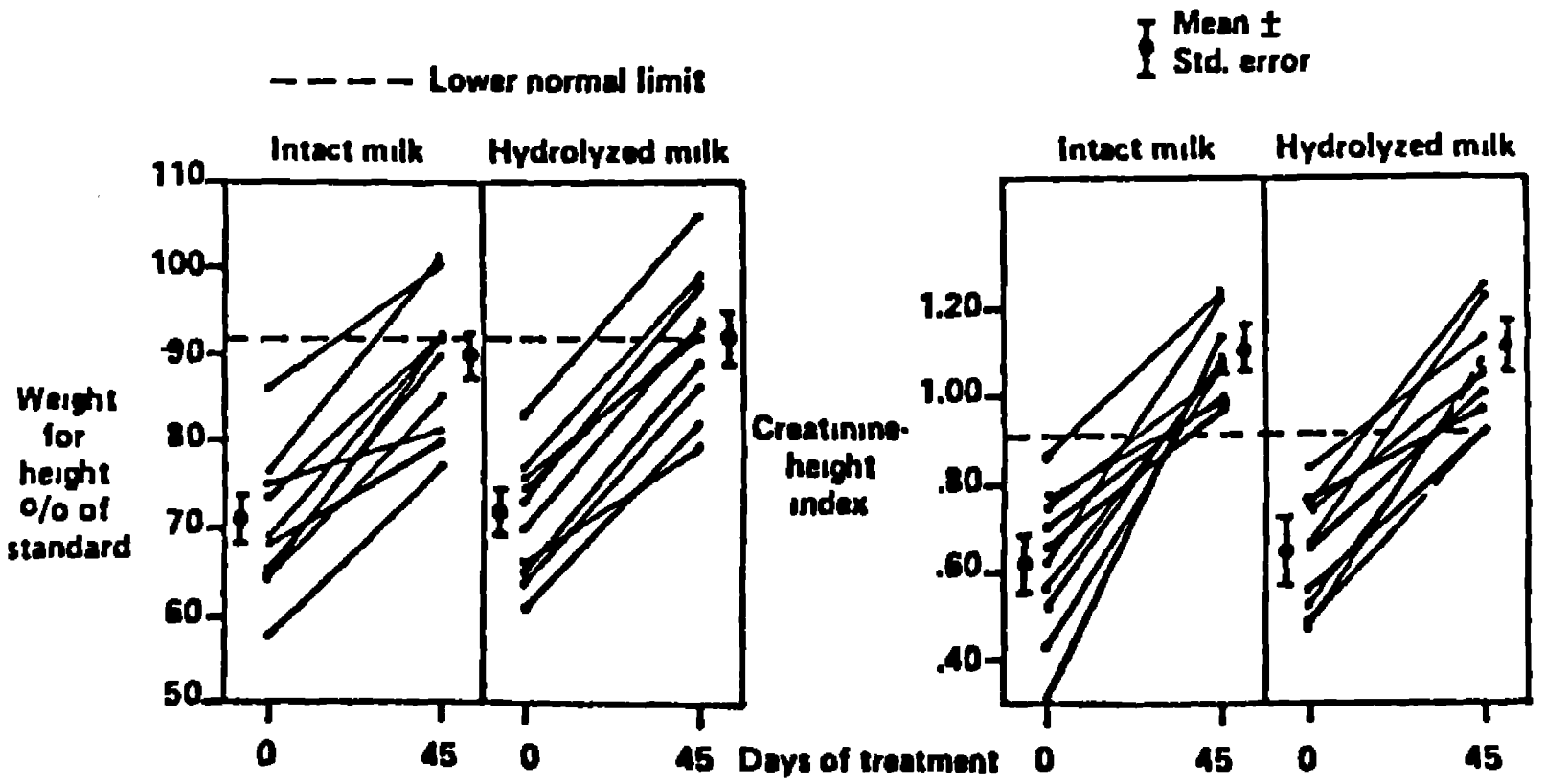



FIG 3 Individual and group (mean ± SEM) changes in two indicators of nutritional recovery after 45 days of treatment with intact or lactose-hydrolyzed milk diets. A minimum of 92% in weight for height and 0.90 in creatinine-height index are considered normal.

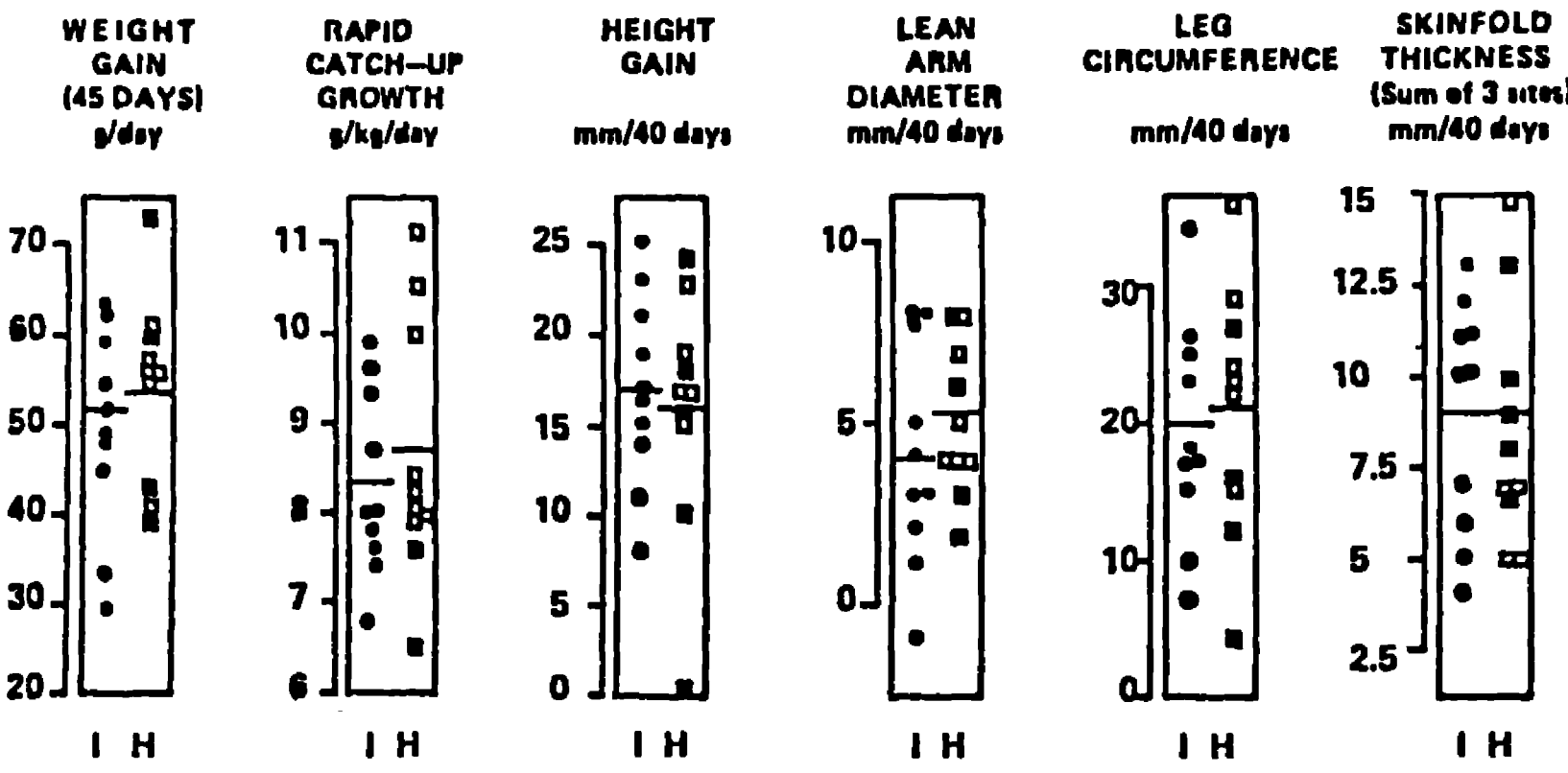



FIG 4. Individual and group (mean) changes in indices of growth after 6 weeks of treatment with intact (*I*) or lactose-hydrolzyed (*H*) milk formulas.

ing the initial 3 days of treatment with IM than with HM. The number of daily stools was also greater in the IM group, although the proportion with abnormal physical characteristics and total fecal weight were similar in both groups. It should be recognized that during this initial postadmission period, no lactose was fed on day 1 and the amount fed on the following 2 days was only 0.3 g/kg/meal, which was one-fourth of the carbohydrate fed from day 8 onward.

Diarrhea disappeared or improved without specific treatment in both groups as nutritional recovery progressed, and no patient required special hydration measures. Stool characteristics and incidence of diarrhea were similar with both treatments at each of the four subsequent metabolic balance periods. Large or abnormal fecal evacuations were not infrequent in two children, but their growth and nutritional recovery were satisfactory. When the children in the HM group were fed intact milk-based formula on days 46 to 50, there were no

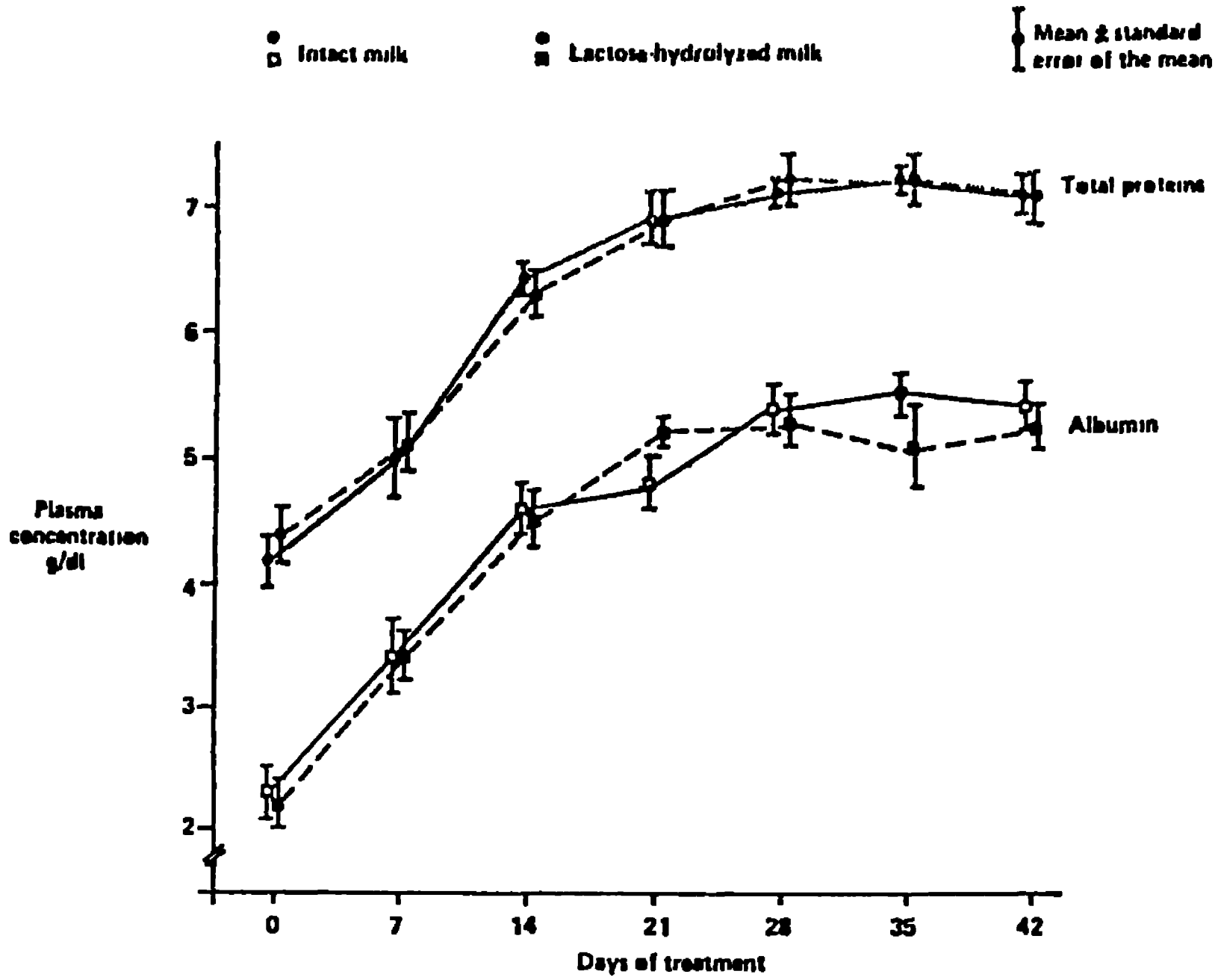



FIG 5. Concentrations of total plasma proteins and serum albumin of children treated with intact or lactose-hydrolyzed milk formulas. Mean ± SEM of weekly measurements; 10 children in each group.

changes in the stool characteristics compared to their last balance period on the hydrolyzed formula.

## Discussion

There is no doubt that the combination of nutritional injury and infectious insults reduces the capacity of the intestinal mucosa to digest lactose in severe PEM (*1*). The use of milk with low lactose content in malnourished populations has led to contradictory and inconclusive results. Zaal (20) reported that lactose-hydrolyzed milk was inferior to whole milk in its effect on growth of school-aged black children in Surinam. The methods of storage and reconstitution of the dried milk used in this study, however, might have altered the quality of the protein in the lactose-hydrolyzed milk via the Maillard reaction. Evaluation of lactose-containing foods in young children with severe PEM syndromes has been conducted in Australia (21, 22), Kenya (23), and Biafra (24). The limitation of these previous attempts to compare lactose-containing and lactose-free diets in the recovery of children with PEM-related to the brief length of observation (21–23), the noncomparability of treatment groups (21, 22), or the noncomparability of nutritional quality of therapeutic diets (24). We addressed these problems by: *1*) instituting a 45-day protocol to permit the quantification of rapid catch-up and overall growth, perhaps the two most sensitive indices of the nutritional adequacy of a recovery regimen; *2*) feeding identical formulas to the two cohorts, except for the addition of a food-grade β-galactosidase to the diet of one (HM) group which effectively hydrolyzed over 90% if the lactose; and *3*) allocating the patients to the alternative dietary

treatments in such a way that the two groups had equivalent characteristics and antecedents of illness at the onset of the feeding trial.

We found a complete overlap of both treatment groups with respect to clinical features, weight gain and other anthropometric improvements, body protein repletion, and overall nutritional recovery. The mean daily weight gain during the initial phase of rapid, catch-up growth was about 13 times greater than the average of healthy children of similar height and age. The difference in stool characteristics observed during the first few days of treatment were not clearly related to the intact milk intake. In any event, the subjects experienced no profuse diarrhea, no impairment of hydration requiring special rehydration measures, and absorption and retention of nutrients was satisfactory (13).

There is no reason to believe that our group of patients did not share the same characteristics of intestinal lactase activity as reported for other series of children with the same type and severity of PEM. The criteria for children as lactose-malabsorbers in other studies has generally been an intestinal mucosal biopsy or a response to a *supra*physiological challenge with lactose in aqueous solution. However, the most reasonable dose and form of lactose with which to assess the absorptive status of malnourished children is probably that which is contained in the amount of milk that provides the protein requirements during recovery; our routine meal delivery of lactose was precisely that amount.

The *gradual* introduction of proteins and energy into the diet of severely malnourished children is an important feature of any well-conceived recovery regimen (14, 25), as the metabolic challenge induced by feeding too much, too soon, may disrupt the labile homeostatic balance of children with PEM (26). In fact, rapid refeeding increases the risk of mortality (27). It is possible that the more conservative approach followed in our dietary therapy reduced the probability of untoward effects of lactose intake in children with a degree of lactase deficiency. If our findings in relation to diarrhea at the beginning of treatment were in fact related to milk intake, they would suggest that the intestine of the recently admitted child with severe PEM might be exquisitely sensitive to lactose. The gradual increase in the delivery of milk protein and therefore of lactose, might result in some form of intestinal adaptation or enzymatic rehabilitation, such that by the time the diet has been appropriately advanced to therapeutic levels, the intestine handles the lactose content of the IM formula as well as the lactose-free carbohydrates of the HM ration.

A prospective follow-up of children convalescing from persistent diarrhea reported from Houston (28, 29), found an inferior immediate growth response in the cohort of patients assigned to receive a lactose-containing formula, as compared to the group fed a soy protein and sucrose regimen. It is difficult to judge whether the extent of the intestinal lesion in that study prevented rapid normalization of lactase activity, or whether the protein source was a factor in the differential response. Such results, however, could suggest that some malnourished patients with intestinal complications might not tolerate lactose during the occurrence or convalescence of the diarrheal disease. In those PEM patients—as in children with severe lactase deficiency and intolerance to lactose following intestinal injury—the reduction of the disaccharide content of milk can be indicated to preserve the use of this highly digestible protein source.

Our data can be interpreted, however, as indicating that lactose hydrolysis or the use of lactose-free diets, are not necessary for the *routine* dietary therapy for preschool children with severe edematous PEM, when sound principles of dietary management and gradual advancement of nutrient density are followed. The additional cost of eliminating the lactose from milk prior to its use for the rehabilitation of malnourished children might have been justified had the clear superiority of our HM regimen been demonstrated. In the absence of such a result, however, we must conclude that available supplies of milk can be used intact to provide the *protein-base* for recovery diets for severely malnourished children, even in the early, postadmission period of recuperation.

The authors extend their gratitude to Ms Milagro de Castillo, Ms Enriqueta de Lopez, Ms Alfonsina Rosales,

and other members of the nursing staff of INCAP's Clinical Research Center, and to Ms Carmen Escalante and the staff of the Metabolic Kitchen for their invaluable assistance in the care of the children. We also thank Dr Ramiro Batres for clinical assistance. We appreciate the analytical contributions of Dr Oscar Pineda, Ms Cristina de Campos, and Ms Silvia Morales. We are grateful to Ms Marie Marcucci of the Core Laboratory of the MIT Clinical Research Center for the creatinine determinations.

## References

1. Torun B, Solomons NW, Viteri FE. Lactose malabsorption and lactose intolerance: implications for general milk consumption Arch Latinoam Nutr 1979,29 445-94
2. Bowie MD, Brinkman GL, Hansen JDL. Acquired disaccharide intolerance in malnutrition. J Pediatr 1965;66 1083–91.
3. Cook GC, Lee FD The jejunum after kwashiorkor. Lancet 1966,2·1263–7.
4. Barbezat GO, Bowie MD, Kaschula RDC, Hansen JDL. Studies on the small intestinal mucosa of children with protein-calorie malnutrition. S Afr Med J 1967,41:1031–6.
5. James WPT. Jejunal disaccharidase activities in children with marasmus and with kwashiorkor. Arch Dis Child 1971,46 218–20.
6. Graham GG, Paige DM. Nutritional implications of low intestinal lactase activity in children. In: Borgstrom B, Dahlqvist A, Hambreaus L, eds. Intestinal enzyme deficiencies and their nutritional implications Uppsala, Sweden. Almqvist and Wiksell, 1973,45–51
7. Lifshitz F. Acquired carbohydrate intolerance in children: clinical manifestations and therapeutic recommendations. In: Paige DM, Bayless TM, eds. Lactose digestion. clinical and nutritional implications. Baltimore, MD. The Johns Hopkins University Press, 1981.80–90.
8. Rand AG Jr. Enzyme technology and the development of lactose-hydrolyzed milk. In: Paige DM, Bayless TM, eds. Lactose digestion: clinical and nutritional implications Baltimore, MD: The Johns Hopkins University Press, 1981.219–30.
9. Sorensen KL, Meersohn WJ, Larsen L, Sonne J, Gudmand-Hoyer E. A new low lactose skimmed milk powder XII International Congress of Nutrition, San Diego, CA, abstracts 1981.72(abstr).
10. Bond JH, Levitt MD. Fate of soluble carbohydrate in the colon of rats and man. J Clin Invest 1976;57 1158–64
11. Bond JH, Currier BE, Buchwald H, Levitt MD. Colonic conservation of malabsorbed carbohydrate, Gastroenterology 1980,78 444–7.
12. Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG Jr. Absorption of short chain fatty acids by the colon. Gastroenterology 1980;78: 1500–4.
13. Torun B, Solomons NW, Caballero B, Flores-Huerta S, Orozco G, Pineda O. The effect of dietary lactose on the early recovery from protein-energy malnutrition. II. Indices of nutrient absorption. Am J Clin Nutr 1984,40.601–610.
14. Torun B, Viteri FE, Tratamiento de ninos hospitalizados con desnutricion proteinico-energetica severa. Rev Col Med (Guatemala) 1976;27:42–52.
15. Stuart HC, Stevenson SS. Physical growth and development. In: Nelson WE, Vaughan VC, McKay RJ, eds Textbook of pediatrics Philadelphia, PA: Saunders, 1969.54–5.
16. Kerry K, Anderson C. A ward test for sugar in feces. Lancet 1964,1.981–2.
17. Viteri FE, Alvarado J. The creatinine-height index: its use in the estimation of the degree of protein depletion and repletion in protein-calorie malnourished children. Pediatrics 1970,46 696–706.
18. Snedecor GW, Cochran WG. Statistical Methods. 2nd ed. Ames, IA. Iowa State University Press, 1967.
19. Alvarado J, Viteri F. Erythropoietic changes during recovery of protein-calorie malnutrition and its relation to lean body mass. XIII International Congress of Hematology, Munich, 1970, Abstracts. Munich. JF Lehmanns Verlag, 1970.300.
20. Zaal J. A study on the prevalence and implications of hypolactasia in Surinam's Bushnegro children. Thesis, University of Amsterdam, 1977.
21. Mitchell JD, Brand JC, Halbisch J. Weight-gain inhibition by lactose in Australian aboriginal children. A controlled trial of normal and lactose hydrolyzed milk Lancet 1977;1.500–2.
22. Brand JC, Miller JJ, Vorbach EA, Edwards RA. A trial of lactose hydrolyzed milk in Australian aboriginal children. Med J Aust 1977,2(suppl 4).10–13.
23. Rothman D, Habte D, Latham M. The effect of lactose on diarrhea in the treatment of kwashiorkor. J Trop Pediatr 1980,26:193–7.
24. Ifekwunigwe AE. Emergency treatment of large numbers of children with protein-calorie malnutrition. Am J Clin Nutr 1975;28.79–83.
25. Picou DM. Evaluation and treatment of the malnourished child. In: Suskind RM, ed. Textbook of pediatric nutrition. New York, NY: Raven Press, 1981.217–28.
26. Viteri FE. Primary protein-energy malnutrition: clinical, biochemical and metabolic changes. In: Suskind RM, ed Textbook of pediatric nutrition. New York, NY. Raven Press, 1981:189–215.
27. Patrick JJ. Death during recovery from severe malnutrition and its possible relationship to sodium pump activity in leucocytes. Br Med J 1977;1:1051–4.
28. Strickland A, Garza C, Nichols BL. Formula effects on growth after diarrhea. Am J Clin Nutr 1979,32.937(abstr).
29. Garza C. Milk versus low-lactose diets for lactose-intolerance children. In· Paige DM, Bayless TM, eds. Lactose digestion: clinical and nutritional implications. Baltimore, MD: The Johns Hopkins University Press, 1981:162–72.

# The effect of dietary lactose on the early recovery from protein-energy malnutrition. II. Indices of nutrient absorption[1-5]

*Benjamin Torun,[6] MD, PhD, Noel W Solomons,[7] MD, Benjamin Caballero,[8] MD, Samuel Flores-Huerta,[8] MD, Guido Orozco,[9] MD, and Oscar Pineda,[10] PhD*

ABSTRACT Absorption of dietary energy, nitrogen, carbohydrates and calcium, and retention of nitrogen and calcium were studied in 20 children with protein-energy malnutrition of the edematous type, using metabolic balance techniques and breath $H_2$ analysis, to assess the advisability of using lactose-containing formulas in the rehabilitation of severely malnourished children. Ten patients received for 45 days a diet formula based on cows' milk (intact milk) and 10 similar children received the same formula pretreated with β-galactosidase to hydrolyze the lactose (hydrolyzed milk). Dietary intakes were gradually increased to reach, on the 8th day, 4 g of protein and 150 kcal/kg. There were no differences between groups with respect to absorption or retention of the index nutrients. Postprandial carbohydrate malabsorption was occasionally observed in two patients with servings of the intact milk formula, and in one with the hydrolyzed milk diet. When the nutritional quality of a diet is assessed, the amount of nutrients that are absorbed and utilized are more important than the small, incompletely absorbed fractions that do not have significant metabolic or clinical implications. Therefore, the use of milk as the protein source for recovery diets is not contraindicated in the routine treatment of PEM. *Am J Clin Nutr* 1984,40 601–610.

KEY WORDS Calcium, dietary therapy, dietary carbohydrates, dietary proteins, intestinal absorption, lactase, lactose intolerance, milk, nitrogen balance, protein-energy malnutrition

## Introduction

Although milk is a source of excellent dietary protein and is often available for the treatment of malnutrition in developing countries in which protein-energy malnutrition (PEM) is highly prevalent, many clinicians have been reticent to advocate the use of whole cows' milk as the basis of recovery diets for severely malnourished children (1–3). The technology for reducing or removing the lactose content of milk provides a potential solution to any difficulties that might be attributable to the carbohydrate. However, before justifying the additional expense of providing low-lactose or lactose-free milk, the cost-effectiveness of the performance of unaltered whole cows' milk should be as-

[1] From the Division of Human Nutrition and Biology, Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala; and the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139.

[2] Supported in part by grants-in-aid from the National Dairy Council, Rosemont, IL and the SugarLo Company, Pleasantville, NJ.

[3] Presented in part at IV Syposium on Gastroenterology and Nutrition, Sophia Antipolis, France, November 1981; at the Congreso Panamericano de la Leche, Buenos Aires, Argentina, April, 1982, and at the Annual Meeting of the American Society for Clinical Nutrition, Washington, DC, May 1982.

[4] INCAP Publication I-1285.

[5] Address reprint requests to: Dr Benjamin Torun, Division of Human Nutrition and Biology, INCAP, Apartado Postal 1188, Guatemala City, Guatemala.

[6] Chief, Division of Nutrition and Health, and Director, Clinical Research Center, INCAP. [7] Associate Professor of Clinical Nutrition, MIT, and Visiting Professor/Affiliated Investigator, INCAP. [8] Fellow, World Hunger Programme, United Nations University/INCAP. [9] Resident physician, Clinical Research Center, INCAP. [10] Biochemist, Division of Nutrition and Health, INCAP.

Received August 9, 1982.
Accepted for publication April 17, 1984.

sessed. In comparing intact milk with low-lactose milk, not only are effects on clinical recovery, growth and stool characteristics of concern, but also the absorption and utilization of nutrients.

Since the decreased transit time and net accumulation of fluid in the intestinal lumen expected to result from major malabsorption of lactose might reduce the efficiency of the digestion and/or absorption of other dietary constituents, it is reasonable to investigate whether the utilization of dietary nitrogen or energy are impaired when lactose-containing foods are consumed by lactose-malabsorbers. Graham and Paige (3) reported an acute, transient decrease in nitrogen absorption in lactose-intolerant children who were fed a formula that had a lactose content of more than twice the lactose/protein proportion in cows' milk. In studies by Bowie (4) and Brown et al (5), in which the delivery of lactose in the diet was more moderate, no reduction in the apparent absorption or retention of macronutrients was observed.

Adequate calcium nutriture is critical to the child recovering from PEM to provide for skeletal mineralization in situations during which longitudinal catch-up growth rates of up to 2 cm per month can be achieved. Lactose promotes calcium absorption in experimental animals (6–11), while lactose-hydrolyzed milk produced inferior calcium bioavailability for human subjects (12–14). A decreased absorption of calcium was reported in lactose-malabsorbers consuming intact milk, however (14–16).

In the context of the exploration of the effects of lactose on clinical features and growth during the early recovery of Guatemalan preschool children with severe PEM of the edematous type (17), we have also examined the efficiency of absorption of protein, calcium, total energy, and carbohydrates in two well-matched cohorts of patients treated with intact milk (IM) or lactose-hydrolyzed milk (HM).

## Patients and Methods

### *Subjects*

Twenty Guatemalan boys of Maya or ladino (Maya-Caucasian) descent, 15 to 36 months old, with severe, acute edematous PEM (kwashiorkor or marasmic-kwashiorkor) participated in the study for 45 to 50 days. They were assigned at the time of admission to either one of two dietary treatment groups by stratified binary-choice allocation. Both groups of children were similar in age, severity of edema, anthropometry, and history of diarrhea. The criteria for selection, the characteristics of the patients and the therapeutic regimens were described in an accompanying article (17). Informed consent was obtained from the children's parents upon admission to the Clinical Research Center. The research protocols were approved by INCAP's Committee on Human Research and MIT's Committee on the Use of Humans as Experimental Subjects.

### *Diets*

As described in the companion article (17), the two groups of children received either a diet based on intact, whole cows' milk as the protein source, or the same formula after adding a β-galactosidase (lactase, LactAid, SugarLo Company, Pleasantville, NJ) to hydrolyze more than 90% of the dietary lactose into its constituent monosaccharides On the 1st day, the children received 0 7 g casein and 70 kcal/kg/day. The experimental diets contained milk protein and total energy, which were increased on days 2, 4, 6, and 8, respectively, to 1 g and 100 kcal, 2 g and 120 kcal, 3 g and 150 kcal, and 4 g and 150 kcal/kg/day. This level of intake was maintained constant for the remainder of the study. Soybean oil was added to the dried, whole cows' milk to provide a total of 40% of dietary energy as fat, and the balance of the prescribed energy was provided by sucrose. The diets were supplemented with adequate amounts of vitamins, minerals, and electrolytes. The diets were divided into five equal meals, fed at 3 h intervals, during the course of the day. On days 2 to 5, the children ingested with each meal, as an average, 0 45 g of either intact or hydrolyzed lactose per kg body weight, and from day 8 onwards, 1.2 g/kg. The group receiving the untreated formula was designated as the intact milk or IM group; the children receiving the lactase-treated formula diet were identified as the lactose-hydrolyzed milk or HM group. After day 45, the 10 children in the HM group were fed the HM formula for 5 more days.

### *Metabolic balance studies*

Complete collection of urine and stools were made in all children during days 2 to 5, 12 to 14, 22 to 24, 32 to 34, and 42 to 44. Collections were also made on days 48 to 50 in those children initially assigned to group HM, but subsequently switched to the intact milk formula after day 45. During the first two balance periods, the children usually did not walk and were confined to a metabolic bed with a weighed fecal basin in place and a plastic urine collector diverting the urine into a separate bottle containing 5 ml of 50% hydrochloric acid as preservative. Once the child was ambulatory and advised the nursing staff about defecating or his pattern of defecation was known, he was allowed to move around freely in the Clinical Research Center facilities and playground, wearing a self-adhesive polyethylene urine collection bag. All stools were collected into preweighed, plastic basins, and the weight of each stool was recorded. Stools were refrigerated during the duration of each balance period, and then pooled proportionately and frozen. Later, stools were dried in

ovens at 90 to 100°C until they reached a constant weight, prior to analysis. Previous experiments showed no differences in fecal fat, nitrogen, or total energy between this procedure and drying in a vacuum oven at 30 to 40°C. Brilliant blue was used as fecal marker to identify the beginning and end of the collection period.

Duplicate samples of diets were collected and frozen for analysis. Accurate records of the weights of diets consumed in each meal were kept by the nursing staff.

*Determination of nitrogen absorption and retention*

The nitrogen contents of diets, urine and feces were determined in duplicate using a micro-Kjeldahl method. "True" nitrogen digestibility and "true" nitrogen retention were calculated by the following equations:

$$\% \text{ true N digestibility} = \frac{\text{ingested N} - (\text{fecal N} - \text{endogenous N})}{\text{ingested N}} \times 100$$

$$\text{true N retention} = \text{ingested N} - [\text{fecal N} + \text{urinary N} + \text{insensible N losses}]$$

Endogenous N, or obligatory fecal N loss, was assumed to be 20 mg N/kg/day (18) Insensible N losses were assumed to be 8 mg N/kg/day during the first balance period and 14 mg N/kg/day thereafter, based on integumental N losses with different amounts of protein intake (19). Nitrogen retention was related to the *average* weight of the child during the metabolic balance period for its final expression as mg N/kg/day.

*Determination of calcium absorption and retention*

An aliquot of 0 5 g of dried stools was incinerated at 650°C for 16 h; this second fecal ash was dissolved in 100 ml of 1 N HCl. Five-gram aliquots of diets were homogenized by sonication, dried at 95°C for 36 h, and incinerated at 650°C for 16 h; the ash was dissolved in 100 ml of 1 N HCl. Aliquots of 200 μl of urine were dilutes 10 1, and 200 μl of the dissolved diets and feces were diluted 20 1 in 0 15% aqueous solution of lanthanum chloride Calcium concentrations were determined by atomic absorption spectrophotometry (model A 775, Varian Techtron Pty Ltd, Melbourne, Australia).

The apparent calcium absorption and apparent calcium retention (ie, without accounting for endogenous or integumental Ca losses) were determined using the equations:

$$\% \text{ apparent Ca absorption} = \frac{\text{ingested Ca} - \text{fecal Ca}}{\text{ingested Ca}} \times 100$$

$$\text{apparent calcium retention} = \text{ingested Ca} - [\text{fecal Ca} + \text{urinary Ca}]$$

This was also related to the average weight of the child during the balance period for its expression as mg Ca/kg/day.

*Determination of total energy absorption*

The energy contents of dried homogenized aliquots of diets and stools were determined in duplicate by adiabatic bomb calorimetry on a Gallenkamp calorimeter, using certified benzoic acid as standard (British Chemical Standard N-190J, Middlesbrough, Teeside, England). Apparent energy absorption was calculated in an analogous fashion to that of calcium absorption, using bomb calorimetry data for ingested and fecal energy. The energy of combustion of 100 g of diet, which contained 4 g protein, 4 4 g fat, and 23 5 g carbohydrate, was, theoretically; 159 kcal (ie, 5 6, 9 4, and 4 1 kcal/g of protein, fat, and carbohydrate, respectively). The "metabolizable" energy of those ingredients, based on the Atwater factors, was 150 kcal. Therefore, the net (or "metabolizable") energy intake was calculated multiplying the bomb calorimetry measurements of the diet (gross energy) by 0 943 (ie, 150/159).

*Hydrogen breath tests*

$H_2$ breath tests were performed serially throughout the study Samples of exhaled air were collected in the rubber bag through a respiratory face mask and a small, low-resistance Rudolph valve (Warren Collins, Braintree, MA), as previously described by Solomons et al (20, 21). Hydrogen concentration was quantified using a thermal conductivity gas-solid chromatograph (MicroLyzer, Quintron Instruments Co, Milwaukee, WI) (22) calibrated with certified gases containing hydrogen at 53 or 99 8 ppm (Supelco, Bellefonte, PA, or Linde Division, Union Carbide, N Chicago, IL). The chromatograph has a coefficient of error of 2% in the 0 to 100 ppm range. Breath samples were collected after an overnight fast and at 3 or 4 h intervals after the administration of breakfast or following an oral dose of 6 7 g of the nonabsorbable disaccharide, lactulose (4-O-β-D-galactopyranosyl-D-fructofuranose), administered as a flavored syrup (Cephulac, Merrill-National Laboratories, Cincinnati, OH) The test with lactulose was performed on admission and 1 wk later in order to determine the competence of the colonic bacterial flora to metabolize unabsorbed carbohydrate with augmented $H_2$ excretion in expired air, since diarrhea (23, 24), antibiotics (22, 25) and the debilitation of severe malnutrition (26) can reduce the rate of production and/or respiratory excretion of $H_2$. If the patient did not have a positive response initially, the lactulose test was repeated 1 day before or after each scheduled, weekly postprandial breath $H_2$ study until the child had demonstrated a colonic flora capable of mounting an $H_2$ response, only in the presence of a positive breath $H_2$ response after an oral of 6.7 g of lactulose would a flat curve be interpreted as truly negative following a feeding of the diet.

The postprandial tests were done after breakfast at 7-day intervals until day 45, and again on days 46 and 50 in the patients of the HM group when switched to IM On day 3, the test-meal contained 0 3 g of lactose (IM formula or of galactose and glucose (HM formula), thereafter it contained 1 2 g of the carbohydrates from milk. The child remained awake during the 3 to 4 h of the test, and was allowed water ad libitum but no further food. To quantify the breath-test response to the various carbohydrates, the total breath $H_2$ concentration and

the maximum increment above fasting levels were determined. A test was considered "positive," that is indicative of carbohydrate malabsorption, when the increase in breath $H_2$ concentration was ≥20 ppm (27, 28).

*Statistical analyses*

The differences between treatment groups were examined using "Student's" *t* test or analysis of variance. The changes over time with respect to dietary treatment were evaluated by analysis of covariance for repeated measures of the same individuals after the method of Winer (29).

## Results

### *Nitrogen absorption and retention*

Table 1 provides results on the "true" nitrogen retention and absorption for both the beginning and during the period of full-strength nutritional treatment. Actual protein intakes on days 8 to 45 were 3.88 ± 0.10 g/kg/day (mean ± SD). No statistically significant differences in absorption related to lactose intake (Table 1) or as a function of time (Fig 1*A*). There were no differences between groups in nitrogen retention (Table 1), and the only significant change in nitrogen retention with time occurred at the beginning of dietary protein repletion with full protein intake (Fig 2).

### *Energy absorption*

Table 1 lists the net (metabolizable) energy intakes and the apparent fractional absorption of energy. Neither between treatment nor as a function of time (Fig 1*B*) were any statistically significant differences found.

### *Calcium absorption and retention*

Table 1 shows the dietary calcium intakes and absorption efficiencies. There were no statistically significant differences between groups nor as a function of time (Fig 1*C*), nor were differences in calcium retentions observed (Fig 3). The within-group variables in absorption and retention of calcium were several times greater than those of nitrogen and energy.

### *Carbohydrate absorption*

The initial lactulose test on the first day of hospitalization was positive in only five patients assigned to the IM group and four of the HM group. The inability to produce a positive response persisted in several children for variable periods after beginning treatment. Also, some children with an initial positive response lost the capacity to respond to lactulose due to treatment of infections with antibiotics. Thus, 33% of all the $H_2$ breath tests after breakfast had an associated negative lactulose test.

The original analytical plan had been to interpret each postprandial $H_2$ breath test as a discrete diagnostic entity, but given the high proportion of tests associated with negative lactulose tests, we performed a global, intergroup comparison of the *pattern* of $H_2$ excretion in those postprandial breath tests in which the $H_2$ producing capacity was assured. In neither the pattern of *absolute* $H_2$ excretion (data not shown) nor that for the postmeal *rise* in breath $H_2$ concentration (Fig 4) was an excess frequency of incom-

TABLE 1
Metabolic balance and intestinal absorption of dietary protein (1 g = 157 mg N), energy, and calcium of malnourished children treated with IM or lactose HM milk formulas

| | | First balance period (days 2 to 5) | | | Composite of second through the fourth balance periods (after day 8) | | |
|---|---|---|---|---|---|---|---|
| | | Intake | Retention | Absorption | Intake | Retention | Absorption |
| | | *mg/kg/day* | *mg/kg/day* | % | *mg/kg/day* | *mg/kg/day* | % |
| Nitrogen | IM | 226 ± 27* | 125 ± 41 | 86 ± 10 | 611 ± 16 | 271 ± 70 | 91 ± 6 |
| | HM | 242 ± 28 | 140 ± 35 | 91 ± 5 | 606 ± 17 | 287 ± 67 | 91 ± 5 |
| Energy | IM | 104 ± 11 | | 88 ± 6 | | | 94 ± 3 |
| | HM | 107 ± 10 | | 91 ± 4 | | | 94 ± 2 |
| Calcium | IM | 87 ± 16† | 36 ± 16 | 43 ± 15 | 147 ± 10 | 54 ± 28 | 39 ± 17 |
| | HM | 86 ± 13† | 37 ± 15 | 45 ± 13 | 144 ± 16 | 53 ± 27 | 39 ± 17 |

* Mean ± SD.
† $CaCO_3$ was added to the formulas on days 2 to 5.

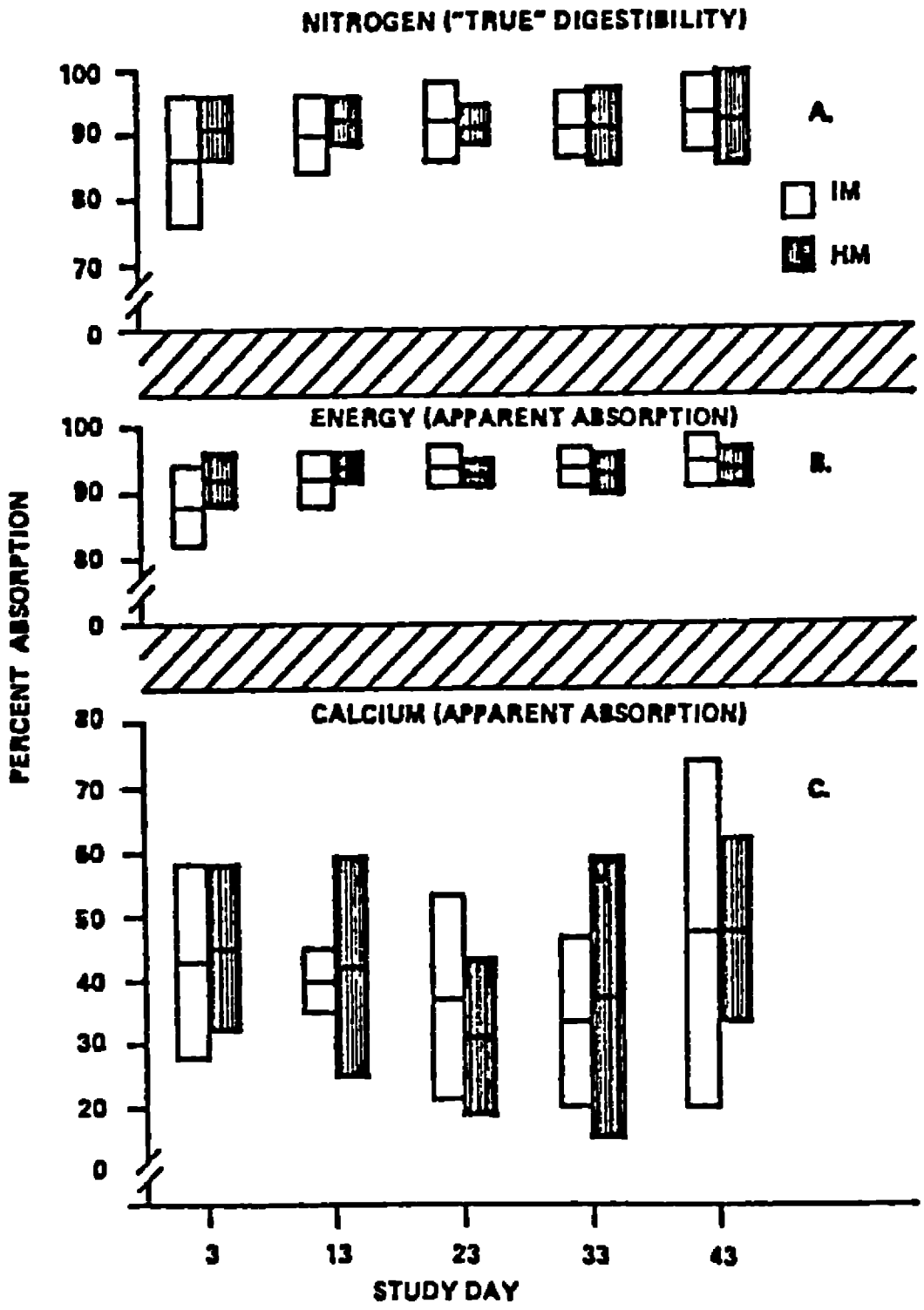



FIG 1. Absorption of dietary nitrogen (*A*), energy (*B*) and calcium (*C*) during 3- or 4-day balance periods at 10-day intervals, with diets based on intact (*IM*) or lactose hydrolyzed (*HM*) milk (mean ± SD). Average intakes were 234 mg N, 106 kcal, and 86 mg Ca/kg/day on the first balance period, and 608 mg N, 143 kcal, and 146 mg Ca/kg/day thereafter, including inorganic Ca supplement given during the first balance.

plete carbohydrate removal in the small intestine evident. On only four occasions in three different patients—two from the IM and one from the HM group—were the increments in postprandial breath $H_2$ concentrations in excess of 20 ppm. The IM group child with a positive response on two occasions and the only HM group child with a positive response, were among the two children in each group who frequently had large or abnormal stools throughout the study. The four positive tests represented 6% of all valid, morning meal experiences. Barr (30) and Bayless (31) have argued that incomplete absorption of carbohydrate substrates can be diagnosed by an increment of only ≥10 ppm. If this criterion were applied to our results, we still would classify ony 12 tests (18% of total) as compatible with incomplete absorption of carbohydrate and, once again, the distribution of positive test were almost identical in the two dietary treatment groups.

*Switch from hydrolyzed to intact milk*

When the children in the HM group were switched to the intact milk diet for 5 days, there were no significant changes in the absorption of nitrogen, energy, or calcium. Only one child manifested a positive $H_2$

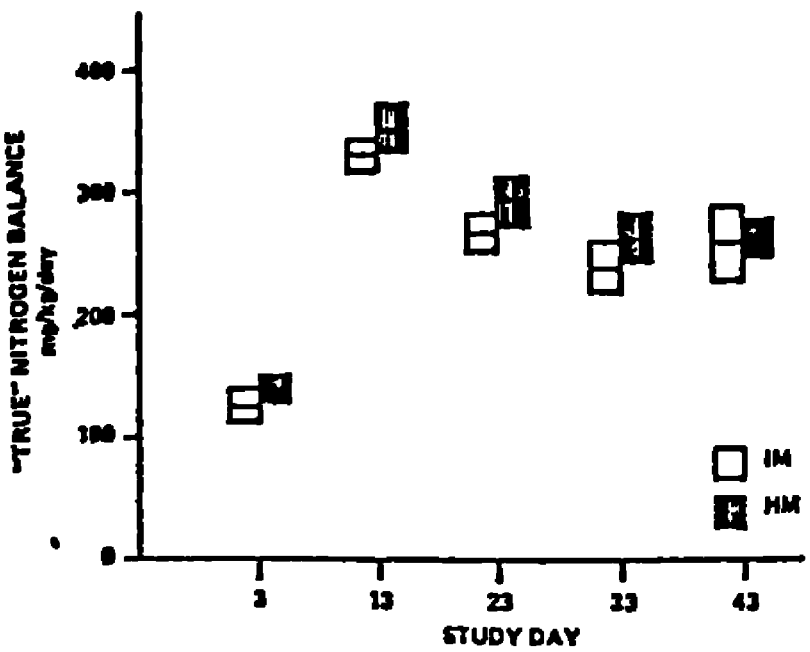



FIG 2. True nitrogen balance, including estimates of insensible N losses, measured for 3 or 4 days at 10-day intervals with intact (*IM*) or lactose-hydrolyzed (*HM*) milk diets (mean ± SEM). Average intakes were 234 mg N (1.49 g protein)/kg/day on the first balance period and 608 mg N (3.88 g protein)/kg/day thereafter.

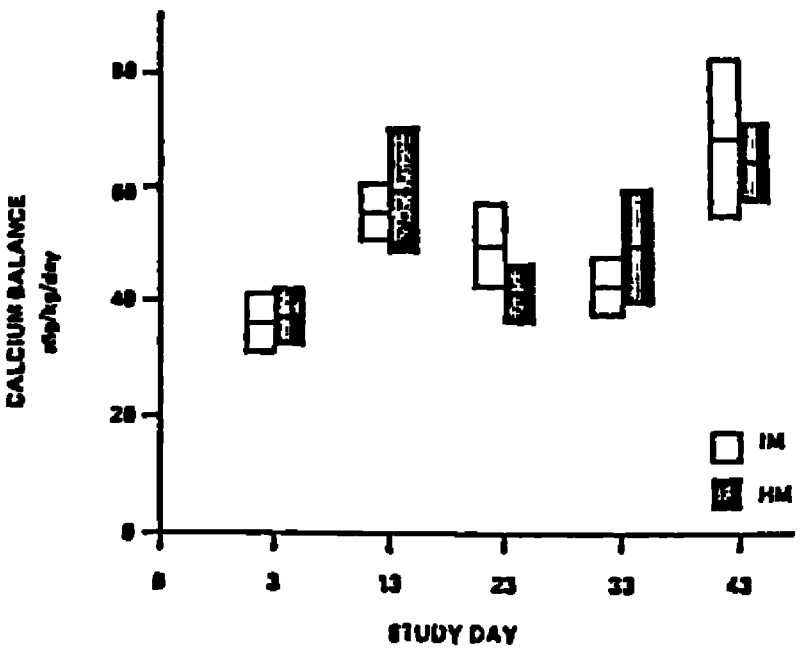



FIG 3. Calcium balance measured for 3 or 4 days at 10-day intervals with intact (*IM*) or lactose-hydrolyzed (*HM*) milk diets (mean ± SEM) Average intakes were 86 mg/kg/day on the first balance period (including inorganic Ca supplement) and 146 mg/kg/day thereafter (no Ca supplement given).

breath test after being switched from HM formula to IM formula. This is in accord with the absence of alterations in diarrhea or fecal characteristics (17). Curiously, a greater retention of nitrogen with the intact milk formula (298 ± 49 mg N/kg/day) occurred than had been seen in the final balance period on the HM diet in this group when compared by paired *t* test ($p < 0.05$) or when analyzed over time by analysis of covariance ($p < 0.05$) (29). Calcium retention did not change with the dietary modification.

## Discussion

The controversey about the possibility of deleterious effects of dietary lactose is important in relation to two nutritional issues: *1*) the loss of dietary energy in the form of lactose that is not absorbed; and *2*) the loss of other nutrients that are incompletely absorbed due to changes in intestinal transit and fluid movements produced by the malabsorption of dietary lactose. Such issues are particularly relevant in the treatment of malnourished patients since it is known that gastrointestinal malfunctions such as low pancreatic enzyme outputs (32), altered micellarization and malabsorption of fats (33–38), and in some instances, lower nitrogen absorption (33, 39) can be present in severe PEM. The therapeutic regimens followed in the present study, however, resulted in very good intestinal absorption of dietary energy, nitrogen, and calcium, independent of the lactose contents of the formula diets. The retentions of these nutrients were highly satisfactory with either the intact or hydrolyzed-milk treatments, coinciding with the equally good clinical and anthropometric results obtained with both regimens (17). Lactose, itself, was also well handled in the intestine, as the diets seldom resulted in increased breath $H_2$ concentrations or excretion of fecal reducing substances or acidic stools (17). The few instances of incomplete carbohydrate absorption were evenly distributed between the lactose-containing and the lactose-free diet groups, and neither regimen produced abdominal discomfort or important diarrhea (17).

Although we did not measure fecal fat excretion, results of a number of investigations indicate that lactose does not impair the absorption of lipid by malnourished children (3, 4). Moreover, the absence of an effect of lactose on total dietary energy absorption in the present study agrees with the results of reports where diets containing either lactose or glucose were fed to black South African children with kwashiorkor (4) and to children from Bangladesh who had weight-for-height from 74 to 108% of standard (5).

Nevertheless, the incomplete absorption of carbohydrate in the small intestine does

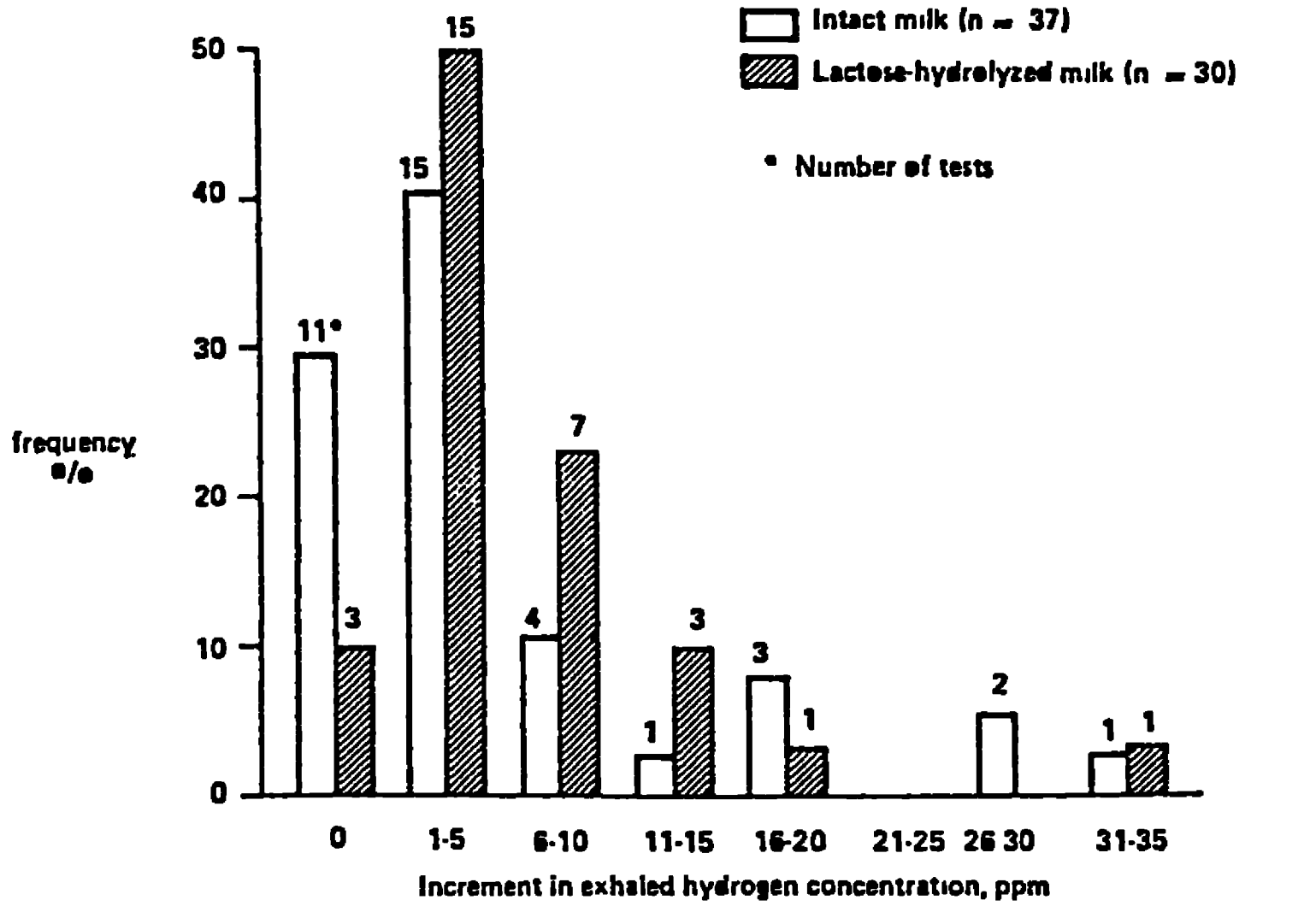



FIG 4. Pattern of maximal increments in exhaled hydrogen concentration above baseline (fasting) levels, within 3 h after breakfast with intact (*IM*) or lactose-hydrolyzed (*HM*) milk diets. The tests were performed during 7 wk of dietary therapy. Only the data of patients with a positive hydrogen response to lactulose are shown.

not necessarily produce a loss of dietary energy equivalent to the amount of nonabsorbed carbohydrate (40, 41). Conclusions about the operation of an effective colonic salvage of carbohydrates that escape absorption in the small intestine derive from experiments by Bond and co-workers (42, 43) on the fecal versus pulonary excretion of radioactivity from $^{14}$C-labelled sugars instilled into the cecum of rats and man, and in human intestinal perfusion studies by Ruppin et al (44) with volatile short-chain fatty acids, the degradation products of fermented sugars. Given the low incidence of positive breath $H_2$ tests after meals, it would appear that the predominant site of intestinal uptake of carbohydrate energy from the formula diet occurred in the small intestine, not in the colon. We are reasonably confident of the conclusions of these $H_2$ breath test data since the appropriate precautions to preclude the pitfalls of interpreting pulmonary $H_2$ excretion responses in severely ill children (45, 46) were observed.

In relation to protein metabolism, our results showed no influence of lactose on nitrogen absorption or retention. Some previous reports do not fully coincide with these results, while others do. Brown et al (5) found no differences in the nitrogen absorptions or retentions of lactose-malabsorbing children fed a mixed rice-vegetable diet containing either glucose or lactose. In contrast, Graham and Paige (3) found better nitrogen absorptions and retentions in four lactose-malabsorbers when fed a sucrose-formula than when they were fed a lactose formula. It should be noted, however, that their diets provided 7.8 g of lactose and a lactose/protein ratio of 4:1, which is much higher than the 1.5:1 ratio in the cows' milk that would normally be used as the base of a dietary formula for preschool children. Bowie's (4) studies in children with kwashiorkor indicated a lower nitrogen absorption with milk than with the glucose-based diet, but there was a compensatory decrease in urinary nitrogen, such that no differences in nitrogen balance were observed overall. The divergence between this last study and our nitrogen absorption results could be due to the fact that our therapeutic diets provided

initially low amounts of energy and protein which were then gradually increased to reach, after 8 days, the levels which Bowie introduced from the very beginning of his treatment regimen. Furthermore, Bowie's studies consisted of three balance periods during only 11 days of observation. It is possible that the gradual advancement of nutrient content and the longer duration of our study allowed better metabolic and gastrointestinal adaptation to therapy, and resulted in better nitrogen absorption as well as less diarrhea than experienced in Bowie's subjects (4, 17).

It is interesting to note the increase in nitrogen retention observed when the children were switched for a few days from the lactose-hydrolyzed to the intact milk formula. This was not accompanied by changes in nitrogen, energy, or calcium absorption, nor altered stool characteristics (17). It is difficult to establish a cause-effect relationship between lactose intake and increased nitrogen retention, because we did not do a complete crossover experiment which would have also involved switching the IM group to hydrolyzed milk. Although the significance of this observation should be further explored, we can conclude that the intake of 1.2 g lactose per kg body weight per meal, after eating essentially no lactose for 45 days, did not impair nutrient absorption or retention, and did not cause intestinal discomfort (17).

The potential relationship between lactose intake and dietary calcium absorption could have had two opposing consequences; on the one hand, lactose malabsorption could have led to *decreased* calcium absorption (14–16), and on the other hand, lactose in the intestinal lumen might have *enhanced* calcium absorption (6–13, 16, 47). We did not find an important lactose malabsorption, while calcium absorption and retention were high and equal in both treatment groups. This latter finding agreed with the short-term observations of Graham and Paige (3) in preschool children. The absence of a lactose effect on calcium absorption in ouf study could be due to the high calcium intake, which was 2 to 4 times higher than current recommendations, and probably overshadowed any potential enhancing influence that the disaccharide might have had on absorption of the mineral. In any event, the high absorption led to high calcium retentions that are closer to the calcium accretion of a rapdily growing 35-wk human fetus (48) than to the 12 to 14 mg/kg/day expected in a child 2 to 3 yr old (49). This could be due to greater demands for calcium in malnourished children who may have some degree of skeletal mineralization deficit and who are trying to catch-up not only in terms of weight, but also of length.

In summary, the amount of lactose accompanying doses of milk protein added as the dietary intake was advanced from 1 to 4 g protein/kg/day, were not associated with any decrease in the absorption of total dietary energy or carbohydrate, nor with any decrease in the digestibility or retention of nitrogen during treatment of severe PEM. The presence or absence of lactose did not produce either an increase or a decrease in the uptake and retention of calcium from the milk-based diet, in comparison with a hydrolyzed-milk preparation. This parity of nutritional response was observed even during the early phase of recovery, when the children on the IM diet had more frequent and more liquid stools. Therefore, we conclude that neither clinical considerations nor nutritional or gastrointestinal considerations are sufficient to justify any proscription of the routine use of intact milk as the base of therapeutic diets for children with severe PEM. Reduction of the lactose content can be reserved for the occasional patient with PEM who manifests severe malabsorption or intolerance of this carbohydrate. It should be borne in mind from a nutritional point of view that the amounts of dietary nutrients *absorbed* and *utilized* are more important than small losses of incompletely absorbed nutrients that do not limit metabolic processes or nutritional repletion. In a practical sense, for consideration in nutritional recovery centers in the developing world, the minor discomfort that loose evacuations might cause during the first few days of dietary therapy with a milk-based formula is not as important as the net effective delivery of nutrients to the malnourished child.

The authors extend their gratitude to Ms Milagro

Castillo, Ms Enriqueta Lopez, Ms Alfonsina Rosales, and other members of the nursing staff of INCAP's Clinical Research Center, and to Ms Carmen Escalante and the staff of the metabolic kitchen for their invaluable assistance in the care of the children. We also thank Dr Ramiro Batres for his clinical assistance, Mr Ruben Dario Mendoza for his laboratory assistance, and Lic Rafael Flores for statistical advise. We appreciate the technical counsel in the $H_2$ breath analysis offered by Mr Lou Betzweiser and Dr Lyle Hamilton.

## References

1. Torun B, Solomons NW, Viteri FE. Lactose malabsorption and lactose intolerance: implications for general milk consumption. Aarch Latinoam Nutr 1979,29.445–94.
2. Friedl J. Lactase deficiency Distribution, associated problems, and implications for nutritional policy. Ecol Food Nutr 1981;17:37–48.
3. Graham GG, Paige DM. Nutritional implications of low intestinal lactase activity in children. In: Borgstrom B, Dahlqvist A, Hambreaus L, eds. Intestinal enzyme deficiencies and their nutritional implications. Uppsala, Sweden: Almqvist and Wiksell, 1973,45–51.
4. Bowie MD. The effect of lactose-induced diarrhea on absorption of nitrogen and fat. Arch Dis Child 1975;50.363–6.
5. Brown KH, Khatun M, Parry L, Ahmed MG. Nutritional consequences of low dose milk supplement consumed by lactose-malabsorbing children. Am J Clin Nutr 1980,33.1054–63.
6. Greenwald I, Gross J. The prevention of tetany of parathyroidectomized dogs. II. Lactose-containing diets. J Biol Chem 1929;82:531–44.
7. Vaughan OW, Filer LJ. The enhancing action of certain carbohydrates on the intestinal absorption of calcium in the rat. J Nutr 1960,71:10–14.
8. Dupuis Y, Fournier PL. Etude comparée de l'action de vitamine D et du lactose sur les échanges calcique durant la vie du rat. CR Acad Sci (Paris) 1964,258D.2906–09.
9. Pansu D, Chapuy MC. Relation chez l'homme, entre l'action sur la calcemie de divers composes glucidique et leur utilisation digestive. CR Acad Sci (Paris) 1970,260:3103–06.
10. Armbrecht HJ, Wasserman RH. The enhancement of $Ca^{++}$ uptake by lactose in the rat small intestine. J Nutr 1976,106·1265–71.
11. Schaafsma G, Visser R. Nutritional interrelationships between calcium, phosphorus and lactose in rats. J Nutr 1980,110.1101–11.
12. Mills RH, Breiter H, Kampster E, McKay B, Pickens M, Outhouse J. The influence of lactose on calcium retention in children. J Nutr 1940,20 467–76.
13. Kobayashi A, Kawai S, Ohbe Y, Nagashima Y. Effect of dietary lactose and a lactase preparation on the intestinal absorption of calcium and magnesium in normal infants. Am J Clin Nutr 1975,28 861–3.
14. Debognie JC, Newcomer AD, McGill DB, Phillips SF. Absorption of nutrients in lactase deficiency. Dig Dis Sci 1979,24.225–31.
15. Condon JR, Nassium JR, Millard JC, Hilbe A, Stainthorpe EM. Calcium and phosphorus metabolism in relation to lactose tolerance. Lancet 1970,1:1027–9.
16. Kocian J, Skala I, Bakos K. Calcium absorption from milk and lactose-free milk in healthy subjects and patients with lactose intolerance. Digestin 1973;9:317–24.
17. Solomons NW, Torun B, Caballero B, Flores-Huerta S, Orozco G. The effect of dietary lactose on the early recovery from protein-energy malnutrition. I. Clinical and anthropometric indices. Am J Clin Nutr 1984,40.591–600.
18. Torun B, Cabrera-Santiago I, Viteri FE. Protein requirements of preschool children: obligatory nitrogen losses and nitrogen losses and nitrogen balance measurements using cow's milk. Arch Latinoam Nutr 1981;31:571–85.
19. Viteri FE, Martinez C. Integumental nitrogen losses of preschool children with different levels and sources of dietary protein intake. Food Nutr Bull 1981; suppl 5:164–8.
20. Solomons NW, Vitri FE, Hamilton LH. Application of a simple gas chromatographic technique for measuring breath hydrogen. J Lab Clin Med 1977;90.856–62.
21. Solomons NW, Viteri FE, Rosenberg IH. Development of an interval sampling hydrogen ($H_2$) breath test for carbohydrate malabsorption in children: evidence for a circadian pattern of breath $H_2$ concentration. Pediatr Res 1978,12.816–28.
22. Christman NT, Hamilton LH. A new chromatographic instrument for measuring trace concentrations of breath-hydrogen. J Chromatogr 1982;229: 259–65.
23. Solomons NW, Garcia R, Schneider R, Viteri FE, Argueta von Kaenel V. $H_2$ breath tests during diarrhea. Acta Paediatr Scand 1979;68.171–2.
24. Brown KH, Black RE, Parry L. The effect of diarrhea on the incidence of lactose malabsorption among Bangladeshi children. Am J Clin Nutr 1980,30:2226–7.
25. Murphy EL, Calloway DH. The effect of antibiotic drugs on the volume and composition of intestinal gas from beans. Am J Dig Dis 1972;17.639–42.
26. Solomons NW, Garcia-Ibanez R, Aycinena P, Torun B, Viteri FE. Lactose intolerance in protein-energy malnutrition: a clinical case study and family study using a hydrogen ($H_2$) breath-analysis test for carbohydrate malabsorption. Arq Gastroenterol 1979,16:137–45.
27. Newcomer AD, McGill DB, Thomas PJ, Hofmann AF. Prospective comparison of indirect methods for detecting lactase deficiency. N Engl J Med 1975,293:1232–6.
28. Metz G, Jenkins DJA, Peters JJ, Newman TJ, Blendis LM. Breath hydrogen as a diagnostic method for hypolactasia. Lancet 1976;1:1155–8.
29. Winer BJ. Multifactor experiments having repeated measurements of the same elements. In: Winer BJ, ed. Statistical principles in experimental design.

2nd ed. New York, NY: McGraw-Hill. 1971:514–603.
30. Barr RG. Limitations of the hydrogen breath test and other techniques for predicting incomplete lactose absorption. In: Paige DM, Bayless TM, eds. Lactose digestion: clinical and nutritional implications. Baltimore, MD: The Johns Hopkins University Press, 1981:110–14.
31. Bayless TM. Lactose malabsorption, milk intolerance, and symptom awareness in adults. In: Paige DM, Bayless TM, eds. Lactose digestion: clinical and nutritional implications. Baltimore, MD: The Johns Hopkins University Press, 1981:117–23.
32. Barbezat GO. The exocrine pancreas and protein-calorie malnutrition. S Afr Med J 1967;41:84.
33. Holemans K, Lambrechts A. Nitrogen metabolism and fat absorption in malnutrition and kwashiorkor. J Nutr 1955;56:477–94.
34. Gomez F, Ramos-Galvan R, Cravioto J, Frenk S, Vasquez Santaella J, de la Peña C. Fat absorption in chronic severe malnutrition in children. Lancet 1956;2:121–2.
35. Robinson W, Behar M, Viteri F, Arroyave G, Schrimshaw NS. Protein and fat balance in children recovering from kwashiorkor. J Trop Pediatr 1957;2:217–23.
36. Dutra de Oliveira JE, Rolando E. Fat absorption studies in malnourished children. Am J Clin Nutr 1964,15.287–92.
37. Underwood DA, Hashim SA, Sebrell WH. Fatty acid absorption and metabolism in PCM. I. Effect of fat-free and fat-containing diets on fecal fatty acids. Am J Clin Nutr 1967;20.226–32.
38. Viteri FE, Schneider RE. Gastrointestinal alterations in protein-calorie malnutrition. Med Clin North Am 1974,58:1487–1505.
39. Hansen JDL, Schendel HE, Wilkins JA, Brock JF. Nitrogen metabolism in children with kwashiorkor receiving milk and vegetable diets. Pediatrics 1960;25:258–82.
40. Levitt MD, Bond JH. Quantitative measurement of lactose absorption and colonic salvage of non-absorbed lactose: direct and indirect methods. In: Paige DM, Bayless TM, eds. lactose digestion: clinical and nutritional implications. Baltimore, MD: The Johns Hopkins University Press, 1981:80–90.
41. Phillips SF. Lactose malabsorption and gastrointestinal function: effects on gastrointestinal transit and the absorption of other nutrients. In: Paige DM, Bayless TM, eds. lactose digestion: clincial and nutritional implications. Baltimore, MD: The Johns Hopkins Press, 1981:51–7.
42. Bond JH, Levitt MD. Fate of soluble carbohydrates in the colon of rats and man. J Clin Invest 1976;57:1158–64.
43. Bond JH, Currier BE, Buchwald H, Levitt MD. Colonic conservation of malabsorbed carbohydrate. Gastroenterology 1980;78:444–7.
44. Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt JC Jr. Absorption of short chain fatty acids by the colon. Gastroenterology 1980,78:1500–7.
45. Solomons NW. The hydrogen breath test and gastrointestinal disorders. Compr Ther 1989,7:7–15.
46. Solomons NW. Diagnosis and screening techniques for lactose maldigestion: advantage of the hydrogen breath test. In: Paige DM, Bayless TM, eds. lactose digestion: clinical and nutritional implications. Baltimore, MD: The Johns Hopkins University Press, 1981.91–109.
47. Ziegler EE, Fomon SJ, Edwards BB, Nelson SE. Mineral absorption from soy-based and milk-based infant formulas. Am J Clin Nutr 1982;35: 823(abstr).
48. Forbes GB. Calcium accumulation by the human fetus. Pediatrics 1976;57.976–7.
49. Leitch I, Aitken FC. The estimation of calcium requirements: a re-examination. Nutr Abst Rev 1959;29:393–407.

# Evaluation of Arm Circumference as a Public Health Index of Protein Energy Malnutrition in Early Childhood

*by* MARIJKE I. VELZEBOER, Dr. PH, *Vice-President of Pueblo to People*
BEATRICE J. SELWYN, RN, ScD, *Assistant Professor of Epidemiology*
FREDERICK SARGENT II, MD, *Professor of Human Ecology*
ERNESTO POLLITT, PhD, *Professor of Nutrition and Behavioural Sciences and*
HERNAN DELGADO, MD, MPH, *Scientific Medical Officer, Division of Human Development*, INCAP

Arm circumference (AC) is an easy and inexpensive way to detect pre-school malnutrition and is, therefore, being used increasingly in lesser-developed nations for rapid and extensive nutrition surveillance and screening programs, as well as for monitoring nutrition rehabilitation (1–7).

It is the purpose of this paper to evaluate the AC measurement as a public health index of protein-energy malnutrition (PEM) as compared with more commonly used anthropometric measures like weight for height and weight for age. The literature is reviewed concerning these relationships, and in the second part of the paper, AC will be compared with weight for height and weight for age by using measurements made on 144 pre-school Guatamalan children.

The AC measure can detect a depletion in muscle tissue (8) and calorie stores, in the form of subcutaneous fat (9,10), and progress in growth (11,12). The AC of pre-school children, excluding infants, has been shown to increase very little with age (11,13) and to vary little between ethnic groups (10) Based on a large sample of healthy Polish children (14), Wolanski demonstrated that the AC of children aged 0 to 12 months increased rapidly, whereas it varied little between 12 and 60 months as well as between the sexes, and that from the second to fifth year fat tissue is replaced by muscle so that overall AC increases only 1.5 cm. Therefore, an age constant measurement of 16 5 cm has been recommended as a standard for use during this 4-year period when age is not known (15). Percentages of this standard have been applied to determine degrees of malnutrition and are as follows (5):

| *Nutritional Status* | *Percent of Standard* | *Arm Circumference* |
|---|---|---|
| Severe malnutrition | Less than 75% | Less than 12 5 cm |
| Moderate malnutrition | Between 75% and 85% | 12 5–13 5 cm |
| Normal | Over 85% | Over 13 5 cm |

Since most cases of malnutrition occur within this 4-year age span, AC seems to be a promising public health index for screening malnutrition

## Review of the Literature

The literature was reviewed concerning the relationship between AC and the more conventional anthropometric indices of malnutrition, e g , weight for height and weight for age. Table 1 gives correlation coefficients in ascending order for various studies in which AC was compared to weight for height, weight for age, and weight alone. Since the author's data concern only AC versus weight for height and weight for age, these two variables will be emphasized in the discussion.

Most of the correlation coefficients between AC and weight for height are similar All but four of the correlation coefficients between AC and weight for height lie in the 0 66 to 0 79 range. Even though the coefficient is low for the Colombian sample, Acciarri *et al.* (17) stated that AC was highly associated with acute malnutrition, as manifested by weight for height, and considered the relationship between weight for height and AC to be good They also believe the relationship between AC and weight for age to be good Likewise both coefficients are low in the Guatemalan study (17). The 0.55 coefficient for

*Please address correspondence to*:
Dr. B. J. Selwyn, The University of Texas Health Science Center, School of Public Health, Post Office Box 20186, Houston, Texas 77025

The work was done with the collaboration of the Division of Human Development of the Institute of Nutrition for Central America and Panama, Guatemala.

weight for height and AC derived by Ruthauser (19) for the 37 to 48-month old Nilotic population of Uganda also falls out of the range, although these coefficients are higher for the younger age groups of this same population. The opposite applies to the Indian population of Hyderabad studied by Visweswera and Singh (20) These investigators recorded a stronger relationship between the AC and weight for height of older children than for the 1 to 2-year age group.

The correlation coefficients for AC and weight for age are similar to those for AC and weight for height and fall in the 0 59 to 0.78 range The low correlation for the Egyptian children at first led El Lozy (23) to place little trust in AC. However, subsequent studies (32) convinced him of its validity. The data collected by Jelliffe and Jelliffe (24) during a nutritional study, where 19 per cent of all households in Barbados were sampled, also revealed a low correlation with weight for age (0.62). The investigators believed this was due to the marked layer of subcutaneous fat exhibited by a substantial number of children. Of children 12 to 59 months old, 47 3 per cent had triceps measurements 80 per cent of the standard, while 18 6 per cent had fatfolds of 100 per cent or more of standard.

The largest range of correlation coefficients reported (0 52 to 0 95) is for AC and weight alone (Table 1) The study done in the United States (31) is the highest of the coefficients, most likely because of the choice of study subjects. Weight alone is less acceptable as a measure of malnutrition in children and was not used in the present study.

Some concern has been expressed over the sensitivity of AC, or its ability to detect malnourished children with respect to weight for height and weight for age. Shakir (15) found that one of the major limitations to using AC was that it was not a very sensitive indicator of moderate or mild malnutrition as determined by weight for age. Based on data collected from 800 Colombian pre-school children, Acciarri *et al.* (33) observed AC to be more sensitive in detecting cases of acute, rather than chronic, malnutrition and in cases of severe, rather than mild, malnutrition. In their national probability samples

TABLE 1

*Studies correlating arm circumference of individuals (Loewenstein and Phillips,[16] updated by authors)*

| Country and (Investigators) | Location or Ethnic Group | No. | Age | Measurements | Correlation Coefficients |
|---|---|---|---|---|---|
| Colombia (Acciarri *et al*)[17] | Cali | 800 | 0 3 yrs | AC vs wt/ht | 0 47 |
| Guatemala (INCAP, unpublished)[18] | Patulul | 2,792 | 1 5 yrs | AC vs wt/ht | 0 53 |
| Uganda (Ruthauser)[19] | Nilotic | 23 | 37 48 mths | AC vs wt/ht | 0 55 |
| India (Viswesware and Singh)[20] | Hyderabad | Total 2,292 | 1 2 yrs | AC vs wt/ht | 0 58 |
| | | | 2 3 yrs | AC vs wt/ht | 0 60 |
| | | | 3 4 yrs | AC vs wt/ht | 0 67 |
| Nepal (Nichaman *et al*)[21] | National probability sample | | 0 5 yrs | AC vs wt/ht % of median | 0 67 |
| Uganda (Ruthauser)[19] | Bantu | 19 | 25 36 mths | AC vs wt/ht | 0 67 |
| | Nilohamatic | 34 | 37 48 mths | AC vs wt/ht | 0.70 |
| | Nilotic | 29 | 13-24 mths | AC vs wt/ht | 0 71 |
| Sri Lanka Nichaman *et al*)[21] | National propability sample | | 0-5 yrs | AC vs wt/ht % of median | 0 72 |
| Uganda (Ruthauser)[19] | Nilohamatic | 72 | 25 36 mths | AC vs wt/ht | 0 73 |
| | Bantu | 36 | 13 24 mths | AC vs wt/ht | 0 75 |
| | Nilohamatic | 85 | 13 24 mths | AC vs wt/ht | 0 76 |

TABLE 1 (*continued*)

| Country and (Investigators) | Location or Ethnic Group | No. | Age | Measurements | Correlation Coefficients |
|---|---|---|---|---|---|
| Togo (Nicholman *et al.*)[21] | National probability sample | | 0 5 yrs | AC vs wt/ht | 0.76 |
| Uganda (Ruthauser)[19] | Nilotic | 25 | 25-36 mths | AC vs wt/ht | 0.77 |
| Uganda (Rabinow and Jelliffe)[22] | Busoga | 156 | 2 33 mths | AC vs wt/ht | 0.79 |
| Guatemala (INCAP, unpublished)[18] | Patulul | 2,972 | 1 5 yrs | AC vs wt/age | 0.59 |
| Egypt (El Lozy)[23] | | | | AC vs wt as % normal | 0.59 |
| Colombia (Acciarri *et al.*)[17] | Cali | 800 | 0 6 yrs | AC vs wt/age | 0.62 |
| Barbados (Jelliffe and Jelliffe)[20] | 1 % House-hold survey | | 12-59 mths | AC vs wt/age | 0 62 |
| Uganda (Cool)[25] | Ankole | 70 | 36 47 mths | AC vs wt/age | 0.68 |
| | | 59 | 28 59 mths | AC vs wt/age | 0.70 |
| | | 77 | 12 23 mths | AC vs wt/age | 0.73 |
| | | 76 | 24 35 mths | AC vs wt/age | 0.78 |
| Tanzania (Kondakis)[26] | Kilimanjaro Region | 211 | 1 48 mths | AC vs wt | 0 52 |
| Malaysia (McKay)[27] | Alu Irangrance | 26 | 4.0 yrs | AC vs wt | 0 60 |
| India (Visweswara and Singhi)[29] | Hyderabad | Total 2,292 | 1 2 yrs | AC vs wt | 0.61 |
| | | | 2 3 yrs | AC vs wt | 0 65 |
| | | | 3 4 yrs | AC vs wt | 0.65 |
| Iran (Froozani)[28] | Estafahan | 40 | 13 24 mths | AC vs wt | 0 68 |
| Zambia (Blankhart)[24] | African | 975 | 6 36 mths | AC vs wt | 0.75 |
| Sierra Leone (Blankhart)[24] | African | 1,351 | 6 36 mths | AC vs wt | 0.75 |
| Malaysia (McKay)[27] | Alu Trangance | 54 | 3 0 yrs | AC vs wt | 0.78 |
| | | 41 | 2 0 yrs | AC vs wt | 0.79 |
| Lebanon (Kanawati *et al.*)[30] | Arab | 1,049 | 3 4% mths | AC vs wt | 0.79 |
| Uganda (Robinow and Jelliffe)[22] | Busoga | 156 | 35 36 mths | AC vs wt | 0.79 |
| Tanzania (Kondakis)[26] | Dodoma | 359 | 1 4% mths | AC vs wt | 0.82 |
| United States (Chowchatanavanich and Kanthavichitra)[31] | Negro and Puerto Rican | 316 | 1 72 mths | AC vs wt | 0.95 |

for Sri Lanka, Nepal, and Togo covering 18,751 pre-school children, Nichaman *et al.* (21) found the specificity of AC (ability to detect those who do not have malnutrition) to be high, but the sensitivity (ability to detect those who do have the disease) to be low and to vary with each age group In this study the median weight for height for each population was used as the criterion for comparison.

Zeitlin (34) described the degree of sensitivity of AC versus weight for age by diagrammatically demonstrating that Filipino cases of malnutrition by weight for age and AC did not completely overlap She discovered, however, that those children shown to be malnourished by weight for age and not by AC were above the critical 16 24 months age period, that their weight for age did not exceed the Harvard third percentile for weight for age, and that weight for age did not differ for the non-overlapping pairs. Thus, her results indicated that 'younger children are selected by AC at an earlier, more easily reversible stage of malnutrition, when weight has fallen off, but length is more normal' (34, p 303).

Margo (35) is more directly critical of the measurement He maintains that, if AC is indeed a valid measurement, the difference between cases detected by the AC or weight for age measurement should be more or less constant for all studies When reviewing the literature, he found this not to be the case (Table 2). Shakir and Morley (5) identified only 3 per cent of their study population as normally AC and below 80 per cent of the Harvard standard (false negative), and 20 per cent as below 85 per cent of the standard for AC and normal for weight (false positive) Cook (25) and Margo (35), on the other hand, encountered much higher false negative rates (13 5 per cent and 70 2 per cent, respectively) in their African pre-school study populations. The discrepancies between results obtained from various studies are summarized in Table 2, and illustrate Margo's skepticism toward the reliability of AC as a surveillance tool The false negative and positive rates for AC versus weight for age based on data collected from 3,801 pre-school Ladino children of migrant workers in Patulul, Guatemala, were 48 9 per cent and 24 3 per cent, respectively (18) These rates were 6 7 per cent and 71 2 per cent when weight for height was used as a criterion

Unfortunately, studies such as these are limited, and their varied results have led to the confusion and skepticism of many nutrition-related workers who might have been able to profit from the use of the AC measurement in field surveillance programs. It is hoped that the increasing amount of work done in this area, using standard techniques, will illuminate the trade-offs of this economically efficient tool for malnutrition detection.

## Materials and Methods

In an effort to develop methodology for rural health systems, the Division of Human Development of the Institute for Nutrition for Central America and Panama (INCAP) has undertaken a project in four Indian villages on the shores of Lake Atitlan in the Department of Solola, Guatemala Since the project employs minimally trained health promoters to work in the community, AC was proposed by the investigator as a simple and inexpensive tool with which to perform continual screening for malnutrition in pre-school children In order to support this choice in this setting, the investigator collected and analyzed relevant data from one village, San Pablo La Laguna. Height, weight, and AC measurements were taken by

TABLE 2

*Studies using arm circumference compared to weight for age*[35]

| Country and (Investigators) | Ethnic Group | Number | Age Months | False Positive | False Negative |
|---|---|---|---|---|---|
| Bagdad, Iraq (Morley and Shakir)[5] | Arab | 777 | 13 72 | 20 | 3 |
| Uganda (Cook)[25] | Ankole | 282 | 12 60 | 21 | 13 5 |
| South Africa (Margo)[35] | Non-white | 621 | 13-60 | 1 3 | 70 2 |
| Sierra Leone (Blankhart)[29] | African | 544 | 7 36 | 16[a]<br>24[b] | 5[a] |
| Guatemala (INCAP)[18] | Guatemalan | 2,801 | 12 60 | 24 3[c] | 48 9[d] |

[a]Figures obtained using 12 0cm cut-off point for malnutrition
[b]Figures obtained using 12 5cm cut-off point for malnutrition
[c]Unpublished data added to table by authors
[d]Figures obtained using 13 5cm cut-off point for malnutrition

the investigator for 144 children, aged 0 through 5 years, who attended the health post voluntarily. Age data were solicited from the mother by the health promoters in Indian dialect and the investigator recorded the data. The children included in the study were believed to be chronically malnourished, as exemplified by their low weight for age (75 per cent of the Harvard standard) and normal weight for height (98 per cent of the standard) (17,36). Although no cross-sectional data exist on the nutritional status of the pre-school population of San Pablo, it is the investigator's opinion, based on the health personnel's experience in the community, that the attenders were somewhat healthier than those who did not attend.

Weight for age and weight for height were chosen as the criteria with which to compare AC, since these are the most commonly used field measures of malnutrition. For the analysis, the Wolanski standards were used for AC, and the Harvard standards were selected because they are the most universally applied and, therefore, lend themselves to international comparison.

The cut-off point for malnutrition used for AC was 13.5 cm. Children were categorized as malnourished if their weight for height and weight for age were 90 per cent and 80 per cent or less, respectively, of the Harvard standards. These cut-off points are commonly used in field surveys (17,19,37) and are recommended by Jelliffe (14). Because it is generally believed that environmental rather than genetic factors influence the size of a young child (38), the Harvard and Wolanski standards may and have been applied to ethnically different populations.

[illegible] manufactured child-meter for height, and a strong plastic measuring tape for AC. All shoes, hair ornaments, and extraneous objects were removed before weighing and measuring. Since it was culturally unacceptable to remove clothes, an estimation of the weight of clothes was used to correct the body weight. The AC measurement was taken at the midpoint of the upper arm between the acromion and olecranon. This point was visually located, a method which has been shown to be quicker and as reliable as locating the midpoint by measurement (24,30,39). All measuring equipment was calibrated daily.

## Results

*Correlation Analysis*

In order to study the relationships between the various measures, correlation analyses were performed for weight for age, weight for height, AC, and AC for age measurements. Age was correlated with all those measures. The results are presented in Table 3 and show that AC was the most highly correlated with weight for height (0.7911). The correlation of AC with weight for age was somewhat lower (0.7127). There is a very strong correlation between AC and AC for age (0.9683) and the relationships of each of these variables to weight for age and weight for height are similar (0.7127 and 0.7911, 0.7573 and 0.7416, respectively). Age was shown to have some relationship with both AC (0.5132) and weight for height (0.3234). Although these coefficients are low, they are significant.

*Sensitivity and Specificity Analysis*

In order to determine how well AC was able to identify groups of malnourished children as defined by a single cut-off point, cross tabulations were made between cases detected by AC and those categorized by weight for height and weight for age as presented in Tables 4 and 5, respectively. Only children 12 through 60 months (n = 109) were used for this analysis, since AC cut-off points based on the con- [illegible] this age group (5,15).

*Weight for Height*

When compared with weight for height, AC had a specificity of 65 per cent and a sensitivity of 90 per cent, with false positive and negative rates of 35 per cent and 10 per cent, respectively (Table 4). The false negative group (those categorized as malnourished by weight for height but not by AC) was made up of only

TABLE 3

*Correlations of anthropometric measures taken in health post children 12 to 60 months (N = 144)*

| | Weight/Age | Weight/Height | AC | AC/Age | Age |
|---|---|---|---|---|---|
| Weight Age | 1.0 | | | | |
| Weight Height | 0.6953 | 1.0 | | | |
| AC | 0.7127 | 0.7911 | 1.0 | | |
| AC/Age | 0.7573 | 0.7416 | 0.9683 | 1.0 | |
| Age | 0.11788* | 0.3234 | 0.5132 | 0.3006 | 1.0 |

* Not significant ($p > 0.05$); all other numbers significant ($p < 0.001$).
AC = Arm Circumference.

TABLE 4

*False negative, false positive, sensitivity and specificity rates for AC compared to weight for height for 109 children 12 to 60 months measured in the health post*

| | Weight for Height | | |
|---|---|---|---|
| AC | Normal >90% Harvard Standard | Malnourished ≤90% Harvard Standard | Total |
| Normal >13.5 cm | True negatives = 58<br>Specificity = 65% | False negatives = 2<br>10% | 60 |
| Malnourished ≤13.5 cm | False positives = 31<br>35% | True positives = 18<br>Sensitivity = 90% | 49 |
| Total | 89 | 20 | 109 |

AC = Arm Circumference

TABLE 5

*False negative, false positive, sensitivity and specificity rates for AC compared to weight for age for 109 children 12 to 60 months measured in the health post*

| | Weight for Height | | |
|---|---|---|---|
| AC | Normal >80% Harvard Standard | Malnourished ≤80% Harvard Standard | Total |
| Normal >13.5 cm | True negatives = 29<br>Specificity = 91% | False negatives = 31<br>40% | 60 |
| Malnourished ≤13.5 cm | False positives = 3<br>9% | True positives = 46<br>Sensitivity = 60% | 49 |
| Total | 32 | 77 | 109 |

AC = Arm Circumference

two cases, one of which was borderline for weight for height (89 per cent of standard), and thus, not severely malnourished

The percentage of false positive cases (those identified as malnourished by AC and not by weight for height) was high (35 per cent). Fifty-two per cent of these children had ages in the critical 6-24 month age period,* and a total of 73 per cent were under three years old. All children categorized as false positive were malnourished by weight for age. All but two cases were shown to be moderately malnourished by AC.**

The predictive value of a normal AC was 97 per cent, which means that of all those cases declared normal by AC, 97 per cent really are normal when weight for height was used as a criterion. The predictive value of a deficient AC, however, was considerably lower (37 per cent).

*Weight for Age*

As seen in Table 5 the sensitivity and specificity rates of AC, when weight for age is the criterion, are 60 per cent and 97 per cent, and the false negative and positive rates are 40 per cent and 9 per cent, respectively.

* Children younger than two years are especially vulnerable to malnutrition in developing countries where food intake is low and sanitation below optimum. During the first two critical years a child utilizes one-third of his food for growth as opposed to 1 per cent for older children (40), he is more susceptible to disease and is not yet eating an adult diet. For these reasons most serious cases of malnutrition occur during this age.

** The two cases were a set of twins who showed classic symptoms of marasmus and were very underweight for their 14-month age (44 per cent and 49 per cent, respectively). Their AC was also very low (10.1 cm) although they had normal weight for heights (93 per cent), which was due to the excessive degree of stunting which had already affected them. The only child in the measured population whom the investigator classified as having symptoms of kwashiorkor also had a normal weight for height (93 per cent). This child's AC, on the other hand, was 12.9 cm and her weight for age only 69 per cent of the standard.

The false negative percentage is very high and cause for concern, since these children would not be detected by AC and hence not referred to the health post for confirmation Therefore, these cases were reviewed to gain insight into their characteristics The mean age of these children was 44 months, only four (13 per cent) were younger than 30 months In other words, most of them were past the critical age period. Moreover, of the four under 30 months, all but one had a weight for age adequacy between 75 per cent and 80 per cent of the standard (degree 1, Gomez) The one exception was a child of 28 months. All of the 43 false negative children had a normal weight for height percentage (above 90 per cent) with a mean of 103 per cent. This group was generally very stunted with a mean height of 82 per cent of the standard, which caused them to be categorized as malnourished by weight for age and normal by weight for height and AC.

With respect to weight for height, the predictive value of a normal AC was only 48 per cent, whereas that of a deficient AC was 94 per cent This trend is the opposite of that shown by AC when it was compared to weight for height

*AC vs. Weight for Height vs Weight for Age*

The ability of each of these measures to identify cases of malnutrition with respect to one another is illustrated in the Venn diagram (Figure 1) Of all the 109 cases measured, none was categorized as being malnourished by weight for height alone. Only 3 (2 8 per cent) were so categorized by AC, as compared to 29 (26 6 per cent) for weight for age All cases identified as being malnourished by weight for height (20 = 18 per cent) were malnourished by weight for age and 18 (90 per cent) of those had a low AC Of the 77 categorized as malnourished by weight for age, less than one-fifth (16 per cent) had weight for height measures below 90 per cent of the standard, whereas 60 per cent (46) had an AC measure of 13 5cm or below Most of the 49 cases with low AC had a low weight for age (94 per cent), whereas for weight for height this was true for only 37 per cent (18) of the cases Weight for age classified 71 per cent of the children as malnourished, which is 37 per cent more than AC (45 per cent) and 75 per cent more than weight for height (18 per cent)

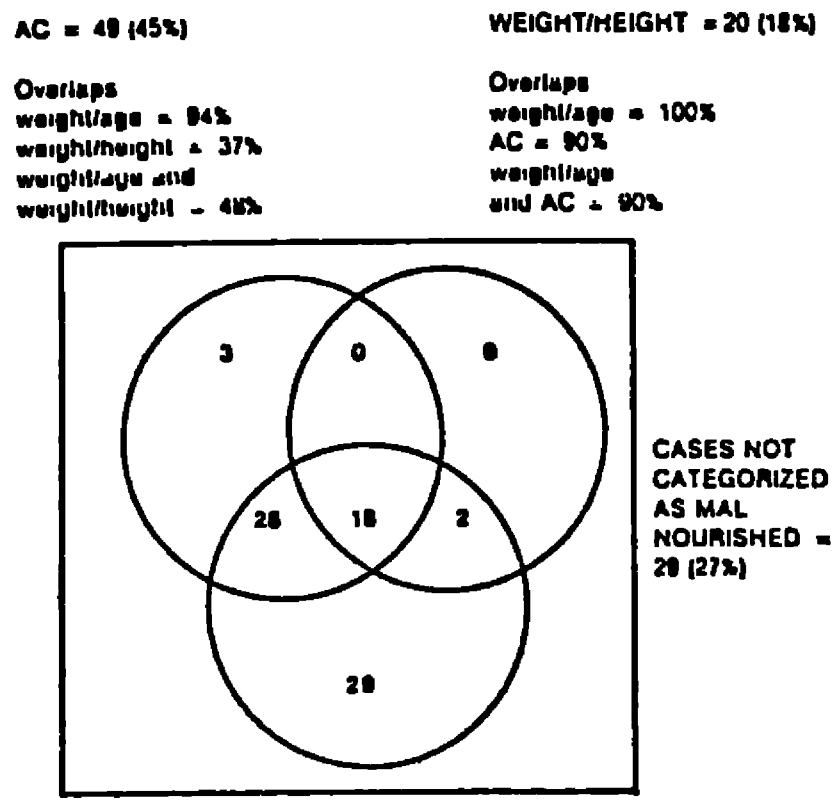



Fig. 1 Venn diagram illustrating relationship of ability of arm circumference, weight/height, and weight/age to detect cases of malnutrition (n = 109) Children 12 to 60 months measured in health post

## Discussion

The analysis demonstrated that there was a strong correlation between AC and weight for height, as well as weight for age The coefficient between AC and weight for height (0 79) was higher than any encountered in the literature reviewed although most of the correlation coefficients derived by Ruthausen (19) for her Ugandan population and all of those described by Nichaman *et al* (21) lie within the 0 67 to 0 76 range. It is interesting, however, that the correlation between the measures taken for Latin American populations is consistently lower (17,25) compared to all studies reviewed Unfortunately, more publications on Latin American populations were not available for review, so that no conclusions can be made concerning correlations within these populations It is, however, very unlikely that they are so ethnically different from those populations reviewed that the relationships between their anthropometric measurement could vary to such a degree (38) This discrepancy is also found when AC was correlated with weight for age The 0 71 correlation derived for the Guatemalan study population of San Pablo, however, is within the range of those found by Cook (25) for a pre-school Ugandan population The San Pablo coefficient also agrees with those listed for AC versus weight in all but one (26) of the studies reviewed

The high correlation coefficient for AC and AC for age and similar correlations with weight for age and weight for height indicate that either measure may be used in the detection of malnutrition among young children The 0 5132 correlation coefficient for AC

and age demonstrates, however, that there is a relationship between these two variables and that AC, therefore, is not entirely age independent for the San Pablo population Martorell *et al* (41) also discovered a relationship between age and AC for 1,240 Ladino Guatemalan pre-school children Whereas these children showed an increase of 38 per cent in the AC during the first 12 months of life, which is in keeping with the 31 per cent increase found in the standard population of Wolanski, the increase for the next 48 months was nearly twice as high for the Guatemalan population (15 per cent) as that encountered for the Polish children of the standard (8 per cent) * It must be kept in mind, however, that both Guatemalan populations have been subjected to some degree of malnutrition which may in itself vary with age (35,40) Various studies (42-44) concerning the AC of healthy standard populations have shown results similar to those of Wolanski If, however, AC were dependent on age, the measure would detect more younger children, which is to their advantage, for they are at a higher risk of malnutrition (4,30)

AC was also shown to identify 90 per cent of the cases categorized as malnourished by weight for height The measure did not perform as well when weight for age was used as the criterion for malnutrition. The Venn diagram, however, demonstrates that weight for height and weight for age do not measure the same thing, the former being an indicator of acute malnutrition and the latter of cumulative episodes of malnutrition (33,45) While all children with low weight for height were also shown to be malnourished by weight for age, the reverse did not apply This is not surprising considering that the study population was chronically malnourished as demonstrated by their low mean weight for age (75 per cent) and height for age (85 per cent) and normal weight for height (98 per cent). In fact, 71 per cent of all the children had weights below 85 per cent of the standard for their ages, and of all the children classified as malnourished by any of the three measures, over one-third had only weight for age deficiencies, as compared to zero for weight for height, and 3 per cent for AC. Only one-fifth of the total population had low weights compared to their standard heights. Even though only 37 per cent of the children with low AC had a low weight for height, 94 per cent of them had a low weight for age Thus, whereas AC with respect to weight for height has a high false positive rate (35 per cent) and low false negative rate (10 per cent), these values are reversed (9 per cent and 40 per cent, respectively) when weight for age is the criterion

* Martorell *et al* (41) also found AC to be sex dependent However, the difference between the AC measurement for the Wolanski males and females (0 51 cm for 0 11 months, 0 31 cm for 12 60 months) is consistently larger than those between the Guatemalan sexes (0 125 and 0 23 cm, respectively)

The cross-tabulation analysis points out, however, that these children identified as being malnourished by AC, but not by weight for height (false positives), may have been at risk of malnutrition They were mostly younger and had low weights for age In a sytem where AC is used to screen and refer cases to a health post for confirmation by weight for height, the false positives would have been attended Given the critical age of these children, attendance is preferred, especially since the assessment by weight for age classified the 31 false positives as malnourished Shakir (5) agrees that this 'misclassification is in favor of the child, and could be useful in crisis situations, for children in this critical age interval' (p 665).

The false negatives, on the other hand, would have been lost to any kind of follow up AC would have failed to screen 40 per cent of the children for confirmation by weight for age Even though the number is large, these children were probably not at a high risk of being severely malnourished, for most of them were beyond the critical age range and all of them had normal weight for heights. The generally stunted nature of this group was most likely responsible for this misclassification.

Similar results concerning classification of children according to AC with respect to weight for height and weight for age were found in the Patulul population of Guatemala (18) The resulting false negative rates (6 7 per cent and 48.9 per cent, respectively) corresponded closely to those counted in San Pablo. Both false positive rates (71 2 per cent and 4 3 per cent), however, were substantially higher. As in the San Pablo study, the Patulul data showed a low false negative rate and high false positive rate when AC was compared to weight for height, while the reverse was true when weight for age was used as the criterion. With the exception of his own investigation of 621 non-white African children, all other studies Margo (35) reviewed (Table 5) had much higher sensitivities (85 per cent to 97 per cent) for AC with respect to weight for age than were encountered in either Guatemalan population

The San Pablo data suggest that AC may have been a more sensitive indicator of malnutrition among younger age groups when compared to either weight for height or weight for age Zeitlin's (34) findings for Filipino cases of malnutrition support this view She found that AC and thigh circumference selected younger children who had experienced weight loss, but who were not yet affected by nutritional stunting Cook (30) also found the correlation between weight for age and AC for age to be higher for children less than 36 months old, although the highest coefficient was found with the 24 to 36-month age group

## Conclusion

These findings have definite implications for nutritional screening and prevention programs Even

though it would be ideal to identify and intervene in all cases of malnutrition, in poor areas with high rates of malnutrition resources simply would not allow it. When compared to weight for age, AC selected a younger and more severely malnourished group of cases that was smaller in number and hence more feasible to treat. AC successfully detected children with acute malnutrition as defined by weight for height, who are the children with the greatest risk of deteriorating nutritional status and even death (46). Moreover, AC also performed well in identifying severely malnourished children whose stunted condition made their weight appear normal for their height.

The results of the study presented herein suggest that in areas where malnutrition affects young children and where nutritional stunting is common, AC is a useful indicator of malnutrition. AC has been shown to be a good surrogate for the more commonly used weight for height and weight for age. Therefore, it is recommended that AC be adopted by nutrition screening and surveillance programs in poor areas of the developing world where severe malnutrition is prevalent and resources limited.

## References


1. Sastry JC, Vijayaranghavan K. Use of anthropometry in grading malnutrition in children. Indian J Med Research 1973; 61:1224 32.
2. Naik PA, Zopf TE, Kakar DN, Singh M, Cood SK. Anthropometric profile of the pre-school child of Punjab. Indian Pediat 1976; 13.919 28
3. Bennett JF. A screening test in emergency child feeding in Kivu, Congo. J Trop Pediat 1969; 15:238 41.
4. McDowell J. The Kayunga Nutrition Scout Project (unpublished paper), 1977.
5. Shakir A, Morley D. Measuring malnutrition. Lancet 1974; 1:758·59.
6. Blankhart D, Latham MC, Schulpen TW. Low arm circumference reporting and nutrition rehabilitation of under fives. Env Child Health 1977; 23:8 11.
7. Ghosh SM, Yayathi T. Quick nutritional screening by mid arm circumference or a bangle· Indian Pediat 1976; 13:915 18.
8. Cheek DB, Habicht JP, Berrall J, Holt AB. Protein calorie malnutrition and the significance of cell mass relative to body weight. Am J Clin Nutr 1977; 30:851 60.
9. Gurney JM. Field experience in Obeokuta, Nigeria: with special reference to differentiating protein and calorie reserves. J Trop Pediat 1969; 15:225 33.
10. Jelliffe DB. The arm circumference as a public health index of protein calorie malnutrition of early childhood. J Trop Pediat 1969; 15:179 89 and 253 59.
11. Jelliffe DB. Field anthropometry independent of precise age. J Pediat 1969; 75:334
12. Fisher MM. Growth in Zambian children Trans R Soc Trop Med Hyg 1976; 70.426 32.
13. Burgess HJL, Burgess AP. A modified standard for mid upper arm circumference in young children. J Trop Pediat 1969; 15:189 93.
14. Jelliffe DB. The assessment of the nutritional status of the community. WHO Monograph Series, No. 53, 1966.
15. Shakir A. Arm circumference in the surveillance of protein·calorie malnutrition in Baghdad. Am J Clin Nutr 1975; 28:661 65.
16. Luewenstein MS, Phillips JF. Evaluation of arm circumference measurements for determining nutritional status of children and its use in an acute epidemic of malnutrition. Owerri, Nigeria, following the Nigerian Civil War. Am J Clin Nutr 1973; 26:226 33.
17. Acciari G, Eckroad JC, Fajardo LF, *et al.* Comparative analysis of some anthropometric measurements. Arch Latin Am de Nutr. 1977; 27:259 75.
18. Division of Human Development (DDH) of the Institute of Nutrition for Central America and Panama (INCAP). Unpublished data, 1979.
19. Ruthhauser HE. Correlations of the circumference of the mid upper arm with weight and weight for height in three groups in Uganda. J Trop Pediat 1969; 15:196 98.
20. Visweswara Rao K, Singh D. An evaluation of the relation between nutritional status and anthropometric measurements. Am J Clin Nutr 1970; 23:83.
21. Nichaman MA, Lane MH, Miller CD, Brink EW, Stachling N. Comparison of weight for height and arm circumference variables as anthropometric indicators. The Am Soc for Clin Nutr-- 18th Annual Meeting, San Francisco, April 1978.
22. Robinow M, Jelliffe DB. Intercorrelations between anthropometric variables. A contribution to nutritional anthropometry of infancy and early childhood in developing countries. Proceedings of VII International Congress of Nutrition, Hamburg, 1966; 4:144.
23. El Lozy M. A modification of Wolanski's standards for the arm circumference. J Trop Pediat 1969; 15:195·96.
24. Jelliffe DB, Jelliffe EFP. Experience in the Caribbean. J Trop Pediat 1969; 15:209 13.
25. Cook R. The arm circumference in a field survey in Ankole, Uganda. J Trop Pediat 1969; 198·201.
26. Kondakis XG. Field surveys in North Greece and Dodema, Tanzania. J Trop Pediat 1969; 15:201 205.
27. McKay D. Experience with the mid arm circumference as a nutritional indicator in field surveys in Malaysia. J Trop Pediat 1969; 15:213 17.
28. Froozani MD. Weight, height and arm circumference of a group of low income Esfahan children. J Trop Pediat 1978; 24:117 19.
29. Blankhart D. Experience in Sierra Leone and Gambia. J Trop Pediat 1969; 15:205 09.
30. Kanawati AA, Haddad N, McClaren DS. Preliminary results with mid arm and mid arm muscle circumference used as nutritional screening procedures for pre-school children in Lebanon. J Trop Pediat 1969; 15:233 38.
31. Chhoovivanathanavich P, Kanthavichitra N. Arm circumference in children. Lancet 1970; 1:44.
32. El Lozy M. Measuring malnutrition. Lancet 1974; 2:175.
33. Acciarri G, Eckroad J, Fajardo L, *et al.* Screening for malnutrition with arm circumference. Arch Latin Am de Nutr 1977; 27:343·58.
34. Zeitlin MF. Breast-feeding and growth status in two pre-school child populations; reference standards for maximum thigh circumference from 0 5 years; thigh circumference- an age independent screening index for pre-school nutrition interventions. Doctoral thesis, Massachusetts Institute of Technology, 1977.

35. Margo G. Assessing malnutrition with the mid arm circumference. Am J Clin Nutr 1977; 30:835 37.
36. Seoane N, Latham MC. Nutrition anthropometry in the identification of malnutrition in childhood. J Trop Pediat 1971; 17:98 104.
37. Beghin ID. Assessment of the effectiveness of a nutrition rehabilitation center at Fond Parisien, Haiti. J Trop Pediat 1969; 15:248 51.
38. Martorell R, Habicht JP, Yarbrough C, Guzman G, Klein RE. Identification and evaluation of measurement variability in the anthropometry of preschool children. Am J Phys Anthrop 1976; 43:347 52.
39. Bray GA. Use of anthropometric measurements to assess weight loss. Am J Clin Nutr 1978; 31:769 73.
40. Forman SJ. Infant nutrition. 2nd ed. Philadelphia: Saunders, 1974; 34 38.
41. Martorell R, Yarbrough C, Lechtig A, Delgado H, Klein RE. Upper arm anthropometric indicators of nutritional status. Am J Clin Nutr 1976; 29:46 53.
42. Robinow M, Jelliffe DB. The use of arm circumference in a field survey of early-childhood. J Trop Pediat 1969; 15:217 22.
43. Karlberg P, Engstrom I, Lichtenstein H, Svenuburg, I. Growth of Swedish children up to 7 years of age. Acta Pediat Scand Suppl 1967; 177:74 5.
44. Tanner JM (Quoted by Burgess and Burgess) J Trop Pediat 1969; 15:189.
45. Cravioto J, De Licardie ER, Birch HC. Nutrition, growth and neuro integrative development. Pediatrics 1966; 38: suppl.
46. Sommer A, Lowenstein MS. Nutritional status and mortality; a prospective validation of the QUAC stick. Am J Clin Nutr 1975; 28:287 92.