Appraisal of the Effect of Nutrition on Biochemical Maturation

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ANTHROPOMETRIC studies have revealed that in both rural and urban communities of underdeveloped countries growth rates of infants during the first five months of life do not differ from those of babies born in countries which are highly developed technologically. From four months of age on, however, the growth rate diminishes progressively, and this deviation from the normal attains its maximum between eighteen and twenty-four months of age. After this, the growth rate increases again, although neither the magnitude nor the duration of the better growth rate is sufficient to enable these children to reach their normal values. At adolescence, therefore, children in the underdeveloped areas are both lighter in weight and shorter in stature than are children of the same age and ethnic group in the more developed areas.[1,2] Preliminary data from cross sectional studies have also shown that this deceleration of somatic growth seems to be accompanied by mental retardation. Inverse correlations between height and/or weight deficit and intelligence have been described.[3–6]

A study of the dietary records of these children is helpful in understanding these findings. It has been shown[7] that beyond the age of four to six months human milk alone is insufficient to maintain an adequate growth rate. More than 50 per cent of the infants living in underdeveloped areas, however, receive no supplementary foods because their parents do not believe that they are needed or consider them unsuitable for the child. When food other than human milk is given to the infant, a gastrointestinal infection usually develops and the parents attribute this to the food itself rather than to the poor sanitary conditions under which it was prepared. Thus, as malnutrition progresses, the infant becomes increasingly susceptible to environmental stresses, until it finally presents the full blown picture of protein-calorie malnutrition. Children suffering from protein malnutrition show two types of signs: (1) those that are universal and occur regardless of the geographic area, ethnic group, specific composition of the protein of the diet or clinical type of the disease, and all of which are associated with deceleration of growth and development; and (2) those called incidental signs which probably result from associated deficiencies and vary with age, skin and hair color, and environmental factors.[8] Regardless then of the type and number of incidental signs present in a given subject, an essential feature of malnutrition is deceleration of growth and development.

CERTAIN EXAMPLES OF ARRESTED BIOCHEMICAL GROWTH IN ANIMALS

During the last decade, reports of arrested biochemical maturation in animals as a result of protein-calorie malnutrition have appeared in the literature. Among the most interesting reports are those of experiments made with pigs that have been fed kwashiorkor-producing diets. Durbin and co-workers[9] first found that glucose-6-phosphatase activity in the liver of an eleven day old pig was negligible; they next demonstrated that glucose-6-phosphatase activity did not develop at all in the liver of pigs

From the Institute of Nutrition of Central America and Panama (INCAP) Guatemala, C. A. INCAP Publication I-244.

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Presented at the Symposium on Recent Advances in the Appraisal of the Nutrient Intake and the Nutritional Status of Man at the Massachusetts Institute of Technology, Cambridge, Massachusetts, on March 6 and 7, 1962, under the sponsorship of The National Vitamin Foundation, Inc., New York, New York.

TABLE I
Glucose-6-Phosphatase Activity in the Liver of Pigs of Various Ages, Fed on Normal or Protein-Deficient Diets[9]

Diet	No. of Animals	Age (days)	Glucose-6-Phosphatase (M of phosphorus/min./100 gm.)
Normal (ad libitum)	1	11	0.1*
Normal (ad libitum)	1	20	5.8
Normal (ad libitum)	7	26–81	4.6
Low protein (250 gm./day)	3	63–109	0
Low protein (250 gm./day plus 75 gm. carbohydrates)	3	64–102	0.54*

* These figures are at the limits of sensitivity of the method and indicate little or no glucose-6-phosphatase activity.

raised for eleven days on a 5 per cent vegetable mixture or on a 5 per cent vegetable mixture plus additional carbohydrates consumed between meals. In contrast, glucose-6-phosphatase activity developed normally in animals fed normal diets or the vegetable mixture plus 5 per cent casein.

Young pigs normally have prolonged hypoglycemia following an intravenous injection of insulin in a dose of 0.1 I.U. per kg. of body weight; they also have a low rate of glucose utilization (0.4 per kg. given intravenously). As the animals grow older, the response to insulin and the rate of carbohydrate utilization changes if they have been fed a normal diet, but these changes are absent in pigs fed low protein diets[10] (Fig. 1 and 2).

In most cases, measurements of free amino acid concentrations in blood plasma of children affected with kwashiorkor show an abnormally high ratio of phenylalanine to tyrosine.[11] A similar finding in urine, previously reported by Cheung et al.,[12] suggests the possibility of a defect in the enzyme system that converts phenylalanine into tyrosine. Using protein-deficient rats as experimental animals, this hypothesis was tested; the preliminary results (Table II) are compatible with the idea of ar-

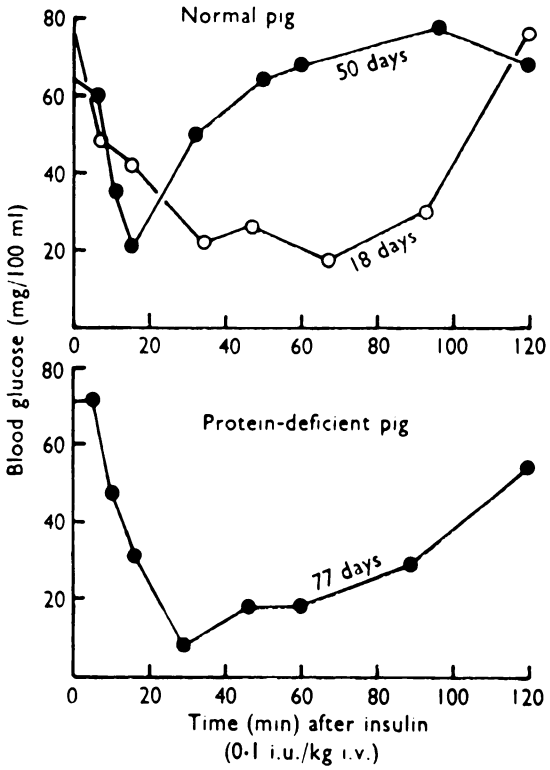

FIG. 1. From HEARD, C. R. C., DURBIN, P. A. J. and PLATT, B. S. *Proc. Nutr. Soc.*, 20 (2): xx, 1961.[10]

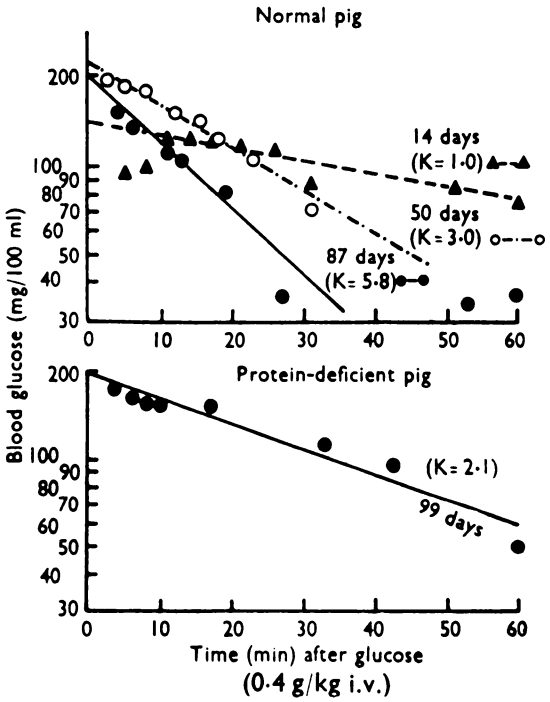

FIG. 2. From HEARD, C. R. C., DURBIN, P. A. J. and PLATT, B. S. *Proc. Nutr. Soc.*, 20 (2): xx, 1961.[10]

TABLE II
Phenylalanine Hydroxylase in Liver of Malnourished Rats

μM of Tyrosine Formed per gm. of Liver	μM Formed in Total Liver	Weight of Animals (gm.)	Liver Weight (gm.)
Control Animals at Twenty-One Days of Life			
0.350	1.05	30	1.27
Control Animals at Thirty-Four Days of Age			
2.13	9.8	93.3	4.6
1.42	5.4	77.1	3.8
1.56	5.8	84.3	3.7
2.26	9.5	83.0	4.2
1.12	5.0	82.0	4.4
2.67	11.5	81.5	4.3
Experimental Animals at Thirty-Four Days of Age			
0.375	0.7	46.9	2.0
1.015	2.03	45.4	2.0
0.513	0.98	43.5	1.9
0.330	0.50	38.3	1.5
0.032	0.06	39.5	1.7
0	0	26.2	1.7

rested biochemical maturation of the phenylalanine-hydroxilase system.

These findings corroborate the results published in 1957 by Ross and Batt.[13] They showed that in the rat the activity of several enzymes in the liver depends upon both age and diet, and that appropriate adjustments of the diet can produce the enzyme pattern characteristic of any given age.

Research on tissue enzymes in human nutrition has been primarily concerned either with the activity of some systems related to the metabolism of specific nutrients or with more complex systems whose activity depends upon the integrity of the mitochondria—Krebs cycle oxidation, oxidative phosphorization and phosphatide system. Waterlow[14] has stated that the results obtained so far are not very illuminating, but these studies have helped to substantiate the concept that protein malnutrition distorts the body pattern and produces changes not only in its gross proportions—the relative size of different organs, the relative amounts of different types of tissue-structure

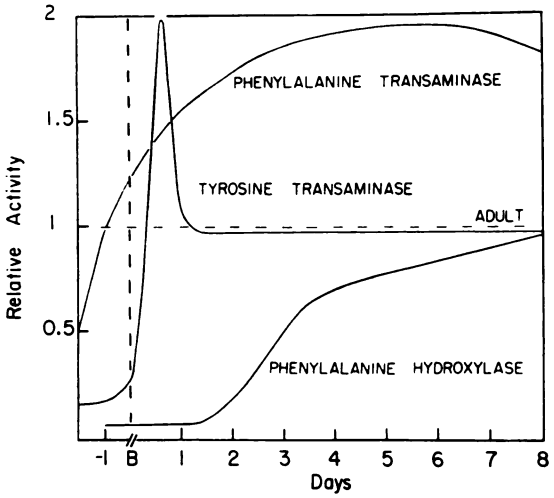

FIG. 3. Changes in activity of phenylalanine transaminase, tyrosine transaminase and phenylalanine hydroxylase in the liver of the rat immediately before and after birth (B). Activity in the liver of the adult is arbitrarily defined as 1 unit. Tyrosine transaminase has exceedingly little activity before birth, but the activity increases sharply at the age of two hours, reaches that of the adult by twelve hours and twice that of the adult by sixteen hours, quickly returning to normal adult levels within the next twelve hours. Activity of phenylalanine hydroxylase in the liver reaches that of the adult at about age eight days. Activity of phenylalanine transaminase is greater in the liver of the newborn than in that of the adult until the rat reaches age thirty days. From KRETCHMER, N. *Pediatrics*, 23: 606, 1959.[15]

and parenchymatous in an organ—but also in the relative amounts of different proteins within the cell.

Recently, Kretchmer[15] has reported that by determining the activity of three enzymes (phenylalanine-transaminase, tyrosine-transaminase and phenylalanine-hydroxilase) it is possible to obtain a measure of the biochemical maturation of the rat during its first weeks of life. The development of these systems apparently follows the same pattern in human liver (Fig. 3).

Studies on the cause of hyperbilirubinemia in the newborn have thrown light on the development of enzyme systems involved in glucoronidation. Brown and Zuelzer have shown[16] that glucoronyl-transferase activity is virtually absent in the liver of the fetus and is markedly reduced in that of the newborn. During the first fifteen to twenty days of postnatal life, an approximately fivefold in-

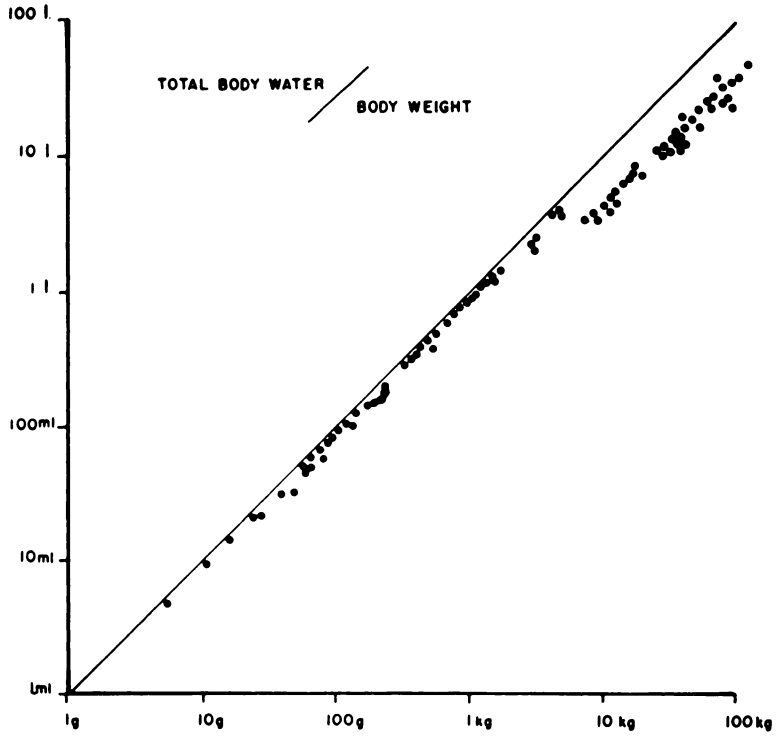

FIG. 4. Changes in body water compartment during growth. From FRIIS-HANSEN, B. *Helvet. Paediat. acta*, 10: 12, 1955.

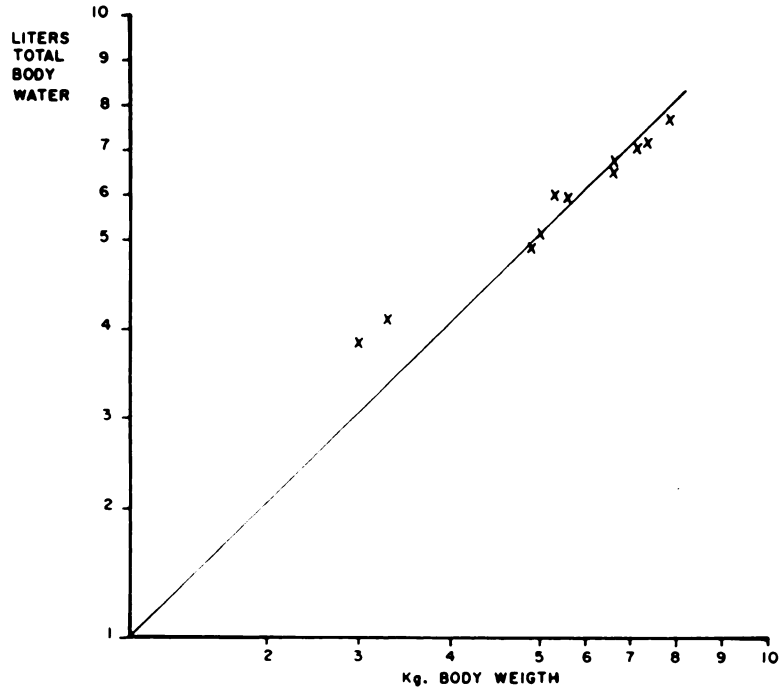

FIG. 5. Relationship between total body water and body weight. Data taken from SMITH, R. *Clin. Sc.*, 19: 275, 1960.[17]

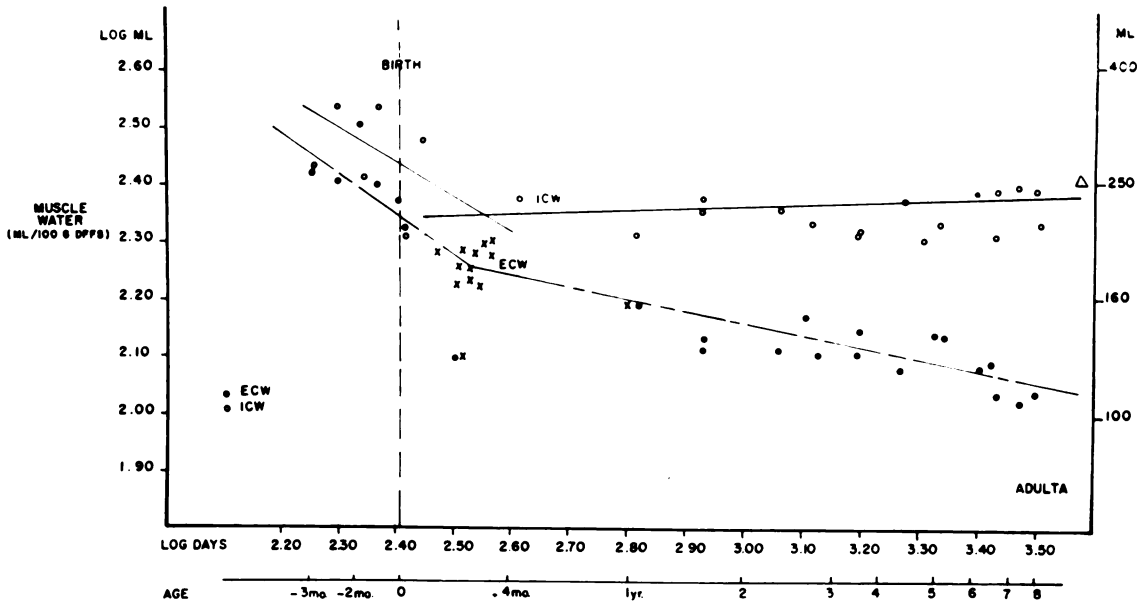

FIG. 6. Distribution of water in human muscle in relation to age. The logarithm of water content is plotted against the logarithm of age from conception. The vertical interrupted line represents the time of birth. The content of water in the intracellular phase (ICW) is represented by open circles and in the extracellular phase (ECW) by closed circles. X = malnourished children "age by weight." Data taken from METCOFF, J. Report of the Thirty-Second Roos Conference on Pediatric Research, p. 93. Chicago, Ill., 1959.

crease occurs; the levels of enzyme activity approximating those found in adults. In a similar way, the activity of uridine diphosphoglucose-dehydrogenase is low in the liver of the fetus and the newborn increases gradually to adult levels during the first two weeks of life.

These data point both to the possibility of defining biochemical maturation of the human child in enzymatic terms and to the probability of using the signs of arrested biochemical growth as indices of the effect of deficient food intake. How soon these signs become evident in measurable changes is one of the problems which will have to be solved.

CERTAIN EXAMPLES OF ARRESTED BIOCHEMICAL GROWTH IN MALNOURISHED CHILDREN

Water

Several authors[17–22] have reported on the content and distribution of water in malnourished children. When these data are recalculated on the basis of the "age" indicated by the actual body weight or height, it is apparent that the general picture presented by severely malnourished children is, in this aspect, like a reversion to an earlier stage of development. Figures 4, 5 and 6 are typical examples of this situation.

Fat Absorption

The figures for absorption of fat derived from cow's milk are low at birth and increase steadily to approximately eighteen months of age when coefficients of 95 per cent or more are finally attained.[23] During severe protein-calorie malnutrition, fat absorption is impaired and values range from 48 to 82 per cent of intake.[24–26] When the absorption coefficients found in malnourished infants are compared with those obtained in normal infants of the same height or weight, there is no significant difference.

Plasma Lipids

Plasma concentrations of total lipids, neutral fat, phospholipids, cholesterol, and alpha and beta lipoproteins are low in children with severe malnutrition. Soon after successful treatment, all the lipid fractions show a progressively significant rise which is followed by stabilization. The magnitude of the rise and the speed at which the plasma lipids attain their maximal

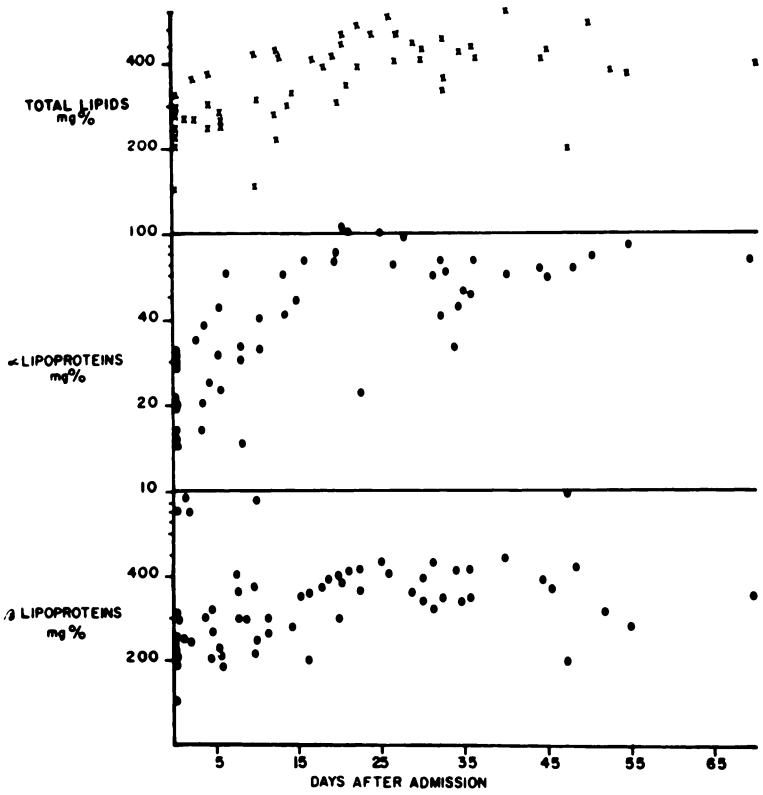

FIG. 7. Total lipids and lipoproteins during recovery from chronic severe malnutrition (kwashiorkor). From CRAVIOTO, J., DE LA PEÑA, C. and BURGOS, G. *Metabolism*, 8: 722, 1959.[28]

values are independent of the composition of the diet ingested.[27,28] Since a strikingly similar phenomenon occurs in normal newborn babies during their first days of life,[29] it seems that these findings in children recovering from severe protein-calorie malnutrition could be considered as another example of reversion to an earlier biochemical age (Fig. 7 and 8).

Creatinine

It has been found that children with kwashiorkor have a reduced output of urinary creatinine.[30–32] During the first days of recovery, most of the patients exhibit a sudden rise, more marked in those with edema, followed by a variable drop. The increase in urinary excretion coincides with a decrease in plasma concentration. These rapid changes in urine and blood concentration have been explained as a result of the reduced renal plasma flow and glomerular filtration rate characteristic of the acute stages of kwashiorkor.[33] Once these rapid changes have disappeared, urinary excretion of creatinine can be used as a measure of protein depletion.

Arroyave[34] has reported that in Guatemala economically and nutritionally underprivileged children without overt malnutrition do not differ radically in magnitude of protein depletion, as measured by the urinary excretion of creatinine per centimeter of body height, from children affected with kwashiorkor. When excretion levels are recalculated on the basis of "age by height," it can be seen (Table III) that the creatinine muscle mass of children with severe protein-calorie malnutrition is similar to that of normal children of a much younger age.

COMMENTS

It has been stated[35] that signs of "biochemical nutritional pathology" appear when the concentration of an essential nutrient in the tissues

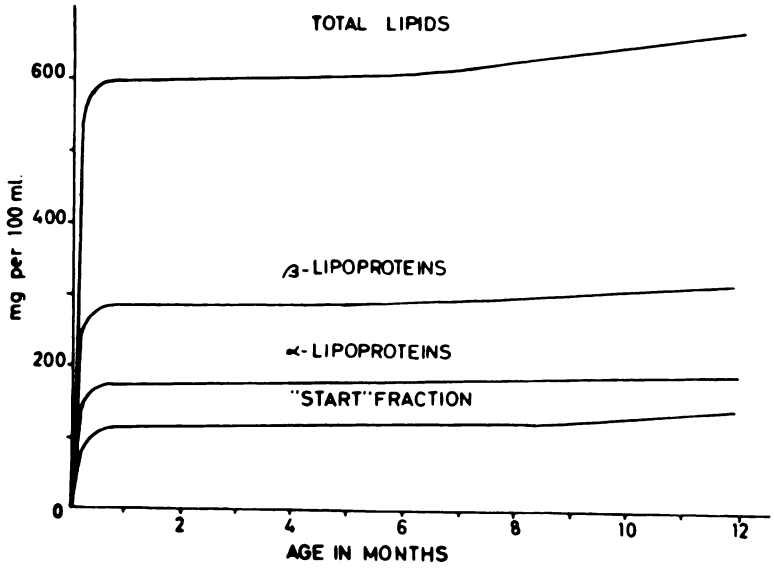

FIG. 8. Quantitative distribution of total lipids, alpha lipoproteins, beta lipoproteins and "start" fraction from birth until the end of the first year of life. From CRAVIOTO, J., DE LA PEÑA, C. and BURGOS, G. *Metabolism*, 8: 722, 1959.[28]

TABLE III*

Urinary Excretion of Creatinine in Normal Children and Children with Kwashiorkor

Chronological Age (yr.)	Twenty-Four Hour Output of Urinary Creatinine	
	Observed Values (mg.)	Calculated for the "Age by Height" (mg.)
4.09	230	224
4.06	216	273
4.02	345	207
4.03	245	260
4.00	317	208
4.07	144	273
3.10	130	178
3.07	144	171
3.07	216	244
3.02	273	202
3.11	273	245
4.03	202	245
4.03	187	173
4.00	230	251
4.10	316	275
3.11	302	223
4.06	236	239
4.01	158	178
4.03	230	188
4.03	259	224
4.06	288	262
4.07	288	263

* From ARROYAVE, G. and DURÁN, E. Unpublished data.

decreases to a point where it interferes with metabolism. A decrease in concentration may result from a dietary deficiency, poor absorption, impaired transport or decreased utilization. The biochemical measurements for evaluating nutritional pathology are based on the recognition of these signs of biochemical pathology. However, the practical use of these methods of evaluation is limited by homeostasis.

The concept of homeostasis implies that at each particular stage of development there is a chemical internal environment best suited to the optimal functioning of the individual. Development itself implies, however, that the chemical setup at a certain age should bring about changes that will cause the individual to progress one step further toward maturation.

Throughout development from the fertilized ovum to the full term infant and to the normal adult, many changes occur in the biochemistry of the body. Growth, associated with continuing alterations of tissue composition, means that each organ at each given time is characterized by a particular composition and that, as man develops, continuing changes in enzyme processes take place. Precise correlations of structure, function and activity of enzyme systems have been made both in isolated studies of some mammalian species and with the chick

embryo. It has been found, for example, that cholinesterase activity correlates closely with neural differentiation and that adenosinetriphosphatase appears at the time the muscle begins to function. The regulated progression of chemical change and differentiation extends into postnatal life with various enzymes appearing active at different times in the development of various tissues. At one age, activity of some enzymes may be less than at another age or it may even not be detectable at all.

In the last few years, knowledge of the biochemical changes which accompany growth and development of the human child has increased tremendously, especially in regard to its enzyme patterns; but there is little information, as yet, about the exact time of the appearance and disappearance of the various enzyme systems during the life cycle.

The purpose of this paper is to present some examples that may help to draw attention to the fact that certain biochemical features shown by children with kwashiorkor are in some ways similar to a reversion to an earlier stage of development.

It has been reported that malnourished infants respond abnormally to certain stimuli which are well tolerated by better nourished infants of the same chronological age.[36,37] Some of the differences in response are a function of size and can be eliminated by proper correction for size. However, in the light of recent findings on the biochemical maturation of the human infant and on the vulnerability of infants to the toxic action of drugs during the postnatal period when the enzyme systems necessary for drug metabolism are not yet fully developed,[38] it is possible that the differences in mortality resulting from the same pathologic process, even with the best treatment available, can be partially explained.

The recognition of altered responsiveness in malnourished children, who comprise the majority of preschool infants living in today's world, and the study of the mechanisms of this altered behavior may provide information which may prove useful for the assessment of their nutritional status and for the development of better methods of treatment which will result in a better chance of survival.

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