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Biochemical Changes in the Skin in Kwashiorkor[1]

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EVER SINCE THE CLASSICAL description of kwashiorkor by Cecilly Williams (1935), it has been recognized that skin changes constitute an important and striking clinical feature of this syndrome. It is surprising, however, that the voluminous literature on the subject of protein–calorie malnutrition contains few detailed studies of the biochemical changes in the skin in this disease. Such studies may be expected to throw light on the precise significance of these skin changes.

The skin constitutes an appreciable proportion of the total body weight—about 8% in the adult and probably more in the child. The crude protein content of the skin is nearly 22% and accounts for about ⅛ of the total body protein. It is obvious, therefore, that in any study of disorders of protein metabolism, the skin must receive attention. Of the skin proteins, collagen, which constitutes 70% of the total nitrogen of the skin and is mainly responsible for its mechanical strength and stability, is specially important.

In this paper the results of an investigation on the nitrogen content, collagen content, and amino acid composition of the dermis of children suffering from kwashiorkor are presented.

MATERIALS AND METHODS

Nineteen children suffering from kwashiorkor were investigated as inpatients. Their ages ranged from 1 to 5 years, and they conformed in all respects to the description given earlier. At the time of admission, skin biopsies were performed; a bit of skin measuring 2.5 × 5 mm was taken from the anterior aspect of the thigh. In 10 of the children the biopsy was repeated after nutritional rehabilitation with the skin taken from an identical area on the opposite thigh. Some relevant data on these children are given in Table I.

Skin biopsies obtained from 10 normal children also aged between 1 and 5 years were analyzed for purposes of comparison.

The tissues were stored at −20 C until analyzed, when a portion of the skin was taken for estimation of total nitrogen. The rest of the skin was processed for dermal amino acid analysis as described by Neldner et al. (1). The dermis was separated from the epidermis by frozen-section technique, dehydrated for 24 hr at 105 C, and then continuously defatted for 8 hr with ether in a Soxhlet apparatus. The defatted, dehydrated bit of the dermis, which generally weighed between 2 and 4 mg was hydrolyzed with 6 N HCl under vacuum at 100 C for 24 hr. After hydrolysis was complete, the acid was evaporated in a vacuum desiccator. The residue was dissolved in pH 2.2 buffer and analyzed for its amino acid composition using a Spinco-Beckman automatic amino acid analyzer. An aliquot of the hydrolysate was taken for estimation of dermal nitrogen by the micro-Kjeldahl procedure. Total nitrogen of the skin was determined after preliminary acid hydrolysis by the same method.

From the total nitrogen, dermal nitrogen, and hydroxyproline values obtained, the following calculations were made:

a) Hydroxyproline × 7.09 = collagen protein (2).

b) (Collagen protein × 18.6)/100 = collagen nitrogen.

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TABLE I

Clinical data of the kwashiorkor children

	Number of subjects	Age	Weight, kg	Height, cm	Total plasma protein, g/100 ml	Albumin, g/100 ml
Kwashiorkor without dermatitis	12	1–4 years	6.0 (5.0–8.0)	70 (52.5–75)	4.71 (3.35–5.70)	1.80 (1.31–2.48)
Kwashiorkor with scaly dermatitis	4	1–4 years	5.0 (4.0–6.0)	80 (65–80)	4.75 (4.42–5.34)	1.90 (1.75–2.01)
Kwashiorkor with crazy-pavement dermatitis	3	1–4 years	6.5 (5.0–7.0)	70	4.19 (3.41–5.36)	1.25 (1.49–1.55)

Numbers in parentheses show range of values.

TABLE II

Nitrogenous[a] constituents of the skin in kwashiorkor

Group	Total nitrogen	Epidermal nitrogen	Dermal nitrogen	Collagen nitrogen	Noncollagen nitrogen	Collagen/noncollagen nitrogen
Normals (8)	18.74 ± 1.05	2.71 ± 0.52	16.03 ± 1.48	10.66 ± 0.75	5.24 ± 1.53	2:1
Kwashiorkor without dermatitis (11)	14.31 ± 1.23	2.56 ± 0.83	12.95 ± 1.37	7.78 ± 2.13	5.17 ± 0.83	1.5:1
Kwashiorkor with dermatitis (6)	10.80 ± 1.34	1.71 ± 0.57	9.02 ± 3.32	5.86 ± 1.72	3.13 ± 1.60	1.9:1
Kwashiorkor after treatment	17.77 ± 1.22	1.77 ± 0.911	15.78 ± 1.06	11.17 ± 1.39	4.45 ± 1.15	2.5:1
Normals versus kwashiorkor without dermatitis	$P < 0.001$	NS	$P < 0.05$	$P < 0.01$	NS	
Normals versus kwashiorkor with dermatitis	$P < 0.001$	$P < 0.02$	$P < 0.001$	$P < 0.001$	$P < 0.02$	
Effect of treatment	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$	$P < 0.05$	

Numbers in parentheses are numbers of subjects. NS = not significant. Values are means ± SEM. [a] Values for nitrogen are expressed as g/100 g dry defatted tissue.

c) Total nitrogen − dermal nitrogen = epidermal nitrogen.

d) Dermal nitrogen − collagen nitrogen = noncollagenous nitrogen.

RESULTS

The results of the study are presented in Tables II and III.

Nitrogen Content

At the time of admission, the total nitrogen content of the skin of children with kwashiorkor was significantly lower than that of normal children of comparable age. Both the dermis and the epidermis showed a reduction in the nitrogen content but the reduction in the dermis was of a greater magnitude. The reduction in the nitrogen content of skin was much more pronounced in children who had skin lesions than in those who did not. In the dermis, both the collagen nitrogen and the noncollagen nitrogen content were lowered; collagen nitrogen was reduced to a greater extent than the noncollagen content. This was reflected in a distortion of the ratio of collagen nitrogen to noncollagen nitrogen, which instead of the normal 2:1 was lowered to 1.5:1.

In children having skin manifestations, however, the loss in noncollagen nitrogen was also considerable and the ratio of collagen to noncollagen nitrogen was 1.9:1.

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TABLE III

Dermal amino acid content expressed as percentage of dermal nitrogen

	Normal	Kwashiorkor	
		Without dermatitis	With dermatitis
Hydroxyproline	5.6	4.1[a]	4.0[a]
Serine	2.6	2.7	2.9[a]
Proline	9.6	8.7	7.9[a]
Glycine	26.9	25.1	19.9[a]
Tyrosine	0.8	0.8	0.6[a]
Arginine	6.3	11.8[a]	11.5[a]
Ammonia	5.5	10.0[a]	14.6[a]

Levels of aspartic acid, threonine, glutamic acid, valine, alanine, leucine, isoleucine, lysine, and phenylalanine were not different from those in normal children.

[a] Significantly different from "normal."

Following treatment and healing of the skin lesions, the total nitrogen content of the skin increased in every case and the ratio of the collagen to noncollagen nitrogen also returned to normal.

Amino Acids

The levels of several amino acids, when expressed per unit of dry defatted dermis, were lowered in the skin of all children suffering from kwashiorkor. The reduction was greater in those children who had dermatitis—particularly of the crazy-pavement type, than in those who had no skin lesions. In children without skin lesions however, when the amino acid concentrations were expressed as a percent of dermal nitrogen, only hydroxyproline showed a significant reduction compared with controls. In children with skin lesions, in addition to hydroxyproline, three other amino acids—proline, glycine, and tyrosine were also significantly reduced. The concentration of two amino acids, arginine and serine, were significantly elevated in children with dermatitis, while the concentration of only arginine was elevated in those without dermatitis. Ammonia, as a percent of dermal nitrogen, was appreciably increased in all cases.

Following nutritional rehabilitation, all these changes were reversed, except in the case of arginine, where the already high levels increased further.

DISCUSSION

A striking feature in the skins of children suffering from kwashiorkor was the marked reduction in the nitrogen content. Although both the epidermis and the dermis showed a reduction, the decrease was particularly marked in the dermis. The low level of hydroxyproline in the dermis would clearly indicate a loss of collagen. The reduction in collagen content was greater than the reduction in total dermal nitrogen, suggesting that in advanced states of protein malnutrition collagen is preferentially lost from among the various fractions of dermal protein. It is also significant that the decrease in collagen content was much greater in children who had skin lesions than in those who did not and that the levels were particularly low in children with crazy-pavement dermatosis. The observation made here that the collagen in skin was reduced is contrary to the conclusions of Picou et al. (3), Halliday (4) and Frenk et al. (5), who have reported that synthesis of collagen in the skin is not affected in children suffering from protein–calorie malnutrition. A careful analysis of the data of Picou and Halliday would, however, show that the skin of malnourished children contained 52.6 g collagen protein and 42.4 g noncollagen protein/kg fat-free skin. These values are not very different from those ovserved here, 42.0 g and 32.0 g, respectively. They are, however, grossly lower than 188 g and 94 g reported by Eisele et al. (6) for normal subjects. It is also clear from the data of Picou and Halliday that the ratio of collagen to noncollagen protein in these undernourished children was 1.3:1, as against a normal 2:1, showing that there was a preferential loss of collagen—a finding similar to that reported here.

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In addition to hydroxyproline, the concentrations of three other amino acids, proline, glycine, and tyrosine, were significantly lower in the dermis of children having skin lesions. The reduction in the hydroxyproline content of the dermis may be due to one or both of two factors, *1*) lowered levels of proline from which hydroxyproline is synthesized and *2*) failure of proline to be hydroxylated to hydroxyproline. The lowering of the proline content in the dermis of children with kwashiorkor would, therefore, acquire some significance and may suggest that this may be causally related to low levels of hydroxyproline. On the other hand, the ratio of proline to hydroxyproline in children suffering from kwashiorkor was much higher (2.1 and 2.0 in cases without and with skin lesions, respectively) than in normal children (1.7) and may be interpreted as indicative of a defect in the formation of hydroxyproline from proline. Among factors known to influence this hydroxylation is the ascorbic acid status of the individual. Our studies on children suffering from kwashiorkor have shown that their tissue levels of vitamin C are not very different from those of children without kwashiorkor (7) and this factor may not be related, therefore, to low levels of hydroxyproline in the skin. There are, however, no data on the levels of enzymes that are concerned with the hydroxylation.

The reduction in the tyrosine content of the dermis would appear to be significant. There is evidence to show that this amino acid is related to the maturation and structural integrity of the collagen fibers. Bensusan (8) and Hodge (9, 10) have shown that tyrosine residues are involved in the proper alignment of tropocollagen, while the work of Bowes et al. (11) has suggested an important role for this amino acid, both in fibril aggregation and in the maturation of collagen. That the level of tyrosine should be significantly lowered in children with skin lesions—the level being greatly lowered in those with crazy-pavement dermatosis compared with scaly skin—but normal in children whose skins were also normal, assumes etiological significance.

These observations on the hydroxyproline and tyrosine content of skin would permit the speculation that the skin lesion in kwashiorkor is causally related to a lowered amount of collagen and a relative structural immaturity. These changes may be expected to lead to impaired integrity of the skin, which would yield under conditions of mechanical stress. The clinical observation that lesions of crazy-pavement dermatosis are most marked in areas of the skin subjected to pressure and stress supports this concept. It would appear from these studies, however, that a reduction in collagen content alone even up to nearly 30% would be insufficient to lead to clinical manifestations in the skin. An associated lowering of tyrosine content seems to be essential for this purpose.

The significance of the changes in the levels of the other amino acids, serine, arginine, and glycine, as well as that of ammonia, is not clear and needs further study.

SUMMARY

The total nitrogen, dermal nitrogen, and collagen nitrogen content of the skin and the amino acid pattern of the dermis were determined in the skin obtained from 10 normal children and in 19 children suffering from kwashiorkor, 7 of whom had characteristic cutaneous lesions.

The skin of children with kwashiorkor had lower levels of total nitrogen, dermal nitrogen, and collagen nitrogen compared with normal children, and the reduction in all these components was of a greater magnitude in children who had cutaneous lesions.

There was a significant reduction in the hydroxyproline content of the dermis in all children and a rise in the arginine and ammonia content. In the dermis of children with cutaneous lesions, hydroxypro-

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line, tyrosine, proline, and glycine levels were lowered. These findings provide a biochemical explanation for the occurrence of cutaneous lesions in kwashiorkor.

REFERENCES

1. NELDNER, K. H., J. D. JONES AND R. K. WINKELMANN. Scleroderma: dermal amino acid composition with particular reference to hydroxyproline. *Proc. Soc. Exptl. Biol. Med.* 122: 39, 1966.
2. ESTOC, J. E. The amino acid composition of mammalian collagen and gelatin. *Biochem. J.* 61: 589, 1955.
3. PICOU, D., D. HALLIDAY AND J. S. GARROW. Total body protein, collagen and non-collagen protein in infantile protein malnutrition. *Clin. Sci.* 30: 345, 1966.
4. HALLIDAY, D. Chemical composition of the whole body and individual tissues of two Jamaican children whose death resulted primarily from malnutrition. *Clin. Sci.* 33: 365, 1967.
5. FRENK, S., J. METCOFF, F. GOMEZ, R. R. GALVAN, J. CRAVIOTO AND I. ANTONOWICZ. Intracellular composition and homeostatic mechanism in severe chronic infantile malnutrition. II. Composition of tissues. *Pediatrics* 20: 105, 1957.
6. EISELE, C. W., AND L. EICHELBERGER. Water electrolyte and nitrogen content of human skin. *Proc. Soc. Exptl. Biol. Med.* 58: 97, 1945.
7. MOHANRAM, M. Ascorbic acid nutrition. *Indian J. Med. Res.* 53: 891, 1965.
8. BENSUSAN, H. B., AND A. SCANU. Fiber formation from solution of collagen. II. The role of tyrosyl residues. *J. Am. Chem. Soc.* 82: 4990, 1960.
9. HODGE, A. J. Quoted in *International Review of Corrective Tissue Research*, edited by D. Hall. New York: Academic, vol. 1, 1963, p. 64.
10. HODGE, A. J. Chemical aspects of collagen fibrillogenesis. *Proc. Natl. Acad. Sci., U.S.* 97: 186, 1960.
11. BOWES, J. H., R. G. ELLIOT AND J. A. MOSS. *Recent Advances in Glue Research*, p. 71. Quoted in *Treatise on Collagen*, edited by G. N. Ramachandran. New York: Academic, 1967, p. 71.

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