NUCLEIC ACID CONTENT OF THE INTESTINAL MUCOSA IN KWASHIORKOR*

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Protein depletion studies in rats have shown that the amounts of RNA and protein of the liver, kidney and muscle are affected rapidly and extensively by the protein content of the diet.[1] It is, however, known that there is no similar effect on the composition of the rat intestinal mucosa.[2] In protein-malnourished children it has been found that the liver content of RNA and protein decreases on the average up to 40%.[3] No information has been found in the literature on the effect of protein-calorie malnutrition on the RNA, DNA and protein contents of the intestinal mucosa of human subjects. However, diarrhoea and malnutrition in young children is a frequent association and is accompanied by histological and enzymatic changes in the mucous membrane of the small intestine.[4] In this communication we present the results of the determination of RNA, DNA and protein composition of small-bowel mucosa in kwashiorkor.

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METHODS AND MATERIALS

The study comprised 6 cases of kwashiorkor in which the RNA, DNA and protein content of the intestinal mucosa was measured, using biopsy specimens. In each patient the biopsy was performed twice, i.e. on admission to hospital and after recovery. Intestinal biopsies were done by the procedure described previously,[4] after starving the patients overnight. The biopsy specimens were frozen with dry ice immediately after they had been obtained, and were stored in a deep-freeze (−20°C) until analysed. The determination of nucleic acids was on the basis of Schmidt-

TABLE I. RNA, DNA, AND PROTEIN CONTENTS OF INTESTINAL MUCOSA IN KWASHIORKOR

Patient	Sex	Age (mths)	Stage at which biopsy was performed	RNA-P (μg./G)	DNA-P (μg./G)	Protein (mg./G)	RNA-P / DNA-P	Protein / DNA	Body-weight (kg.)	Serum protein (albumin) (G/100 ml.)
T.A.	M	12	Admission	378	371	81·6	1·02	15·4	5·88	4·46 (2·62)
			Recovery	393	421	87·8	0·96	14·9	6·10	6·91 (4·10)
E.D.	F	9	Admission	387	434	70·0	0·89	11·3	5·80	4·86 (2·84)
			Recovery	397	414	88·8	0·96	15·0	6·25	7·34 (4·09)
M.S.	M	22	Admission			81·2			9·21	3·46 (1·71)
			Recovery	322	412	89·0	0·79	15·1	8·52	6·92 (4·18)
T.O.	F	26	Admission	365	466	88·5	0·77	13·3	8·31	5·65 (1·69)
			Recovery	496	478	107·2	1·03	15·8	9·03	8·51 (3·69)
H.L.	M	27	Admission	396	462	103·2	0·86	15·6	9·90	7·26 (3·73)
			Recovery	310	276	68·7	1·08	17·3	9·66	4·48 (2·24)
W.W.	M	17	Admission	434	443	86·0	0·98	13·6	8·63	4·07 (2·03)
			Recovery	367	371	95·2	0·99	17·9		8·51 (3·69)
Mean ± SD		19	Admission	392	435	85·1	0·90 ± 0·1	13·8±1·75	7·91	4·49 (2·18)
Mean ± SD			Recovery	381	395	89·5	0·97 ± 0·1	16·0±1·18	7·96	7·57 (3·91)

Thanhauser's method modified by Munro,[5] which was scaled down in order to fit the small biopsy specimens (about 10 mg.). After weighing, the specimen was homogenized in 0·6 ml. of ice-cold distilled water, and 0·25 ml. of ice-cold 0·6 N $HClO_4$ was added to 0·5 ml. of the homogenate. The mixture was allowed to stand at 0°C for 10 minutes and then centrifuged. The precipitate was washed twice with 0·5 ml. of cold 0·2 N $HClO_4$. After draining off the excess acid by inverting the tube briefly over filter paper, 0·4 ml. of 0·3 N KOH was added and incubated at 37°C for one hour in a water-bath. After cooling in ice, protein and DNA were precipitated by adding 0·25 ml. of 1·2 N $HClO_4$. After standing for 10 minutes in ice, the precipitate was centrifuged and washed twice with 0·5 ml. of 0·2 N $HClO_4$. The supernatant and washings were decanted and made up to 5 ml. with 0·2 N $HClO_4$ and distilled water, giving a solution of ribonucleotide in 0·1 N $HClO_4$. The ultraviolet absorption of the solution was read at 260 and 230 mμ and RNA content was calculated with the following formula described by Fleck and Begg:[6]

CRNA 3·40 A 260 mμ - 1·44 A 232 mμ

This gives the RNA-P/ml. in solution in terms of μg. The precipitate obtained above was dissolved in 0·2 ml. of 0·3 N KOH by warming briefly at 37°C. The solution was made up to 4 ml. with distilled water. To estimate DNA content 0·5 ml. of 2·5 N $HClO_4$ was added to 2·0 ml. of this solution and heated at 70°C for 15 minutes. After cooling the solution was centrifuged and 1·0 ml. of the supernatant was mixed with 2·0 ml. of 2% diphenylamine reagent.[7] The colour was developed by incubating at 30°C for 16 - 20 hours. The optical density at 600 μ is measured against a blank and compared with the values obtained with the standard DNA (calf thymus, BDH). The protein content was estimated by the Lowry method.[8]

RESULTS AND DISCUSSION

The content of nucleic acids and protein of the intestinal mucosa determined on admission was compared with that estimated in the same patient when completely recovered. The results obtained are shown in Table I. No significant differences in the amounts of RNA, DNA and protein were found between admission and recovery. This implies that the cell composition of the intestinal mucosa in kwashiorkor does not change. This is in contrast to the liver, in which RNA and protein content decreases to a considerable extent.[3]

It is deduced from animal experiments that in the case of the intestinal mucosa, protein depletion expresses itself by a change in the rate of cell division without any alteration in cell composition.[1,2] It thus shows a quite different response to that of other organs or tissues. From the data presented this appears to hold true in the protein-depleted human; this is of interest because the characteristic morphological changes of the intestinal mucosa in kwashiorkor are atrophy of the intestinal villi and a decrease in number and size of the columnar cells.[4]

SUMMARY

The content of nucleic acid and protein of the intestinal mucosa was measured in children with kwashiorkor using serial biopsy specimens. It was found that RNA, DNA and protein content and ratios did not change to a significant degree between admission to hospital and recovery.

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