METABOLISM OF HISTIDINE IN PROTEIN MALNUTRITION

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Studies on the specific defects in the metabolism of amino acids in protein malnutrition are of recent origin. Whitehead(1961, 1962) has reported the increased excretion of histidine and Urocanic acid in children with protein malnutrition. The cause for this abnormal excretion of histidine and urocanic acid in kwashiorkor is not known at the present time. The present investigations are concerned with the metabolism of histidine in albino rats under conditions of experimental protein malnutrition.

Experimental

A kwashiorkor like syndrome was produced in rats by the method of Sidransky (1960). Albino rats of 60-70 gram weight were force fed by stomach tube 6-7 grams of a maize diet* in two portions daily for a period of three days. Some of the rats were fed the same diet ad lib for a period of one week or two weeks. Albino rats of the same weight from the laboratory stock colony served as controls. At the end of the experimental period the rats were exsanguinated after light ether anaesthesia. Portions of the livers were taken for determination of gross composition

* One kg. of the diet contained 370 gms. of maize flour, 430 gms of sucrose, 160 gms. of peanut oil, 20 gms. of mineral salt mixture (Hubbel, Mendel and Wakeman 1937) 10 gms. of vitaminised starch(Chapman, Castillo and Campbell 1959) and 10 gms.of vitaminised peanut oil (containing optimal amounts of vitamins, A,D,E & K).

and histopathological examination. The gross composition of the livers was determined as described by Sidransky (1960).

Ten per cent homogenates of the livers were prepared in 0.25 M sucrose using a glass homogeniser with a Teflon Pestle. The histidase and urocanase activity of the supernatant fractions (1 hr.at 16,500 x g.) were determined by the method of Tabor and Mehler (1955). A plot of enzyme activity (increase or decrease in optical density at 277 mμ with either histidine or urocanic acid as substrate) against time was made in all cases and the linear portion of the graphs (indicating zero order kinetics) was used to determine the enzyme activity. Activities of the enzymes have been expressed on the dry weight basis of liver (the moisture content was assumed to be 70 per cent) as well as on the protein content of the supernatant fractions. Protein was determined by Nesslerisation after digestion as described by Johnson (1941).

Results and Discussion

The gross composition of the livers of the various experimental groups is given in Table I. The livers of force fed animals revealed a marked increase in lipid content. Rats fed ad lib on the low protein maize diet also showed an increase in the lipid content. The protein content of the livers of experimental animals of various groups showed slight changes when compared to control animals. Histopathological examination of the livers of force fed animals showed that there was severe periportal fatty infiltration which is similar to that found in the livers of kwashiorkor children. These results in general are in good agreement with those noted by Sidransky (1960) who force fed rats with similar experimental diets.

TABLE I

LIVER COMPOSITION OF ALBINO RATS FED ON A LOW-PROTEIN MAIZE DIET

Experimental details are described in text. Values indicated are mean ± S.E.M.

Group	No. of rats	Weight of liver (gms)	Protein (%)	Total protein (mg)	Lipid (%)	Total lipid (mg)
Control rats	4	2.45±0.01	14.06±0.85	343.3±12.84	3.71±0.78	65.7±8.42
Force fed rats (3 days)	6	3.28±0.17	13.39±0.63	436.8±18.89	10.84±0.88	358.4±37.9
Ad lib fed rats (7 days)	6	1.87±0.16	16.17±0.35	302.2±16.49	7.09±0.48	131.0±7.23
Ad lib fed rats (14 days)	8	1.96±0.02	14.98±0.68	295.6±21.95	5.16±0.39	91.09±16.06

The most striking changes that were noticed were concerned with the enzymes of histidine metabolism involved in urocanic acid pathway (Table II). There seems to be a rough parallel in the activities of histidase and urocanase activity in normal animals but there is a significant change in the urocanase activity of animals force fed and ad lib fed on a low protein diet. Urocanase activity is more strongly influenced by protein deficiency than histidase activity. Three days of forced feeding appeared to produce a significant change in urocanase activity but not in histidase activity. The degree of enzyme depletion was dependent on the duration of ad lib feeding of maize diet. The levels of histidase were considerably more than urocanase even after seven days ad lib feeding although there was a significant difference in levels of both enzymes when compared to

that of normal animals. Ad lib feeding a low protein diet for a period of two weeks resulted in an almost complete loss of both histidase and urocanase activity.

TABLE II

LEVELS OF HISTIDASE AND UROCANASE ACTIVITY IN ALBINO RATS FED ON A LOW-PROTEIN MAIZE DIET

Experimental details are described in text. Each group consisted of six female rats except group C which had eight female rats. Values indicated are mean ± S.E.M.

Group	Period of feeding (days)	Histidase activity (a)	Histidase activity (b)	Urocanase activity (a)	Urocanase activity (b)
A. Force fed rats	3	0.184±0.005	0.035±0.001	0.072±0.010*	0.014±0.002*
B. Ad lib fed rats	7	0.108±0.019*	0.015±0.003*	0.095±0.011*	0.012±0.002*
C. Ad lib fed rats	14	0.035±0.003*	0.006±0.001*	0.041±0.003*	0.007±0.001*
D. Control rats	-	0.207±0.013*	0.030±0.003*	0.216±0.016*	0.031±0.003*

(a) Micromoles of urocanic acid formed(Histidase) or utilised (Urocanase) per minute per gram dry weight of liver.

(b) Micromoles of urocanic acid formed(Histidase) or utilised (Urocanase) per hour per milligram protein of liver supernatant.

* Fisher's (t) test was applied for comparison of results in the various groups. Compared with group 'D' these values showed significant difference ($P < 0.01$). Test between groups C and D showed very high significance ($P < 0.001$).

The present experiments indicate that in experimental protein malnutrition in albino rats there is an enzymic deficiency of urocanase in the initial stages and continued protein malnutrition leads to enzymic deficiencies of both histidase and urocanase. It is possible that in kwashiorkor children

a similar enzymic deficiency exists which causes the excretion of histidine and urocanic acid (Whitehead, 1961, 1962). Direct verification of this hypothesis should await the development of specific micromethods for the determination of histidase and urocanase activity in liver biopsy specimens of kwashkorkor children.

Low levels of urocanase have been reported in conditions of ethionine induced liver damage(Silverman, Gardiner and Bakerman, 1960) and folic acid deficiency (Baldridge, 1957, 1958, 1960). Urocanic acid excretion in hepatic coma is presumably due to deficiency of urocanase (McIssac and Page, 1961). Recently Robinson and Gupta (1961) have claimed that urocanase has a pyridoxal requirement but no confirmation of this report has yet appeared. A genetic defect in the metabolism of histidine possibly at the histidase stage has recently been uncovered (Auerbach et al., 1962). It is now apparent that conditions of protein malnutrition have also an influence on the metabolism of histidine.

The nature of the factors responsible for the induction of the synthesis of various enzymes involved in the catabolic pathway of histidine as well as the metabolic fate of isotopic histidine and the probability of alternate metabolic pathways of histidine (Sen, McGeer, and Paul, 1962) in protein malnutrition are under investigation.

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