Histidine Metabolism in Experimental Protein Malnutrition in Rats

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(Received 7 December 1964)

1. The activities of the enzymes histidase, urocanase and histidine-pyruvate transaminase were studied in rats under conditions of protein malnutrition. Urocanase and histidase activities in liver were markedly lowered in experimental protein malnutrition, but the activity of histidine-pyruvate transaminase was unaffected. There is a metabolic control *in vivo* of the enzymes involved in the catabolism of histidine. 2. Significant changes in the urinary excretion of histidine, composition of liver and serum were apparent in the protein-malnourished rat. 3. The changes in the activities of the enzymes and other parameters were of a reversible nature and dependent on the nature of the dietary protein. 4. The significance of these findings is discussed in relation to abnormal histidine metabolism in kwashiorkor.

Recent interest in the metabolism of histidine in protein malnutrition arose from the observations of Dean (1961), Whitehead & Arnstein (1961), Whitehead (1962) and Dean & Whitehead (1963) that there was a large excretion of histidine and urocanic acid in the urine of children suffering from kwashiorkor. It appeared likely that this defect in histidine metabolism in kwashiorkor was due to an impairment of the function of the key enzymes, namely, histidase (L-histidine ammonia-lyase, EC 4.3.1.3) and urocanase, which are almost exclusively located in the liver (Miller & Waelsch, 1957; Zannoni & La Du, 1963). Further, it is known that the liver in kwashiorkor is abnormal histologically (Trowell, Davies & Dean, 1954) and in some biochemical functions (Waterlow, 1959, 1961).

Although an accurate measure of the activity of these enzymes in liver biopsy specimens of children with kwashiorkor would indicate a metabolic defect, it appeared desirable to study these aspects initially in experimental protein malnutrition in rats. The present paper deals with the changes in the activity of enzymes of histidine metabolism, the urinary excretion of histidine, the gross composition of liver and the electrophoretic pattern of serum proteins in rats under conditions of experimental protein malnutrition. A preliminary note has been pub-

lished (Rajagopal Rao, Deodhar & Hariharasubramanian, 1963).

EXPERIMENTAL

Materials. Analytical grade reagents (British Drug Houses Ltd., Poole, Dorset, or E. Merck A.-G., Darmstadt, Germany) were used for the preparation of buffers for use in enzyme assays. L-Histidine monohydrochloride monohydrate was Merck A.R. grade. Urocanic acid dihydrate, 1-methylhistidine, 3-methylhistidine, anserine, carnosine and the barium salt of formiminoglutamic acid were all products of California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A. Imidazolylacetic acid was a product of K & K Laboratories, New York, N.Y., U.S.A. It was also prepared by the action of L-amino acid oxidase from *Crotalus adamanteus* on L-histidine in the absence of catalase and purified by ion-exchange chromatography on Dowex 50 (H⁺ form; 8% cross-linked; 200–400 mesh) resin (Meister, 1957; Baldrige & Tourtellotte, 1958). Imidazolylpropionic acid was prepared by the catalytic hydrogenation of urocanic acid (Kraml & Bouthillier, 1955). Solvents used for paper chromatography were distilled before use. Vitamins used in the various experimental diets were obtained from F. Hoffmann-La Roche Ltd., Basle, Switzerland. Commercial cane sugar was used as sucrose in the diets.

Animals and diets. Female albino rats (Wistar strain) weighing about 40–50 g. were obtained from the breeding colony of this Institute. They were maintained on a stock diet of casein for 2 weeks or more until the weight of the animals was in the range 70–90 g. Corn (*Zea mays*) and ragi (*Eleusine coracana*) were obtained commercially and were first reduced to a coarse powder in a conventional flour mill and later pulverized in a micro-atomizer to give a very fine uniform flour. Sucrose was also powdered in a similar way. Vitamin-supplemented starch used in the various diets had the composition described by Chapman, Castillo & Campbell

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(1959). The mineral salt mixture was the same as that used by Hubbel, Mendel & Wakeman (1937). The vitamin-supplemented peanut oil contained (per g.): vitamin A, 200 i.u.; vitamin D, 100 i.u.; vitamin E, 10 i.u.; vitamin K, 500 μ g. The experimental diets contained 37% of corn or ragi flour, 43% of sucrose, 16% of peanut oil, 2% of mineral salt mixture, 1% of vitamin-supplemented starch and 1% of vitamin-supplemented peanut oil. The protein contents of the diets were 3.6 and 3.8% respectively. For the protein-free diet, the protein component was replaced with sucrose. The milk diet contained 30% of skimmed milk powder, 51% of sucrose, 16% of peanut oil, 1% of salt mixture, 1% of vitamin-supplemented starch and 1% of vitamin-supplemented peanut oil. The protein content of this diet was 10%. The compositions of these diets are similar to those described by Sidransky (1960). Stock casein diet used for pre-feeding or re-feeding the rats contained 20% of casein, 70% of corn starch, 5% of peanut oil, 2% of salt mixture, 2% of vitamin-supplemented starch and 1% of vitamin-supplemented peanut oil. The protein content of this diet was 16%.

Rats were force-fed through a rubber catheter with the mouthpiece described by Gillespie & Lucas (1957). The diets were homogenized with water so that 1 ml. of the suspension contained 0.5–0.7 g. of the diet. The amount of diet force-fed was determined after drying portions at 80–90°. At least three determinations were made and the average was calculated. The rats were force-fed twice daily at 9 a.m. and 4 p.m. The average daily intake of diet per rat was approx. 1 g./10 g. initial body wt. Force-feeding animals of low body weight resulted in heavy mortality. Considerable difficulty was experienced in force-feeding with the ragi diet as ragi contains fibrous material. Feeding *ad lib.* was carried out in the usual manner. The duration of feeding is described in each experiment. All animals had access to water. They were starved overnight before the experimental diets were given and were housed in individual wire cages with raised bottoms.

Rats of approximately the same age and weight were distributed into several groups by simple randomization. Each group consisted of a minimum of six rats. Fisher's *t* test was applied for comparison of results in various groups.

Metabolic experiments in vivo. An oral load of L-histidine (500 mg./100 g. body wt.) was administered through a stomach tube to the rats that had been deprived of food overnight, and the animals were then placed in metabolism cages. A 17 hr. urine specimen (4 p.m.–9 a.m.) was collected over 2 ml. of 6N-HCl. The urine collections were made up to a known volume (25–50 ml.), filtered and frozen until analysed.

Analytical methods. Most of the urine samples obtained from experimental animals were examined as a routine for imidazole compounds by paper chromatography after treatment with HgCl_2 as described by Baldrige & Tourtellotte (1958).

For the examination of serum proteins, rats were killed by heart puncture while under light ether anaesthesia. Blood was collected in centrifuge tubes, allowed to clot and the serum separated by centrifugation. Serum was stored at -15° until analysed. The total proteins in serum were determined by the method of King & Wootton (1959). Electrophoresis of serum proteins was done on agar gel by the method of Giri (1956).

Lipid and protein contents of the liver were determined as

described by Sidransky (1960). Frozen sections of liver were stained with oil red O and microscopically examined for fatty infiltration.

Microbiological assays of histidine. Histidine was determined in the urine by a microbiological procedure (Barton-Wright, 1952), with *Leuconostoc mesenteroides* P 60 as the test organism. Recovery of added histidine to unprocessed urine samples was poor (70–80%), and consequently portions of neutralized urine samples (20 ml. out of a total volume of urine made up to 25–30 ml.) were passed through short columns (10 cm. $\times$ 1.8 cm.) of Dowex 50 cation-exchange resin (H^+ form; 8% cross-linked; 200–400 mesh). After a water wash (20 ml.) to remove interfering materials, the amino acids were eluted with 2N- NH_3 and the eluate was freeze-dried. The residue was dissolved in water (2.0 ml.) and diluted portions (0.5 ml. diluted to 50 ml.) were assayed for free histidine. An equal portion (0.5 ml.) of the concentrated eluate from the column was hydrolysed with 4N-HCl for 1 hr. by autoclaving at 15 lb./in.², neutralized and diluted to 50 ml., and assayed for bound histidine as described above. Anserine, carnosine, 1-methylhistidine and 3-methylhistidine gave no growth response with this organism. Acid hydrolysis of authentic carnosine under conditions used for the determinations of bound histidine gave the expected amount of histidine.

Enzyme assays. Liver homogenates (10%, w/v) were prepared in 0.25M-sucrose by using a Potter-Elvehjem homogenizer with a Teflon pestle. Histidase and urocanase activities were determined in the supernatant fractions remaining after centrifugation at 10000g for 40 min. by the spectrophotometric methods of Tabor & Mehler (1955). These methods were suitable for normal livers, but in fatty livers the supernatant fraction was opalescent and considerable difficulties were encountered in spectrophotometry. Consequently the method was modified as follows.

The assay system for histidase contained 30 μ moles of sodium pyrophosphate buffer, pH 9.2, 20 μ moles of neutralized glutathione, 2 μ moles of L-histidine and a suitable amount of enzyme (0.2–0.5 ml.) in a total volume of 2.9 ml. The assay system for urocanase contained 50 μ moles of potassium phosphate buffer, pH 7.4, 1 μ mole of urocanic acid and a suitable amount of enzyme (0.2–0.5 ml.) in a total volume of 2.9 ml. The reaction was initiated by adding substrate (histidine or urocanic acid) and the tubes were incubated at 37° for 20 min. At the end of the incubation period, 0.1 ml. of 30% (v/v) HClO_4 was added to the tubes to inactivate the enzymes. The blanks contained all components and HClO_4 was added initially.

After centrifugation, a portion (0.1–0.2 ml.) was taken from the clear supernatant and diluted to 3 ml. with 0.1M-phosphate buffer, pH 7.4. The extinction at 277 m μ was determined in a Beckman model DU spectrophotometer. In all the calculations ϵ 18600 for urocanic acid was used. Under these conditions of assay, the reaction was linear with respect to time for at least 30 min. and proportional to enzyme concentration. Histidase and urocanase activities are expressed as μ moles of urocanic acid formed or utilized/hr./mg. of protein respectively.

Histidine-pyruvate-transaminase activity was determined in dialysed liver homogenates (Rowell, 1962) and the alanine formed was estimated after paper-chromatographic separation as described by Vaidyanathan, Radhakrishnan & Giri (1952). The pH of the reaction system was 7.4 as described by Rowell (1962), but it was found that the pH

Table 1. *Effect of various dietary regimens on the composition of liver and serum in experimental rats*

Experimental details are given in the text. Values are given as means $\pm$ s.e.m. of results from six rats. Fisher's *t* test was applied for comparison of the results in the various groups: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001. Comparisons were made with the group of rats on stock casein diet which were killed at the start of the experiment and served as controls.

Expt. no.	Diet	Initial wt. (g.)	Mode of feeding	Food intake (g./day)	Duration (days)	Weight loss or gain (g.)	Total liver lipid (mg.)	Total liver protein (mg.)	Total serum protein (g./100 ml.)	Serum albumin (g./100 ml.)	Albumin/globulin ratio	Histopathology of liver
1	Stock casein diet (controls)	94	—	—	—	—	177.4 $\pm$ 12.1	492.3 $\pm$ 14.8	6.2 $\pm$ 0.09	4.09 $\pm$ 0.07	1.96 $\pm$ 0.13	Normal
	Maize diet	93	Force-fed	7.0	4	-3	255.0 $\pm$ 31.8	567.8 $\pm$ 29.7	5.43 $\pm$ 0.26*	3.15 $\pm$ 0.16*	1.12 $\pm$ 0.11***	Mild to moderate periportal fatty infiltration
	Maize diet	94	Force-fed	7.0	5	-4	226.0 $\pm$ 5.1**	492.3 $\pm$ 26.6	4.78 $\pm$ 0.13***	2.30 $\pm$ 0.05***	0.93 $\pm$ 0.03***	Typical periportal fatty infiltration of protein deficiency type
2	Stock casein diet (controls)	74	—	—	—	—	109.7 $\pm$ 6.7	329.8 $\pm$ 30.7	5.25 $\pm$ 0.10	3.41 $\pm$ 0.09	1.83 $\pm$ 0.07	Normal
	Maize diet	73	Fed <i>ad lib.</i>	9.7	15	+6	227.8 $\pm$ 29.2**	602.6 $\pm$ 76.8*	4.40 $\pm$ 0.13***	1.78 $\pm$ 0.07***	0.69 $\pm$ 0.01***	Mild to moderate periportal fatty infiltration
	Maize diet; later re-fed with casein diet	75†	Fed <i>ad lib.</i>	9.0	15	+27	200.2 $\pm$ 21.6**	658.9 $\pm$ 32.3***	5.20 $\pm$ 0.09	3.12 $\pm$ 0.19	1.71 $\pm$ 0.06	Normal
3	Stock casein diet (controls)	73	Fed <i>ad lib.</i>	11.5	17	+51	178.3 $\pm$ 17.3	717.8†	5.90 $\pm$ 0.15	3.55 $\pm$ 0.05	1.51 $\pm$ 0.05	Normal
	Stock casein diet	73	—	—	—	—	210.7 $\pm$ 16.9	493.7 $\pm$ 35.1	—	—	—	Normal
	Maize diet	70	Force-fed	7.8	4	-2	238.7 $\pm$ 16.5	584.2 $\pm$ 55.3	—	—	—	Mild to moderate periportal fatty infiltration
	Maize diet	71	Fed <i>ad lib.</i>	6.7	15	-11	144.3 $\pm$ 5.24	324.4 $\pm$ 47.7*	—	—	—	Mild to moderate periportal fatty infiltration
4	Stock casein diet (controls)	81	—	—	—	—	119.8 $\pm$ 10.4	547.2 $\pm$ 21.1	7.01 $\pm$ 0.08	4.62 $\pm$ 0.10	1.95 $\pm$ 0.10	Normal
	Ragi diet	87	Force-fed	3.6	4	-7	174.0 $\pm$ 17.9*	603.0 $\pm$ 25.9	6.28 $\pm$ 0.11***	3.86 $\pm$ 0.18	1.68 $\pm$ 0.18	Mild periportal fatty infiltration
	Ragi diet	88	Fed <i>ad lib.</i>	8.0	15	-17	321.2 $\pm$ 30.30	449.7 $\pm$ 60.6	5.45 $\pm$ 0.29***	3.40 $\pm$ 0.22	1.74 $\pm$ 0.20	Mild periportal fatty infiltration
	Ragi diet; later re-fed with casein diet	71†	Fed <i>ad lib.</i>	8.0	15	+14	134.2 $\pm$ 24.5	672.8 $\pm$ 43.3	6.22 $\pm$ 0.11***	3.67 $\pm$ 0.09**	1.46 $\pm$ 0.11**	Normal
	Protein-free diet	89	Fed <i>ad lib.</i>	7.0	15	-23	182.7 $\pm$ 17.8**	393.5 $\pm$ 24.5***	5.32 $\pm$ 0.20***	—	—	Moderate to severe periportal fatty infiltration
5	Protein-free diet; later re-fed with casein diet	66†	Fed <i>ad lib.</i>	7.5	15	+12	206.9 $\pm$ 23.3**	610.4 $\pm$ 44.3	6.41 $\pm$ 0.14**	—	—	Mild fatty infiltration
	Stock casein diet (controls)	79	—	—	—	—	165.9 $\pm$ 17.9	471.2 $\pm$ 21.4	5.67 $\pm$ 0.06	3.72 $\pm$ 0.17	1.97 $\pm$ 0.22	Normal
	Milk diet	73	Force-fed	5.8	4	+5	253.2 $\pm$ 68.6	509.5 $\pm$ 26.5	5.63 $\pm$ 0.19	3.52 $\pm$ 0.11	1.70 $\pm$ 0.08	Normal
	Milk diet	75	Fed <i>ad lib.</i>	10.9	15	+18	151.6 $\pm$ 21.8	467.4 $\pm$ 30.4	5.72 $\pm$ 0.19	—	—	Normal

† Result of only one determination as other samples were lost.
‡ Weight of rats at the end of feeding with the experimental diets and before re-feeding with the casein diet.

optimum of this enzyme was 8.5 (D. Rajagopal Rao & A. D. Deodhar, unpublished work; Balldridge & Spolter, 1963, 1964). Histidine-pyruvate-transaminase activity was expressed as μ moles of alanine formed/hr./mg. of protein.

Protein was determined by the micro-Kjeldahl procedure.

RESULTS

The main object of our work was a study of the enzymes involved in histidine metabolism in conditions of protein malnutrition in rats and the possible relevance of this study to abnormal histidine metabolism in children with kwashiorkor. A kwashiorkor-like syndrome was produced in rats by the method of Sidransky (1960). In addition, a diet based on ragi (*Eleusine coracana*) was used in some of the experiments as it is a major dietary component of some of the population in Mysore, India.

Composition of liver and serum. Force-feeding rats with diets containing low-quality protein (maize or ragi as sole source of protein) but adequate in other components for a short duration (4–5 days) caused fatty livers (Table 1). Feeding *ad lib.* with the same diets caused similar changes, but the

period needed for this effect was longer. The severity of fatty infiltration as revealed by lipid content and histological examination was consistently greater and more reproducible with a maize diet than with a ragi diet. The findings on liver composition and histological findings on liver agree well with those of Sidransky (1960). The force-feeding procedure had little effect on liver composition, as force-feeding with a diet based on skim milk powder caused no fatty infiltration or abnormal histological changes in liver (Table 1). Platt, Halder & Doell (1962) have observed that force-feeding rats with low-protein diets has considerable influence on carbohydrate metabolism, the histopathology of liver and its composition. Fatty infiltration of liver, changes in total serum protein, albumin concentration and albumin/globulin ratio were observed in both the groups (ragi and maize diets), but the changes were more significant in the group given the maize diet. The changes observed in the gross composition of liver or the electrophoretic pattern of serum were reversed by re-feeding the experimental rats with a casein diet (Table 1).

Changes in enzyme activity. The nature of dietary

Table 2. *Effect of various dietary regimens on the activities of histidase, urocanase and histidine-pyruvate transaminase in experimental rats*

Experimental details are given in the text. Values are given as means $\pm$ S.E.M. and the numbers of determinations are shown in parentheses. Histidase and urocanase activities are expressed as μ moles of urocanic acid formed or utilized/hr./mg. of protein respectively. Histidine-pyruvate-transaminase activity is expressed as μ moles of alanine formed/hr./mg. of protein. Fisher's *t* test was applied for comparison of the results in the various groups: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Comparisons were made with the group of rats on stock casein diet which were killed at the start of the experiment and served as controls. The dietary regimen and composition of liver and serum of corresponding experiments were as given in Table 1. Histidine-pyruvate-transaminase activity was determined in Expt. 4 only.

Expt. Group no. no.	Diet	Histidase	Urocanase	Histidine-pyruvate transaminase
1	1 Stock casein diet	0.061 $\pm$ 0.008 (5)	0.072 $\pm$ 0.009 (5)	
	2 Force-fed with maize diet (4 days)	0.049 $\pm$ 0.008 (6)	0.051 $\pm$ 0.011 (6)	
	3 Force-fed with maize diet (5 days)	0.037 $\pm$ 0.010 (6)	0.025 $\pm$ 0.005 (6)**	
2	1 Stock casein diet	0.053 $\pm$ 0.012 (6)	0.055 $\pm$ 0.010 (6)	
	2 Fed <i>ad lib.</i> with maize diet	0.030 $\pm$ 0.005 (6)	0.046 $\pm$ 0.003 (6)	
	3 Fed <i>ad lib.</i> with maize diet; later re-fed with casein	0.084 $\pm$ 0.006 (6)***	0.080 $\pm$ 0.011 (6)*	
	4 Fed <i>ad lib.</i> with stock casein diet	0.058 $\pm$ 0.007 (5)	0.073 $\pm$ 0.004 (5)***	
3	1 Stock casein diet	0.134 $\pm$ 0.014 (6)	0.059 $\pm$ 0.005 (6)	
	2 Force-fed with maize diet	0.056 $\pm$ 0.005 (6)**	0.022 $\pm$ 0.004 (6)***	
	3 Fed <i>ad lib.</i> with maize diet	0.036 $\pm$ 0.017 (6)**	0.036 $\pm$ 0.006 (6)**	
4	1 Stock casein diet	0.170 $\pm$ 0.026 (5)	0.266 $\pm$ 0.031 (5)	0.317 $\pm$ 0.021 (6)
	2 Force-fed with ragi diet	0.219 $\pm$ 0.029 (5)	0.181 $\pm$ 0.018 (6)*	0.219 $\pm$ 0.041 (5)
	3 Fed <i>ad lib.</i> with ragi diet	0.117 $\pm$ 0.007 (6)	0.146 $\pm$ 0.016 (9)**	0.286 $\pm$ 0.026 (6)
	4 Fed <i>ad lib.</i> with ragi diet; later re-fed with stock casein diet	0.217 $\pm$ 0.011 (6)	0.310 $\pm$ 0.038 (6)	0.211 $\pm$ 0.008 (6)***
	5 Fed <i>ad lib.</i> with protein-free diet	0.105 $\pm$ 0.023 (5)	0.166 $\pm$ 0.029 (5)*	0.308 $\pm$ 0.052 (6)
	6 Fed <i>ad lib.</i> with protein-free diet; later re-fed with stock casein diet	0.208 $\pm$ 0.013 (4)	0.377 $\pm$ 0.026 (5)*	0.180 $\pm$ 0.021 (5)**

protein influences the enzymes involved in histidine metabolism (Table 2). Decreased histidase and urocanase activities were observed in rats force-fed with maize diets rather than those given ragi diets. Rats given a protein-free diet showed changes in urocanase activity ($P < 0.05$). Urocanase activity was always markedly ($P < 0.01$) decreased in conditions of protein malnutrition; the response of histidase was somewhat variable, although it did show a definite downward trend. The pattern of change with histidine-pyruvate transaminase was different (Table 2). The activity of the enzyme was essentially the same in conditions of protein deficiency when compared with the controls, but a significant decrease ($P < 0.01$) was observed when the rats were re-fed with a good-quality protein.

Metabolic experiments in vivo. The importance of the routes of histidine catabolism in experimental protein malnutrition was examined by 'load experiments' in which histidine was given orally to measure the urinary excretion of histidine and its metabolites. The urinary excretion of histidine in normal or malnourished rats is relatively low. After a 'load' of histidine, part of the urinary histidine was in a bound form that could be released by acid hydrolysis. In the normal rat on a stock casein diet, the bound histidine was about 50% of the total histidine in urine (Table 3). The urinary excretion of free and bound histidine by rats in experimental protein deficiency was higher ($P < 0.05$) than in the normal (Table 3). It appears that the major part of histidine is utilized by the rat, as only a fraction (2–5%) of the test dose could be accounted for in the 17 hr. urine samples. When malnourished rats were re-fed with a casein diet, the urinary excretion of

free or bound histidine was lowered (Table 3). A qualitative comparison of imidazole compounds by paper chromatography of the various experimental samples of urine from the rats in protein malnutrition also indicated that histidine was the major imidazole compound present. No urocanic acid was detected under conditions of protein malnutrition in our experiments, although measurements *in vitro* indicated low urocanase activity. The degree of protein deficiency was possibly not severe enough to produce a total loss of urocanase activity in the liver, and alternate pathways of metabolism of urocanic acid may operate in protein malnutrition (Sen, McGeer & Paul, 1962).

DISCUSSION

In groups of rats maintained on ragi and maize diets, a lowering of the total serum protein and albumin concentrations were observed. Fatty infiltration of the liver was evident in both groups. However, these changes were more significant in the group given the maize diet. The degree of changes noticed with ragi and maize diets may be due to differences in the dietary intake of proteins and their amino acid composition. Ragi has almost twice as much of methionine, cystine and tryptophan as maize has (Balasubramanian, Ramachandran, Viswanatha & De, 1952*a,b*; Balasubramanian & Ramachandran, 1957). It is well known that methionine has a lipotropic effect, and this may be the cause for the low degree of fatty infiltration of liver in rats given the ragi diet.

Enzymes involved in histidine metabolism, histidase and urocanase, showed decreased activity in

Table 3. *Effect of various dietary regimens and an oral load of histidine on its urinary excretion in experimental rats*

Experimental details are given in the text. Values are given as means $\pm$ s.e.m. and the numbers of determinations are shown in parentheses. Fisher's *t* test was applied for comparison of the results in the various groups: * $P < 0.05$; ** $P < 0.01$. In Expt. 2, comparisons were made between groups 1 and 2, and between groups 3 and 4. The dietary regimen and composition of liver, serum and enzyme activity of corresponding experiments were as given in Tables 1 and 2. In Expt. 1 the urine specimens were not analysed for total histidine after acid hydrolysis.

Expt. no.	Group no.	Diet	Histidine excreted (mg./17 hr. urine collection)	
			Free histidine	Total histidine
1	1	Stock casein diet	1.05 $\pm$ 0.15 (8)	—
	2	Force-fed with maize diet	1.35 $\pm$ 0.62 (6)	—
	3	Fed <i>ad lib.</i> with maize diet	3.47 $\pm$ 0.69 (8)**	—
2	1	Fed <i>ad lib.</i> with ragi diet	22.70 $\pm$ 3.90 (6)**	26.80 $\pm$ 4.70 (6)**
	2	Fed <i>ad lib.</i> with ragi diet; later re-fed with stock casein diet	2.90 $\pm$ 0.70 (5)	6.10 $\pm$ 1.40 (5)
	3	Fed <i>ad lib.</i> with protein-free diet	14.80 $\pm$ 3.80 (6)*	—†
	4	Fed <i>ad lib.</i> with protein-free diet; later re-fed with stock casein diet	2.50 $\pm$ 0.50 (6)	6.30 $\pm$ 0.80 (6)

† Only two urine specimens showed an increased amount of histidine after acid hydrolysis.

the group given maize diets more consistently than in the group given ragi diets. Histologically the 'kwashiorkor liver' is similar to the one in which fatty infiltration is caused by feeding with ethionine (Popper & Schaffner, 1957), and, moreover, Silverman, Gardiner & Bakerman (1960) have reported that abnormal histidine metabolism in ethionine-induced fatty liver is due to decreased urocanase activity. Instances of impairment in histidine metabolism (urocanic acid pathway) not necessarily involving a fatty liver are: lowering of activity of the enzymes in vitamin deficiency (Baldrige, 1958*a,b*; Baldrige & Burket, 1960), genetic disorders such as histidinaemia (Auerbach, DiGeorge, Baldrige, Tourtellotte & Brigham, 1961*a,b*; Ghadimi, Partington & Hunter, 1962; La Du, Howell, Jacoby, Seegmiller & Zannoni, 1962, 1963), severe liver damage as in hepatic coma (McIsaac & Page, 1961) and neoplastic diseases (Dymock, 1964). Variation in histidase and more particularly of urocanase activities were observed even on animals maintained on the same stock casein diet (controls). Makoff & Baldrige (1964) reported that histidase activity, barely detectable at birth, varied with the age of the rat, whereas urocanase activity was higher at the time of parturition and remained fairly constant up to 50 days of age. However, our results (Table 2) indicate that in rats (40–60 days old) histidase and urocanase activities are of similar magnitude and an increase in the urocanase activity was concomitant with an increase in histidase activity in control animals (Table 2). Histidine-pyruvate transaminase activity was essentially unaffected in conditions of protein deficiency when compared with controls, in contrast with the decreased activities of histidase and urocanase. When the rats were re-fed with a good-quality protein, histidase and urocanase activities were restored but a significant decrease in histidine-pyruvate-transaminase activity was observed. It is indicative of metabolic control *in vivo* of the several routes of catabolism of histidine (urocanic acid pathway, transaminase pathway and other routes) that conditions of protein malnutrition seem to influence the urocanic acid pathway predominantly by affecting the activities of histidase and urocanase. The relative activities of the enzymes (urocanic acid pathway and transaminase pathway) also vary in different species. In ox, sheep and rabbit livers, the transaminase activity is negligible, whereas the urocanase and histidase activities are considerable (D. Rajagopal Rao & A. D. Deodhar, unpublished work; Rowsell & Carnie, 1964). According to Baldrige & Spolter (1963), the transaminase pathway is relatively more important in the rat. However, isotopic studies and experiments with isolated enzyme systems indicate that the urocanic acid pathway is predominant (Tabor, 1954, 1955; Greenberg, 1961).

The urinary excretion of free and bound histidine after a test 'load' of histidine was significantly greater by the rats in experimental protein deficiency. On re-feeding the malnourished rats with a casein diet, the urinary excretion of free or bound histidine was lowered. These results supplement and also indirectly confirm the findings of Sauberlich & Baumann (1946) on the effect of dietary proteins on the urinary excretion of amino acids in rats. It is possible that a part of histidine released by acid hydrolysis may be from N^{α} -acylhistidine or peptides. Indirect evidence for such a hypothesis is found in a genetic disorder histidinaemia, where the urocanic acid pathway of histidine is blocked, and N^{α} -acetylhistidine is excreted in the urine (Shaw, Boder, Gutenstein & Jacobs, 1963). Occasionally, we have noticed that a part of the histidine in the urine of children with kwashiorkor is in the bound form and can be released by acid hydrolysis (D. Rajagopal Rao & K. Hariharan, unpublished work). The abnormal excretion of histidine and urocanic acid in clinical protein (kwashiorkor) can be partly explained by our present investigations. It has been possible to detect and measure an increased urinary excretion of histidine in experimental protein malnutrition and also to find that these urinary amounts of histidine can be reversed to normal values by re-feeding with a good-quality protein diet. The present investigations, however, strongly indicate that histidase and urocanase are affected in conditions of experimental protein malnutrition and that this change is reversible. It is therefore possible that the variations in the urinary excretion of urocanic acid observed by Dean & Whitehead (1963) in children with kwashiorkor during treatment with milk-protein diets may be due to a gradual increase in the urocanase activity in liver during treatment.

Further evidence for possible alternate pathways of metabolism of histidine and impairment of renal function in protein malnutrition might be obtained by measurements *in vivo* of the activity of the multi-enzyme sequence involved in the complete catabolism of histidine.

This work was supported by the Public Law 480 funds of the U.S.A. A. D. D. thanks the Williams Waterman Foundation for a fellowship during these investigations. We thank Mr A. Paul Jayaraj for assistance in histopathology, Mr Govinda Rao in the forced-feeding experiments and Mrs D. Rajalakshmi for statistical analysis of the data. We acknowledge the kind interest and helpful discussions of Dr M. Swaminathan and Dr P. B. Rama Rao.

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