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Distribution of α-Aminoisobutyric-C^{14} Acid in Rats Deficient in Thiamine, Pantothenate, Fatty Acids, or Potassium[1]

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A nutritional deficiency of fatty acids in young rats resulted in a marked elevation of the steady-state serum level of an injected dose of α-aminoisobutyric acid, and in a decrease in the transport of this model amino acid into skeletal muscle, heart, and intestine. The liver level was greatly increased. Adrenalectomy of the deficient rats reduced the uptake in both liver and kidney. These changes were similar to those reported earlier for rats deficient in vitamin B_6. No consistent pattern of change in distribution of the model amino acid was found in rats deficient in thiamine, pantothenate, or potassium. The extremely high liver levels of the amino acid seen in thiamine-deficient rats could be returned toward normal by removing the adrenal glands. All of the changes found in the K^+-deficient animals were brought essentially to normal by bilateral adrenalectomy. No consistent relationship was found between the uptake of the amino acid and the K^+ level in the heart or skeletal muscle of the rats in the control, K^+-deficient, and the K^+-deficient adrenalectomized groups.

The accumulation of amino acids by Ehrlich mouse ascites tumor cells *in vitro* has been found to be increased by a number of unrelated biological agents, including pyridoxal and pyridoxal phosphate (1), estradiol disulfate and estrone sulfate (2), and several auxins (1). In addition, the accumulation is dependent upon the normal K^+ and Na^+ content of the tumor cells (3). The first two sets of agents, the forms of vitamin B_6 and the soluble estrogens, produce their effect by slowing the transport efflux of the amino acids from the cells (4, 2). The similar action by compounds with markedly different biological functions suggests that vitamin B_6 and the estrogens alter amino acid accumulation in the tumor cells nonspecifically and in a way that is not related to their normal roles in the animal body. Nevertheless, pyridoxal has been found important for normal amino acid transport in Ehrlich ascites tumor cells taken from vitamin B_6-deficient mice (1), and for normal uptake of the model amino acid, α-aminoisobutyric acid, by all tissues examined in rats *in vivo* (5; cf. Ref. (6) for a more detailed discussion of the relations between vitamin B_6 and amino acid transport). Estradiol-17β, on the other hand, alters the distribution of model amino acids *in vivo* only in those tissues that are susceptible to normal estrogenic control (for example, the uterus) (7-9).

The above facts leave unclear the nature of the effect of vitamin B_6 on amino acid accumulation *in vivo*. If the effect is nonspecific, other nutritional changes might be expected to alter amino acid distribution in the rat in the same way as does a deficiency of vitamin B_6. Of special interest among the possible deficiencies is that of the essential fatty acids, since vitamin B_6 has been reported necessary for the synthesis of arachidonic from linoleic acid by the rat (10, 11), or for other, incompletely identified reactions in fat metabolism (12, 13). This last observation may explain why the skin

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lesions produced in a nutritional deficiency of the essential fatty acids are strikingly similar to those seen in vitamin B_6 deficiency (14).

The present paper reports results on the distribution in the rat of the model amino acid, α-aminoisobutyric acid (AIB), in four separate nutritional deficiencies. Deficiencies of thiamine and pantothenate were examined to determine a possible nonspecificity among the vitamins on AIB distribution. Fatty acid deficiency was studied to determine a possible relation of depressed fatty acid formation to the vitamin B_6 effect, and K^+ deficiency was produced to evaluate the role of cellular K^+ and Na^+ in AIB transport *in vivo*.

EXPERIMENTAL

Albino rats (Sprague-Dawley-Holtzman) of both sexes were used in different experiments. Control animals were maintained on a purified diet as described previously (5). Deficiencies of individual nutrients were produced by altering the complete diet as follows: deficiencies of vitamin B_1 or pantothenate, by omitting the thiamine chloride or calcium pantothenate, respectively; deficiency of fatty acids, by replacing the corn oil with glucose and cutting the amount of cod liver oil to one half that given to the control animals; and K^+-deficiency by substituting the normal salt mixture with the following salts (%) $CaCO_3$, 1.16; Na_2HPO_4, 1.02; $CaHPO_4 \cdot 2H_2O$, 0.29; $MgSO_4 \cdot 7H_2O$, 0.356; ferric citrate $\cdot 7H_2O$, 0.106; KI, 0.003; $MnSO_4 \cdot H_2O$, 0.015; $CuSO_4 \cdot 5H_2O$, 0.0012; $ZnCl_2$, 0.010. Animals were placed on each diet at weaning, except those that were adrenalectomized, which were somewhat older. Table I presents a summary of the length of time animals were kept on each diet, their weights at use, and other objective and subjective evidence for the presence and severity of each experimental condition used. Comparisons were made in each case between control and deficient rats of about the same body weights. We have shown earlier (5) that uptake of AIB in nutritionally deficient rats is more closely related to weight than to age.

Distribution of AIB-C^{14} in the rats was determined at either 24 or 39 hours after injecting 1 mg (5×10^6 cpm) of this amino acid per kilogram of body weight. All animals were fasted for 15–24 hours before being killed. Adrenalectomized rats were used 3–6 days after bilateral removal of their adrenal glands. They were maintained on 0.9% NaCl as their drinking water. Other details of the methods of handling and analyzing the samples have been described (5). Results are in some cases expressed as a distribution ratio, which is the ratio of the concentration of the amino acid in the tissue cell water to that in the serum.

RESULTS

Distribution of AIB in rats with the uncomplicated deficiencies. For these experiments the animals were killed 39 hours after

TABLE I

SUMMARY OF CONDITION OF RATS IN DIFFERENT NUTRITIONAL STATES AT TIME THAT AIB-C^{14} DISTRIBUTION WAS MEASURED

Deficiency	Days on diet at use	Wt. at use (gm)	Other test
Thiamine	17–25	49–61	Typical external symptoms
Thiamine, adrenalectomized	22–24[a]	93–108[b]	Typical external symptoms
Pantothenate	23–34	58–92	Acetylated 30–49% of 1 mg PAB[c]
Fatty acid	29–49	81–98	Typical external symptoms
Fatty acid adrenalectomized	42–62[a]	100–159[b]	Typical external symptoms
Potassium	13–20	48–92	Skeletal muscle $[K^+]$ = 46–68 meq/kg Heart $[K^+]$ = 60–70 meq/kg[d]
Potassium, adrenalectomized	7–15[a]	87–161	Skeletal muscle $[K^+]$ = 71–80 meq/kg[d]
Adrenalectomized	—[a]	95–134	Fasting blood glucose, 58–77 mg%

[a] Rats adrenalectomized 3–6 days before use.

[b] Compared with deficient intact rats weighing 71–79 gm (thiamine deficient) or 140–162 gm (fatty acid deficient).

[c] Normal rats acetylate 60–80% of a 1 mg dose of *p*-aminobenzoic acid (PAB) (15).

[d] Normal rats had skeletal muscle $[K^+]$ of 107 meq/kg, heart $[K^+]$ 83 meq/kg. These decreases are all statistically significant ($p < 0.05$).

they were injected with the AIB-C^{14}, and the amino acid was analyzed in skeletal muscle, heart, liver, kidney, intestine, and serum, as well as in urine collected over the entire 39 hours. Statistical analyses (Student's t-test) were made comparing results from groups of deficient animals with those from groups of controls of the same size. Table II presents typical distribution ratios, tissue and serum levels, and urinary excretions as found with one group of control rats, i.e., on the complete purified diet. Table III summarizes the changes that were found in the distribution ratios in each of the four deficiencies, and Table IV gives the data for changes in AIB levels in the five tissues, in serum, and the total radioactivity excreted in the urine. In these latter two tables, the percentage changes are given only in those cases in which the differences from normal were statistically significant ($p < 0.05$).

Effects of adrenalectomy on AIB distribution in thiamine, fatty acid, or potassium deficient rats. In these three deficiency states, the uptake of AIB by the liver was increased markedly above the normal, the elevation being as high as 400% in thiamine-deficient animals (Table III). A similar increase in AIB uptake by liver in vitamin B_6-deficient animals was shown to be a result of secretions of the adrenal gland, since bilateral adrenalectomy reduced the high liver values, and injection of hydrocortisone to either normal or B_6-deficient rats produced large elevations (5). α-Aminoisobutyric acid distribution was therefore tested in bilaterally adrenalectomized rats that had been previously fed the diets deficient in thiamine, fatty acids, or potassium. Serum, liver, muscle, the kid-

TABLE II

DISTRIBUTION OF AIB-C^{14} BETWEEN SERUM AND TISSUES, AND EXCRETION INTO THE URINE OF THE RAT ON A COMPLETE PURIFIED DIET.[a]

Tissue	Distribution ratio	Cpm/μl cell water
Muscle	4.99 ± 0.60	4.52 ± 0.64
Heart	7.00 ± 0.53	6.26 ± 0.58
Liver	9.34 ± 1.59	8.16 ± 1.37
Kidney	42.3 ± 4.2	34.12 ± 2.85
Intestine	14.9 ± 1.4	13.45 ± 1.47
Serum	—	0.90 ± 0.06
Urine	—	916 ± 127[b]

[a] AIB injected intraperitoneally 39 hours before samples taken. Urine values represent amount excreted over the entire 39 hours. Values are averages ± the standard error for 8 rats weighing 39–94 gm, average 63 gm.

[b] Total excreted in 39 hours, cpm/gm body weight.

TABLE III

PERCENTAGE CHANGE IN DISTRIBUTION RATIOS OF AIB PRODUCED BY A DEFICIENCY OF THIAMINE, PANTOTHENATE, FATTY ACIDS, OR POTASSIUM.[a]

Tissue	% Change in deficiency of			
	Thiamine	Pantothenate	Fatty Acids	Potassium
Muscle	−44	−41	−60	−42
Heart	+81	n.s.[b]	−60	+89
Liver	+372	n.s.	+52	+65
Kidney	+73	n.s.	n.s.	−37
Intestine	n.s.	−37	−49	n.s.

[a] Typical control values are given in Table II. Differences from controls are significant ($p < 0.05$) in those cases in which numerical values are shown. 6–8 rats per group. Changes in AIB levels in the 5 tissues and serum, and in urinary excretion values, are shown in Table IV.

[b] Difference from normal controls not significant ($p > 0.05$).

TABLE IV

PERCENTAGE CHANGE IN AIB LEVELS IN TISSUES AND SERUM, AND IN 39-HOUR URINARY EXCRETION OF AIB, PRODUCED BY A DEFICIENCY OF THIAMINE, PANTOTHENATE, FATTY ACIDS, OR POTASSIUM.[a]

Tissue	% Change in deficiency of			
	Thiamine	Pantothenate	Fatty acids	Potassium
Muscle	−39	n.s.[b]	−32	n.s.
Heart	+101	+36	−37	+128
Liver	+389	+131	+163	+105
Kidney	+118	+49	+80	n.s.
Intestine	n.s.	n.s.	n.s.	+30
Serum	n.s.	+56	+70	+24
Urine	−48	−66	−58	−79

[a] Changes in distribution ratios in tissues of these same animals are given in Table III.

[b] Difference from normal controls not significant ($p > 0.05$).

TABLE V

EFFECTS OF ADRENALECTOMY ON AIB DISTRIBUTION RATIOS IN TISSUES OF RATS DEFICIENT IN THIAMINE, FATTY ACIDS, OR POTASSIUM.[a]

Tissue	% change in distribution ratios produced by adrenalectomy in deficiency of		
	Thiamine	Fatty acids	Potassium
Muscle	+27	n.s.[b]	n.s.
Liver	−52	−27	−49
Kidney	—	−35	n.s.

[a] Adrenalectomy did not change any of the values in these tissues taken from rats on the control diet. Values are changes produced by adrenalectomy of deficient rats compared with intact animals on the same deficient diet. Details of status of animals given in Table I; 4–7 rats per group. Percentage changes are given only in those cases in which the alteration was statistically significant ($p < 0.05$). The serum AIB was changed significantly only in the K^+-deficient adrenalectomized rats (an increase of 21%). Muscle and kidney levels increased correspondingly in these animals so that distribution ratios in these tissues were not altered significantly.

[b] Difference from intact deficient rats not significant ($p > 0.05$).

neys (in two cases), and heart (K^+-deficiency only) were measured for their concentrations of AIB-C^{14} 24 or 39 hours after injection of a standard dose of the amino acid. Table V shows the effects of the adrenalectomy on AIB distribution in the three deficiency states. Adrenalectomy of rats on the complete diet did not in itself produce changes in liver, muscle, kidney, or serum AIB levels, although it did result in an increase in the heart level. In each of the three deficiency states, the high distribution ratios found in the livers of intact animals were reduced markedly by the adrenalectomy. In the fatty acid-deficient group the AIB level in liver was the same as found in intact rats on a nutritionally complete diet. The K^+-deficient adrenalectomized animals had serum AIB levels even higher than found in the intact deficient ones, but the absolute levels in liver and heart did not change significantly, so that distribution ratios in these tissues were not satistically different from those given by the adrenalectomized rats on the complete diet. Removing the adrenal glands also elevated the distribution ratio of AIB in skeletal muscle of the thiamine-deficient group toward the normal value, and brought the kidney distribution ratio down significantly in the fatty acid-deficient rats.

DISCUSSION

Of the different nutritional states studied, only fatty acid deficiency produced changes in AIB distribution similar to those seen in a deficiency of vitamin B_6. Both states resulted in increased serum levels of AIB, decreased urinary losses, and decreased distribution ratios in skeletal muscle, heart, and intestine. In both cases, also, the liver distribution ratio was greatly elevated, and this increase could be eliminated by removal of the adrenal glands. The major difference between the two groups was in the uptake of AIB by the kidney. Uptake by this tissue, as measured by distribution ratio, was not changed in the fatty acid-deficient rats, whereas it was decreased by nearly 50% in the B_6-deficient animals (5). When the fatty acid-deficient rats were adrenalectomized, however, the kidney level was found to be reduced significantly (Table V). The decreases in the other tissues, on the other hand, were more marked in the fatty acid deficiency than in the vitamin deficiency. In vitamin B_6 deficiency, the absolute levels of AIB in the various tissues were not changed significantly (except in the liver of the intact, deficient rat); the depressed distribution ratios were a result entirely of the increased serum levels (5). In fatty acid-deficient rats, in contrast, both heart and skeletal muscle showed significant decreases in absolute levels of AIB compared with the normal animals, and the liver and kidneys showed decreases in the adrenalectomized rats. The results therefore suggest that normal essential fatty acid metabolism may be more directly responsible than vitamin B_6 itself in maintaining normal AIB uptake by the tissues examined. Lipid has long been recognized as a necessary component for maintaining the structural and functional integrity of the cell membrane. It is not possible to say at present, however, whether the effect of vitamin B_6 on amino acid

transport *in vivo* is related to the role of the vitamin in lipid metabolism.

Individual deficiencies of the two vitamins thiamine and pantothenate led to changes in AIB distribution that were markedly different from each other and from those found in the other deficiencies. Furthermore, in neither case was a meaningful pattern of changes apparent. In pantothenate deficiency, increased serum AIB levels led to increased levels in heart, liver, and kidney without changing the distribution ratios. Skeletal muscle and intestine, in contrast, showed no significant changes in absolute levels in this deficiency; as a consequence, distribution ratios in these two tissues were decreased significantly because of the elevated serum levels (Table III). Since none of the tissues in the deficient rats had less AIB than their controls, the increased serum level found in pantothenate-deficient animals probably results from the decreased urinary loss of the amino acid (a 66% reduction in AIB excretion over the 39 hours of the test).

Pantothenate deficiency is the only abnormal nutritional condition studied that did not alter AIB uptake by the liver. Deficiency of this vitamin leads to adrenal necrosis and hemorrhage (16–18 and others), to a decrease in the formation of cholesterol and other steroids (19, 20, for example), and to a low content and release of steroid hormones from the adrenal cortex (21–23). Corticosteroid production is therefore presumably low in our rats in this deficiency. This fact could account for our failure to find an increased liver uptake of AIB in pantothenate deficiency.

The pattern of change in AIB distribution in vitamin B_1 deficiency is especially interesting. In contrast to all of the other abnormal situations studied here, the serum level was not changed significantly from normal. Kidney and heart uptakes were elevated significantly, skeletal muscle was decreased, and liver AIB levels in the intact rats were the highest yet observed in this tissue. Only intestine remained unchanged. The increased body AIB, reflected by decreased urinary loss, may be accounted for as increases in such tissues as liver, kidney, and heart, which showed 100 to nearly 400% elevations in their absolute levels of the amino acid. Adrenalectomy resulted in a return toward normal of the high distribution ratios in both liver and skeletal muscle (Table V). However, this effect on the muscle is not likely a result of removal of adrenal steroids. We did not find an effect of adrenal steroids or adrenalectomy on skeletal muscle uptake of AIB in earlier studies *in vivo* (7, 5). In addition, Wool (24) and Kostyo and Schmidt (25) have found that glucocorticoid administration, not its removal, decreases uptake of amino acids by muscle tissue studied *in vitro*.

Rats on the K^+-deficient diet had 43% lower K^+ levels in their skeletal muscle than did comparable rats on a normal diet (see Table I). The AIB level in the muscle was not changed significantly from normal, but the distribution ratio was decreased 42% because of a 24% elevation in the serum AIB level. The changes in K^+ level and AIB distribution ratio were therefore the same in this tissue. A similar relationship was shown earlier in the Ehrlich ascites tumor cells (3). However, the uptake of AIB by muscle tissues could not be correlated directly with their K^+ content. The heart showed an average of a 128% increase in absolute level of AIB and an 89% increase in its distribution ratio in the K^+-deficient animals, even though the average K^+ level had *decreased* by 21% (Table I). After K^+-deficient rats were adrenalectomized, the skeletal muscle K^+ was elevated, but was still 29% below normal. α-Aminoisobutyric acid distribution ratios in skeletal muscle, as well as in all other tissues of the K^+-deficient adrenalectomized rats, were not significantly different from normal. No significant correlation could be found between $[K^+]$ in skeletal muscle and the distribution ratio in this tissue when statistical analysis was made of values given by control, K^+-deficient, and K^+-deficient adrenalectomized animals ($r = 0.441$ at 16 degrees of freedom, $p > 0.05$). This finding is in agreement with the results of Kostyo and Schmidt (25), who have presented evidence that AIB uptake by the rat diaphragm *in vitro* is not related directly to the K^+ content of the tissue. In the present experiments, the potassium level was used

as a measure of the relative Na^+-K^+ values in the tissues.

A change in distribution ratio in a tissue can result from increases or decreases in the AIB level in the tissue itself, or from changes in the serum level. The various possibilities are illustrated by the changes in the skeletal muscle under the various conditions. The distribution ratio of AIB in this tissue was decreased significantly in each deficiency. In vitamin B_1 deficiency, the absolute muscle level was decreased but the serum level remained unchanged. In both pantothenate and K^+ deficiencies, the muscle level was not changed, and the decreased distribution ratio was a reflection of elevated serum levels produced in these two conditions. In fatty acid deficiency, the skeletal muscle AIB level was decreased in the presence of elevated serum levels. However, in all of these situations AIB transport was decreased since, at the AIB levels used, skeletal muscle levels in the normal rat will respond to changes in serum levels so that distribution ratios are maintained (see 26, 27).

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