Some Effects of Vitamin B_6 Deficiency on Fat Metabolism in Rats

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In a number of studies on the role of vitamin B_6 in fat metabolism in the rat, attention has been given to the effect of the vitamin deficiency on the content of fat in the liver, carcass, blood and kidneys. Data on the composition of liver with respect to total fatty acids, neutral fat, cholesterol or phospholipids have been provided by the work of several investigators; the literature has been reviewed by Carter & Phizackerley (1951). Comparison of results obtained by the various workers regarding the lipotropic property of pyridoxine is difficult because some of the diets were incomplete with respect to lipotropic factors. Restricted or liberal supply of food may also be a cause for the variation in the data. Experiments on the total lipid concentration of the carcass as affected by pyridoxine have yielded consistent results when fat-free diets were fed. McHenry & Gavin (1938, 1941) showed that, on a fat-free diet, pyridoxine-supplemented animals have a higher content of body fat than deficient groups. This was so whether the supplemented groups were fed *ad lib.* or were pair-fed with the vitamin-deprived groups. When fat was included in the diet, results have been conflicting. Carter & Phizackerley (1951) could not detect a statistically significant difference in the body fat between deficient and control groups. Greater unsaturation of carcass fat, as measured by its iodine value, has been found in pyridoxine deficiency (Quackenbush & Steenbock, 1942). The total lipids in the blood, liver and kidneys of deficient animals had a lower iodine value (Tulpule & Patwardhan, 1950).

The work described in this paper relates to the role of pyridoxine in the metabolism of fat in the rat. The content and fatty acid distribution of the body fat of pyridoxine-deficient rats as compared with pair-fed controls has been investigated. The ability of the pyridoxine-deficient rat to convert ^{14}C-labelled glucose into fatty acids has been studied. Since it has been demonstrated (Quackenbush, Steenbock, Kummerow & Platz, 1942) that linoleic acid and pyridoxine have a complementary action in the cure of acrodynia, the effect of supplementation with linoleic acid *per se* and in corn oil has also been studied.

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EXPERIMENTAL

Albino rats of the Wistar strain were used throughout, and they were housed individually in screen-bottom cages with water provided *ad lib.* There were two diets with the following compositions:

(*a*) *Casein diet.* This consisted of vitamin- and fat-free casein, 90; vitaminized casein, 4; agar, 2; salt mixture (Steenbock & Nelson, 1923), 4; choline chloride, 0·2; inositol, 0·2%.

(*b*) *Sucrose diet.* This consisted of sucrose, 74; vitamin- and fat-free casein, 16; vitaminized casein, 4; agar, 2; salt mixture, 4; choline chloride, 0·2; inositol, 0·2%.

Vitaminized casein was obtained by mixing into 800 g. vitamin- and fat-free casein the following: thiamine hydrochloride, 100 mg.; riboflavin, 100 mg.; calcium pantothenate, 400 mg.; nicotinic acid, 400 mg.; *p*-aminobenzoic acid, 400 mg.; biotin, 20 mg.; pteroylglutamic acid, 20 mg.

Preparation of tissue lipids. Livers were extracted by the procedure of Carter & Phizackerley (1951), and phospholipids were separated by initial treatment with acetone followed by $MgCl_2$. Bodies from each group of rats were frozen in liquid air and ground to a fine paste in a power mincer. After samples had been removed for determination of moisture and of nitrogen, the balance of the pooled carcasses was dehydrated with cold ethanol. Fat was extracted with boiling ethanol and then with ether in a percolator. The combined extracts were concentrated *in vacuo*, the residue mixed with light petroleum, and the mixture was filtered after standing overnight. A sample of the filtrate was taken for phosphorus estimation and the balance was freed of solvent by evaporation. The residue was extracted with acetone and a conventional $MgCl_2$ precipitation was carried out to separate phospholipids. Neutral fat and unsaponifiable material were recovered from the acetone filtrate.

Analytical methods. Total nitrogen was determined by the Kjeldahl method. Moisture was estimated by drying tissue samples to constant weight at 110°. Phosphorus was determined by the procedure of Fiske & SubbaRow (1925), and the content of phospholipids calculated on the assumption that rat phospholipids contain 4% P. Iodine number was determined by the method of Yasuda (1931). Fatty acids were separated as lead salts by the procedure of Hilditch (1949). Fractionation of methyl esters of fatty acids was done in a 50 ml. distilling bulb using a copper coil as a fractionating column as described by Lovern (1934).

Animals on casein diet

Sixty rats, having an initial average weight of 120 g., were divided into six groups comparable with regard to average weight and sex and they were maintained on the casein diet.

Table 1. *Composition of liver and of carcass of rats fed on a casein diet*

Group and no. of animals	Supplements	Average wt. (g.)		Average food intake (g./rat/day)	Moisture (%)		Protein* (%)		Total lipids (%)		Phospholipids (%)		Iodine value of total lipids	
		Liver	Carcass		Liver	Carcass	Liver	Carcass	Liver	Carcass	Liver	Carcass	Liver	Carcass
A (10)	None	5·3	79	6·2	72	70	21·8	24·1	4·8	1·0	2·1	0·73	67	81
B (10)	Pyridoxine	6·7	122	6·2	72	68	20·7	22·7	5·1	2·7	2·5	0·54	61	64
C (9)	Linoleic acid	6·0	77	6·2	71	71	20·7	23·1	5·2	1·9	2·4	1·48	71	72
D (10)	Pyridoxine + linoleic acid	5·8	121	6·2	73	69	20·8	22·4	4·9	3·9	2·4	0·69	67	55
E (8)	8 weeks, none; last 4 weeks, pyridoxine	9·0	149	8·1	72	67	23·5	21·2	4·7	2·5	2·4	0·28	66	66
F (8)	12 weeks, pyridoxine	8·7	146	8·1	72	68	22·7	21·6	4·8	3·2	2·3	0·31	64	69

* $N \times 6{\cdot}25$.

Table 2. *Composition of liver and of carcass of rats fed on a sucrose diet*

Group and no. of animals	Supplements	Average wt. (g.)		Average food intake (g./rat/day)	Moisture (%)		Protein* (%)		Total lipids (%)		Phospholipids (%)		Iodine value of total lipids	
		Liver	Carcass		Liver	Carcass	Liver	Carcass	Liver	Carcass	Liver	Carcass	Liver	Carcass
N (10)	Normal stock diet	—	110	—	—	63	—	18·5	—	7·3	—	0·61	—	79
1 (9)	None	7·3	142	9·7	72	66	18·9	21·4	1·3	4·6	1·8	0·54	75	64
2 (9)	Pyridoxine	6·8	174	9·7	71	63	19·6	19·2	1·2	8·9	1·9	0·36	65	61
3 (9)	Linoleic acid	5·5	141	9·7	72	67	21·1	20·6	1·1	6·1	2·2	0·48	66	64
4 (9)	Pyridoxine + linoleic acid	6·9	164	9·7	70	64	21·9	19·7	1·4	8·3	2·8	0·30	65	62
5 (9)	None, 16 weeks	6·9	106	8·2	73	68	19·2	22·1	2·0	3·6	1·8	0·62	59	72
6 (9)	Pyridoxine	6·7	168	8·2	74	66	19·4	21·4	1·0	8·2	1·9	0·56	65	66
7 (5)	None	7·8	168	8·2	72	66	20·2	22·3	1·1	6·8	2·4	0·74	86	92
8 (5)	Pyridoxine	6·5	215	8·2	71	62	20·1	21·9	1·4	12·6	3·0	0·64	91	91

* $N \times 6{\cdot}25$.

Four groups were given for 8 weeks the following treatment: *A*, no supplement; *B*, 50 μg. pyridoxine hydrochloride/rat/day; *C*, 20 mg. linoleic acid/rat/day; *D*, same amounts of pyridoxine and of linoleic acid. At the end of 8 weeks, animals in these groups were killed for analysis. Groups *B–D* were fed isocalorically with group *A*, which was fed *ad lib.* In addition there were two more groups, *E* and *F*, which received the same treatment as *A* and *B*, respectively, but the treatment was continued for 12 weeks; during the last 4 weeks group *E* was given the pyridoxine supplement. Group *F* was pair-fed with *E*. After 12 weeks animals in groups *E* and *F* were killed for analysis. It had been intended originally to give deoxypyridoxine to all rats in order to expedite the deficiency state and 100 μg. desoxypyridoxine hydrochloride/rat/day was included in all feedings. After 1 week the antivitamin was found to have reduced food consumption drastically and its use was stopped. Analytical data for all groups are given in Table 1.

Animals on sucrose diet

Rats with initial weight 120–130 g. were maintained on the sucrose diet to provide a comparison with those given the casein diet. Groups 1–4 had the same respective treatment as *A–D* (casein diet) but were maintained for 10 weeks before killing for analysis. Group 5 was continued for 16 weeks without supplement to determine the effect of prolonged deficiency and group 6 was a pair-fed, pyridoxine-supplemented control for group 5.

To determine the effect of the inclusion of a reasonable amount of fat in the basal diet, two groups were maintained for 10 weeks on the basal sucrose diet to develop a deficiency state characterized by acrodynia and were fed for 6 subsequent weeks on a diet in which 15% by weight of sucrose was replaced by an equal weight of corn oil. Group 7 was deprived of pyridoxine throughout the entire period, while group 8 was supplemented with pyridoxine and pair-fed with group 7 during the last 6 weeks. These two groups were killed for analysis at the end of the 16th week.

Analytical results for groups 1–8 are shown in Tables 2 and 3. Both tables include a group of rats of the same weight as the initial weight of groups 1–8 but which had received no experimental diet or treatment. This group is designated as *N*.

Administration of [^{14}C]*glucose*

Three groups of rats, six animals/group, with an average initial weight of 100 g., were fed the sucrose diet for 6 weeks. Group I received no pyridoxine and all rats in this group had acrodynia in the sixth week. Each rat in groups II and III received 50 μg. pyridoxine hydrochloride/day. Group II was pair fed with group I, and group III was fed *ad lib.* All rats received 100 μg. desoxypyridoxine/day. During the six weeks the rats were habituated to insertion of a stomach tube.

After 6 weeks all rats received by stomach tube 1 ml. solution containing 60–75 mg. [^{14}C]glucose. The labelled glucose was kindly supplied by Prof. G. Krotkov, Queen's

Table 3. *Distribution of fatty acids in the carcass fat, sucrose diet*

Values represent the individual fatty acids as a percentage of the mixed fatty acids.

Group and no. of animals	Supplements	Saturated fatty acids				Unsaturated fatty acids			
		C_{14}	C_{16}	C_{18}	C_{20}	C_{16}	C_{18}	C_{18} dienoic	C_{20-22}
1 (9)	None	2·6	19·4	1·1	—	13·3	58·3	2·5	2·8
2 (9)	Pyridoxine	3·4	23·8	2·2	—	13·2	56·6	0·8	0·6
3 (9)	Linoleic acid	1·8	22·0	0·8	—	20·1	52·1	3·1	Trace
4 (9)	Pyridoxine + linoleic acid	1·9	24·1	0·7	—	19·1	49·1	3·6	—
5 (9)	None	8·1	21·5	—	—	30·2	36·1	4·1	—
6 (9)	Pyridoxine	7·3	20·7	—	—	30·0	39·3	2·8	—
7 (5)	Corn oil	6·2	10·1	—	—	45·4	27·3	11·0	—
8 (5)	Pyridoxine + corn oil	6·0	11·1	—	—	45·6	27·5	11·9	—
N (10)	Normal on stock diet	1·6	19·6	2·5	1·8	4·3	55·9	12·2	2·1

Table 4. *Incorporation of* ^{14}C*-labelled glucose into rat-carcass fat*

Group I (deficient)*			Group II (pair-fed control)*			Group III (control fed *ad lib.*)†		
Wt. of rat (g.)	Activity of glucose fed (counts in million/min.)	Incorporation of activity into fat (%)	Wt. of rat (g.)	Activity of glucose fed (counts in million/min.)	Incorporation of activity into fat (%)	Wt. of rat (g.)	Activity of glucose fed (counts in million/min.)	Incorporation of activity into fat (%)
80	4·78	0·49	110	4·78	0·28	172	4·78	0·42
100	4·78	0·45	110	4·78	0·24	217	4·66	0·31
80	4·66	0·38	115	4·78	0·38	257	4·66	0·28
102	4·66	0·39	117	4·66	0·35	257	4·66	0·37
128	4·66	0·82	136	4·66	0·42	190	2·88	0·35
100	2·88	0·35	140	4·66	0·46	210	2·88	0·35
	Average	0·48 ± 0·17‡			0·36 ± 0·08‡			0·35 ± 0·05‡

The '*t*' values for significance at the 5% level were 1·51 between groups I and II, and 1·75 for groups I and III.

* Average daily food intake 7·2 g./rat.

† Average daily food intake 13·4 g./rat.

‡ Values represent S.D. for the data in the group.

University, Kingston, Canada. The glucose had been prepared by a photosynthetic method using tobacco leaf and $^{14}CO_2$; it contained ^{14}C equally in all positions, and it had an activity of 1·21 mc/g. During the 26 hr. after glucose administration the above feeding regimen was continued and the animals were kept in vacuum-type desiccators to which CO_2-free oxygen/nitrogen was passed and from which CO_2 was absorbed in 10% KOH solution. After the 26 hr. period the rats were killed by stunning, and neutral fat samples were prepared from each rat. After measurement of activity in the individual fat samples, pooled samples of fat, obtained by mixing one-fourth of the fat extracted from each rat in a particular group, were hydrolysed and the unsaponifiable matter as well as the free fatty acids were recovered for radioactivity measurements. The fatty acids were separated into solid and liquid lead-salt fractions and the activity in the liquid lead-salt portion was determined. The activity of the original glucose solution was checked. Data from the experiment with labelled glucose are given in Table 4.

DISCUSSION

The total amount of fat in the livers of rats fed the casein diet was greater than in the livers of rats maintained on the sucrose diet, but deprivation of pyridoxine had no observable effect upon liver fat and in no case was there sufficient fat to warrant designation of a liver as fatty. A supply of linoleic acid did not apparently alter the quantity or quality of liver lipids.

All rats fed the casein diet had less carcass fat than did rats on the sucrose diet and this may have been due to a difference in food consumption. With both diets, lack of pyridoxine in the diet caused a decrease in the percentage of total fat in the carcass, an effect which was partially prevented by a supply of linoleic acid. The proportion of phospholipids and the iodine value of total lipids were greater in deprived than in control animals. The data suggest that vitamin B_6-deficient rats conserve phospholipids and unsaturated fatty acids.

Since the experimental diets were basically free of fat, it could be concluded that the control rats, particularly those on the sucrose diet, were able to synthesize fat. However, the markedly different amounts of fat in deprived and control animals cannot be taken as evidence that fat synthesis did not occur since an increase in fat storage may have been prevented by rapid utilization. The experiment with labelled glucose indicated that the incorporation of ^{14}C into carcass lipids was at least as much in deficient as in control rats. However, the amount of incorporation was small and it is inadvisable to draw a definite conclusion.

SUMMARY

1. Using isocaloric feeding of two fat-free basal diets, one high in protein and the other in carbohydrate, rats supplied with pyridoxine showed an increase in carcass fat with a decreased iodine value.

2. Rats deprived of vitamin B_6 contained markedly less carcass fat with an apparent conservation of phospholipids and of unsaturated fatty acids. There were no marked differences in the amount or characteristics of liver lipids. Data on the incorporation of ^{14}C (from fed glucose) suggest that fat synthesis proceeded equally in control and deficient rats. The decreased quantity of fat in deprived rats may have been due to rapid utilization.

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