reconcile quantitatively with the present results, since it would be expected to lead to smaller differences than are found. A part of the discrepancy may be due to small changes in the activity of the reference ion with changes in chloride concentration.

The results are as characteristic of the Cl^- anion as they are of the other participants. If another anion had been chosen such that the activity coefficients of the substituted ammonium salts had increased, rather than decreased, from NH_4^+ to Me_3NH^+ ion, the present series could possibly have been considerably changed, if not entirely reversed. The competing cations can be considered as being 'partitioned', in a sense, between the polyanion and the ambient anion, the balance being decided by their relevant affinities.

SUMMARY

1. The concentration of sodium chloride needed to dissolve complexes of the polyanions heparin, deoxyribonucleate, hyaluronate, alginate and dextran sulphate with long-chain aliphatic ammonium detergents decreased with increasing degrees of methylation of the cationic nitrogen.

2. The concentration of ammonium chloride and its methyl derivatives needed to dissolve the cetyl-

pyridinium complexes with the same polyanions increased with increasing degrees of methylation of the cationic nitrogen. Guanidinium chloride and ammonium chloride were comparable except that the deoxyribonucleate complex dissolved in a more dilute solution of the former.

3. It is assumed that there is competition between the cations of solvent salt and detergent, and the results are discussed on this basis.

4. The relevance of these results to systems in which there is binding to polyanions of substituted ammonium compounds is discussed.

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Liver Lipids and Ketone-Body Formation in Rats Deficient in Pantothenate

BY C. W. CARTER AND T. D. R. HOCKADAY*

Department of Biochemistry, University of Oxford

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Pantothenic acid is a constituent of coenzyme A (Lipmann, Kaplan, Novelli, Tuttle & Guirard, 1947; Novelli, Kaplan & Lipmann, 1949), and deficiency of this dietary factor has been shown to lower the concentration of the coenzyme in the tissues. Olson & Kaplan (1948) reported an immediate effect of deficiency of the vitamin on the concentration of the coenzyme in the adrenal of the rat, followed later by a 35–40% decrease in kidney, heart and liver. Such changes are associated with widespread disturbances of metabolism. Thus decrease of the concentration of the coenzyme in the cardiac muscle of ducklings (Olson & Stare, 1951), and of the liver of ducks and rats (Olson &

Kaplan, 1948) results in an impaired utilization of pyruvate.

There is also evidence of an impairment of acetate metabolism in pantothenate deficiency. Klein & Lipmann (1953*a*), using slices of rat liver incubated with carboxyl-labelled acetate, observed a marked decrease in the rate of synthesis of fatty acids and of cholesterol after 6 weeks of deprivation of the vitamin. Klein & Lipmann (1953*b*) also demonstrated a correlation between the concentration of coenzyme A and the capacity to synthesize lipid and cholesterol in *Saccharomyces cerevisiae* grown in pantothenate-deficient media.

Pantothenic acid has therefore been implicated in various aspects of lipid metabolism. The present paper reports observations on the analysis of liver

* Present address: Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford.

and carcass lipids and on the capacity of liver slices to oxidize octanoate and to form ketone bodies after dietary depletion of pantothenate in the rat.

METHODS

Animals. The animals used in pairs were an inbred strain of white rats reared in the Department. Each pair consisted of litter mates of the same sex and, as nearly as possible, of the same weight when they started to receive the experimental diet. The rats were weaned when approximately 28 days old (body weight 25–30 g.) and transferred to a synthetic diet when they weighed 55–60 g.

Diet. The rats received one of two diets, referred to below as the 'low-fat' (casein, 19%; sucrose, 62%; cod-liver oil, 5%; salt mixture, 4%; water, 10%; by weight) and 'high-fat' (casein, 19%; margarine, 19%; sucrose, 48%; cod-liver oil, 5%; salt mixture, 4%; water, 5%) diets. The casein was 'Glaxo vitamin-free casein' which had been further extracted, twice with 50% (v/v) ethanol and then twice with 95% (v/v) ethanol. McCollum's salt mixture no. 184 was used [composition given in full by Carter & Phizackerley (1951)]. The basal diet was supplemented with daily doses of vitamins: thiamine, 20 μ g.; nicotinamide, 100 μ g.; riboflavin, 40 μ g.; inositol, 500 μ g.; choline, 1 mg.; *p*-aminobenzoic acid, 250 μ g.; biotin, 0.5 μ g.; pyridoxin, 40 μ g.; arachis oil, 3 drops. In addition, one rat in each pair received 240 μ g. of calcium pantothenate daily. The pantothenate-fed rat was pair-fed with its deficient mate. Water intake was not restricted.

Chemical methods. When the deprived rat showed clear signs of deficiency [see Morgan & Simms (1939); Daft, Sebrell, Babcock & Jukes (1940)] after an average of 80 days on the synthetic diet, it was starved for about 17 hr. and killed by stunning and decapitation. The liver and adrenals were rapidly removed and, when required for chemical analysis, dropped into liquid air. When liver slices were to be used, the liver was weighed on a torsion balance and sliced free-hand with a razor blade. The carcass was added to 200 ml. of ethanolic 30% (w/v) potassium hydroxide. Pieces of liver were taken for measurement of dry weight and nitrogen content.

The lipids of the carcass were estimated by refluxing the carcass with ethanolic potash, filtering the mixture, and extracting in a Soxhlet apparatus with ether both the residue from the filtration and the solids obtained by evaporating a sample of the filtrate. The ethereal extracts were then evaporated to dryness and shaken with 100 ml. of chloroform, used in four portions. The resulting solution was filtered into a weighed beaker, evaporated to dryness, and dried in a vacuum desiccator. The amount of lipid was obtained by reweighing.

The liver lipids were extracted and fractionated by the methods used by Carter & Phizackerley (1951). Free and total cholesterol were then measured by the Leibermann-Burchard reaction, in the modifications of Sperry & Brand (1943) and of Kelsey (1939). The phosphorus contents of both the original light-petroleum extract and the ethereal extracts were measured colorimetrically (Berenblum & Chain, 1938; Ennor & Stocken, 1950). These results were then used to determine, by difference, the amount of neutral fat after samples of the lipid solutions in both acetone and in ether had been treated by the oxidative

method of Bloor (1928, 1929). In all cases estimates were performed on two portions of the light-petroleum extracts.

The adrenal lipids were extracted similarly and cholesterol, free and total, was determined as with the liver.

Ketone bodies were measured by the method of Greenberg & Lester (1944).

Nitrogen was determined by a micro-Kjeldahl procedure.

Manometric experiments. Nine Warburg flasks were set up for each liver. Each contained 25–80 mg. wet wt. of liver slices and 2.5 ml. of β -glycerophosphate buffer (Jowett & Quastel, 1935) in the main chamber. In the side arm of each flask was 0.5 ml. of β -glycerophosphate buffer which contained 1.5 μ moles of sodium hydrogen malate. In three flasks 9.9 μ moles of sodium octanoate were also dissolved in the side-arm buffer. The oxygen uptake was measured for 1 hr. after gassing with oxygen and equilibration in a bath at 38°. Three flasks were removed for determination of ketone-body concentration at zero time. After removal of a flask from the bath, 1 ml. of ZnSO₄ soln. and 1 ml. of Ba(OH)₂ soln. (Greenberg & Lester, 1944) were added and it was corked and placed in ice for 15 min. (Cohen & Stark, 1938).

In experiments with acetoacetate as the added substrate, 5 μ moles of acetoacetic acid dissolved in 0.5 ml. of Krebs-Ringer phosphate, pH 7.4, were added to the side arm of each flask.

RESULTS

Rats deprived of pantothenate grew less rapidly than the pair-fed controls and showed the characteristic dermatological and other signs of deficiency described by Morgan & Simms (1939) and by Daft *et al.* (1940). Table 1 shows the decrease in body and liver weight. The adrenal glands were relatively enlarged and often haemorrhagic, as reported by Supplee, Bender & Kahlenberg (1942) and by Deane & McKibbin (1946).

Analysis of lipid fractions

Liver lipids. Analysis of the liver lipids (Table 1) showed that on the low-fat diet there was a fall in the concentration of neutral fat from 3.9 g./100 g. fat-free dry wt. for the controls to 1.6 g./100 g. for the pantothenate-deficient animals. On the high-fat diet the control value was 10.1 and the deficient value 2.3, so that the difference was accentuated.

In contrast, the pantothenate deficiency was without effect on the concentration of phospholipid. Also the concentration of free cholesterol in the liver remained unchanged in deficiency on either diet (Table 1). There was a decrease in the concentration of esterified cholesterol in deficient animals when the high-fat diet was fed, but the concentration was the same in both control and deficient animals on the low-fat diet. These findings are in accord with the reports of Guehring, Hurley & Morgan (1952) and of Boyd (1953) which indicate that the amount of fat in the diet may be more important than the presence of pantothenate in determining any differences in liver cholesterol.

Table 1. *Lipids and weights of the carcass, liver and adrenals of rats with pantothenate deficiency*

Experimental details are given in the text. Values are given $\pm$ s.e.; the numbers of animals are given in parentheses. L, Low-fat diet; H, high-fat diet; P, pantothenate.

Diet	Body wt. (g.)	Body fat (g./100 g.)	Liver wt.		Liver lipids (g./100 g. fat-free dry wt.)				Adrenal wt.		Adrenal cholesterol (g./100 g. wet wt.)	
			(g.)	(g./100 g.)	Neutral fat	Total cholesterol	Free cholesterol	Phospholipid	(g.)	(g./100 g.)	Total	Free
Low fat + pantothenate (6)	129 $\pm$ 4	9.8 $\pm$ 1.3	4.8 $\pm$ 0.3	3.7 $\pm$ 0.2	3.9 $\pm$ 0.6	1.0 $\pm$ 0.1	0.8 $\pm$ 0.1	14.4 $\pm$ 0.8	33.4 $\pm$ 2.7	27.4 $\pm$ 1.0	8.7 $\pm$ 0.6 (4)	0.59 $\pm$ 0.07 (3)
High fat + pantothenate (4)	174 $\pm$ 7	11.7 $\pm$ 1.1	5.5 $\pm$ 0.5	3.2 $\pm$ 0.2	10.1 $\pm$ 2.0	1.6 $\pm$ 0.1	1.1 $\pm$ 0.1	17.4 $\pm$ 2.1	32.9 $\pm$ 2.8	19.3 $\pm$ 2.0	6.4 $\pm$ 1.0 (5)	0.32 $\pm$ 0.02 (2)
Low fat, no pantothenate (6)	77 $\pm$ 5	3.4 $\pm$ 0.4	3.2 $\pm$ 0.2	4.1 $\pm$ 0.1	1.6 $\pm$ 0.3	1.3 $\pm$ 0.1	1.1 $\pm$ 0.1	13.7 $\pm$ 0.7	29.1 $\pm$ 0.3	40.9 $\pm$ 4.3	1.0 $\pm$ 0.1 (4)	0.43 $\pm$ 0.01 (3)
High fat, no pantothenate (4)	84 $\pm$ 5	3.3 $\pm$ 0.6	2.9 $\pm$ 0.2	3.5 $\pm$ 0.2	2.3 $\pm$ 0.6	1.4 $\pm$ 0.1	1.2 $\pm$ 0.1	17.1 $\pm$ 1.7	24.3 $\pm$ 1.8	31.4 $\pm$ 4.4	3.2 $\pm$ 0.7 (5)	0.45 $\pm$ 0.05 (2)
L + P against L - P	—	0.02-0.01	0.01-0.001	0.2-0.1	0.02-0.01	0.05-0.02	0.05-0.02	—	0.6-0.5	0.2-0.1	0.001	For all + P against all - P, P = 0.6-0.7
L + P against H + P	—	0.3-0.2	0.3-0.2	—	0.01-0.001	0.001	0.05-0.02	0.02-0.01	—	0.01	0.1-0.05	
H + P against H - P	—	<0.001	0.01-0.001	—	0.05-0.02	0.4-0.3	—	—	0.05-0.02	0.05-0.02	0.05-0.02	
L - P against H - P	—	—	0.5-0.4	0.02-0.01	0.3-0.2	—	—	0.1-0.05	0.05-0.02	0.2-0.1	0.05-0.02	

Carcass lipids. There was a significant decrease in the concentration of total carcass lipids in pantothenate-deficient animals and this was relatively uninfluenced by the dietary fat (Table 1).

Adrenal cholesterol. There was a marked fall in the total cholesterol concentration in the adrenals of pantothenate-deficient animals (Table 1). This was greater on the low-fat than on the high-fat diet. The fall occurred in the esterified cholesterol fraction and the concentration of free cholesterol was apparently unchanged in the small number of rats investigated.

Oxygen uptake and ketone-body formation

The average values for the oxygen uptake of liver slices are given in Table 2 and the individual points from which they were calculated in Fig. 1. With no added substrate there was no significant difference in the respiration of control and pantothenate-deficient tissue, in agreement with other studies (Olson & Kaplan, 1948; Klein & Lipmann, 1953*a*). In the presence of octanoate, however, liver slices from deficient animals had a lower oxygen uptake than those from normal animals, both on the high-fat and low-fat diets.

A similar pattern was seen for the accumulation of ketone bodies by the incubated liver slices. In the absence of substrate there was a slight but insignificant decrease in ketone-body production by pantothenate-deficient slices. But, in the presence of octanoate, ketone-body accumulation was significantly less in the deficient than in the control tissue, again both for all animals and for the high-fat group alone.

Ketone-body accumulation by incubated liver slices is determined by its rate of production, and little or no oxidation of acetoacetate occurs in the liver (Stern, Coon & del Campillo, 1953; Langdon, 1960). This is confirmed by the results given in Table 3. Incubation of liver slices with acetoacetate at a concentration of 1.67 mM (or approximately 5 m-moles of acetoacetate/g. of tissue nitrogen) caused no increase in oxygen consumption and there was no difference in ketone-body disappearance between control and pantothenate-deficient tissue.

In all the conditions tested—control, pantothenate-deficient, substrate added, no substrate—there was an increase in ketone-body production with increase in respiration (Fig. 1). The slope of

Table 2. *Respiration and ketone-body production of liver slices from pantothenate-deficient rats*

Experimental details are given in the text. Values are expressed as m-moles/g. of nitrogen $\pm$ s.e., with numbers of animals in parentheses. O, Octanoate; P, pantothenate.

	With pantothenate		Without pantothenate	
	No substrate	Octanoate added	No substrate	Octanoate added
All animals				
Oxygen uptake	3.86 $\pm$ 0.43 (13)	5.14 $\pm$ 0.31 (13)	3.01 $\pm$ 0.53 (8)	3.16 $\pm$ 0.60 (8)
Ketone production	0.46 $\pm$ 0.06 (13)	1.12 $\pm$ 0.14 (13)	0.33 $\pm$ 0.06 (8)	0.49 $\pm$ 0.12 (8)
Six high-fat pairs				
Oxygen uptake	4.09 $\pm$ 0.57	4.90 $\pm$ 0.72	3.44 $\pm$ 0.55	3.70 $\pm$ 0.65
Ketone production	0.49 $\pm$ 0.08	1.08 $\pm$ 0.16	0.40 $\pm$ 0.04	0.63 $\pm$ 0.12
Probability of differences for all animals				
	(+ O + P) against (+ O - P)	(+ O + P) against (- O - P)	(+ O + P) against (- O + P)	(+ O - P) against (- O + P)
Oxygen uptake	0.01-0.001	0.01-0.001	0.05-0.02	—
Ketone production	0.01-0.001	<0.001	<0.001	0.3-0.2 0.2-0.1

Table 3. *Oxygen uptake and ketone-body accumulation for liver slices incubated with and without acetoacetate*

Experimental details are given in the text. Values are expressed as m-moles/g. of tissue nitrogen $\pm$ s.e., with numbers of animals in parentheses.

	No substrate		Acetoacetate added	
	Oxygen uptake	Ketone production	Oxygen uptake	Ketone production
Without pantothenate	3.6 $\pm$ 0.6 (5)	0.21 $\pm$ 0.07 (5)	3.9 $\pm$ 0.02 (5)	-0.36 $\pm$ 0.06 (5)
With pantothenate	3.9 $\pm$ 0.5 (4)	0.33 $\pm$ 0.07 (5)	3.7 $\pm$ 0.4 (5)	-0.48 $\pm$ 0.04 (5)

the line drawn to relate ketone-body production to oxygen consumption is steepest when octanoate was added to slices from animals given pantothenate and is significantly different from the slope for the other three groups. Also, with the deficient animals, the slope is greater in the presence of octanoate than in its absence (Table 4 and Fig. 1).

DISCUSSION

The decreased body weight and the conspicuous loss of fat in the body and liver of pantothenate-deficient rats as compared with pair-fed controls is an indication of metabolic inefficiency. The tissues are wasted to different degrees. Thus, although there is a decrease in the absolute weight of the

deficient liver, when its weight is expressed as a percentage of body weight the loss of liver substance is less marked than that of the body as a whole.

This does not happen in the adrenals. Pantothenate deficiency does not decrease even the absolute weight of the glands on a low-fat diet, and their weight relative to that of the whole body increases markedly on either diet. Though the fall in the concentration of cholesterol esters in the adrenal may indicate a specific metabolic defect as suggested by Dumm, Gershberg, Beck & Ralli (1953), the adrenal hypertrophy is in part the expression of a non-specific nutritional stress (McCarrison, 1919); in pyridoxin deficiency at least, it is accompanied by a depletion of adrenal cholesterol (Hockaday, 1953) similar to that found in pantothenate deficiency by Morgan & Lewis (1952) and here. The concentration of free cholesterol and of phospholipid is unchanged in the liver, although deficiency results in a decreased concentration of neutral fat.

The concentration of neutral fat in the liver represents a balance between the opposing processes of synthesis and degradation. Klein & Lipmann (1953*a*) demonstrated a decreased synthesis of fatty acids and cholesterol from carboxyl-labelled acetate by pantothenate-deficient liver and that the decrease is associated with the fall in coenzyme A concentration. The present experiments reveal an impairment in the capacity of the liver to oxidize octanoate.

The catabolism of octanoate may be represented by the scheme:

Reactions (1) plus (2) give a theoretical ratio of ketone-body production to oxygen utilization of 0.67, and for equal disposal of octanoate between

Fig. 1. Oxygen uptake and ketone-body production of liver slices (from pantothenate-deficient and control rats) incubated with or without octanoate (3.3 mM) for 60 min. A, Control rats, plus octanoate: correlation coefficient, r 0.92. B, Pantothenate-deficient rats, plus octanoate: r 0.92. C, Control rats, no octanoate: r 0.62. D, Pantothenate-deficient rats, no octanoate: r 0.87.

Table 4. Ratio of ketone-bodies produced to oxygen uptake of liver slices from pantothenate-deficient rats

Experimental details are given in the text. O, Octanoate; P, pantothenate.				
	With pantothenate		Without pantothenate	
	No substrate	Octanoate added	No substrate	Octanoate added
All animals	0.11±0.01	0.21±0.02	0.09±0.01	0.15±0.02
Six high-fat pairs	0.12±0.01	0.22±0.01	0.09±0.01	0.16±0.02
Probabilities of difference				
	All animals		Six high-fat pairs	
(+ O + P) against (+ O - P)	0.05-0.02		0.05-0.02	
(+ O + P) against (- O - P)	>0.001		>0.001	
(+ O + P) against (- O + P)	>0.001		0.01-0.001	
(+ O - P) against (- O - P)	0.02-0.01		0.01-0.001	
(+ O - P) against (- O + P)	0.10-0.05		0.10-0.05	
(- O + P) against (- O - P)	0.3-0.2		0.3-0.2	

reactions (2) and (3) the ratio would be 0.15. The average ratios observed in these experiments vary from 0.09 to 0.22, but the ratio of change in ketones to change in oxygen (on addition of octanoate) is 0.76 for slices from the pantothenate-supplemented rats. The ratio is also high for the deficient rats, but the absolute changes are so small that the ratio has little meaning.

This introduces the main difference between pantothenate-deficient and control slices: the lack of alteration, on addition of octanoate, in the average rates of oxygen uptake and ketone-body formation of the former compared with the marked response of the latter. This suggests a gross defect of reaction (1) or of all three reactions. However, the average ratio of ketone-body production to oxygen utilization does rise significantly on adding octanoate to deficient slices and so reaction (2) would seem still to have a reserve capacity.

If the lines in Fig. 1 are extrapolated towards the base-line, those for pantothenate-supplemented animals indicate an oxygen uptake between 2 and 3 m-moles/g. of nitrogen when there is no ketone-body production, suggesting that, unless this level of oxidative activity be exceeded, ketone bodies are not formed. By contrast, for the deprived animals, similar extrapolation indicates that ketone-body production begins as soon as oxygen is consumed.

Krebs (1960), in reviewing the problem of ketosis, concluded that, in conditions such as starvation and diabetes mellitus, the accumulation of ketone bodies is an expression of over-production rather than of under-utilization. The direct proportionality of oxygen consumption and ketone-body formation in all the conditions studied in the present experiments is consistent with this view. The failure of ketone-body accumulation on addition of octanoate to the deficient slices would seem to provide an illustration of the corollary to Krebs's conclusion: that decreased appearance of ketones is essentially due to under-production.

SUMMARY

1. Rats deficient in pantothenic acid grew less than pair-fed controls and had smaller livers with lower concentrations of neutral fat, but unchanged concentrations of phospholipid and free cholesterol. Carcass lipid was reduced. Their enlarged adrenal glands had a lower cholesterol concentration.

2. The main difference between liver slices from pantothenate-supplemented animals and from those deficient in the vitamin was in the lack of

change in oxygen uptake and ketone-body production on addition of octanoate to deficient slices by comparison with the increases observed with control slices.

3. With both groups of slices, and in presence or absence of added octanoate, ketone-body production was proportional to oxygen uptake. In the presence of octanoate, the ratio of ketone-body production to oxygen uptake was decreased by pantothenate deficiency.

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