unsaturated cod-liver oil, which is free from ascorbic acid, shows an intense black deposit after immersion in acidified silver nitrate in the dark (although not *in situ* in the liver), and to a limited extent this is also true of oil removed from salmon yolk.

In the late larval stage of 9 March, the various organs are almost completely differentiated. Although no ascorbic acid was found by estimation at this stage, there appears to be some deposition of metallic salts in the tissue. In section the issue is confused by the presence of black pigment, but there are certain discrete deposits near the vertebral column and in cells of the yolk-sac.

SUMMARY

1. It was found by the titrimetric method of Mapson (1943) that ascorbic acid was present in early

stages of developing salmon (8 and 14 days after fertilization), but absent in a late larval stage in which most of the yolk had been absorbed. It was contained both in the blastoderm region and in the yolk and yolk-sac.

2. Sections obtained from material fixed in acidified silver nitrate show that ascorbic acid may be present in blastoderm cells, in the underlying periblast and also in adjacent yolk. In this respect the distribution is much the same as in early stages of chick development as recorded by Barnett & Bourne (1940-1).

3. There are intense deposits of metallic silver where oil globules have been situated in the main part of the yolk. These may be due, in part, to ascorbic acid present in the yolk, although there is also evidence in favour of a reaction between the silver nitrate solution and the oil globules.

REFERENCES

- Barnett, S. A. & Bourne, G. (1940-1). *J. Anat., Lond.*, **75**, 251.
 Barnett, S. A. & Bourne, G. (1942). *Quart. J. micr. Sci.* **83**, 259.
 Barnett, S. A. & Fisher, R. B. (1944). *J. exp. Biol.* **20**, 14.
 Daniel, R. J. (1946). *J. exp. Biol.* **24**, 123.
 Long, C. N. H. (1947). *Recent Progress in Hormone Research*, **1**, 99.
 Lugg, J. W. H. (1942). *Aust. J. exp. Biol. med. Sci.* **20**, 273.
 Mapson, L. W. (1943). *J. Soc. chem. Ind., Lond. (Trans.)*, **62**, 223.
 Zwemer, R. L., Lowenstein, B. E. & Pines, K. L. (1940). *Endocrinology*, **27**, 945.

Studies in Biochemical Adaptation. Effect of Diet on the Intestinal Phosphatase of the Rat

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(Received 14 April 1949)

Verzár & McDougall (1936) suggested that the absorption of glucose and fructose is accompanied by phosphorylation by the intestinal mucosa. Part of the evidence upon which this theory was based was that iodoacetate decreases the rate of absorption of glucose, and this evidence has been criticized by several later writers (e.g. Klinghoffer, 1938). Again, Verzár supposed that an adrenal cortical hormone was responsible for the phosphorylation, and this has been criticized (Cori & Cori, 1941). Nevertheless, most workers accept the view that phosphorylation of glucose and fructose is an important stage in their absorption.

It has also been suggested that fats are phosphorylated before absorption (e.g. Sinclair, 1929). Verzár & McDougall (1936) claimed that this was controlled

by the adrenal cortex, but, as with their theory of carbohydrate absorption, this view has since been severely criticized. Frazer (1948; see also Frazer, 1946) has suggested that fat may be absorbed as the unchanged triglyceride, although this is not universally accepted (Lundbaek & Maaløe, 1947). Most workers are agreed, however, that at least some phosphorylation of fat occurs during absorption (Schmidt-Nielsen, 1946).

It might be considered that diets with varying proportions of carbohydrate and fat might possibly stimulate the small intestine to produce varying amounts of phosphatase, since it is known that the amounts of some digestive enzymes are affected by variations in the degree to which they are called upon to carry out their work. A study was therefore

made of the amount of intestinal phosphatase in animals fed on diets containing different amounts of carbohydrate and of fat.

EXPERIMENTAL

Piebald rats of the Hartwell strain were placed on one of four diets for 6–7 months from weaning. The diets were constructed so as to contain varying proportions of protein, carbohydrate and fat (Table 1). Each diet was fed to ten rats, five male and five female, each rat being housed in a separate cage.

Table 1. *Composition of diets*

Diet	Casein (‘Light white’ Glaxo) (%)	Sucrose (%)	Lard (%)
Basal	15	55	15
Fat-free	15	70	0
High fat	15	0	70
High protein	70	15	0

In addition, each diet contained dried yeast (10%) and salt mixture (5%). Each animal was given cod-liver oil (5 drops twice weekly).

Preparation of intestinal extract. Animals were killed by exsanguination. The small intestine was dissected out, as free as possible from adherent tissue, from pylorus to caecum. The contents were removed by blowing a gentle stream of saline through the lumen. Since the activity of phosphatase decreases progressively from duodenum to caecum (Kay, 1932; Folley & Kay, 1936), it was thought desirable to assay the enzyme separately in the proximal and distal portions of the intestine. The intestine was therefore divided into as nearly as possible equal proximal and distal lengths. Each length was slit open, dried gently between filter paper and weighed. It was then ground with about twice its weight of washed silver sand. Water saturated with CHCl_3 was added to a volume exactly ten times the original wet weight of the intestine and the suspension kept for 24 hr. at laboratory temperature with occasional shaking.

Estimation of phosphatase activity. The alkaline phosphatase was assayed separately in the extracts from the proximal and distal parts of each intestine. The extracts, after standing for 24 hr., were further diluted with CHCl_3 water so that they contained 1 g. of wet tissue in 1000 ml. for the proximal part of the intestine and 1 g. in 250 ml. for the distal part. These dilutions were found to give enzymic activities such that conveniently measurable amounts of phosphate ions were liberated in the period of incubation with the substrate.

The phosphatase digest was set up as follows: 1 ml. of the diluted extract was added to 10 ml. of buffered sodium β -glycerophosphate (0.5 w/v), and the mixture incubated at 37° for 1 hr. Of this mixture, 1 ml. was added to 4 ml. of water; to this was added 5 ml. of trichloroacetic acid (12% w/v) and the whole well mixed. It was not necessary to centrifuge or filter at this stage since, owing to the high dilution of the enzymic preparation, the amount of protein precipitated was so little that no significant turbidity occurred.

Inorganic phosphate was estimated in the preparation by placing 5 ml. in a colorimeter tube, adding 4 ml. of acid molybdate reagent and 1 ml. of freshly prepared dilute

SnCl_2 solution, the colour being measured in the Evelyn photoelectric colorimeter after 10 min. at laboratory temperature. An exactly similar analysis, carried out upon a 1 ml. sample of the digest taken immediately after adding the enzyme preparation, gave the initial inorganic phosphate content of the digest. The solutions of sodium β -glycerophosphate, molybdate and SnCl_2 were prepared as described by Bodansky (1932–3).

The activity of the enzyme is expressed in ‘units’ (mg. P liberated/g. tissue/hr. at pH 9.2 and 37°). No attempt was made to obtain possible activation of the enzyme by, for example, the addition of salts of Mg or Zn.

RESULTS

The mean weights of the animals and of the whole of their small intestines are given in Table 2. The differences between the different groups of animals

Table 2. *Effect of diet on weight of rat and of small intestine*

Diet	Average wt. of rats (g.)	Average wet wt. of small intestine (g.)
Basal	187	6.45
Fat-free	186	6.74
High fat	168	6.45
High protein	193	6.76
Average, all groups	184	6.62

are statistically not significant. As was to be expected, the average weights of the female animals and of their small intestines were lower than those of the males. Also, the average weights of the proximal portions of the intestines were higher than those of the distal portions. Table 3 shows these values for all groups combined.

Table 3. *Weight of rats and of small intestine. Comparison of male and female rats, and of proximal and distal portions of intestine*

	Average wt. of rats (g.)	Average wet wt. of small intestine (g.)		
		Whole	Proximal portion	Distal portion
Male	202	7.40	4.03	3.37
Female	166	5.83	3.17	2.66
Average, all rats	184	6.62	3.60	3.02

The values for phosphatase activities are given in Table 4. It seems reasonable to conclude that there is the same amount of intestinal phosphatase in the rats on the basal and fat-free diets and also the same amount in the rats on the high fat and high protein diets. On the other hand, there is more intestinal phosphatase in rats on either of the latter two diets than in those on either of the former two diets. For example, rats on the basal and fat-free diets had intestinal phosphatase values of 52 and 58 units/g.; rats on the high fat and high protein diets had values of 77 and 78 units/g.

Table 4. *Phosphatase activity of small intestine of rats on different diets*(Ten rats on each diet. Results (mean $\pm$ s.e.) expressed as 'units', i.e. mg. P liberated in 1 hr. at pH 9.2 and 37°.)

Tissue		Diet			
		Basal	Fat-free	High fat	High protein
Whole intestine	Total units	(1) 330 $\pm$ 46.5	(2) 391 $\pm$ 41.7	(3) 489 $\pm$ 39.3	(4) 538 $\pm$ 42.6
	Units/g.	(5) 52 $\pm$ 7.1	(6) 58 $\pm$ 4.8	(7) 77 $\pm$ 6.3	(8) 78 $\pm$ 6.4
Proximal portion	Total units	(9) 271 $\pm$ 40.7	(10) 328 $\pm$ 31.3	(11) 375 $\pm$ 33.0	(12) 448 $\pm$ 37.2
	Units/g.	(13) 77 $\pm$ 10.6	(14) 87 $\pm$ 6.0	(15) 113 $\pm$ 8.3	(16) 119 $\pm$ 10.9
Distal portion	Total units	(17) 58 $\pm$ 7.8	(18) 62 $\pm$ 13.3	(19) 114 $\pm$ 10.8	(20) 90 $\pm$ 16.1
	Units/g.	(21) 20.3 $\pm$ 3.4	(22) 20.3 $\pm$ 3.9	(23) 34.5 $\pm$ 3.5	(24) 29.0 $\pm$ 3.7

Significant differences ($P < 0.05$) exist between the following groups: (1) and (3), (1) and (4), (2) and (4), (5) and (7), (5) and (8), (6) and (7), (6) and (8), (9) and (11), (9) and (12), (10) and (12), (13) and (15), (13) and (16), (14) and (15), (14) and (16), (17) and (19), (17) and (20), (18) and (19), (21) and (23), (22) and (23).

DISCUSSION

The chief difference between the basal and fat-free diets on the one hand and the high fat and the high protein diets on the other is the presence of much carbohydrate (55 or 70 % sucrose) in the former and its absence in the latter. It would seem therefore that the presence of sucrose leads to a reduction in the amount of phosphatase in the intestinal tissue. References to similar work are very meagre. Bellini & Cera (1940) found an increase in the intestinal phosphatase of the rat during fat absorption. Weil & Russell (1940) found a decrease in plasma phosphatase in the rat during fasting, and an increase during the feeding of lard and of cephalin but not of lecithin. Lundbaek & Goranson (1948) showed that the

phosphorylase of rat muscle is increased during starvation.

We can at present suggest no reason why the presence of sucrose in the diet should lead to a decrease in intestinal phosphatase, and until more data are available it is profitless to speculate.

SUMMARY

The effect was studied of varying the proportions of dietary protein, fat and carbohydrate on the alkaline phosphatase of the small intestine of the rat. Diets containing 55 or 70 % of sucrose produced a significantly lower amount of the enzyme than diets in which the sucrose was replaced by fat or protein.

REFERENCES

- Bellini, L. & Cera, B. (1940). *Pathologica*, **32**, 195 (see *Chem. Abstr.* **34**, 7350).
 Bodansky, A. (1932-3). *J. biol. Chem.* **99**, 197.
 Cori, C. F. & Cori, G. T. (1941). *Ann. Rev. Biochem.* **10**, 151.
 Frazer, A. C. (1946). *Physiol. Rev.* **26**, 103.
 Frazer, A. C. (1948). *J. R. Soc. Arts*, **96**, 582.
 Kay, H. D. (1932). *Physiol. Rev.* **12**, 384.
 Folley, S. J. & Kay, H. D. (1936). *Ergebn. Enzymforsch.* **5**, 159.
 Klinghoffer, K. A. (1938). *J. biol. Chem.* **126**, 201.
 Lundbaek, K. & Goranson, E. S. (1948). *Nature, Lond.*, **162**, 1002.
 Lundbaek, K. & Maaløe, O. (1947). *Acta physiol. scand.* **13**, 247.
 Schmidt-Nielsen, K. (1946). *Acta physiol. scand.* **12**, Suppl. no. 37.
 Sinclair, R. G. (1929). *J. biol. Chem.* **82**, 112.
 Verzá, F. & McDougall, E. V. (1936). *Absorption from the Intestine*. London: Longmans, Green & Co.
 Weil, L. & Russell, M. A. (1940). *J. biol. Chem.* **136**, 9.

The Reactions of Haems with Cyanides and isoCyanides

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(Received 2 May 1949)

By determining the minimum amount of pyridine required to give a haemochromogen with a very strong solution of haem (ferrous protoporphyrin), Hill (1926) demonstrated conclusively that pyridine haemochromogen contains 2 molecules of organic base per molecule of haem or per atom of iron. This result was in agreement with that of von Zeynek

(1910) who found 2.2 mol. of pyridine per mol. of haem in a solid pyridine haemochromogen.

Potassium cyanide, however, unlike other nitrogenous substances, was found by Anson & Mirsky (1928) to give two distinct compounds with haem. The first, containing 1 mol. of cyanide per molecule of haem, was named by them 'cyan-haemochromogen'