Regulation of intestinal alkaline phosphatase levels in the rat

Role of the adrenal cortex

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SYNOPSIS Bilateral adrenalectomy produces a reduction in the alkaline phosphatase concentration in rat intestine, which is not prevented by the administration of saline, but is prevented by the administration of cortisone. The administration of A.C.T.H. to normal rats leads to a rise in intestinal alkaline phosphatase levels associated with a small increase in the weight of the adrenals, while the administration of hydrocortisone produces large increases in the enzyme, even with doses which cause significant hypoplasia of the adrenal glands.

The significance of these findings in relation to mechanisms of fat absorption and the treatment of steatorrhoea is discussed.

Verzar and Laszt (1935) and Laszt and Verzar (1936) first demonstrated that fat absorption was impaired in the adrenalectomized rat, and that it could be improved by the administration of eucortone. They claimed that fat transport was impaired in these animals, and suggested that this was due to impaired phosphorylation. Barnes, Miller, and Burr (1941) disputed their findings and stated that adrenalectomized rats absorbed fat at least as well as normal animals, and while they agreed that there was some deficiency in fat transport, their data seemed to show that phosphorylating mechanisms were unaffected.

Recently we have confirmed that fat absorption is diminished in the hypo-adrenal rat (Watson and Murray, 1966) and shown that this is not due to impaired intraluminal hydrolysis of dietary fat or to inadequate intracellular glyceride synthesis. We have suggested that the defect may lie in the actual movement of lipid molecules across the juxta-luminal membrane of the intestinal epithelial cell.

For reasons to be discussed later, it was decided to investigate the role of the adrenal cortex in the regulation of small bowel alkaline phosphatase, as a possible mechanism by which the adrenal controlled fat absorption.

Work on this subject has hitherto been scanty and conflicting, probably because most of it has depended upon histochemical techniques. Thus Tissières (1948)

claimed that the adrenal had no effect on rat intestine alkaline phosphatase, and Hebert (1950) claimed that it had. She stated that the alkaline phosphatase staining reaction was diminished in rats which had been both adrenalectomized and castrated, made more positive in such animals by the administration of cortical hormone, and increased in normal rats given cortical hormones. Adrenaline had no effect.

The main quantitative studies on intestinal alkaline phosphatase have been those of Kutscher and Wust (1942) and Tuba and his colleagues (Tuba and Dickie, 1954; Dickie, Robinson, and Tuba, 1955; Triantaphyllopoulos and Tuba, 1959a and b). Tuba and his co-workers have written on the distribution and kinetics of intestinal alkaline phosphatase and on the effects of protein, amino-acid, carbohydrate, and fatty acid absorption upon the tissue levels.

Kutscher and Wust (1942) studied intestinal and renal alkaline phosphatase in the guinea-pig. They stated that adrenalectomy caused a reduction in alkaline phosphatase in both organs which was corrected in the kidney by the administration of desoxycorticosterone acetate, but only slightly improved in the intestine by similar treatment. Unfortunately they did not use control animals in these studies.

METHODS

Alkaline phosphatase activity (K.A. units/g. wet tissue) in

intestinal homogenates was determined by the method of Kind and King (1954) as modified by Marsh, Fingerhut, and Kirsch (1959) and Axelsson, Ekman, and Knutsson (1965) for AutoAnalyzer techniques. The entire batch of samples from each separate experiment was analysed on the same run.

About 70% of the alkaline phosphatase in an intestinal homogenate is in the microsomal fraction, so that it is mainly in a particulate form rather than in solution (Morton, 1954; Triantaphyllopoulos and Tuba, 1959a). The constant agitation of the sample during its passage through the AutoAnalyzer keeps the microsomes in a state of suspension and assures that near maximum values are obtained. This was demonstrated by comparing determinations on standard homogenates with homogenates which had been incubated for one hour at 37°C. with butanol, thereby bringing the alkaline phosphatase of particulate matter into solution (Morton, 1954).

PREPARATION OF HOMOGENATES Early in the present study we confirmed the findings of Triantaphyllopoulos and Tuba (1959a) that alkaline phosphatase activity was greatest in the proximal small bowel and fell progressively distally. Therefore intestinal homogenates were prepared in a standard manner, as follows.

The rat was killed by decapitation, the small bowel separated at the pyloro-duodenal junction, and about 20 cm. removed while the heart was still beating. This was washed through with 10 ml. of tap water, laid straight, without stretching, on a sheet of dry blotting paper, and the segment of bowel 8 to 12 cm. from the pylorus separated, opened along its length with fine scissors and 'dried' in a uniform manner as previously described (Watson and Murray, 1966).

The piece of tissue was weighed to the nearest milligram on an Oertling torsion microbalance, homogenized in tap water using a ground glass homogenizer, and brought to a uniform concentration of 5 mg./ml. Homogenates were stored at -20°C. until the determinations were carried out.

REPRODUCIBILITY OF METHOD A series of ten replicates on a high value sample gave a mean value of 360 ± 12 for 2 standard deviations.

ANIMAL TECHNIQUES All experiments were performed on

male, Wistar rats obtained from the same source, and in separate batches for each experiment. Bilateral adrenalectomy was performed through a single dorsal incision. A sham operation duplicated the procedure, except for the removal of the glands. In experiments which involved injection schedules, control animals were given injections of saline. Since fasting affects the amount of alkaline phosphatase in the bowel, the animals were offered food up to the moment of sacrifice.

The hypoadrenal state of adrenalectomized animals was confirmed as previously described (Watson and Murray, 1966).

RESULTS

The results of the first group of experiments are summarized in Table I.

EXPERIMENT 1 In this experiment the animals were sacrificed six days after adrenalectomy. The adrenalectomized rats were given tap water to drink. The average initial weights of the control and adrenalectomized rats were 126 and 127 g. respectively.

Adrenalectomy resulted in a highly significant fall in the alkaline phosphatase levels of comparable segments of the upper small intestine.

EXPERIMENT 2 The effect of a sham operation was studied. Both groups of animals were given food and water as usual. The average initial weights of the two groups were 82 and 81 g., and the rats were killed after six days.

Sham operation had no effect on the intestinal alkaline phosphatase levels.

EXPERIMENT 3 Adrenalectomized rats were given 1% saline to drink after the operation, and the animals killed after six days. The average initial weights of the groups were 90 and 91 g.

The administration of saline did not offset the effect of adrenalectomy, and the fall in alkaline

TABLE I
RESULTS OF EXPERIMENTS ON ADRENALECTOMIZED AND SHAM OPERATED RATS

Experiment No.	Condition	No. of Rats	Average Weight Gain (g.)	Intestinal Alkaline Phosphatase (K.A. units/g.)		Statistics
				Mean	S.D.	
1	Normal	6	37	277	69	t = 4.5; p < 0.002
	Adrenalectomy	6	9	135	34	
2	Normal	6	32	199	49	t = 0.38; p > 0.25
	Sham operation	6	33	209	41	
3	Normal	5	26	201	19	t = 3.66; p < 0.01
	Adrenalectomy + 1% saline	6	18	126	42	
4	Normal	6	29	119	11	t = 1.76; p > 0.1
	Adrenalectomy + cortisone acetate (2.5 mg./day)	5	-1	140	27	

phosphatase was again significant. This time the fall occurred in a situation in which the difference in weight gain between the groups was not significant ($t = 1.26$; $p > 0.1$).

EXPERIMENT 4 Adrenalectomized rats were given tap water and 2.5 mg. cortisone acetate daily by injection. The animals were killed after seven days. The initial average weights of the two groups were 77 and 78 g.

The condition of the adrenalectomized rats was poor and one of them died. In spite of this, and the fact that there was a gross difference in weight gain, the intestinal alkaline phosphatase levels were higher in the operated animals, although the difference did not reach formal significance.

From these experiments the following conclusions seem reasonable. Operative stress comparable to that of adrenalectomy does not affect rat intestinal alkaline phosphatase levels. In the adrenalectomized rat, however, the levels fall significantly and are not maintained by the administration of saline. Supplementary cortisone does maintain the enzyme levels in these animals.

Changes in the intestinal alkaline phosphatase levels cannot be attributed simply to alterations in the animal's weight.

The next group of studies was planned to investigate the effect of hormone administration to the normal rat. The results of experiments 5 and 6 are summarized in Table II.

cortisone acetate subcutaneously for 10 days. The average initial weights were 68 g. for both groups. The cortisone-treated rats became very unhealthy about three days after treatment began, and all of them lost weight. The adrenals were not weighed in this experiment, but in every treated rat they were grossly hypoplastic.

This was not a satisfactory experiment. The intestinal alkaline phosphatase levels were lower in the treated animals, although the difference does not achieve formal significance. It is not certain how far the poor condition of the rats and the involution of the adrenals contributed to this result. All that can be stated is that a large dose of cortisone did not cause a rise in the intestinal alkaline phosphatase levels of the normal rat. The experiment would have been repeated with a smaller dose of cortisone, but the subsequent experiments seemed to make this unnecessary.

EXPERIMENT 6 Normal rats were given 4 units of A.C.T.H. subcutaneously for 14 days. They remained in good condition and the two groups gained weight almost equally. Initial average weights were 80 and 76 g. The adrenals were slightly larger in the treated animals, but the mean difference of 6.8 mg. for paired glands is not significant.

The intestinal alkaline phosphatase levels were significantly higher in the A.C.T.H.-treated rats than in the controls.

EXPERIMENT 5 Normal rats were given 12.5 mg.

EXPERIMENT 7 In the final experiment three groups

TABLE II

RESULTS OF EXPERIMENTS ON NORMAL RATS GIVEN CORTISONE (12.5 MG./DAY) AND A.C.T.H. (4 UNITS/DAY)

Experiment No.	Condition	No. of Rats	Average Weight Gain (g.)	Alkaline Phosphatase (K.A. units/g.)		Statistics
				Mean	S.D.	
5	Normal	6	40	175	47	$t = 1.91$; $p < 0.05$
	Cortisone	6	-6	122	29	
6	Normal	5	64	136	37	$t = 3.55$; $p < 0.01$
	A.C.T.H.	5	59	210	29	

TABLE III

RESULTS OF EXPERIMENT ON RATS GIVEN SUBCUTANEOUS HYDROCORTISONE IN DOSES OF 1 AND 10 MG./DAY

Experimental Conditions	No. of Rats	Average Initial Weight (g.)	Mean Weight Gain (g.)	Weight of Paired Adrenals (mg.)		Intestinal Alkaline Phosphatase (K.A. units/g.)	
				Mean	S.D.	Mean	S.D.
Normal (a)	8	77.4	33.0	36.9	5.3	153	41
1 mg./day (b)	6	77.1	46.8	35.0	4.2	245	39
10 mg./day (c)	8	77.0	37.5	25.2	4.2	345	65

Adrenal weight statistics: comparing (a) and (c): $t = 4.75$; $p < 0.001$

Alkaline phosphatase statistics: comparing (a) and (b): $t = 4.21$; $p < 0.002$

(a) and (c): $t = 7.07$; $p < 0.001$

(b) and (c): $t = 3.48$; $p < 0.01$

of rats were used: controls (7a), normal rats given hydrocortisone 1 mg./day (7b), and 10 mg./day (7c) by subcutaneous injection for 10 days. All animals remained in good condition, except two of those in group 7b which seemed to be unwell from the start of the study and were excluded from the data. Both animals were necropsied and had bilateral renal lesions, probably inflammatory.

The results of this experiment are recorded in Table III. The difference between the average paired adrenal weights of groups a and c is significant. In spite of this the degree of increase in intestinal alkaline phosphatase in these animals was the greatest of the series, and was considerable. Even the 1 mg. dose of hydrocortisone produced a significant rise, and the further increase from the larger dose was significantly higher than this.

Hydrocortisone has a powerful effect in raising the level of intestinal alkaline phosphatase in the rat, even when its administration causes a significant degree of adrenal involution.

DISCUSSION

This series of experiments has shown that the adrenal cortex has an important, though not necessarily exclusive, role in the regulation of intestinal alkaline phosphatase levels in the rat, and that hydrocortisone is a powerful stimulant to increased concentration of the enzyme in this location. It is also clear that it is not necessary, as claimed by Hebert (1950), to castrate male rats, as well as removing their adrenals, to achieve a significant reduction in the intestinal alkaline phosphatase. This is not to deny that other hormones may affect the enzyme, and further studies on its regulation are in progress.

This study provides information which may explain, in part or in whole, the impaired fat absorption which follows adrenalectomy, and the absorptive difficulties associated with untreated Addison's disease. There seems little doubt from the biochemistry of alkaline phosphatase and its location at the brush border of the intestinal epithelium, that it plays an important part in absorption mechanisms. Wiseman (1964) summarizes current views on the part played by phosphorylation in the intestinal transport of sugars. But while it is known with some certainty how this affects carbohydrate absorption, there is no similar consensus on the effect of phosphorylation on the mechanism of fat absorption, and the role of intestinal phospholipid as a vehicle for the absorption and cellular transfer of fatty acids remains unsettled.

Goldacre (1952) advanced an interesting hypothesis, which suggested that the presence of

contractile proteins at certain cell surfaces could effect the transfer of molecules. He made a comparison with the contractile protein system of muscle, where the myosin-catalysed hydrolysis of adenosinetriphosphate (A.T.P.) releases energy which is utilized by the actin-myosin system to perform mechanical work in a reversible contraction-relaxation cycle.

Evidence bearing on Goldacre's hypothesis is scanty, but cytochemical studies have shown that the surfaces of cells concerned in active transport frequently, perhaps invariably, have associated with them the enzyme alkaline phosphatase, and Danielli (1952) has suggested that this is the enzymic contractile protein postulated by Goldacre. Much needs to be done before this can be accepted. For example it needs to be shown that these phosphatases are indeed contractile, and that they are situated in the membrane in such a manner as to be able to transport molecules by contraction.

From a consideration of our data (Watson and Murray, 1966), we felt that the primary reason for impaired fat absorption in the hypo-adrenal rat was diminished movement of lipid across the intestinal epithelial membrane. We would now suggest, tentatively, that impaired fat absorption in the hypo-adrenal rat is related to the diminished amount of alkaline phosphatase in the intestinal epithelium, and that the specific defect may be one of diminished biomechanical molecular transfer.

These considerations, if correct, have practical implications, in particular whether it is possible to increase the level of alkaline phosphatase in diseased human jejunum, especially in those conditions associated with mucosal atrophy, by the administration of hydrocortisone or other steroids. Applying quantitative techniques to the study of alkaline phosphatase in human jejunum we have found wide differences in the concentration of the enzyme related to structural disorders of the bowel and also certain systemic conditions not associated with mucosal changes (Ferguson, Watson, Murray, and Fell, 1966). We believe that a better understanding of the function and control of intestinal alkaline phosphatase will lead to a significant advance in our understanding of normal and abnormal function in the small intestine.

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REFERENCES

- Axelsson, H., Ekman, B., and Knutsson, D. (1965). Personal communication.

- Barnes, R. H., Miller, E. S., and Burr, G. O. (1941). *J. biol. Chem.*, **140**, 241.
- Danielli, J. F. (1952). *Symp. Soc. exp. Biol.*, **6**, 1.
- Dickie, N., Robinson, M. I., and Tuba, J. (1955). *Canad. J. Biochem.*, **33**, 83.
- Ferguson, A., Watson W. C., Murray, E. S., and Fell, G. S. (1966). In preparation.
- Goldacre, R. J. (1952). *Int. Rev. Cytol.*, **1**, 135.
- Hebert, S. (1950). *Arch. Biol. (Liège)*, **61**, 235.
- Kind, P. R. N., and King, E. J. (1954). *J. clin. Path.*, **7**, 322.
- Kutscher, W., and Wust, H. (1942). *Hoppe-Seylers Z. physiol. Chem.*, **273**, 235.
- Laszt, L., and Verzar, F. (1936). *Biochem. Z.*, **288**, 351.
- Marsh, W. H., Fingerhut, B., and Kirsch, E. (1959). *Clin. Chem.*, **5**, 119.
- Morton, R. K. (1954). *Biochem. J.*, **57**, 595.
- Tissières, A. (1948). *Acta anat. (Basel)*, **5**, 224.
- Triantaphyllopoulos, E., and Tuba J. (1959a). *Canad. J. Biochem.*, **37**, 699.
- , — (1959b). *Ibid.*, **37**, 711.
- Tuba, J., and Dickie, N. (1954). *Ibid.*, **32**, 621.
- Verzar, F., and Laszt, L. (1935). *Biochem. Z.*, **276**, 11.
- Watson, W. C., and Murray, E. S. (1966). *J. Lipid Res.*, **7**, 236.
- Wiseman, G. (1964). *Absorption from the Intestine*. Academic Press, London.

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