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THE EFFECTS OF ADRENALECTOMY AND FASTING ON INTESTINAL FUNCTION IN THE RAT

By R. J. LEVIN, H. NEWEY* AND D. H. SMYTH
From the Department of Physiology, University of Sheffield

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Early studies on adrenalectomized rats suggested that the adrenal cortex played a specific role in carbohydrate absorption (see Verzar & McDougal, 1936). Later workers (Deuel, Hallman, Murray & Samuels, 1937; Althausen, Anderson & Stockholm, 1939; Clark & MacKay, 1942) showed that feeding sodium chloride restored glucose absorption to normal and it was suggested that the depressed absorption found in adrenalectomy was secondary to a disturbed electrolyte balance. There are, however, a number of reports in the literature where saline treatment did not restore normal absorption of sodium chloride (Stein & Wertheimer, 1941) or of glucose (Verzar & Sailer, 1952).

The above investigations were carried out in the whole animal, where the results obtained depended not only on the activity of the intestinal epithelial cells, but also on such factors as blood flow, gastric emptying, etc. A re-evaluation of the effect of adrenalectomy on the absorptive and metabolic functions of the small intestine was therefore undertaken using recent *in vitro* and *in vivo* techniques. During these investigations it became obvious that the digestive ability of the intestine was affected by adrenalectomy and studies were also made on intestinal enzyme activity. A preliminary account of this part of the work has been given by Levin, Newey & Smyth (1962).

METHODS

Adrenalectomy. When rats are bilaterally adrenalectomized many animals survive indefinitely even when given tap water as the drinking fluid. The usual explanation of their survival is the existence of accessory cortical tissue which supplies enough corticosteroids to support a normal electrolyte balance (Gaunt, 1933; Weisz, Horvath & Kadas, 1959). It is therefore necessary to adopt some definable criterion of satisfactory adrenalectomy, and the following routine was used.

White male rats (180–220 g) of the Sheffield strain maintained on a stock diet (Oxoid 86) were used, and the operation was carried out via the lumbar approach under light ether anaesthesia. Both adrenals were removed, each by a separate incision, together with a large portion of the surrounding perirenal fat, in order to remove as much accessory adrenal tissue as possible. The skin incisions were closed with 12 mm Michel clips. Post-operatively

* J. G. Graves Fellow.

the rats were maintained for 6–7 days on 1 % NaCl and the stock diet *ad libitum* and were usually weighed every day. On the seventh post-operative day the weight of the rats was checked and the 1 % NaCl was replaced by tap water. The animals were allowed free access to both diet and tap water for a further 6–9 days during which time the body weights were checked every day. A rat was considered to be functionally adrenalectomized if its body weight did not increase during this period of drinking tap water. Gaunt (1933) considered that for mature animals body weight was the most reliable check on the early progress of adrenal insufficiency. Rats that were used during the tap-water period are referred to as 'uncompensated adrenalectomized' rats.

Usually any rat that lost weight over the tap-water period could be maintained in a healthy condition by replacement of the tap water with 1 % NaCl. If this saline solution was fed for 7 days such animals generally gained weight. Rats that were so treated are called 'compensated adrenalectomized'. Sham-operated animals were prepared by removing a small amount of fat from the perirenal fat pads and manipulating the kidneys to the same extent as was done in the adrenalectomized rats. Almost all sham-operated rats increased their body weight over the tap-water period.

Fasting. In order to have controls for the loss of intestinal weight produced by adrenalectomy, other experiments were carried out in which rats were fasted for 3 days but allowed free access to water. These are referred to subsequently as 'fasted' animals.

Absorption studies. In vivo, rats were anaesthetized with intraperitoneal pentobarbitone sodium and absorption was studied by the technique of Sheff & Smyth (1955), in which saline solutions containing the substance to be absorbed are circulated through the lumen of the intestine.

In vitro, the technique was that of the everted sac of rat small intestine (Wilson & Wiseman 1954), as used by Barry, Matthews & Smyth (1961). The rats were anaesthetized with intraperitoneal pentobarbitone sodium, the combined jejunum and ileum washed through with 0·9 % NaCl at room temperature, then stripped from the mesentery, everted, stretched and divided into five equal segments. Sacs, 16–18 cm long, were made from the middle segment, and incubated in bicarbonate-saline solution (Krebs & Henseleit, 1932) in equilibrium with 5 % CO_2 and 95 % O_2. The sacs were filled with 1 ml. of saline solution, and were incubated in 50 ml. of this solution for 30 min at 38° C. Sugars, amino acids or peptides were present in the mucosal or serosal fluids as described subsequently. At the end of the experimental period determinations of sugars, amino acids or peptides were made in the mucosal fluid, serosal fluid and gut wall, and transports were calculated as described by Parsons, Smyth & Taylor (1958) or when peptides were used as described by Newey & Smyth (1959).

Metabolism studies. In order to determine how much glucose was metabolized by the gut, a technique was used similar to the 'metabolic experiments' described by Parsons *et al.* (1958). In these experiments 500 mg glucose % was present in the mucosal fluid (15 ml.) and serosal fluid (1 ml.). At the end of the experimental period the sac was homogenized, the homogenate pooled with the final mucosal and serosal fluids and the total amount of glucose determined. The difference between this and the amount initially present was taken as the amount metabolized.

Estimation of intestinal enzyme activity. Rats were anaesthetized with pentobarbitone sodium and the combined jejunum and ileum washed through with 0·9 % NaCl at room temperature, stripped from the mesentery, blotted on filter paper, weighed and homogenized for 3 min in 100 ml. of 0·9 % NaCl. Peptidase activity was measured by determining the ability of the homogenates to hydrolyse 15 mM glycyl- or leucyl-glycine. When glycyl-glycine was used a 25 ml. sample of the homogenate was added to 25 ml. of 0·9 % NaCl containing 100 mg of the peptide, while with leucyl-glycine 10 ml. of homogenate was added to 40 ml. of 0·9 % NaCl containing 142 mg of the peptide. These solutions were incubated for 30 min at 38° C and the free glycine determined. Maltase activity was measured by incubating, for 30 min at 38° C, 10 ml. of the intestinal homogenate with 15 ml. of

0·9 % NaCl to which had been added 250 mg maltose. The free glucose formed was estimated by the glucose oxidase method. Preliminary studies showed that with the substrate concentrations used both peptidase and maltase activities of the homogenates were approximately linearly related to enzyme concentration over an eightfold range of concentration. Other experiments showed that homogenates incubated in Krebs's bicarbonate-saline solution gave qualitatively similar results to those incubated in 0·9 % NaCl for both peptidase and maltase activities.

Chemical estimations. Glucose and other reducing sugars were estimated by the method of Nelson (1944) as modified by Somogyi (1945), or by the glucose oxidase method of Huggett & Nixon (1957) as modified by Dahlqvist (1961). Glycine was estimated after de-proteinization by the method of Alexander, Landwehr & Seligman (1945) as modified by Christensen, Riggs & Ray (1951). Glycyl-glycine was determined by estimating free and bound glycine as described by Newey & Smyth (1959).

Expression of results

The mucosal fluid is the fluid in which sacs are suspended, and the serosal fluid is that inside the sacs. As a measure of absorptive activity *in vitro* the mucosal transfer is used, i.e. the amount of fluid (ml.) or solute (in μ-moles) leaving the mucosal fluid in 30 min. We consider this parameter corresponds most closely to absorption *in vivo*, which is measured as the amount of substance leaving the lumen in 15 min.

Levin & Smyth (1963) have pointed out the possible fallacies of not taking into consideration changes in gut weight, and where necessary transfers *in vitro* have been calculated both in absolute amounts transferred per sac of gut and in amounts transferred per initial wet weight of gut. In experiments involving changes in concentration of solutes various parameters have been used to express these changes. The *final serosal-mucosal concentration difference* is the final concentration of solute in the serosal fluid minus the final concentration of solute in the mucosal fluid; the *serosal concentration increase* is the final serosal concentration minus the initial serosal concentration. The significance of these is discussed in the text.

The enzyme activities were measured as the amount of peptide hydrolysed or the amount of glucose formed in 30 min at 38° C per sample of intestinal homogenate. This activity was then expressed either as 'total activity per whole intestine' (absolute) or as 'activity per gram initial wet weight of intestine'.

RESULTS

Transfer in vitro

Table 1 shows the absolute transfers of glucose, glycine and fluid and the initial intestinal wet weight of gut for normal, sham-operated, uncompensated adrenalectomized and fasted rats. The figures for fasted rats will be discussed in a later section. In the uncompensated adrenalectomized rats the intestinal weight is decreased by 26 % compared with the sham-operated group, and the difference between the mean weights in these two groups is significant ($P < 0{\cdot}001$). The transfers of fluid, glucose and glycine are all significantly lower in the uncompensated adrenalectomized animals than in the sham-operated animals, the extent and significance of the depression being for fluid 19 % and $P < 0{\cdot}01$; for glucose 38 % and $P < 0{\cdot}05$; and for glycine 22 % and $P < 0{\cdot}001$. If the transfers for glucose, glycine and fluid are expressed on a gram initial wet weight basis the differences between adrenalectomized and sham-operated rats become insignificant.

TABLE 1. Mucosal transfer of glucose, glycine and fluid by sacs of rat everted small intestine incubated for 30 min at 38° C. Mucosal fluid: 50 ml. bicarbonate-saline containing 28 mM glucose and 15 mM glycine. Serosal fluid: 1 ml. bicarbonate-saline containing 28 mM glucose only. Gas phase: 95% O_2, 5% CO_2. The results are expressed as the mean ±S.E. The figures in brackets denote the number of animals

	Normal	Sham-operated	Uncompensated adrenalectomized	Fasted
Wet wt. (g)	1·09±0·08 (8)	1·19±0·05 (12)	0·88±0·04 (14)	0·78±0·05 (5)
Mucosal fluid transfer (ml.)	1·35±0·09 (8)	1·27±0·04 (12)	1·03±0·07 (14)	0·69±0·05 (5)
Mucosal glucose transfer (μ-mole)	94±6·2 (5)	103±10·3 (5)	64±11·8 (7)	42±10·0 (5)
Mucosal glycine transfer (μ-mole)	58±3·8 (8)	55±2·2 (12)	43±1·8 (14)	38±1·2 (5)

TABLE 2. Metabolism of glucose by sacs of rat everted small intestine incubated 30 min at 38° C. Mucosal fluid; 15 ml. bicarbonate-saline containing 28 mM glucose. Serosal fluid; 1 ml. bicarbonate-saline containing 28 mM glucose. Gas phase: 95% O_2, 5% CO_2. The results are expressed as the mean ±S.E. The figures in brackets denote the number of animals

	Sham-operated (6)	Uncompensated adrenalectomized (10)	Fasted (6)
Intestinal wet wt.	1·09±0·05	0·87±0·04	0·67±0·03
Glucose metabolized (μ-moles)	66±6·4	45±5·9	10±2·9
Glucose metabolized (μ-moles/g wet wt.)	60±4·5	50±4·9	15±4·3

Glucose metabolism

The amount of glucose metabolized by sacs of intestine made from control animals, uncompensated adrenalectomized animals and fasting animals was determined by the 'metabolic experiment' described in the methods section, and the results are seen in Table 2. The absolute amount of glucose metabolized by sacs from the adrenalectomized group is reduced by 32% when compared to the sham-operated group and this reduction is significant ($P < 0·05$). When the results are expressed per gram wet weight the 17% difference between the two groups is not significant.

Transfer against a concentration gradient

The figures in Table 1 give no information about movement against a concentration gradient for although glucose was initially present in both mucosal and serosal fluids, glycine was present in the mucosal fluid only. Further experiments were therefore done to study transfer against a gradient, and in these glycine, glucose and galactose were used. In one group of experiments glucose and glycine were present together each in the same initial concentration in mucosal and serosal fluids; in a second

group of experiments galactose was present in the same initial concentration in mucosal and serosal fluids. Glucose was omitted in this case because Fisher & Parsons (1953) have shown that it competes with galactose for the transfer mechanism. The results of these experiments are shown in Table 3, and the parameters selected deserve some consideration. They are important not only in the present context, but in the interpretation of *in vitro* experiments generally, as many authors have used concentration changes to measure transfer capacity. The final serosal-mucosal concentration difference if positive suggests that movement has occurred against a concentration gradient and also shows the magnitude of the final gradient. This value is therefore given in Table 3. This positive gradient

TABLE 3. Mucosal transfer of glycine, glucose and galactose against a concentration gradient by sacs of rat everted small intestine incubated for 30 min at 38° C. Mucosal fluid: 50 ml. bicarbonate-saline containing 28 mM galactose or 28 mM glucose and 15 mM glycine. Serosal fluid: 1 ml. bicarbonate-saline containing 28 mM galactose or 28 mM glucose and 15 mM glycine. Gas phase: 95% O_2, 5% CO_2. Results are expressed as the mean ± S.E. The figures in brackets denote the number of animals

	Initial wet wt. (g)	Initial mucosal concn. (mM)	Final serosal concn. (mM)	Final serosal–mucosal concn. difference (mM)	Serosal fluid transfer (ml.)
			Glycine transfer		
Sham-operated (4)	1·23 ± 0·03	15	27 ± 0·8	13·2 ± 0·9	1·07 ± 0·15
Uncompensated adrenalectomized (4)	0·99 ± 0·03	15	27 ± 0·9	13·2 ± 0·9	0·86 ± 0·11
			Glucose transfer		
Sham-operated (4)	1·23 ± 0·03	28	30 ± 1·3	5·1 ± 1·7	1·07 ± 0·15
Uncompensated adrenalectomized (4)	0·99 ± 0·03	28	32 ± 0·8	5·6 ± 1·0	0·86 ± 0·11
			Galactose transfer		
Sham-operated (4)	1·38 ± 0·03	28	37·5 ± 1·8	10·7 ± 1·9	0·13 ± 0·03
Uncompensated adrenalectomized (4)	0·90 ± 0·06	28	43·4 ± 3·6	16·5 ± 3·7	0·20 ± 0·05

is not, however, completely unequivocal evidence of movement against a gradient in the case of substances metabolized by the intestine or bound to some constituent in the intestine, as a greater removal from the mucosal fluid than from the serosal fluid could create such a final gradient without any uphill transport occurring. More certain evidence of uphill transport is an increase in the serosal concentration, but even here attention must be paid to fluid transfer. Decrease in serosal volume is unlikely, but cannot be completely ruled out and the only certain evidence of uphill transfer is increase in the serosal concentration when fluid is not leaving the serosal side. Table 3 in fact shows an increase in serosal concentration, combined with fluid movement towards the serosal side, both in control animals and with uncompensated adrenalectomy. These results are the first unequivocal demonstration that the active transport mechanisms for glucose,

galactose and glycine are able to operate in the intestine of adrenalectomized rats.

Transfer of glycine from glycyl-glycine in vitro

Newey & Smyth (1962) considered that when glycyl-glycine is initially present in the mucosal fluid, the glycine formed intracellularly may use a transfer mechanism which is different from that concerned with entry of glycine into the cell from the mucosal fluid. Experiments were therefore done to see whether this mechanism dealing with intracellular glycine is affected by adrenalectomy. The transfer of glycine from glycyl-glycine present in the mucosal fluid is shown in Table 4. Although there was a 20% diminution in the amount of peptide hydrolysed by the adrenalectomized intestine when compared with that hydrolysed by the sham-operated intestine, the difference was not statistically significant ($P > 0{\cdot}1$).

TABLE 4. Transfer of glycine from glycyl-glycine by sacs of rat everted small intestine incubated for 30 min at 38° C. Mucosal fluid: 50 ml. bicarbonate saline containing 28 mM glucose and 15 mM glycyl-glycine. Serosal fluid: 1 ml. bicarbonate-saline containing 28 mM glucose only. Gas phase: 95% O_2, 5% CO_2. The results are expressed as the mean ± S.E. The figures in brackets denote the number of animals

	Sham-operated (6)	Uncompensated adrenalectomized (8)
Intestinal wet wt. (g)	1·00 ± 0·07	0·89 ± 0·06
Mucosal fluid transfer (ml.)	1·27 ± 0·06	1·11 ± 0·10
Glycyl-glycine hydrolysed (μ-mole)	86 ± 8·9	69 ± 2·5
Glycine transfer (μ-mole)	81 ± 6·9	70 ± 2·3

The amount of glycine transferred from glycyl-glycine hydrolysed intracellularly was also not statistically different from the sham-operated group ($P > 0{\cdot}2$). These results contrast with experiments on the *in vivo* absorption of glycyl-glycine in uncompensated adrenalectomized rats to be described later.

Absorption in vivo

The effects of uncompensated adrenalectomy on the absorption of a number of substances *in vivo* is shown in Table 5. This shows that in uncompensated adrenalectomy, intestinal absorption of glucose, galactose and glycine was greatly inhibited. Glucose, which is both actively transferred and metabolized by the epithelial cells, was inhibited by 45% compared with the sham-operated animals and this inhibition was significant ($P < 0{\cdot}001$). Fructose, a non-actively absorbed but metabolized hexose was inhibited to a similar degree (43%), and the inhibition was significant ($P < 0{\cdot}001$). In the case of galactose, a hexose that is not appreciably metabolized but is transferred by the same mechanism that

carries glucose, absorption was only inhibited by 23%. The difference between inhibition of galactose and that of either glucose or fructose is significant. With glycine, an actively transferred but non-metabolized amino acid, the absorption was depressed by 30%, a depression which is significant ($P < 0.05$) and similar to that of galactose. Xylose absorption was depressed by 39%, an effect which is significant ($P < 0.01$) and the 21% inhibition of sorbose was also significant ($P < 0.01$). It must be borne in mind that the absorption of both these substances is relatively small. For example, in the case of sorbose, approximately 73 μ-moles are absorbed from a circulating amount of 694 μ-moles. Small errors in estimation can thus produce large differences in absorption. Although it would be possible to increase the absorption by increasing the circulation

TABLE 5. Absorption of substances *in vivo*. 50 ml. of 0·9% NaCl containing the substance shown in the first column was circulated through the intestinal lumen for 15 min, except in the cases of xylose and sorbose when only 25 ml. was used. The results are expressed as the mean ± S.E. The figures in brackets denote the number of animals

	Initial concn. (mM)	Absorption (μ-moles/15 min)				
		Normal	Sham-operated	Uncompensated adrenal-ectomized	Compensated adrenal-ectomized	Fasted
Glucose*	28	455±18 (6)	406±18 (9)	223±22 (7)	446±31 (4)	261±28 (6)
Galactose*	28	321±10 (6)	312±20 (9)	241±13 (7)	—	242±23 (6)
Fructose*	28	—	152±2 (5)	87±12 (5)	—	130±11 (9)
Xylose*	28	—	95±5 (8)	58±8 (6)	—	—
Sorbose*	28	—	73±4 (9)	58±4 (8)	—	—
Glycine†	30	403±23 (6)	351±29 (9)	245±25 (7)	339±14 (4)	251±15 (6)
Glycyl-glycine†	30	—	359±22 (4)	163±21 (4)	365±36 (3)	—

* = 30 mM glycine also present. † = 28 mM glucose also present.

period from 15 min to 1 hr it is an undesirable change in technique as uncompensated adrenalectomized rats are more sensitive to anaesthesia and surgical trauma than normal or sham-operated rats.

As glycine absorption was depressed by adrenalectomy the absorption of the peptide glycyl-glycine was studied. The 54% depression obtained in adrenalectomized rats was highly significant ($P < 0.001$), and greater than that of any other substance tested. It will be remembered that *in vitro* the entry of glycyl-glycine was decreased by only 20% which was not significant. This striking difference stimulated further studies on the peptidase activity of the intestine in various conditions, which are discussed later.

The effect of loss of intestinal tissue on absorption in vitro *and* in vivo

In vitro. The decrease in intestinal weight which occurred during uncompensated adrenalectomy might be due in part to a lowered food intake.

In order to mimic this fall in intestinal weight and to study its effects on intestinal function a number of normal rats were fasted for 3 days but were allowed *ad libitum* access to tap water. The weights of intestine under various conditions are shown in Tables 1, 6 and 7. In all cases the weight loss produced by fasting was as great as that produced by adrenalectomy. The transfer capacity of the intestine from fasted rats is shown in Table 1 where it can be seen that fluid, glucose and glycine transfers are greatly depressed when compared with those of normal intestine. If, however, the results are compared with those from the intestine of adrenalectomized rats the only significant difference ($P < 0{\cdot}001$) is in the greater depression of fluid transfer in the fasted intestine, the differences in glucose (34 %) and glycine transfer (12 %) being insignificant, $P > 0{\cdot}2$ and $P > 0{\cdot}1$, respectively.

TABLE 6. Dry weights of small intestine from groups of rats under various conditions. Values are given as the mean ± S.E. The figures in brackets denote the number of animals

Group	Intestinal dry weight (g)
Normal	0·96 ± 0·02 (12)
Fasted	0·75 ± 0·02 (9)
Compensated adrenalectomized	0·96 ± 0·04 (4)
Uncompensated adrenalectomized	0·74 ± 0·04 (5)

The metabolism of glucose by intestine from fasted rats is included in Table 2. There is a dramatic fall in the amount metabolized compared with the sham-operated group whether the results are expressed on the absolute (85 % decrease) or gram wet-weight (75 % decrease) basis. As part of the fluid transport in rat intestine is dependent on glucose metabolism it would seem likely that the large inhibition of glucose metabolism in fasted intestine could account for the low fluid transfers observed in Table 1.

In vivo. The results obtained *in vivo* in fasted animals are included in Table 5. The absorption of glucose in such animals when compared to normal rats was reduced by 43 %, while that of galactose and glycine was reduced by 25 and 38 %, respectively. All these depressions are significant. These reductions of absorption were concomitant with a fall in intestinal weight that occurs over the 3-day fast (Tables 1, 6 and 7).

Intestinal enzyme changes during adrenalectomy

In Tables 7, 8 and 9 are presented the effects of adrenalectomy on the peptidase and maltase activity of the small intestine together with the effects of fasting which will be discussed in the next section. The tables show the enzymic activity expressed both in amounts per whole intestine and amounts calculated per gram intestinal wet weight. As the results for

the two peptidase activities, glycyl-glycine and leucyl-glycine, are qualitatively similar, only those for glycyl-glycine will be discussed in detail. The conclusions based on these results are also valid for the leucyl-glycine data.

TABLE 7. Glycyl-glycine peptidase activity of rat small intestine. The results are expressed as the mean ± S.E. The figures in brackets denote the number of animals

	Initial wet wt. (g)	Glycyl-glycine hydrolysed μ-mole/30 min	
		Absolute	Per g wet wt.
Normal (8)	6·75 ± 0·21	299 ± 22	44 ± 3
Fasted (9)	4·24 ± 0·18	301 ± 16	71 ± 3
Sham-operated (13)	6·94 ± 0·15	339 ± 13	49 ± 1
Uncompensated adrenalectomized (13)	5·20 ± 0·19	179 ± 5	35 ± 1
Compensated adrenalectomized (10)	6·81 ± 0·14	326 ± 17	48 ± 3

TABLE 8. Leucyl-glycine peptidase activity of rat small intestine. The results are expressed as the mean ± S.E. The figures in brackets denote the number of animals

	Initial wet wt. (g)	Leucyl-glycine hydrolysed (μ-mole/30 min)	
		Absolute	Per g wet wt.
Normal (3)	6·46 ± 0·13	2587 ± 103	401 ± 19
Fasted (4)	4·54 ± 0·24	2297 ± 155	509 ± 39
Sham-operated (5)	6·87 ± 0·38	2274 ± 122	332 ± 14
Uncompensated adrenalectomized (6)	5·31 ± 0·18	1280 ± 102	243 ± 22
Compensated adrenalectomized (5)	7·22 ± 0·21	2128 ± 119	295 ± 16

TABLE 9. Maltase activity of homogenates of rat small intestine. Results are expressed as the mean ± S.E. The figures in brackets denote number of animals

	Initial wet wt. (g)	Glucose formed μ-mole/30 min	
		Absolute	Per g wet wt.
Normal (12)	6·61 ± 0·21	2749 ± 161	418 ± 23
Fasted (11)	4·67 ± 0·14	1923 ± 140	415 ± 33
Sham-operated (18)	6·98 ± 0·12	3253 ± 175	466 ± 24
Uncompensated adrenalectomized (17)	5·16 ± 0·18	2516 ± 110	493 ± 22
Compensated adrenalectomized (10)	7·12 ± 0·18	3027 ± 156	425 ± 19

In uncompensated adrenalectomy the intestinal glycyl-glycine peptidase activity shows a highly significant fall when compared to the sham-operated group (Table 7). This is so whether the activity is expressed in absolute values (47 % fall) or per gram wet weight (29 % fall). In the case of leucyl-glycine peptidase activity the figures are 44 and 27 %, respectively (Table 8). In the case of maltase (Table 9) the activity expressed in absolute values fell in uncompensated adrenalectomy by 23 %, but when

expressed per gram intestinal weight there was no significant difference compared with the sham-operated group. In all cases the feeding of 1 % saline for 7 days (compensated adrenalectomy) restored the activity to the level of the sham-operated groups both on the whole-intestine basis and on the gram intestinal wet-weight basis.

Intestinal enzyme changes during fasting

Although a 3-day fast caused an intestinal weight loss similar to that found in uncompensated adrenalectomy it produced very different effects on the levels of peptidase and maltase activities. Surprisingly the amount of peptidase activity per whole intestine remained at the level of the fed controls (Tables 7 and 8). If this activity, however, was expressed on a gram wet-weight basis there was a 61 % increase in glycyl-glycine peptidase activity and a 27 % increase in leucyl-glycine peptidase activity. With maltase the results were very different. Expressed in absolute amounts the activity fell significantly by 30 % (Table 9) but when this was placed on the gram intestinal wet-weight basis there was no significant difference from the fed controls.

DISCUSSION

Although early experiments on the effect of adrenalectomy indicated a depression of the absorptive activity of the intestine, they did not give any information about its effect on transport mechanisms, as not only could the high concentrations of substances used make special transport mechanisms relatively unimportant compared with diffusion, but also absorption could be affected by extraneous factors such as changes in gastric emptying, intestinal motility and splanchnic blood flow. One possible exception is the study by Wix, Fekete, Bonta & Horvath (1951), who showed that saline-feeding partially restored glucose absorption when this was measured by circulating 100 mg % glucose in 0·9 % NaCl through the lumen of the rat intestine *in vivo*. Even this work however did not give unequivocal evidence that the active glucose-transport system could still function after adrenalectomy, for the loss of glucose from the luminal fluid might have been due to metabolism of glucose by the mucosal cells. Only an *in vitro* technique capable of demonstrating movement against a concentration gradient can show whether adrenalectomy does or does not affect the active transport systems of the intestine and our results *in vitro* show that intestine from adrenalectomized rats can transfer glucose, galactose and glycine against a concentration gradient. This is conclusive evidence that the active transport mechanisms in the epithelial cells can still function even in uncompensated adrenalectomy. Our results show that the transfers of glucose, glycine and fluid are depressed when the

results are expressed on an absolute basis, but that no significant difference from the intestines of sham-operated controls is noted when the transfer is expressed per unit wet weight of gut. Similar results are seen in the case of *in vitro* metabolism of glucose. This is depressed if the absolute amount of glucose metabolized is compared with that of the sham-operated controls but this is not so if the results are expressed per gram initial wet weight of intestine.

These results could be interpreted as indicating a decreased amount of mucosal tissue capable of undertaking transfer in the intestine of adrenalectomized rats, and this is a possible explanation of decreased transfers in adrenalectomized animals.

In considering the effects of decrease in intestinal weight on transfer capacity, it is necessary to take into account what part of the gut contributes most to the weight loss. If this is mainly parts other than the epithelium, then transfer values expressed per unit weight of gut could be enhanced, even if there were no real increase in transfer capacity. We are not able to say definitely which parts of the gut contribute most to this weight loss, but histological sections certainly suggest a thinning of the lamina propria. It is interesting in this context to note that in germ-free reared rats (a condition that induces a weight reduction in the small intestine) Heneghan (1963) found that *in vitro* transfers of xylose when expressed per milligram dry weight were increased more than twofold compared with normal animals. Reduction in weight of the small intestine does not therefore necessarily imply a corresponding reduction in its transferring ability. These results together with our own observations make it obvious that great care should be taken when assessing absorption data from rats in conditions that change the weight of the intestine, and support the recommendation of Levin & Smyth (1963) that in such cases transfers should be expressed both in absolute values and per g weight of intestine.

The *in vivo* absorption results presented in this paper were obtained with the use of much lower concentrations in the gut lumen than has hitherto been the case, and with a technique that obviates the effects of any changes in the motility of the gastro-intestinal tract. They show conclusively that uncompensated adrenalectomy causes a reduction in the intestinal absorption of a number of substances and in this respect confirm some of the early studies on absorption of glucose (Verzar & McDougall, 1936) and glycine (Laszt, 1938). Furthermore, our results show that adrenalectomy affects much more the absorption of substances which are metabolized by the intestine, e.g. glucose and fructose, and we take this to indicate that uncompensated adrenalectomy inhibits certain metabolic pathways in the mucosal cells, a result in agreement with our observations on intestinal glucose metabolism in adrenalectomized animals. This conclusion also

agrees with that of Vidal-Sivilla (1961), although he thought that the cause of the depressed metabolism was the shock-like state of the adrenalectomized animals brought about by the trauma of the operative procedures used to measure absorption.

In order to assess the importance of non-specific factors that could depress absorption in uncompensated adrenalectomized rats we used, as have previous workers, substances that are presumed not to require a specific mechanism for transport. For this purpose xylose and sorbose were chosen, two substances widely reported to be passively absorbed (see Wilson, 1962, for references). We assumed that decrease in absorption of these would be due to non-specific factors, such as a decrease in surface area or a reduction in blood flow to the intestine. Our results differ from previous studies (Verzar & McDougall, 1936) in that we find significant depressions of absorption of these substances in uncompensated adrenalectomy. It should be stressed, however, that our experimental conditions were very different from previous work. We used a sugar concentration of 28 mM in 0·9 % NaCl whereas the early experiments used isotonic solutions of the sugars. In interpreting our results with xylose it must be pointed out that Salomon, Allums & Smith (1961), Csaky & Lassen (1964) and Alvarado (1964) have all produced evidence that xylose absorption involves some kind of carrier mechanism, and it is possible that uncompensated adrenalectomy may interfere with xylose absorption by affecting this mechanism. As yet no studies have been published to show that sorbose is absorbed by any mechanism other than diffusion. Because sorbose absorption was reduced by approximately 20 % in adrenalectomy it seems likely that *in vivo* absorption in general will be reduced by a similar amount due to reductions in surface area and changes in blood flow.

An interesting point arising from the *in vivo* results is that absorption of glucose in the normal unoperated controls was found to be on average 42 % higher than that of galactose. This contrasts with the often quoted results of Cori (1925), where galactose was absorbed faster than glucose, and other workers have found a similar result (see Wilson, 1962, for further references). We measure absorption *in vivo* by circulating a fluid containing a low concentration of sugar (28 mM) through the whole intestine (but excluding the duodenum). In most other techniques either concentrated sugars were fed by stomach tube to conscious animals or concentrated sugars were injected into tied-off loops of intestine. It is possible that both the differences in technique and in the concentration of sugars account for the differences between our results and those of other workers.

The feeding of 1 % NaCl to adrenalectomized rats for 7 days resulted in the recovery of glucose, glycine and glycyl-glycine absorption and intestinal weight, and this raises again the fundamental problem whether

adrenalectomy produces its intestinal effects by causing electrolyte imbalance. This is of particular interest in the intestine as Riklis & Quastel (1958) showed that absence of sodium ions resulted in a reduction of sugar transfer. Csaky (1961) has also shown that lowered concentrations of sodium depress amino acid transfer. Sodium lack could affect intestinal function by having either an acute effect or a chronic effect. In the normal animal Na ions are required for the active absorption of hexoses and amino acids, and the absence of Na could be described as having an acute effect on transfer. By a chronic effect we mean that the exposure of the tissue to a lowered concentration of Na for some time might damage cellular processes, an effect quite distinct from the need for Na during the transport of hexoses or amino acids. It seems to us likely that it is the chronic effect which is important in uncompensated adrenalectomy as in our experiments the intestine from adrenalectomized animals was bathed in a solution of normal Na concentration, and yet transfer was depressed. Furthermore, although the transfer of glucose, galactose and glycine was reduced, the intestines from adrenalectomized animals were still able to move these against a concentration gradient. On the other hand the large depression of absorption *in vivo* of fructose, a metabolized but not actively transferred sugar, and the reduction of glucose metabolism *in vitro* could be interpreted as an effect of chronic Na imbalance on the general metabolism of the epithelial cells. In this respect it is interesting that Lüthy & Verzar (1954) found that ATP-ase activity was reduced in the intestine of adrenalectomized rats. Such effects would reduce the amount of available energy and hence depress transfer capacity. What is clear from our results is that although uncompensated adrenalectomy reduces the capacity of the intestine to transfer substances it does not abolish its ability for active transport of a number of these substances.

Effects on intestinal enzymes

It is surprising that although many alterations in enzyme activities after adrenalectomy and during starvation have been recorded for a variety of tissues (Knox, Auerbach & Lin, 1956) little information is available about such changes in the intestine (Spencer & Knox, 1960). Our results with intestinal peptidase and maltase have shown some interesting differences in their behaviour. Peptidase activity fell during uncompensated adrenalectomy but it is striking that the even greater decrease in weight caused by the 3-day fast did not bring about any diminution in peptidase activity. With maltase activity, although there was a fall during uncompensated adrenalectomy per whole intestine this was not apparent when the results were expressed per gram wet weight. It is possible that the decrease in intestinal tissue accounts for the decrease in maltase

activity. As all the enzyme activities were restored to normal by the feeding of 1% saline (compensated adrenalectomy) it appears that they are not under the primary control of the adrenals but are sensitive to the electrolyte imbalance caused by adrenalectomy. During fasting the peptidase activity remained about the normal level so that, when the activity was expressed per gram wet weight intestine, there was a large increase. Maltase in contrast fell in absolute amount during starvation but this fall was not significant when the results were expressed per gram wet weight. Maltase in some respects is similar to other intestinal carbohydrases in that it falls during starvation. Blair, Yakimets & Tuba (1963) recorded that intestinal sucrase decreased during fasting while Ju & Nasset (1959) found that intestinal amylase fell. This difference between maltase and peptidase may be an adaptation to the presence or absence of substrate. Maltase is presumably only concerned with the hydrolysis of ingested carbohydrate and therefore has little or no function during starvation. The intestinal peptidases may have functions other than digestive ones. They may be involved in the break-down of body proteins, not only of the intestinal cells themselves but of plasma proteins and non-dietary protein derived from shed epithelium and secreted enzymes in the intestinal lumen. These sources of substrate are still present during fasting and may thus maintain peptidase activity. Peptidase activity has been reported to be increased in other tissues in conditions that cause atrophy of the organs. Rose, Robertson & Schwartz (1959) found that the activity of leucyl-glycine peptidase in rat diaphragm increased during starvation while Hopsu, Riekkinen & Luostarinen (1962) recorded that the peptidase activity of the accessory reproductive tract of the rat increased when the glands atrophied. These results, together with our own findings on intestinal peptidases, suggest that there may be a physiological role for peptidases during tissue atrophy.

SUMMARY

1. The effects of bilateral adrenalectomy and fasting on the transfer of sugars, glycine, glycyl-glycine and fluid have been studied in the rat small intestine.

2. The transfer *in vitro* of glucose, glycine and fluid is reduced by adrenalectomy, and the reduction is in proportion to the loss in intestinal weight. Glucose, galactose and glycine can still be moved against a concentration gradient.

3. Absorption *in vivo* of sugars, glycine and glycyl-glycine is reduced in adrenalectomized animals. This reduction was greater in the case of those substances metabolized (glucose and fructose). Feeding 1% NaCl to adrenalectomized rats restored both intestinal weight and absorption.

4. Fasted animals, in which a similar loss of intestinal weight occurred, also showed a reduction of sugar and glycine transfer similar to that found in adrenalectomy.

5. The activity of the digestive enzymes, maltase and peptidase, was studied after fasting and adrenalectomy. Adrenalectomy reduced the activity of both enzymes. Fasting, however, did not reduce peptidase activity but did reduce maltase activity. A possible explanation for these differences is discussed.

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