

Fat digestion and absorption in the adrenalectomized rat

WILLIAM C. WATSON and ELSPETH MURRAY

University Department of Medicine, Royal Infirmary, Glasgow, Scotland

ABSTRACT Malabsorption of fat in the adrenalectomized rat has been confirmed. The criteria for adrenal hypofunction were more rigorous than in previous studies. The malabsorption is not due to impaired intestinal lipolysis or inadequate intestinal intracellular glyceride synthesis. Delayed gastric emptying is probably secondary to the malabsorption rather than a cause. It is postulated that impaired intestinal fatty acid activation may be the key defect and that in some way this inhibits fatty acid transport across the mucosal membrane.

KEY WORDS hypoadrenal · rat · fat malabsorption · lipolysis · gastric emptying · fatty acid activation · transport · intramucosal esterification

VERZAR AND LASZT (1, 2) first demonstrated impaired fat absorption in the adrenalectomized rat. They showed that hypoadrenal rats absorbed about 10% of the lipid absorbed by normal animals and that this could be corrected by the administration of eucortone. They also showed that fat transport was impaired and suggested that the fault in both mechanisms was impaired phosphorylation.

Barnes, Miller, and Burr (3) disputed this. They claimed that the adrenalectomized rat absorbed fat as well as, and perhaps better than, the normal rat. While they agreed that there was some deficiency in fat transport, their experimental data seemed to show that phosphorylating mechanisms were unaffected.

Bavetta, Hallman, Deuel, and Greeley (4) supported Verzar's work, and suggested that Barnes et al. had failed to demonstrate a difference because they had used old animals, in which adrenocortical deficiency is not so critical as in the young. Bavetta et al. also showed that the impaired fat absorption was not corrected by giving saline. Bavetta (5) showed that the absorption of certain short-chain fatty acids and their glycerides, namely

tricaproin, sodium caproate and tricapyrin, was unaffected by adrenalectomy. He postulated that the decreased absorption of long-chain fatty acid was due to a failure of the intestinal mucosa to remove fatty acid at a normal rate.

In 1948 Verzar (6) had nothing to add to these facts and opinions and there seems to have been no further work since then.

In the present study normal and hypoadrenal rats are compared not only with respect to fat absorption, but also in their ability to hydrolyze triglyceride and esterify free fatty acid. The results confirm Verzar's view that fat absorption is impaired in the hypoadrenal rat, but reasons for this other than impaired phosphorylation are advanced.

MATERIALS AND METHODS

Young male Wistar rats weighing 100–120 g were divided into two groups. Bilateral adrenalectomy was carried out on all animals in one of the groups. A single posterior incision was used. The animals were returned to their cages and the drinking water was replaced by 1% saline. The experimental procedures were carried out two months later and therefore sham operations were not performed on the control group. The adrenalectomized animals remained in good condition, although they gained less weight than the control group.

Preliminary experiments showed that the best experimental conditions were obtained by giving tap water to the adrenalectomized rats for 5 days before the experiment. If this period was extended, some of the animals died; if reduced, the electrolyte readings were not significantly different from the normal values in other rats. The animals were fasted for 24 hr before the experiment was begun.

Glyceryl tri(oleate-1-¹⁴C) and oleic acid-1-¹⁴C were obtained from the Radiochemical Centre, Amersham,

England. The purity of the samples was checked by thin-layer chromatography and in each case was greater than 99%. The labeled oleic acid and triolein were mixed with unlabeled oleic acid and triolein, respectively, to give specific activities of 1 $\mu\text{C}/\text{ml}$.

The oil or fatty acid in a dose of 0.6 ml was administered by stomach tube to the unanesthetized animals, which were sacrificed in groups at 1, 3, and 6 hr thereafter. In the triolein experiments there were three rats in each subgroup, 18 rats in all, and in the oleic acid experiment there were four rats in each subgroup, a total of 24 rats.

At the time of sacrifice the animals were lightly anesthetized with ether and exsanguinated from the abdominal aorta. This blood was used for the estimation of serum sodium and potassium. The alimentary tract from lower esophagus to distal colon was removed, and the contents of the stomach, small bowel and colon were transferred separately to flasks containing 48 ml of chloroform-methanol 2:1, the volume being made up with water if necessary to 50 ml.

The small bowel contents were recovered by gently syringing the intact gut, separated at the pylorus and terminal ileum, with 1.5 ml and then 0.5 ml of tepid water, followed by blowing air through several times. This double wash with small volumes of water gave recoveries of labeled fat not less than 95% of that obtained by much larger volumes of water, and simplified the extraction procedure.

Mesenteric fat was stripped from the gut, which was then slit open and extracted in chloroform-methanol 2:1.

After the flasks had been shaken thoroughly and sealed, they were left overnight. Next day 10 ml of 0.05% sulfuric acid was added and the chloroform (lipid-containing) phase taken off into fresh containers and dried over anhydrous sodium sulfate. Aliquots (5 ml) of each sample were taken to dryness in special glass vials, redissolved in 15 ml of 0.5% 2,5-diphenyloxazole (PPO) in toluene, and assayed for radioactivity in a scintillation counter. The standard was obtained by making up 0.6 ml of the administered lipid to 100 ml with PPO-toluene and diluting 1 ml of this mixture to 15 ml in a counting vial. This was equivalent to 1% of the dose given.

From these figures it was possible to calculate the percentage amount of the dose in stomach, small bowel lumen, small bowel mucosa, and colon at different time intervals—and, since no radioactive feces were lost, the percentage of the dose absorbed.

Thereafter, depending on the amount of radioactivity in the various samples, 5- or 10-ml aliquots of the lipids of the stomach, small bowel lumen, and mucosa were taken to dryness under nitrogen and separated into the main lipid fractions by thin-layer chromatography on

glass plates coated with Silica Gel G. Multiple spots were applied, and 10–50 mg of lipid were developed in single runs. The developing solvent was petroleum ether-diethyl ether-acetic acid 60:40:1 (7). The spots were identified by UV fluorescence and comparison with known standards. The various lipid fractions were recovered by scraping the bands of silica from the plates and washing the scrapings three times with 15 ml of ether. The ether washings were pooled, filtered, and taken to dryness in tared counting vials. The lipid weight was obtained by reweighing the vials on an Oertling microbalance and 15 ml of PPO-toluene was added for counting.

A number of whole thickness sections were taken at various levels of the small bowel and examined by routine histological methods.

RESULTS

Triolein- ^{14}C Studies

The mean weights and levels of serum sodium and potassium of the rats on the day of the experiment are given in Table 1. The figures are in keeping with the electrolyte

TABLE 1 WEIGHTS AND SERUM SODIUM AND POTASSIUM LEVELS OF NORMAL AND ADRENALECTOMIZED RATS GIVEN TRIOLEIN- ^{14}C

	Normal (9)	Adrenalectomized (9)	
			<i>g</i>
Weight	301 \pm 29.8*	215 \pm 16.9	n = 16 <i>t</i> = 7.5 <i>P</i> < 0.001
			<i>meq/liter</i>
Na ⁺	142 \pm 1.9	124 \pm 4.7	n = 15† <i>t</i> = 10.0 <i>P</i> < 0.001
K ⁺	5.1 \pm 0.6	6.8 \pm 1.1	n = 15† <i>t</i> = 3.9 <i>P</i> < 0.002

Numbers in parentheses designate number of animals.

* SD.

† One blood sample from a normal rat hemolyzed.

biochemistry of adrenal failure. The adrenalectomized rats were significantly lighter than the controls, and the amount of peritoneal fat was considerably less. None of the animals had diarrhea, and, apart from the "excitement defecation" which most of them manifested during gastric intubation, there was no further loss of bowel content during the experiment.

The mean rates of absorption and of gastric emptying are recorded in Table 2. After 6 hr the normal (N) rats had absorbed 89.9% of the dose of oil and the adren-

TABLE 2 DATA ON ABSORPTION OF TRIOLEIN IN NORMAL AND ADRENALECTOMIZED RATS

Time, hr	Normal			Adrenalectomized			"6 hr" statistics
	1	3	6	1	3	6	
Absorption*, % dose	43.8	64.8	89.9	48.8	57.0	53.4	n = 4 t = 5.35 P < 0.01
Gastric emptying, % dose in stomach	43.9	16.3	3.9	33.8	35.5	33.4	n = 4 t = 4.87 P < 0.01
Lumen lipid, % dose	12.2	18.1	4.9	17.1	7.6	7.1	
Mucosal lipid, % dose	12.1	28.5	20.5	10.5	20.1	6.8	
Colonic lipid, % dose	—	1.0	1.3	—	2.1	4.2	

Dose: 0.6 ml of triolein-¹⁴C, by intubation. Each figure is the mean of three results.

* These values were obtained by subtracting the amounts recovered in stomach, small bowel lumen, and colon from the total. They include, therefore, the figure given for mucosal lipid.

alectomized (A) rats 53.4%. There was also a significant difference in the rates of gastric emptying.

Table 2 also gives the percentage of the dose found in the small bowel lumen and mucosa and in the colon. After 3 and 6 hr the amount of labeled fat in the colonic contents of the A rats is two or three times that in the N rats. The amount in the small bowel lumen is variable.

The better fat absorption by the N rats is reflected in the larger amount of labeled lipid in the mucosa, particularly at 3 and 6 hr after administration. The difference at 6 hr is significant (P < 0.01). There is, however, no simple lumen/mucosa ratio which relates the amount of lipid available for absorption to the amount absorbed, and which distinguishes the two groups of rats.

Fig. 1 shows that a certain amount of gastric lipolysis has occurred in both groups. This is slightly greater in the N rats after 6 hr, but not significantly so. No evidence is offered as to whether this is due to gastric lipase or to regurgitated pancreatic lipase.

Fig. 2 compares the degree of intestinal lipolysis at 1 and 3 hr. Initially this was equal for the two groups, but after 3 hr there was considerably greater lipolysis in the A rats. This is not likely to be due to more efficient lipolysis, but to delayed absorption and longer exposure to the lipolytic process.

Fig. 3 shows that after absorption the radioactivity is equally and almost completely incorporated in the triglyceride fraction of the mucosal lipids in both groups of rats. This means that within the epithelial cell the re-esterification of absorbed fatty acid is at least qualitatively similar in the normal and hypoadrenal rats. Because the results in the samples at 1 and 3 hr were almost identical, they were combined to give the data for Fig. 4, which shows that although the labeled, and therefore recently absorbed, fatty acid is equally distributed among the lipid fractions, the specific activity of the triglyceride fraction in the A group is much higher than in the N. This is probably due to a reduction in the

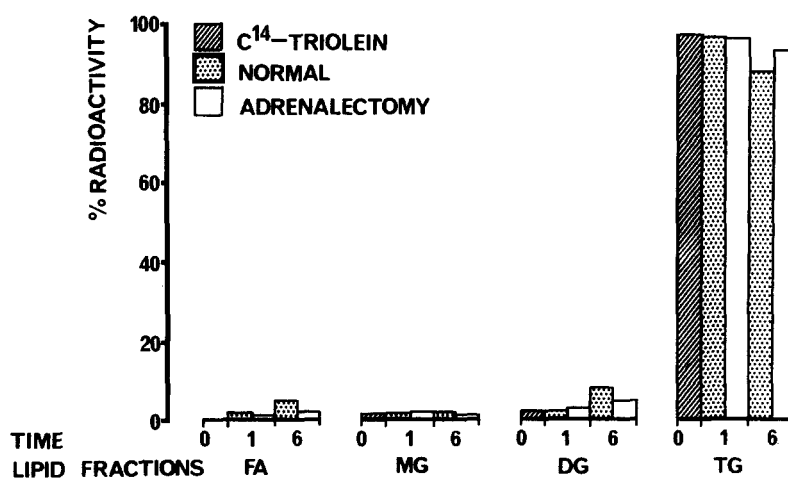


FIG. 1. Gastric lipolysis of triolein-¹⁴C in normal and adrenalectomized rats 1 and 6 hr after feeding. A small amount of gastric lipolysis occurred after 1 hr; this increased only slightly after a further 5 hr. FA, fatty acid; MG, monoglyceride; DG, diglyceride; TG, triglyceride.

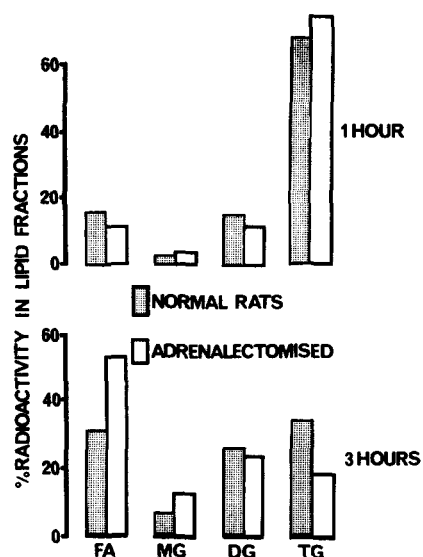


FIG. 2. Diagrammatic representation of the degree of lipolysis of administered triolein at 1 and 3 hr in the intestinal lumen of normal and hypoadrenal rats. Lipolysis is more nearly complete in the hypoadrenal animals. Abbreviations as in Fig. 1.

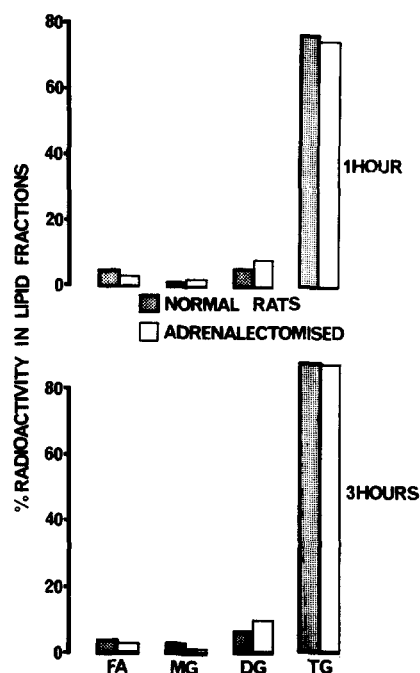


FIG. 3. Pattern of reesterification of ^{14}C -labeled fatty acid after absorption of triolein- ^{14}C into the intestinal mucosa. This is similar for both normal and hypoadrenal rats and shows almost complete incorporation into the triglyceride fraction of the cell lipid. Abbreviations as in Fig. 1.

amount of endogenous triglyceride of the intestinal epithelium.

Oleic Acid- ^{14}C Studies

The mean serum sodium and potassium levels of the

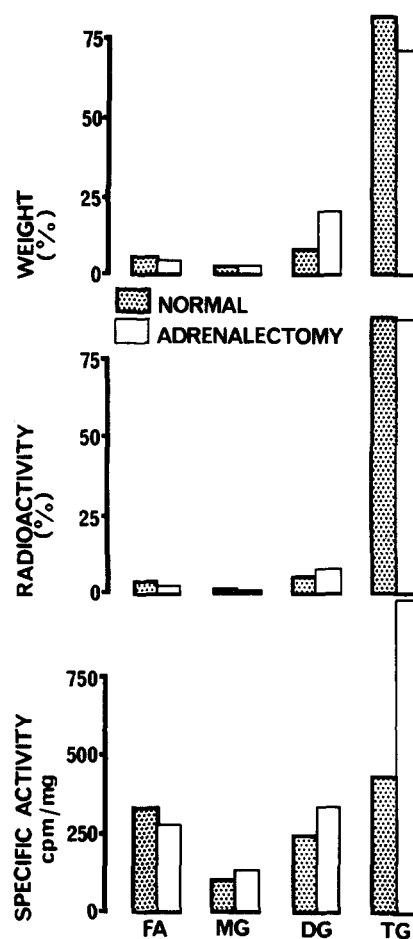


FIG. 4. The upper part of the figure shows the percentage distribution of the lipid fractions by weight, the middle part by radioactivity, and the lowest part the specific activities of the epithelial cell lipids of normal and hypoadrenal rats given triolein- ^{14}C . Abbreviations as in Fig. 1.

rats on the day of the experiment are given in Table 3, and again the values are consistent with the electrolyte biochemistry of the hypoadrenal state. These animals were not weighed, but again the A rats were obviously lighter than the others, and on dissection considerably less fat was found in the peritoneal cavity.

The mean rates of absorption and gastric emptying are shown in Table 4. The differences are less marked in this experiment because the relatively large amount of free oleic acid is less well absorbed by the normal animals, as one would expect.

There was no histological abnormality of the small bowel mucosa in the adrenalectomized rats, at least as judged by means of light microscopy.

DISCUSSION

After the administration of triolein a small and equal amount of gastric lipolysis occurs in both the normal and

TABLE 3 SERUM ELECTROLYTES IN NORMAL AND ADRENALECTOMIZED RATS GIVEN OLEIC ACID-¹⁴C

	Normal (12)	Adrenalectomized (11)	
	<i>meq/liter</i>		
Na ⁺	140 ± 2.7*	132 ± 5.5	n = 21 t = 4.15 P < 0.001
K ⁺	4.8 ± 0.4	6.6 ± 0.8	n = 21 t = 6.6 P < 0.001

Numbers in parentheses designate number of animals.
* SD.

hypoadrenal rats. The rate of gastric emptying is similar in both groups during the first hour, which indicates that there is no initial inhibition of gastric motor activity in the adrenalectomized rats. Thereafter gastric emptying is delayed, probably by regulated inhibition rather than by ileus. If this is so, the inhibiting substance may be enterogastrone.

Intestinal lipolysis proceeds normally at first in both groups of rats, but in time it becomes more complete in the hypoadrenal rats because of the smaller turnover of gastric chyme within the small bowel. There is no impairment of intestinal lipolysis in these animals. It may be the accumulation of unabsorbed free fatty acid within the bowel which causes the delayed gastric emptying.

The absorption of fat also progresses at an equal rate in both groups of animals during the first hour, but thereafter it becomes progressively less in the hypoadrenal rats. In our opinion it is an actual failure to transport fatty acid across the intestinal membrane which is the primary defect, and it is some such membrane transfer mechanism which is impaired by the hypoadrenal state. Once the ingested fatty acid has been taken up by the mucosal cell it is reesterified as completely as in the normal rats. The higher specific activity of the mucosal triglyceride in the hypoadrenal animals is likely to be due to the smaller amount of endogenous triglyceride secondary to chronic malabsorption of fat.

When oleic acid was administered malabsorption was again demonstrated, but less markedly, since the amount of free fatty acid given was relatively large and created absorption difficulties even for normal animals.

This study confirms that fat absorption is impaired in the hypoadrenal rat. This is not due to impaired lipolysis during small bowel digestion, nor is it indirectly attributable to impaired glyceride synthesis in the mucosal cells. The accumulation of fatty acid in the bowel seems to imply impaired transport across the mucosal membrane.

Fatty acid transport in this situation may be linked in some way with fatty acid activation. Although the gut fatty acid activating enzymes are found principally within the microsomes of the small bowel epithelial cell (8) it is not certain where fatty acid activation occurs, whether within the cell or at the cell surface. Should it be the latter, it is possible that fatty acid activation and fatty acid transport are interdependent, so that limitation of activation automatically inhibits transport. Vidal-Sivilla (9) attributes whatever absorption defects investigators have found in adrenalectomized rats to hypoadrenal shock. But we are confident that this explanation does not apply to the present study. His experiments on glucose absorption were carried out on anesthetized animals which were already in poor condition. By preliminary experimentation we discovered the optimum period of saline deprivation which aggravated the hypoadrenal state and yet left the animals fit enough to withstand gastric intubation without the use of an anesthetic, and alert and mobile until the end of the experiment.

We did not attempt to demonstrate in this study whether the malabsorption of fat was related to salt depletion alone. Bavetta et al. (4) showed that fat absorption was not improved in their adrenalectomized rats by giving saline and there is no evidence that salt depletion as such leads to malabsorption in humans. There is certainly no known role for the sodium or chloride ion in the biochemical pathway of fat absorption.

TABLE 4 DATA ON ABSORPTION OF OLEIC ACID IN NORMAL AND ADRENALECTOMIZED RATS

Time, hr	Normal			Adrenalectomized			"6 hr" statistics
	1	3	6	1	3	6	
Absorption, % dose	46.9	64.7	78.6	47.5	53.9	63.9	n = 5 t = 2.4 P > 0.05
Gastric emptying, % dose in stomach	46.2	19.6	12.0	40.4	30.9	29.4	n = 5 t = 2.77 P < 0.05
Lumen lipid, % dose	3.5	6.8	4.1	11.1	13.1	4.4	
Colonic lipid, % dose	3.4	8.9	5.3	1.4	2.1	2.3	

Dose: 0.6 ml of oleic acid-¹⁴C. Each figure is the mean of four results, except for adrenalectomized, 6 hr (three animals).

There was no histological abnormality of the intestinal epithelium in the adrenalectomized rats revealed by light microscopy. Rather surprisingly there seems to be no account of the histology of the small bowel of such animals in the literature. The only paper in this field (10) gives a detailed description of gastric, upper duodenal, and colonic histology, but does not include jejunum or ileum. Tissieres (11) has reported that adrenalectomy has no effect on the alkaline phosphatase content of the villous epithelium, but this awaits corroboration. Indeed the whole field is ripe for investigation by the electron microscopist, histochemist, and molecular biologist.

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