10525

Effect of Adrenalectomy on Rate of Fat Absorption.

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It has been thought for some time that the rates of absorption of glucose and fat from the intestinal tract are decreased after extirpation of the adrenals.[1, 2] This effect has been interpreted by Verzar and his coworkers[3] as being due to the absence of the hormone of the adrenal cortex which causes a postulated decrease in phosphorylation of both glucose and fat by the intestinal mucosa. According to this hypothesis, phosphorylation is necessary for the normal absorption of these 2 foodstuffs. Deuel, *et al.*,[4] and more recently MacKay and Clark[5] have found that adrenalectomy has no influence on the rate of absorption of glucose. Because of these differences it seemed desirable to reinvestigate the effects of adrenalectomy on the absorption of fats. Miller, *et al.*,[6] in a study of fat* absorption by means of "tagged" fat, found no significant decrease in the amount of phosphorylation of fed fat in the intestinal mucosa of adrenalectomized rats. This was accompanied by no decrease in absorption rate. As the fat fed in this study was in the form of methyl esters of fatty acids and as the amounts absorbed were only determined at 2 absorption periods, the following study was made, first to see if there is any difference between the rate of absorption of methyl esters of the fatty acids of corn oil and the natural fat,

1 Judovits, N., and Verzar, F., *Biochem. Z.*, 1937, **292**, 182.

2 Verzar, F., and Laszt, L., *Biochem. Z.*, 1935, **276**, 11.

3 Verzar, F., and McDougall, E. J., *Absorption from the Intestine*, Longmans, Green and Co., 1936.

4 Deuel, H. J., Jr., Hallman, L. F., Murray, S., and Samuels, L. T., *J. Biol. Chem.*, 1937, **119**, 607.

5 MacKay, E. M., and Clark, W. G., unpublished data.

6 Miller, E. S., Barnes, R. H., Kass, J. P., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1939.

* Custom has more or less limited the use of the term "fat" to the glycerol esters of fatty acids. However, the glycerol esters of fatty acids which do not occur in nature are often referred to as fats and we have used the term "fat" here in the same broad sense (esters of naturally occurring fatty acids) rather than in reference to a compound of nature.

and second, to determine the effect of adrenalectomy on the absorption of these 2 substances.

Eighteen adult male rats, weighing from 200 to 270 g were bilaterally adrenalectomized. These animals and a comparable group of unoperated controls were fed on our stock diet and given 1.0% sodium chloride *ad lib.* for 4 days. Although their food intake was lower than that of the controls the operated animals were in good condition and exhibited no signs of adrenal "intoxication" such as a subnormal body temperature, malaise and so on. Other adrenalectomized rats to whom water instead of salt solution was supplied for drinking had their food intake reduced much more and the clinical condition of these animals was such as to make them unsuitable for the administration of fat. Even the apparently normal salt-treated adrenalectomized rats were prostrated by the administration of corn oil. The methyl esters of the corn oil fatty acids had a less deleterious effect. In view of the toxicity of these fats for the adrenalectomized organism the results obtained in our experiments become even more striking.

Feeders were removed while the salt solution for drinking was still supplied, and after 24-hour fasting, each rat was given 0.5 cc of fat per square decimeter body surface by stomach tube. Body surface was calculated by the formula of Carman and Mitchell.[7] Half of the animals received corn oil (Mazola) and the other half the methyl esters of the fatty acids of corn oil. Both of these 2 groups were further subdivided into adrenalectomized and unoperated control groups.

At 2, 4, and 6 hours after giving the fat each animal was anesthetized with ether and the abdomen opened. A hemostat was clamped at the junction of the esophagus and stomach and another at the pyloric junction of the duodenum. A third was then clamped at the cecal end of the small intestine and the intestine cut on the ileum side. This free end of the intestine was then stripped free of mesentery for about 3 inches and placed in an open flask. A large bore, dull needle which was connected by rubber tubing to a 5-gallon bottle containing 1.0% sodium chloride was then inserted into the duodenum just below the pyloric clamp. Pressure was introduced into the system by means of a pressure bulb connected to the sodium chloride reservoir, and the intestinal contents washed into the flask with about 150 cc of salt solution. The stomach and large intestine were then stripped open and washed separately. In some

[7] Carman, G. G., and Mitchell, H. H., *Am. J. Physiol.*, 1926, **76**, 380.

studies it was found necessary to make sure that none of the fed fat was retained in the intestine. Under such conditions the above described washing was followed with approximately 50 cc of 95% alcohol and this followed by 50 cc more of the salt solution. All of this procedure was carried out while the animals were anesthetized but alive. It required approximately 3 minutes including anesthetization for each animal.

In order to test whether any fat was absorbed by the villi, or trapped in the intestine, a loop was made by tying off the intestines of 3, live anesthetized rats. The loops were washed with warm saline and 1.5 cc of the methyl esters of conjugated fatty acids of corn oil, developed as a "tagged" fat by Miller, *et al.*,[6] injected into each. After 5 minutes the loops were washed as described above, the intestines stripped lengthwise and the mucosa scraped off. No "tagged" fat was found to be present in the content or adsorbed on the intestinal mucosa after this treatment.

The figures in Table I show the rates of absorption of methyl esters of the fatty acids of corn oil and unaltered corn oil in adrenalectomized, and unoperated control rats at 2, 4, and 6 hours after feeding. The results calculated on the basis of the weight of fat absorbed per square centimeter body surface during the different periods show no significant differences between the rates of absorption of the methyl esters and unaltered corn oil. Further, there is no significant difference between the rates of absorption for these fats in adrenalectomized and unoperated control rats. If the results are calculated on the basis of the approximate amount of fat absorbed per hour, as is shown in the last column in Table I, there is again no difference between any of the groups and the rate of absorption is the same for each hour after feeding. It is interesting to note the differences in the stomach emptying times. In all cases the methyl esters left the stomachs more rapidly than the corn oil. Even with this difference in emptying time the rates of absorption remained the same. This is contrary to the absorption of glucose where MacKay, Bergman and Barnes[8] found that the rate of absorption was roughly proportional to the amount available for absorption from the intestine. It is unfortunate that the data in Table I does not cover enough absorption periods to afford accurate calculations of the amounts of fat available for absorption each hour after feeding.

It is difficult to understand the difference between this study and

[8] MacKay, E. M., Bergman, H. C., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 323.

TABLE I.

Absorption of Corn Oil (Mazola) and Methyl Esters of Fatty Acids of Corn Oil in Adrenalectomized and Unoperated Control Rats.*

Abs. Time hr	Fat fed	Treatment	Body surface, cm^2	g fed	Fat left in stomach, g	Fat left in intestine, g	Fat abs., g	Fat abs./ cm^2 B.S., mg	Fat abs./ cm^2 B.S./hr, mg
2	Corn oil	control	459	2.11	1.18	.58	.35	.76	.38
2	" "	adrenalectomized							
2	Me. ester	control	426	1.84	.52	1.07	.25	.59	.29
2	" "	adrenalectomized	415	1.84	.34	1.11	.40	.96	.48
4	Corn oil	control	424	1.93	.70	.60	.62	1.46	.37
4	" "	adrenalectomized	413	1.93	.76	.70	.47	1.35	.34
4	Me. ester	control	408	1.75	.54	.56	.64	1.57	.39
4	" "	adrenalectomized	409	1.75	.18	1.02	.54	1.32	.33
6	Corn oil	control	413	1.93	.32	.57	1.05	2.54	.42
6	" "	adrenalectomized	399	1.84	.46	.42	.97	2.43	.40
6	Me. ester	control	422	1.84	.26	.65	.93	2.20	.37
6	" "	adrenalectomized	410	1.84	.12	.52	1.19	2.90	.48

*Each figure is an average of a group of 3 animals.

those published by others on the effects of adrenalectomy on the absorption of fat. One essential difference does exist and that is the type of fat used. In most of his work, Verzar has employed olive oil. In the experiments presented here and in the study by Miller, *et al.*,[6] corn oil has been used. Steenbock, Irwin and Weber[9] have reported a great variation in the absorption of different fats. They found that 65% of fed olive oil was absorbed in 4 hours. Palm oil and oleo stock which were the slowest were only absorbed to about 36%. The data in Table I show approximately 31% corn oil absorbed in 4 hours. Other studies in this laboratory have also shown that olive oil is much more prone to cause diarrhea than corn oil and furthermore, doses of corn oil up to 3 cc have been given to adrenalectomized rats without any harmful effects. Verzar, and Laszt[2] have found that adrenalectomized rats receiving olive oil usually die within 12 to 24 hours after feeding. It might be that some such differences in the type of fat used will explain the apparent discrepancies in the effects of adrenalectomy on fat absorption. It is also possible that the secondary effects of adrenal insufficiency, not present in this series because of the salt regimen, account for the slower absorption of fat in adrenalectomized animals noted by earlier workers.

Summary. The methyl esters of the fatty acids of corn oil are absorbed at the same rate as unaltered corn oil. Adrenalectomy has no significant effect on the absorption of either of these two fats. The rate of absorption of both methyl esters of corn oil fatty acids, and unaltered corn oil is the same for each hour up to 6 hours after feeding.

[9] Steenbock, H., Irwin, M. H., and Weber, J., *J. Nutrition*, 1936, **12**, 103.