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Lipid excretion by bile fistula dogs on a lipid-free diet.

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Previous work[1] has indicated that, contrary to the generally accepted view, the fecal lipids (particularly the sterols) do not reach the intestine by way of the bile, but enter it below the absorbing portion. In order to shed further light upon this question, bile fistula operations have been performed upon 3 of the normal dogs used in previous work with essentially lipid-free diets[1, 2] and these animals have been employed in similar experiments, the results obtained while the dogs were still normal, serving as controls. In the case of 2 of the animals, 6 such control experiments had been carried out, while 13 were available in the case of the 3rd. No attempt was made to keep the bile tract sterile in one of the animals, and there was considerable infection during the experiment. The dog ate well, however, appeared normal, and maintained its weight. There was no essential difference in the results from this animal and those from the others in which the bile tract was kept sterile. During the experiments the dogs were prevented from licking the bile either by means of a muzzle (in one experiment with the infected dog) or by collection of the bile in a balloon kept in place by a binder. There was no marked loss of weight in any case.

The diet was based on the work of Cowgill[3] and contained casein (23.2 parts), sugar (50.0 parts), bone ash (1.4 parts), Liebig's meat extract (1.8 parts), salt mixture (1 part), and Vitavose* (2.2 parts). The casein was continuously extracted with hot alcohol for several days, and that used in most of the experiments contained less than 2 parts of lipids in 10,000 as determined by digestion of the protein with strong alkali for 24

[1] Sperry, W. M., *J. Biol. Chem.*, 1926, lxviii, 357.

[2] Sperry, W. M., and Bloor, W. R., *J. Biol. Chem.*, 1924, lx, 261.

[3] Cowgill, G. R., *J. Biol. Chem.*, 1923, lvi, 725.

* The author is indebted to the Ward Baking Company for the Vitavose used in these experiments.

hours, followed by acidification and extraction with ether and petroleum ether. The small amounts of lipid material in the meat extract and Vitavose were considered negligible in view of the results obtained.

The analytical procedure consisted briefly, in dividing the feces excreted by the dogs on the above diet, into weekly periods by charcoal demarcation, and separating the lipids obtained after complete saponification into unsaponifiable, non-volatile fatty acid, solid fatty acid, and liquid fatty acid fractions. In all, 12 weekly experiments were carried out, of which 11 formed parts of a series in which the animals were kept on the diet for periods of 3 or 4 weeks. The dogs were fed the experimental diet for 3 or 4 days, and a water enema was given before the marker was fed, in order to eliminate the possibility of stagnation of fat from previous diets in the intestine. An enema was administered on the day following the feeding of the marker and good separation into weekly periods was always obtained.

The most striking feature of the results was a marked increase in the total lipid excretion over that obtained when the animals were normal, from about 2 to 4 times as much material being excreted, without exception, by the animals with the bile fistulas. The amounts varied from 3.076 to 8.663 gm., while the normal average excretion was about 1.7 gm. Continuing the experiments over long periods of time appeared to have no effect. The largest excretion was obtained during the 4th week of one of the series. As in the case of the normal animals, the excretion was quite constant in composition, and the ratio of unsaponifiable material to total fatty acids was almost identical with that found in lipids excreted by normal dogs. The ratio of liquid to solid acids was somewhat smaller in most cases and the solid acids had a higher melting point than those from the normal animals. These differences were not large, however, and in general it may be stated that there is markedly larger excretion of lipids by bile fistula than by normal dogs on essentially lipid-free diets, and that the composition is very nearly the same in both cases.

It has been suggested that these results may be explained by an increase in intestinal bacteria and experiments are being planned which it is hoped will throw light on this point. Perhaps a more plausible explanation is that there is a secretion of lipids throughout the length of the intestine, which in normal animals is largely absorbed but which escapes absorption in bile

fistula animals. At any rate it seems to be strongly indicated that fecal lipids do not necessarily enter the intestine by way of the bile.

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A preliminary study of conditioned motor reflexes in thyroidectomized sheep.

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The investigation of habit formation in thyroidectomized sheep and goats by the maze method has yielded results difficult to analyze because of the complicated responses elicited. The conditioned motor reflex method of Bekhterev has, therefore, been adopted.

In a preliminary experiment a conditioned reflex to a tactile stimulus was established in three animals, two thyroidectomized sheep, aged three and four years respectively, and the twin control of the three year cretin. Tactile stimuli were applied to a spot on the rump, at the rate of thirty per minute, for periods varying from two to ten seconds. With the final stimulus, a faradic current was applied to the left foreleg of sufficient intensity to evoke a defensive movement. The first definite leg movement, in response to the tactile stimulus alone, occurred at the tenth combination in the control and in the three year cretin, and at the seventeenth combination in the older cretin. At the end of the ninth day and forty-fifth combination, one milligram of thyroxin was administered to each thyroidectomized animal. In spite of this, one and one half months later the younger cretin died and the training of the other animals was then discontinued, after two hundred thirty-eight combinations of conditioned and unconditioned stimuli.

After an interval of six and one half months the tactile stimulus without reenforcement evoked a vigorous conditioned reflex in both normal and cretin sheep. This is shown in Fig. 1, the

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