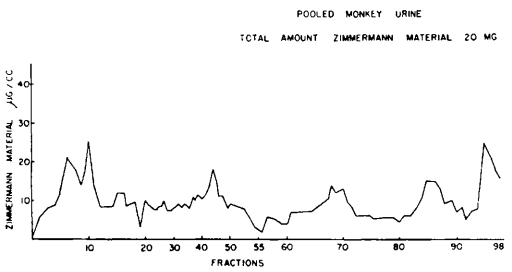

FIG. 1. Distribution of Zimmermann chromogen from monkey urine following chromatography on alumina.

of the ether, residual moisture was removed with benzene. The crude extract thus obtained was chromatographed on alumina in a gradient elution system (Lieberman and Lakshmanan(4)) in which 200 ml benzene and 6% ethanol was added slowly to 800 ml benzene during development of the chromatogram. Removal of more polar steroids from the column was assured by subsequent elution with 20 ml each of 5%, 10%, and 20% ethanol in benzene. The eluate was collected in 100 fractions. Aliquots of 1 ml were taken for Zimmermann reaction (Holtorff and Koch).

Results. Five distinct peaks were obtained (Fig. 1) throughout the chromatograms. Only 2.8 mg of purified Zimmermann reacting material were recovered from the crude extract of the small pool (total 12 mg), while the extract of the larger pool, containing 20 mg of crude material, yielded 5.8 mg. These values were within the range of Dorfman's findings. However, when identification of the material in the peak tubes was attempted by UV, sulfuric acid spectra and infrared spectrophotometry, none of the spectra resembled those of 17-ketosteroids or other steroids. Moreover, no carbonyl absorption was obtained at 1800-1580 cm^{-1} and no peaks were observed at 311 mμ in H_2SO_4. This led us to believe that the color obtained by the Zimmermann reaction was not specific. It is suggested that the technic used for determination of 17-ketosteroids in humans cannot be applied to monkey urine. This does not imply that the monkey does not excrete 17-ketosteroids, but merely that values given in the literature based on the Zimmermann reaction alone are misleading and have led to erroneous conclusions.

Summary. An attempt was made to study the excretion of 17-ketosteroids in Rhesus monkey urine. No 17-ketosteroids could be detected by ultraviolet adsorption, sulfuric acid and infra-red spectra following chromatography of Zimmermann-material. This suggests that values quoted in the literature, based on Zimmermann reaction alone have led to erroneous conclusions.

POSTSCRIPTUM: Since this paper was submitted a similar discrepancy between Zimmermann reactive material and true 17-ketosteroid content has been reported in pregnant goat's urine by Klyne *et al.* (Klyne, W., Wright, A. A., *Biochem. J.*, 1957, v66, 92.)

1. Marker, R. E., Hartman, C. G., *J. Biol. Chem.*, 1940, v133, 529.

2. Dorfman, R. I., Horwitt, B. N., Shipley, R. A., Fish, W. R., Abbott, W. E., *Endocrinol.*, 1947, v41, 470.

3. Holtorff, A. F., Koch, F. C., *J. Biol. Chem.*, 1940, v135, 377.

4. Lakshmanan, T. K., Lieberman, S., *Arch. Biochem. and Biophysics*, 1954, v53, 1.

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Acetal Phosphatides in Adipose Tissue and Livers of Starved Rats. (23288)

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Previous work reported from this laboratory(1) showed that in the adipose tissue of newborn rats, where this tissue is rapidly filling with fat, the acetal phosphatides and phospholipids, initially elevated, decreased to only trace amounts by the fifth to eighth day of life. This finding posed the question: would phospholipid and the tissue lipid aldehyde fraction, presumably acetal phosphatides, in the reverse situation, as in starvation, also become elevated during the period of rapid mobilization of depot fat from adipose tissue.

Such evidence might be indicative that acetal phosphatides and phospholipids are involved in some manner in mobilization of fat to and from adipose tissue. For these reasons, changes in acetal phosphatide content of adipose tissue in relation to phospholipid and total lipid content of the same tissue were investigated in rats subjected to starvation and recovery from starvation.

Materials and methods. Twenty-nine female rats, weighing 130 to 160 g of the Carworth Farms strain, were divided into groups of 3, except the first group consisting of 2 animals which served as controls. During an observation period of 5 days, the animals were fed a stock diet of Purina Chow, and their body weights checked daily. The average weight gain/day was about 3 g. After the observation period, all animals, except the control group, were placed on a starvation regime. The animals received nothing to eat, but were given water *ad libitum*. The control animals were sacrificed with no previous period of starvation. At intervals of 1, 2, 3, 4 and 6 days after beginning of starvation period, groups of animals were sacrificed by a blow on the head. While the animal's heart was still beating, the jugular vein was opened and the animal exsanguinated. The liver and perirenal adipose tissue were dissected out, trimmed free of foreign tissue, washed under the tap and blotted dry with paper towel. In dissecting out the perirenal adipose tissue, care was taken to remove the same general area of tissue in each case. At the end of sixth day of starvation period, the remaining groups of rats were presented with food. During the period of recovery from starvation, groups of animals were sacrificed at intervals of 1, 2, 3 and 5 days after first being presented with food. Adipose tissue and livers were removed as described above. As soon after removal of tissue from the body as possible, tissue lipids were extracted by the procedure of Rice and coworkers(2), and the extracts made up to a suitable volume. Aliquots of the extracts were taken for analysis. Acetal phosphatides were determined by the method of Feulgen, Boguth and Andresen(3). This method in our hands, and with palmital dimethyl acetal as a standard for comparison,

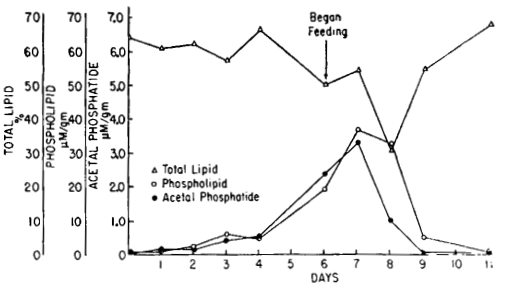

FIG. 1. Lipid changes in adipose tissue of rats subjected to starvation and recovery from starvation.

has given reproducible results with excellent recovery values. Total lipid phosphorus was determined by the procedure of Fiske and Subbarow(4). Total lipids were determined gravimetrically. During the experiment, 3 animals died and some samples of tissue were lost. However, groups of animals were adjusted so that each group consisted of at least 2 animals.

Results. The weight loss of animals during starvation averaged about 10 g/day over the first 4 days, with a total weight loss of 50 to 60 g by the end of the 6 day starvation period. Upon recommencement of feeding, the animals regained the lost weight at the rate of 10 to 15 g/day over the first 3 days, and had regained some 90% of the lost weight by the end of 5 day feeding period.

The changes in liver lipids in starved rats and in rats recovering from starvation are somewhat variable. Total lipid was essentially within normal limits, ranging from 2.86 to 5.07% of the wet weight of tissue. Phospholipids were essentially constant (2.26 to 3.84% wet wt of tissue). Although acetal phosphatides showed a trend toward elevated values during the starvation period, the range of acetal phosphatide concentrations obtained in this experiment (0.112 to 0.725 μmole/g of wet tissue) was within the normal range for liver: 0.365 $\pm$ 0.123 μmole/g wet tissue. On the basis of these results, little can be said of the role of acetal phosphatides in liver, although it is possible they may participate in liver lipid metabolism through a turnover mechanism.

In adipose tissue (Fig. 1), the percent total lipid remained essentially constant until the sixth day of the starvation period when

a decrease of 30 to 40% was noted. In this regard, however, the total weight of perirenal adipose tissue available for analysis decreased steadily throughout the starvation period. During the recovery phase, the total lipid content had returned to normal by the end of the fifth day of feeding.

Acetal phosphatides were detectable in only trace amounts (less than 0.05 μmole/g of tissue) in the adipose tissue of control animals and in animals after one day of starvation. They gradually increased to values of about 0.5 μmole/g of tissue by the end of the fourth day of starvation, then increased sharply to a peak value of 4 μmoles/g of tissue on the first day of the recovery period. At the end of the second day following recommencement of feeding, there was a pronounced drop in acetal phosphatides which continued to decrease to only trace amounts by the third to fifth day of the recovery period.

Total phospholipids behaved in a manner similar to acetal phosphatides, rising to a peak value on the first day of the recovery period and then falling sharply to only trace amounts at the end of the recovery phase.

The changes in acetal phosphatide and phospholipid in adipose tissue occurring during the first 4 days of the starvation period or the last 3 days of the recovery phase may only represent apparent changes, brought about by changes in other lipid constituents in the adipose tissue, such as neutral fat. However, the level of phospholipid and acetal phosphatides obtained on the last day of the starvation period and on the first day of the recovery phase represents increases ranging from 200 to 1000% for the phospholipid and 400 to 700% for the acetal phosphatides as compared to the levels noted in the control and experimental animals over the first 4 days of the experimental period. These levels represent real increases since the expected increase due to decrease in total lipid content would be on the order of 100%.

That the mobilization of fat to or from adipose tissue is an active process has been pointed out by Shapiro and coworkers(5). The finding that acetal phosphatides and phospholipids are elevated during the period of most rapid mobilization of fat to or from adipose tissue indicates that they are in some manner involved in this process. Acetal phosphatides could be involved directly in this process, or they could be precursors of other phospholipids which could then be involved in the mobilization of fat. Since mobilization of fat in adipose tissue has been shown to be an active process, acetal phosphatides could possibly also be involved in the production of the necessary energy for transporting the fat across the cell membrane.

Summary. Acetal phosphatides, total phospholipid and total lipids have been followed in the adipose tissue and livers of rats undergoing starvation and recovery from starvation. Whenever fat was being rapidly mobilized to or from adipose tissue, acetal phosphatides and total phospholipids were found to be greatly increased.

1. Yarbro, C. L., and Anderson, C. E., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 408.

2. Rice, L. I., Shotz, M. C., Alfin-Slater, R. B., and Deuel, H. J., Jr., *J. Biol. Chem.,* 1953, v201, 867.

3. Feulgen, R., Boguth, W., and Andresen, G., *Z. physiol. Chem.,* 1951, v287, 90.

4. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.,* 1925, v66, 375.

5. Shapiro, B., Weissman, D., Bentor, V., and Wertheimer, E., *Metabolism,* 1952, v1, 396.

Received April 23, 1957. P.S.E.B.M., 1957, v95.