in the normal rabbit much of the lipid phosphorus and cholesterol of the serum is bound to the serum proteins, while in the hyperlipaemia of alloxan diabetes in the rabbit the increase of these lipids is principally in the fractions not closely associated with the serum proteins.

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Increased Glyceride Content of Brown Fat by Treatment with Adrenocorticotropin.* (17674)

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Although the important role played by adrenocorticotropin (ACTH) and the hormones of the adrenal cortex in the metabolism of carbohydrate and protein is well established, less is known concerning their effects on the metabolism of fat. However, it is becoming apparent that ACTH may modify the distribution of visible fat in the body. Thus, it has been demonstrated previously that ACTH may cause the disappearance of almost all of the fat of the tela subcutanea(1) while increasing the fat content of the liver (2,3) and marrow of the tibia(4). During the course of autopsies on animals treated with ACTH, it was observed that the brown fat of the interscapular gland had taken on somewhat the appearance of white adipose tissue. Therefore, we have undertaken to analyze this change with microscopic techniques.

Methods. The interscapular gland was studied from 10 adult male rats given 1 mg of ACTH daily for 21 days and fed a high fat diet by stomach tube and 3 castrated rats given the same dosage but a medium carbohydrate diet. For each treated rat there was 1 control. In addition 6 hypophysectomized rats were given 3 mg of ACTH daily for 21 days by *continuous* subcutaneous injection and fed *ad libitum*. In addition, brown fat in other locations was available for study in microscopic preparations made from many animals treated with ACTH as reported in our previous publications. These areas included the brown fat in the region of the kidneys, adrenal glands, superior mediastinum and neck. Samples of the interscapular gland were fixed in Bouin's fluid, 10% neutral formalin and chilled 80% alcohol and Carnoy's fluid. Lipids were stained with Sudan red, Sudan black B, Schiff reagent, Nile blue sulphate, subjected to the Schultz reaction, and also studied for auto-fluorescence and birefringence. The Gomori(5) procedure was used for alkaline phosphatase with sodium glycerophosphate and fructose diphosphate serving as substrates. Best's carmine and the Hotchkiss(6) periodic acid-leucofuchsin techniques were utilized for the demonstration of glycogen. Staining for cytoplasmic basophilia

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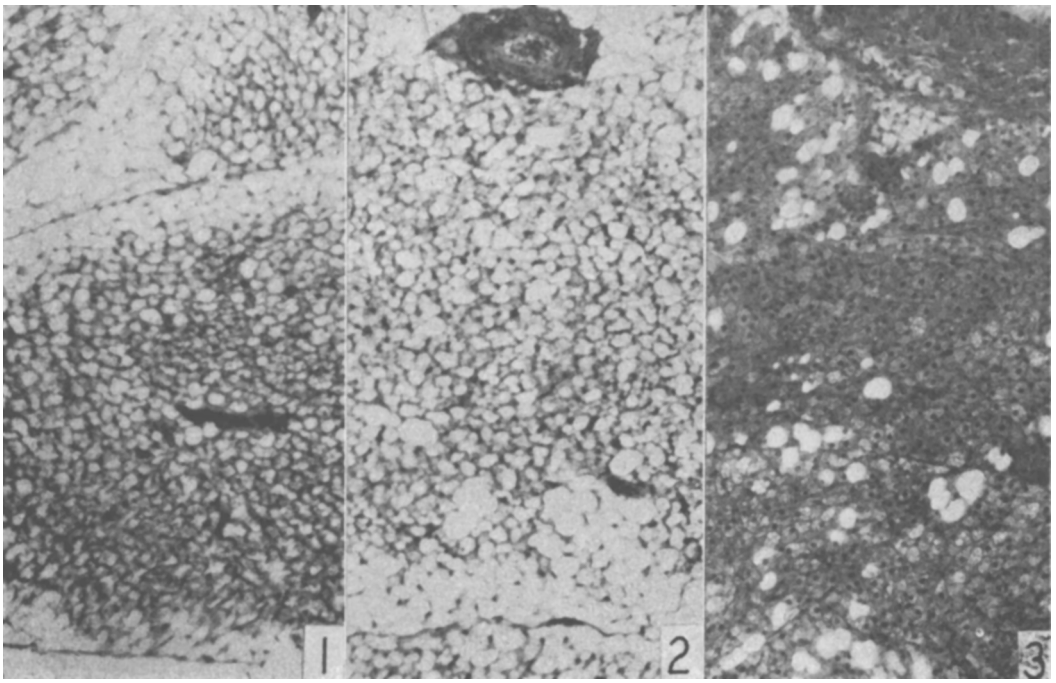

FIG. 1. Interscapular gland of castrated male rat, medium carbohydrate diet. ×110.
FIG. 2. Interscapular gland of castrated male rat, medium carbohydrate diet, 1 mg ACTH daily for 21 days. The vacuoles left by solution of glyceride are increased greatly. ×110.
FIG. 3. Interscapular gland of hypophysectomized male rat, fed *ad libitum*, 3 mg ACTH daily by continuous injection for 21 days. No increase in glyceride content has occurred. ×110.

was attempted with eosin and methylene blue (disodium phosphate-citric acid buffers).

Observations. The interscapular gland of the control animals was subdivided into lobules, with the typical brown fat tissue being concentrated around central blood vessels while a rim of cells similar to white fat in appearance formed a shell around the periphery of some lobules (Fig. 1.) Most of the central cells were multivacuolated after removal of lipid, were void of basophilia, and rarely revealed traces of glycogen. With the Gomori procedure little alkaline phosphatase activity was demonstrated with a glycerophosphate substrate, it being most evident in the nucleolus and occasionally in the blood vessel walls. With fructose di-phosphate as a substrate, phosphatase activity was more evident in both nuclei and cytoplasm. The lipid occurred in the form of multiple droplets and stained rose with Nile blue sulphate indicating that it was chiefly an unsaturated glyceride(7). The tissue in general gave a blue-green fluorescence which may have been due to the presence of some cholesterol or related substances in the cytoplasm of the brown fat cells. The cytoplasm of these cells stained intensely with the Schiff reagent (not preceded by treatment with mercuric chloride). The Schultz reaction for cholesterol was negative and, also, when thick frozen sections were extracted with warm chloroform, the typical blue color of the Lieberman-Burkhardt reaction failed to develop when acetic anhydride and sulfuric acid were added to the solution. However, observation of frozen sections of formalin-fixed tissue with the polarizing microscope revealed the presence of abundant birefringent material which was most concentrated in the central cells of the lobule. This birefringence disappeared completely when the slide was warmed, and upon cooling, a few spherocrystals giving the black cross of polarization appeared. These optical

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phenomena might be suggestive of the presence of cholesterol or its esters(7). Cramer(8) likewise experienced more difficulty in demonstrating cholesterol in the interscapular gland than in brown fat located in other regions of the body. It was apparent that the diet in itself modified the lipid content of brown adipose tissue. For example, this tissue contained less lipid in the controls of the castrated series fed a medium carbohydrate diet than in many of the intact series given a high fat diet.

Treatment of intact and castrated rats with adrenocorticotropin caused a regular increase in amount of sudanophilic lipid in the cells of brown fat as is illustrated by the vacuolation of the constituent cells from which the fat had been dissolved (Fig. 1 and 2). The smaller droplets of the normal cells had enlarged and coalesced to form one large droplet in many cells. In preparations from which fat had been removed, these cells had somewhat the appearance of white fat. It was concluded that this lipid was an unsaturated glyceride since it stained with a fat-soluble oxazone form of Nile blue sulfate and was generally isotropic. All of the other reactions characteristic of the controls remained in the compressed cytoplasm. The striking increase in vacuolation which characterized the brown fat from many other parts of the body after ACTH indicated that the increase in glyceride content was not limited to the brown fat of the interscapular gland. Likewise, the response was not conditioned entirely by the diet since evidence of increased fat content was secured regardless of whether rats were force-fed diets high in carbohydrate, fat or protein.

On the contrary, in the absence of the hypophysis, 3 times the dose of ACTH used in the preceding experiment failed to cause an increase in the glyceride content of brown fat (Fig. 3). In fact, this tissue contained less fat than in the control animals fed a high fat or medium carbohydrate diet by stomach tube.

Fawcett(9) has demonstrated recently that re-feeding of starved rats or treatment with insulin causes an increase in the amount of glycogen in brown adipose tissue. He pointed out further the probable importance of this glycogen in the synthesis of fat. In view of his work, it was of interest to find that ACTH caused no apparent increase in glycogen under the conditions of our experiments.

Discussion. It is not clear from our studies whether the ineffectiveness of ACTH in the hypophysectomized animal was due to the absence of an essential hypophyseal hormone or the unsatisfactory state of nutrition of these rats. Not only were they immobilized but, also, in eating *ad libitum,* they did not secure a normal food intake. In contrast to our findings, Fawcett and Jones(10) have reported that injection of ACTH into mice failed to increase the neutral fat content of interscapular adipose tissue. They also reported that ACTH prevented the loss of fat which occurred after hypophysectomy. Under the conditions of our experiments with rats, very large doses of ACTH were ineffective in preventing the loss of fat from the interscapular gland of hypophysectomized rats. Further, Selye and Timiras(11) reported recently that subjection of rats to cold resulted in depletion of the lipid content of the interscapular gland. At the present time, their findings cannot be reconciled with the effects which we observed after treatment with ACTH. However, they also observed the importance of the nutritional factor since starvation accentuated the loss of lipid from the interscapular gland due to exposure of rats to cold.

Summary. Treatment of intact or castrated rats with ACTH caused an increase in the glyceride content of brown adipose tissue. Treatment of hypophysectomized rats with 3 times the dose used above failed to maintain the fat content of the interscapular gland at a normal level.

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