Reports

Storage of Steroid Hormones by Adipose Tissue in Two Experimental Obesities

Abstract. It appears that obese animals retain proportionally more steroid hormones than nonobese animals. The retention of these hormones does not appear to be a function of the nature of the obesity syndrome but simply a function of the increased fat content.

It has been shown that obesity in mice can be grouped into two general classes: "regulatory" obesity, in which the primary lesion lies in the nervous centers regulating food intake, and "metabolic" obesity, in which the hyperphagia is secondary to metabolic disorders of carbohydrate and fat metabolism (*1*). Since these two classes of obese mice differ markedly with respect to lipogenesis and cholesterologenesis (*2*) as well as enzymatic reactivity (*3*), feeding patterns (*4*), and so on, it seemed to be of interest to determine the characteristics of retention of administered steroid hormone in the two types of obesity.

If retention of administered hormone were entirely due to the amount of fat present, one would expect a direct correlation between retention and fat content, whether in normal animals, in animals with regulatory obesity, or in animals with metabolic obesity. A deviation from this pattern would suggest that specific factors influence steroid retention of fat.

Accordingly, mice with goldthioglucose hypothalamic (regulatory) (*5*) obesity and mice with the hereditary obese-hyperglycemic (metabolic) syndrome (*6*) and their controls were used to study the retention of testosterone and progesterone. A wide range of weights was used in each group. The steroids retained by adipose tissue were identified by chromatography, and retention was determined by measurements of radioactivity. The technique was similar to that used by Plotz and Davis (*7*) and by Zander (*8*) in their study on the retention of progesterone in the fatty tissue of pregnant women.

Ten female goldthioglucose obese mice (36 to 58 g), six nonobese female controls (20 to 32 g) and eight obese hyperglycemic mice (four males, four females, weighing 45 to 91 g) with their nonobese controls (4 males, 4 females, weighing 20 to 35 g), were used in the testosterone study.

Three obese-hyperglycemic mice (one male, two females, weighing 48 to 71 g) with their nonobese controls (one male, two females, weighing 20 to 30 g), as well as four goldthioglucose obese mice (females, 32 to 40 g) and four nonobese controls (females 22 to 29 g) were used in the progesterone experiment.

The mice were injected intramuscularly with either 5 or 10 μc of testosterone-4-C^{14} (1.21 mg or 2.42 mg) or with 10 μc of progesterone-4-C^{14} (0.33 mg) dissolved in sesame oil. The mice were placed in individual metabolism cages for 18 hours. Urine and feces were collected during this time interval. At the end of 18 hours, the mice were killed by decapitation, and adipose tissue was removed and extracted for 12 hours with ether/95 percent ethanol (3:1). The extracted fat was partitioned between heptane and 70 percent methanol (heptane fractions were counted to check the completeness of the extraction). Carrier steroids were added to the methanol extracts. These extracts were chromatographed by the Zaffaroni (*9*) system with heptane/propylene glycol as the solvent system. Testosterone, etiocholanolone, and androsterone zones (testosterone-C^{14} study) and progesterone (progesterone-C^{14} study) were eluted from the chromatogram. The radiochemical purity of the C-19 steroids and of the progesterone was determined by Bush-type wash-out chromatogram (*10*), acetylation and chronic acid oxidation (C-19 steroids), and dinitrophenylhydrazine derivative (progesterone). Retention was determined by measurement of radioactivity.

The majority of the methanol extracts from adipose tissue were treated in the manner described above. However, a few samples were chromatographed with the toluene/propylene glycol system. In this solvent system, corticoids and estrogens would be retained on the paper. However, since only negligible activity was found in these fractions, the remainder of the samples were directly chromatographed in the heptane/propylene glycol system for isolation of C-19 steroid and progesterone.

In the testosterone-C^{14} experiment the major portion of the activity retained in adipose tissue was present in the testosterone (96 percent), etiocholanolone (2 percent) and androsterone (1 percent) fractions. Progesterone accounted for most of the retained activity (93 percent) in the progesterone-C^{14} experiment, with the corticoid fractions accounting for about 1 to 3 percent of the activity and 1 percent in the fractions containing substances less polar than progesterone.

Most of the administered testosterone or progesterone is excreted in the urine and feces of the mice at the end of 18 hours. Similar results were obtained by Gallagher *et al.* (*11*) in studies on recovery of administered testosterone-4-C^{14} and progesterone-21-C^{14} in excreta and tissues of normal mice. However, the activity retained by adipose tissue

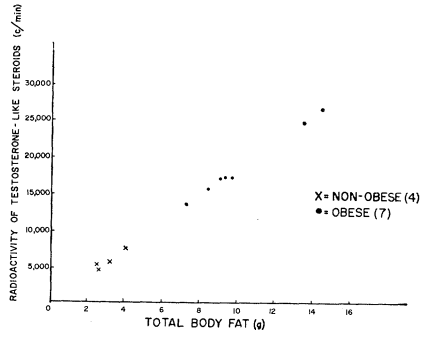

Fig. 1. Steroid hormone retention in adipose tissue after administration of testosterone-4-C^{14} to goldthioglucose obese and nonobese mice.

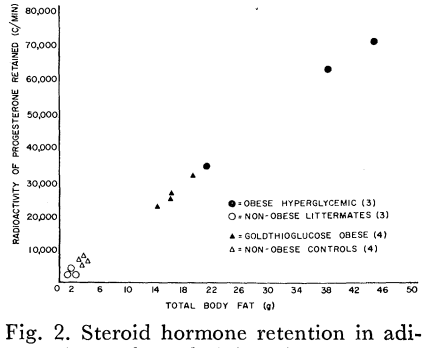

Fig. 2. Steroid hormone retention in adipose tissue after administration of progesterone-4-C^{14} to two kinds of obese mice.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

ranges from 0.24 to 5.2 percent of administered testosterone-4-C^{14} activity and from 0.19 to 4.3 percent of administered progesterone-4-C^{14} activity. This activity is correlated with the amount of fat present in the animals.

Figure 1 shows the total radioactivity of C-19 steroids plotted against grams of fat for goldthioglucose obese mice and nonobese controls given 10 μc of testosterone-4-C^{14}. This activity was measured 18 hours after injection. It is evident that retention of labeled hormone is proportional to the amount of fat, or to obesity per se. The same picture was found in goldthioglucose obese mice given 5 μc of testosterone.

Similar results were obtained with the obese-hyperglycemic mice—that is, retention of testosterone-C^{14} was proportional to the amount of fat present in obese and nonobese mice. In addition, C^{14} retention per gram of fat (1800 count/min per gram of fat for animals given 10 μc) was the same for both types of obese mice and their nonobese controls. For the various types of mice at the two doses studied after 18 hours, retention of testosterone was represented by the formula: percentage retention $= 1.3 + 0.05 \times 10^{-3} f$, where f is total body fat in grams.

Figure 2 shows the radioactivity present in the progesterone fraction plotted against the grams of fat for goldthioglucose obese mice, obese hyperglycemic mice, and their respective nonobese controls measured 18 hours after the injection of progesterone-4-C^{14}. As in the testosterone study, retention of labeled hormone is correlated with the amount of fat present regardless of the type of obesity or whether the mice are obese or nonobese. Carbon-14 retention per gram of fat is the same for all animals (1600 count/min per gram of fat in the progesterone study). Thus, although the obese-hyperglycemic mice and goldthioglucose obese mice differ markedly in fatty acid and cholesterol synthesis, retention of administered steroid hormone appears to be a physical phenomenon common to both groups because of their increased fat content. A large amount of excess fat in obese animals favors the retention of injected steroid hormones. This may have physiological consequences in that obese animals (and obese patients) may retain larger amounts of their own steroid hormones. It may also have therapeutic implications in that it appears likely that steroid hormones administered to obese patients may be stored in appreciable amounts in their fat depots (*11*).

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5. Goldthioglucose hypothalamic mice become hyperphagic and obese as a result of the destruction of the ventromedial area of the hypothalamus by a single injection of 1 mg of goldthioglucose per gram of body weight (*2*). No abnormality of carbohydrate or fat metabolism (outside of those secondary to hyperphagia) is present.
6. The hereditary obese hyperglycemic syndrome is a recessive condition characterized by obesity, hyperglycemia, hypercholesterolemia, increased hepatic glycogen turnover and phosphorylase, hyperplasia of the Islets of Langerhans, insulin resistance, sensitivity to cold and to growth hormone, increased lipogenesis and cholesterogenesis even under fasted conditions, increased pancreatic and circulating insulin, abnormalities in the metabolism of adipose tissue, and so forth (*2*).
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Nervous Pathways of Cutaneous Pains

Abstract. In discussing the reality of a peripheral duality of pain-afferent systems, which she attributes to an artifact [*Science* 128, 713 (1958)], M. H. Jones rejects as valueless data concerning differences in the velocity of conduction of nerve impulses as determined by reaction times. Yet precise data tend to show the reality of these differences in velocity.

I found it very strange that Margaret Hubbard Jones should maintain, on several occasions (*1*), solely on the basis of subjective data, that the duality of cutaneous pains is an artifact.

For a long time now the dissociation of cutaneous pain systems has been established, as I reported in detail in 1935, in an article in *Traité de Physiologie* (*2*).

Supporting data obtained from an analysis of reaction times were deliberately rejected by Jones, who cited a study by Lele, Sinclair, and Weddell (*3*) in support of her position.

Answering Libet (*4*), she declares, "Libet's emphasis on reaction time is unfortunate. None of the studies meet the minimum requirements for work in the field."

Actually, research studies of Lele *et al.* have shown that these reaction times do vary—something which has been known for a century. It was through variations in reaction time that Helmholtz measured, for the first time, in 1850, the velocity of the nerve impulse in afferent tracts, with an accuracy which afterwards proved satisfactory.

Of course, measurements should be planned under very precise conditions and made on trained and sufficiently coherent subjects (this was not the case in the research of Lele *et al.*); it is then possible to get mean values which are stable enough to be significant. A most important point lies in the use of constant physiological intensities, for reaction times depend on the intensity of the sensation, showing rapid variability around the threshold. For touch, a margin of about 230 msec between threshold intensity and an intensity 150 times greater (*5*) is found; narrower margins are sometimes met with, however, (*6*).

But Lele *et al.*, when comparing reaction times to stimulation consisting of equal pressures on a finger and on a toe, took no account of differences in the sensibility of their subjects and did not establish threshold values; hence, their comparison loses all its significance and their conclusions all their value.

In 1930 (*7*) I made a series of measurements of reaction times to such stimulations as painful pricking, burning, and pinching, the stimulations being so graded as to cause sensations of seemingly equal pain on the forehead (or the temple), the wrist, and the ankle.

Under such conditions (the dispersion indices of means computed on series of 20 being usually less than 10 percent), I found, for two nerve pathways each 80 cm long (between forehead and hand and between hand and foot), reaction-time differences of 235 and 216 msec for burning (by contact for forehead and hand, by immersion for hand and foot); differences of only 49 msec for pricking (either between forehead and hand or between hand and foot); and, finally, differences of 135 msec for pinching between temple and foot (that is, 68 msec for a pathway 80 cm long).

The resulting probable velocities of nerve impulses are about 4.50 m/sec for burning, 16 m/sec for pricking, and 12 m/sec for pinching, whereas for touch the velocity is about 40 m/sec, according to determinations which von Wittich established as early as 1868 (*8*) through study of reaction times.

In 1939, Zotterman (*9*), using tactual percussion, contact, pricking, and burning as stimulations on a cat's tegument innerved by the saphena, recorded four types of action potentials in this nerve: the fastest (30 to 60 m/sec) were correl-