Influence of Cortisone upon Brown Fat of Hamsters and Mice.* (20834)

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In the course of studies on the extraneural progression of parenterally inoculated poliomyelitis virus (strain MEF_1) into cortisone-treated Syrian hamsters, a selective affinity for brown (hibernating) fat was noted. White fat was not involved. The differential lipotropism was exemplified by microscopic lesions confined exclusively to brown fat even in areas where the two forms of adipose tissue were in intimate contact. Viral assay further confirmed the selectivity of involvement(1-4). To further the understanding of the role played by brown fat in experimental poliomyelitis, an investigation was undertaken to evaluate the action of cortisone upon brown fat of hamsters and mice.

Experimental. In the first series of experiments, 200 untreated male hamsters were sacrificed at varying ages in order to establish normal growth curves for the two types of lipid-containing tissues. Preliminary dissections demonstrated that the mass of brown fat, situated between the mesial aspects of the scapulae, remained a constant fraction of the total mass of body brown fat, irrespective of age. The interscapular brown fat body (ISB) is a discrete, encapsulated, bilobate structure which is superficially located and readily resected with accuracy. The demarcated inguinal fat pad in the hamster is composed almost exclusively of white fat. These two fat masses were dissected out and their weights used as the representation of total body brown fat and white fat respectively. A number of ISB, thus removed from normal animals of different ages were examined histologically for the presence of infiltrating white fat cells. Counts were made repeatedly in order to determine the ratio of the two varieties of lipid cells. The second series of experiments was designed to investigate the effect of cortisone upon the weight and histological appearance of brown and white fat in hamsters and mice. Forty prepubescent, male hamsters between 25-30 g were each injected intramuscularly with 12 mg of cortisone (3 mg, 4 times, given over a period of 2 days), and groups of 10 animals were killed at 2, 4, 7 and 10 days after commencement of cortisone administration. Forty uninjected prepubescent male hamsters, serving as controls, were sacrificed at the same times. An additional 54 male hamsters of widely varying ages, weighing from 12-80 g, were treated with cortisone in total doses ranging from 4-24 mg, and sacrificed 7 days later. Forty male Swiss mice weighing between 14-17 g received 9 mg of cortisone intramuscularly (3 mg, 3 times, over a period of two days), and were sacrificed in groups of 10, at the time intervals stated above. Forty untreated mice served as controls. The interscapular brown fat body and the inguinal white fat pad were dissected out in all animals, weighed on a Roller-Smith balance, and equated to mg/100 g body weight.

Growth pattern of brown and white fat in the normal hamster. Up to a certain age the inguinal fat pad (white fat) increases proportionately to the weight of the body, *i.e.*, about 250 mg/100 g body weight in the 25 g male hamster and about 1,250 mg/100 g body weight in the 75 g animal. Beyond this stage, the proportion of inguinal fat to body weight maintains a relatively stationary ratio. The *interscapular brown fat body* rises sharply in weight to a level of approximately 260 mg/100 g body weight in the 20 g hamster. Beyond this body weight there is no further increase in the relative weight of the brown fat body. In the 75 g hamster, this structure weighs about the same, *i.e.*, 260 mg/100 g body weight (Graph 1). In hamsters weighing 20 g or less, the interscapular brown fat body is composed exclusively of brown fat cells, as determined by histologic sections. These cells are smaller in diameter than adult

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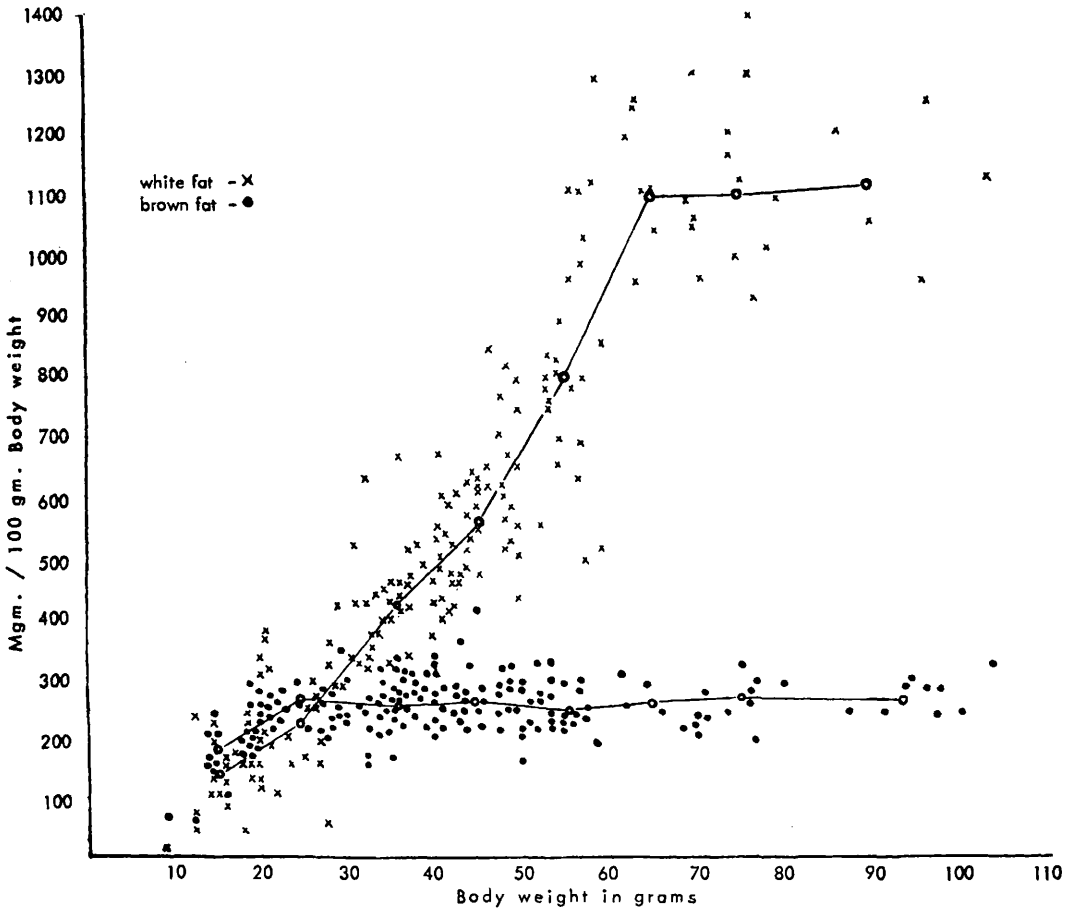
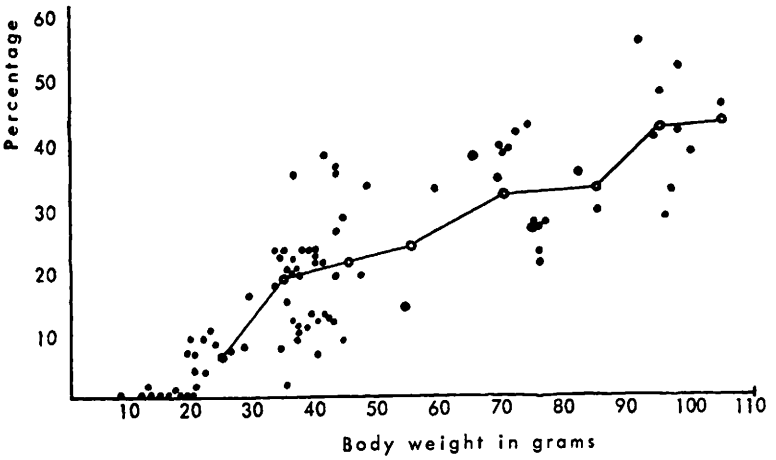


GRAPH 1. Growth curves of brown fat (ISB) and white fat (inguinal fat pad) in the normal male Syrian hamster.

fat cells and are characterized by polygonal contour, centrally situated nuclei and polylocular cytoplasmic vacuolization. With progressive increase in body weight, a greater number of typical white fat cells appears within the confines of the ISB capsule. The emergent white fat cells are generally restricted to the peripheral subcapsular region. In the 80 g hamster, white fat elements constitute about 50% of the ISB cell population (Graph 2).

Effect of cortisone upon brown and white fat of hamsters and mice. On the second day after cortisone inoculation into the prepubescent male hamster, a moderate increase in the volume and weight of the ISB is noted. Subsequently this enlargement becomes more striking until the seventh day when a 2-fold increment is noted concurrently with a pronounced thymic atrophy. There is a regression of the ISB weight to normal values by the tenth day (Table I). Cortisone has a similar effect upon hamster brown fat located elsewhere (*i.e.*, ventrally to the scapulae, in the posterior cervical region and within the axillae).

The peak of ISB hypertrophy which is consequent to cortisone therapy occurs 7 days following administration of the hormone. There is some retardation of somatic growth under the influence of the hormone, cortisone-treated hamsters weighing after 7 days about 21 g as compared to 29 g control weight. Nevertheless, an absolute increase in brown fat in the treated groups is evident when calculated in terms of mg/100 g of body



GRAPH 2. Percentage of white fat cells within ISB according to body weight.

weight (Table I) or in terms of the amount of the fat per animal irrespective of body weight. While hamsters of all ages exhibit some measure of ISB enlargement, the degree of reactive hypertrophy diminishes with increasing body weight. Animals above 80 g respond with 25% ISB hypertrophy as compared to 200-300% in the 20 g hamster.

Histologically, there is no evidence of cellular hyperplasia to account for the rapid elevation in weight. The individual brown fat cells, however, show a striking increase in diameter achieving an average of 28 μ 7 days after cortisone injection, in contrast to normal values of 18 μ. The lipid vacuoles coalesce and the nucleus is compressed peripherally (Fig. 1). There is no microscopic evidence of undue congestion or edema to account for the elevated weight. In fact, there is a significant decrease in water content of the ISB during the period of cortisone-induced hypertrophy.

A reduction of doubtful significance in the weight of the inguinal white fat pad is produced by cortisone. Similarly, microscopic examination discloses no variation in white fat cell diameter as a consequence of cortisone administration.

Cortisone-treated mice submitted to a similar procedure also show an appreciable ISB hypertrophy as compared to control groups. The increase in weight, however, is not as striking as in the hamster, and the peak of response occurs on the fourth day (Table I).

Discussion. The morphologic and distributive distinctions between brown and white fat have stimulated considerable speculation as to possible functional divergency between the two forms of lipid tissue(5,6). Significant biochemical differences have also been recorded(7). The affinity of the MEF_1 virus for brown fat can be accepted as further evi-

TABLE I.
Effect of Cortisone upon Weight of Interscapular Brown Fat and Inguinal White Fat.

	Days after cortisone injection			
	2	4	7	10
Interscapular brown fat				
Hamsters (cortisone)	373* ± 13.6†	433 ± 25.6	632 ± 36.5	369 ± 46.1
" (control)	244 ± 14.6	259 ± 13.8	304 ± 17.4	320 ± 12.9
Mice (cortisone)	826 ± 59.4	928 ± 31.1	789 ± 47.7	686 ± 35.8
" (control)	665 ± 36.6	671 ± 18.6	637 ± 37.5	621 ± 26.7
Inguinal white fat				
Hamsters (cortisone)	250 ± 11.2	229 ± 15.4	328 ± 23.4	399 ± 20.9
" (control)	196 ± 23.7	223 ± 17.8	367 ± 29.2	510 ± 38.9

* mg/100 g body wt. † Stand. errors.

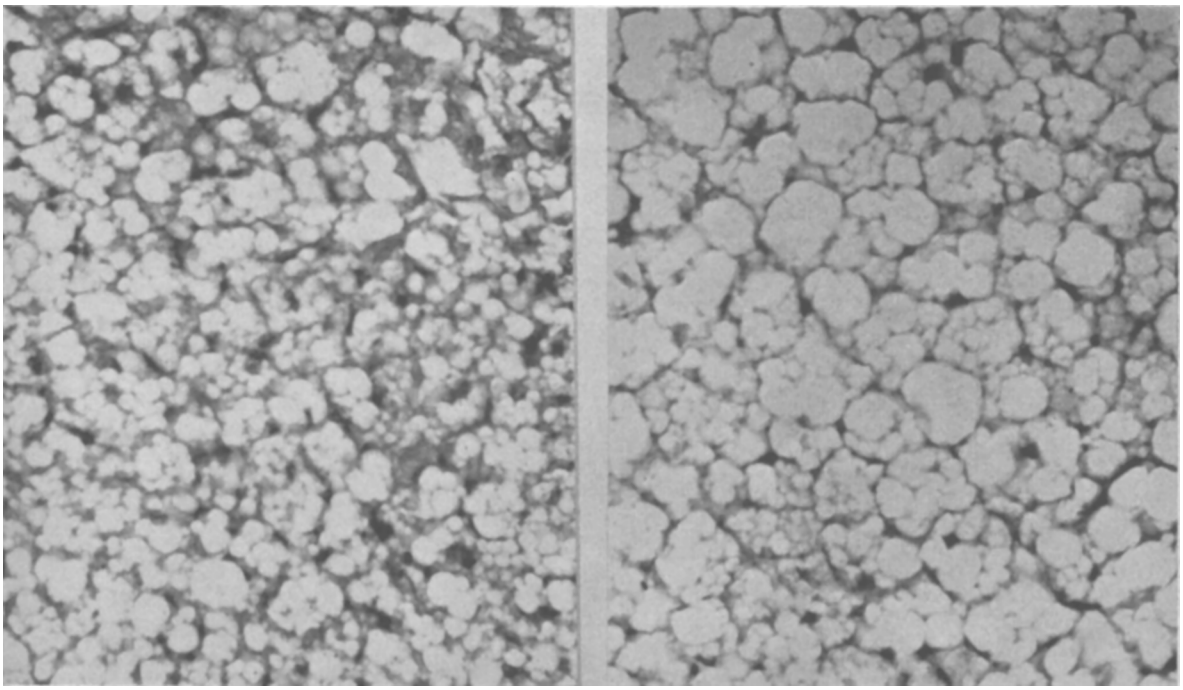


FIG. 1. (Left) Microphotograph (× 70) showing interscapular brown fat in the normal hamster. (Right) Microphotograph (× 70) showing increased cell diameter, coalescence of lipid vacuoles and peripheral migration of nuclei of interscapular brown fat in the cortisone-treated hamster.

dence of a profound distinction between the two tissues.

The effects of cortisone, described in this paper, indicate a possible relationship between brown fat and adrenocortical function. Selye and others(8,9) have noted that ACTH or stress result in a rapid reduction in ISB size. Other observers have described a primary increase in brown fat following ACTH administration(10). Antopol(11) has observed an atrophy of the ISB in mice following large doses of cortisone. The discrepancies between these reports and the observations recorded herein may be attributable in part to the varied time intervals of observation and possibly to differences in the ages of the animals studied.

Within the time limits of observation in the present report, there was noted a reduction of doubtful significance in white fat weight. Other authors noticed a decrease in the amount of this tissue after stress(12).

The degree of ISB hypertrophy after cortisone diminishes with increasing age (body weight). The reduced responsiveness is partially explained by the progressive infiltration of white fat cells within the ISB, thus decreasing the reactivity of the structure. Investigations in progress in this laboratory indicate that other factors *e.g.*, testicular activity, may play a role in mitigating cortisone-responsiveness of brown fat in older animals. Further work on the physiology of hamster brown fat, now in progress, suggests a seasonal variation in the weight and a reactivity of this tissue to a wide range of endocrine disturbances.

Summary. 1. Beyond an early age (about 3 weeks), the proportion of brown fat within the hamster maintains a relative constancy. In contrast, the ratio of white fat to body weight increases precipitously with age until full maturity of the hamster. 2. Cortisone treatment results in a prompt hypertrophy of brown fat in hamsters and mice, reflected microscopically by measurable expansion of cell diameter. White fat is possibly somewhat reduced in weight. 3. The reactive hyper-

trophy of brown fat in cortisone-treated hamsters diminishes with increasing age, apparently caused in part by the increasing numbers of unresponsive white fat cells within the borders of brown fat masses.

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Effect of Ergotamine Tartrate Upon Systemic and Peripheral Blood Sugar in Rats.* (20835)

RACHEL COSGROVE. (Introduced by Raymond W. Cunningham.)

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Shepherd and Buchanan(1) have reported the lowering of blood sugar values by ergotamine tartrate. Their blood sugar determinations were made on peripheral blood samples obtained from the tip of the rat tail. Evidence has been obtained in this laboratory to the effect that the tail blood (peripheral blood) sugar level after ergotamine tartrate treatment is not representative of the systemic blood sugar level.

In the course of blood sugar determinations on rats treated with ergotamine tartrate, it was noted that the powerful vasoconstrictive action of this agent made it difficult to bleed the rats from their tails. To overcome this difficulty blood samples were obtained by cardiac puncture. It was found that the blood sugar determinations from heart blood samples differed greatly from determinations on blood taken from the tail. Comparisons were then made between tail blood and heart blood samples taken at the same time. The results indicated a great divergence in the blood sugar level of systemic and peripheral blood. Komrad and Loew(2) have suggested that certain other vasoconstrictive agents such as Pitressin, Pitocin, and ephedrine may possibly cause a lowering of epinephrine-induced hyperglycemia by prevention of an even distribution of this hyperglycemia due to circulatory changes.

Methods. All animals used in these experiments were normal adult male Wistar rats ranging in weight from 182 to 355 g. No animal was used more than once. After a fasting period of 16 hours, the animals were placed in bleeding cages, warmed by means of a heating lamp to facilitate bleeding, and control samples taken using potassium oxalate as an anticoagulant. Control blood samples were taken only from the tail, because the high incidence of mortality following cardiac puncture outweighed the information obtained by heart blood controls. Moreover, the lack of significant difference between heart and tail blood samples subsequently demonstrated in the starch control group justified this procedure. The animals were divided into groups of 8 or 9 rats for treatment. One and one-

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