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Adipokinetic Activity of Oxycel-Purified Corticotropin.* (20224)

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The administration of crude extracts of anterior pituitary to mice, rats, and guinea pigs was found by Best and Campbell(1,2) to produce a rapid increase in the quantity of lipid in the liver and a decrease in carcass fat. It was subsequently shown that the source of the lipid which accumulated in the liver under these circumstances was the fat storage depots (3,4) and that the increment of hepatic lipid was composed largely of glycerides(5); it was concluded that pituitary extracts promoted the mobilization of depot fat to the liver. Since only a fraction (20-50 per cent) of the fat disappearing from the depots could be accounted for in the liver(2,5), the utilization as well as the migration of fat appeared to have been enhanced. The descriptive term adipokinin has been applied to the pituitary principle responsible for this metabolic effect(6). A number of studies have shown that pituitary extracts rich in one or another of the well-defined anterior pituitary principles (gonadotropins, corticotropin, thyrotropin, growth hormone) may produce various effects on fat metabolism of experimental animals, and it has sometimes been implied that one of these might be the pituitary factor causing these effects. Fatty liver and lipemia have been induced by thyrotropic preparations(7-9), and pronounced adipokinetic activity has been reported in preparations rich in growth hormone(10,11) and prolactin(12). Payne(13), studying a variety of crude and partially purified pituitary extracts, found no correlation between adipokinetic activity and that of any one of the well-characterized hormones, and concluded that the fat-metabolizing factor was a distinct principle.

In the present study estimates were made of the adipokinetic activity of some of the extracts obtained in the course of fractionation of hog anterior pituitary according to the procedures devised in this laboratory for the extraction and purification of corticotropin and growth hormone(14-18). The results indicate that a fraction potent in adipokinin is obtained by these methods and lend support to the idea that the fat-mobilizing principle is a factor distinct from other pituitary hormones.

Experimental. Assays were performed by a modification of the method of Campbell(19). Female mice of the Swiss Webster strain (obtained from Taconic Farms and local dealers) varying in weight from 18 to 24 g were used; in most experiments, mice of nearly uniform weight were selected. The animals were deprived of access to food one hour before pituitary extracts were to be administered, and after injection they were kept in cages containing water but no food. In the earlier experiments the test materials were injected subcutaneously in 0.5 cc volume at pH 4 and the mice were killed seven hours later; in later experiments, particularly when potent extracts

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were tested, the materials, dissolved in 0.9% saline solution adjusted to pH 9, were injected intraperitoneally in 0.2 cc volume and the animals were sacrificed 3 to 3½ hours later. Control mice, injected with saline solution alone, were used in each experiment. An impression was gained that the test was more sensitive and the results more reproducible when the injected material was given in alkaline rather than in acid solution. The mice were killed by skull fracture and exsanguinated by a cervical incision; in such bled animals one may roughly estimate the quantity of liver fat from the degree of yellowness of the liver. A portion of liver (470-520 mg) was removed, blotted, weighed and transferred to a Potter-Elvehjem homogenizing tube. The lipids were extracted by a modification of the method of Folch and his associates(20) by homogenizing the tissue in 15 cc of a mixture of chloroform-methanol (2:1 by volume). The homogenate was then filtered through Munktell's No. 00 filter paper and a 10-cc aliquot of the filtrate was transferred to a tared beaker and evaporated to dryness at 100-110°, the weight of the dried lipid then being obtained by difference. Results were expressed as percentage of lipid in the liver. In several preliminary experiments it was found that approximately 1.5 mg of the crude lipid extracted from 500 mg of liver was insoluble in petroleum ether, and therefore, as a correction for non-lipid materials in the lipid extract, 0.3 was arbitrarily subtracted in all experiments from the value obtained for the percentage of lipid, and the values for percentage lipid indicated in the tables and figures have been corrected in this way. The presence of non-lipid materials in lipid extracts of brain was noted by Folch *et al.*(20). The completeness of lipid extraction from the liver by the method used was indicated by the failure to extract by prolonged Soxhlet extraction (chloroform) significant quantities of lipid from previously ground, extracted liver residues.

Results. The following preparations were studied: Hog anterior pituitary powder (acetone dried). Preparation A, or crude corticotropin, was the material prepared by glacial acetic acid extraction of hog anterior pituitary as described by Payne, Raben, and Astwood (14) and designated by them "crude corticotropin." Preparation D, or oxycel-purified corticotropin, was the extract obtained by acid elution from oxycellulose which had been stirred with preparation A under the conditions devised by Astwood, Raben, Payne, and Grady (15). "Oxycel-unadsorbed material" refers to that part of preparation A which remained after the latter had been twice-treated with oxycellulose, and was the "acetic acid fraction" of Raben and Westermeyer(17). "Glacial acetic acid extracted growth hormone" was the preparation of growth hormone described by Raben and Westermeyer(17,18). Some experiments were also made with Armour growth hormone preparations, mainly lot No. R285128.

The data (derived from many experiments performed over a period of months) relating the concentration of hepatic lipid to the dose of extract administered seven hours previously is summarized in Fig. 1 for four preparations, which, in order of decreasing potency, were preparation D, preparation A, anterior pituitary powder, and the oxycel-unadsorbed material. Since the slopes of the curves were not uniform and the response was not linear over the entire dosage range, precise quantitative estimates of relative potency was difficult. However, if a comparison was made of the smallest dose which produced an average value of 10% for the hepatic lipid concentration (the control values for this series being 6.9%), the approximate effective dose for each preparation was: preparation D, 15 μg, preparation A, 500 μg, anterior pituitary powder, 2 mg, oxycel-unadsorbed material, 15 mg, indicating that preparation D was about 30 times as potent in adipokinin as preparation A, 150 times as active as the starting anterior pituitary powder, and 1000 times the oxycel-unadsorbed fraction. Since the corticotropic activity of preparation D has been found to be 40 times that of preparation A and 200 times the anterior pituitary powder while the oxycel-unadsorbed material is greatly depleted(16), the parallelism between adipokinetic and corticotropic activity in the fractionation procedure is apparent. Preparation D constitutes 0.25% by weight of the anterior pituitary powder serving as starting material, and approximate-

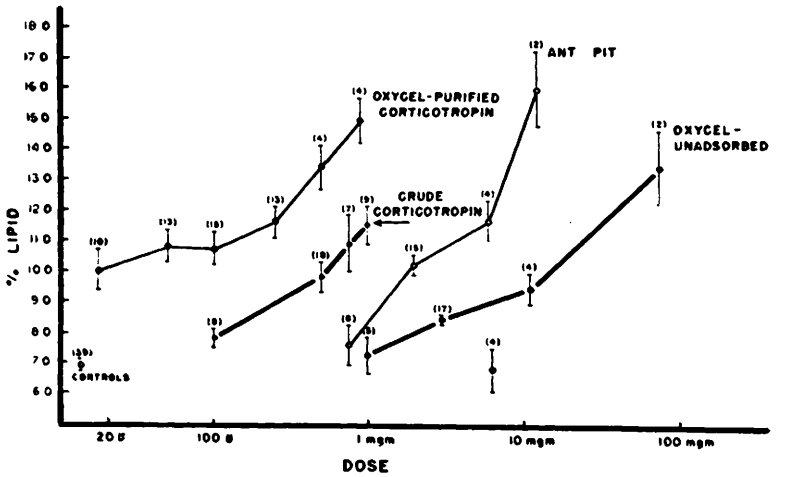

FIG. 1. Relationship between dose and response (% hepatic lipid 7 hr after injection) for 4 pituitary preparations. In this and the other figures the numbers in parentheses indicate the number of mice used to determine the mean represented by each point, and the limits of the vertical line through each point indicate the stand. error of the mean. The value for the control mice used in these experiments is shown in the lower left corner of the chart.

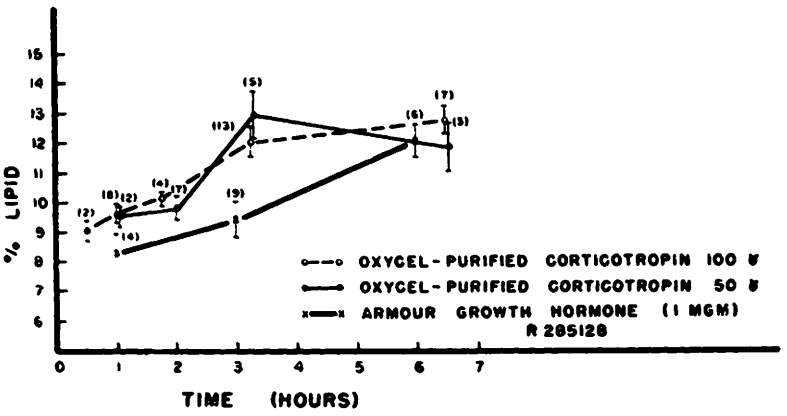

FIG. 2. The percentage of lipid in the liver at different times after injection of preparation D and Armour growth hormone. The extracts were administered intraper. in alkaline solution.

ly one-third of the adipokinin in the powder is recovered in Preparation D. The oxycel-unadsorbed fraction, greatly depleted of adipokinin, is used as starting material for the preparation of growth hormone by the procedure of Raben and Westermeyer; the growth preparation constitutes 12-15% of the total solids of the unadsorbed fraction and is equal in growth activity to the purified preparations currently in use(17,18). Even if all the adipokinin in the oxycel-unadsorbed fraction were concentrated in the growth hormone preparation the product would still be expected to be perhaps only 1% as active in adipokinin as preparation D. and adipokinetic activity of this order has been noted (Fig. 4).

That part of preparation A which was not adsorbed by oxycellulose in the quantity (8% by weight) used to obtain preparation D was found to be considerably more potent in adipokinin before than after it had been treated with a second, larger quantity of oxycellulose (20% by weight). The fraction eluted by hydrochloric acid from the second batch of oxycellulose was potent in adipokinin, perhaps half as active as preparation D. Corticotropin and intermedin remaining unadsorbed after the first oxycel treatment have similarly

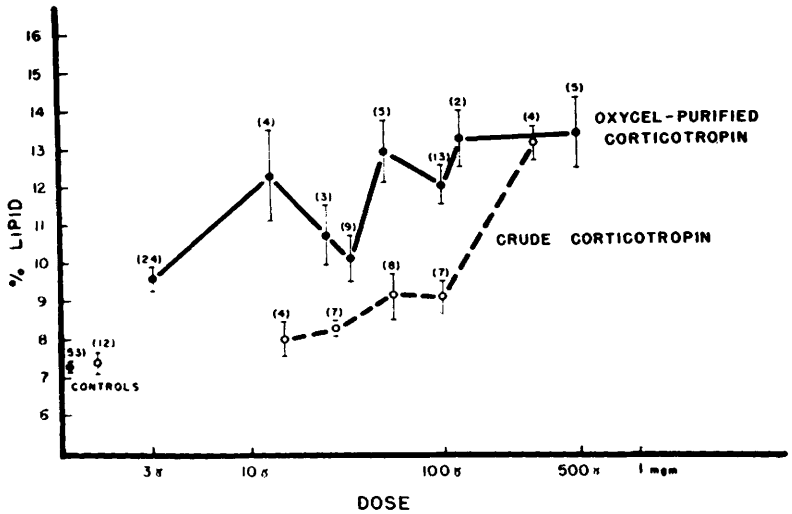

FIG. 3. Percent hepatic lipid 3 hr after administration of various doses of preparations A and D. The points are the average of separate experiments performed over a period of months and different lots of the preparations were used. Variation in the sensitivity of the animals and in the potency of the extracts may be factors in the variability of the results. The 4 values averaged to yield the point at 300 μg crude corticotropin were obtained in one experiment with the same batch of preparation A. Control values for each series are indicated on the left.

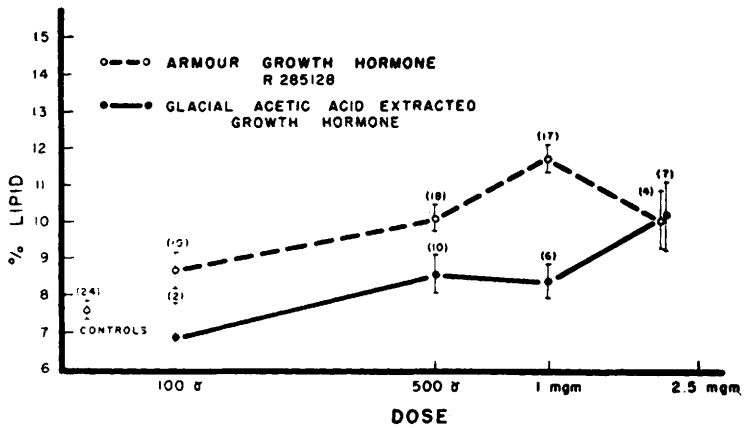

FIG. 4. Comparison of adipokinetic potency of Armour growth hormone and of Raben-Westermeyer growth hormone. Seven-hour test period.

been found to be extracted by retreatment with oxycel(21). The results of injecting mice with alkaline solutions of oxycellulose which had been stirred with preparation A and from which preparation D had been eluted, indicated that insignificant adipokinetic activity was retained by the adsorbent after acid elution.

The accumulation of lipid in the liver consequent to the intraperitoneal administration of preparation D was found to occur at a surprisingly rapid rate. Doses of 10-100 μg produced some increase over control values in hepatic lipid concentration within 30 to 60 minutes; the peak value was reached at about 3 hours and changed little between then and 7 hours (Fig. 2). With less potent adipokinetic extracts (Armour's growth hormone and oxycel-unadsorbed material) the accumulation of lipid was more gradual, the values 6 hours after injection being considerably higher than at 3 hours. It is not clear whether this difference between the stronger and weaker preparations is attributable to factors such as different rates of absorption and duration of action or

whether there is a qualitatively different mechanism of fat accumulation. The 3-hour period is convenient for the comparative assay of potent preparations. The 3-hour values for liver lipid concentration obtained with various doses of preparation A and D are shown in Fig. 3, and the results may be interpreted as showing approximately the same order of comparative potency as the 7-hour tests.

Direct comparisons were made of the adipokinetic activity of 2 growth hormone preparations and of preparation D with the results shown in Fig. 2 and 4, which indicate that adipokinin is not growth hormone. The data of Fig. 2 show that at 6 hours 50 μg preparation D and 1 mg Armour growth hormone (R285128) caused approximately the same increase in liver fat, while at 3 hours, in the same doses, preparation D was more potent than the growth hormone preparation. Fig. 4 illustrates the adipokinetic activity at several doses of 2 pituitary extracts of equal growth activity, the Armour preparation and glacial-acetic acid extracted growth hormone(18); the former preparation would appear to be about 5 times the latter in fat mobilizing activity.

Since the most active adipokinin preparation studied, preparation D, is rich in corticotropin and intermedin(21), the possibility that the fat-mobilizing factor is identical with one of these must be considered, as well as the possible modification of the adipokinetic by the corticotropic effect. Preparation D did not produce an increase in the liver lipid of the adrenalectomized mouse, but if such animals were maintained postoperatively with cortisone, (0.5 mg cortisone acetate subcutaneously daily), the adipokinetic response to preparation D was observed, confirming the observations made with other pituitary extracts that the fat-mobilizing effect is not mediated by the adrenal but requires the presence of cortical hormones(13,22). The comparative adipokinetic potencies of preparation D, Armour growth hormone, and the oxycel-unadsorbed material, as judged by assays on adrenalectomized mice maintained with cortisone, were of the same order as in the intact animal (Table I), suggesting that adipokinetic assays in intact animals were not seriously

TABLE I. Adipokinin Effects in Adrenalectomized Mice.

	Preparation and dose		No. animals	% lipid in liver ± S.E.
A	Preparation D	100 μg	(11)	9.9 ± .35
	Controls	0	(11)	6.4 ± .32
B	Preparation D	50 "	(3)	11.4 ± .66
	Armour growth hormone	500 "	(3)	10.2 ± .36
C	Preparation D	25 "	(2)	9.1 ± .24
	Oxycel–unadsorbed material	3 mg	(3)	7.5 ± .05

Mice adrenalectomized 2-3 days before injection with pituitary extracts and post-operatively received 0.5 mg cortisone acetate daily subcut. up to and including the day the tests were made. The data of B and C were derived from single experiments; that of A from a number of separate experiments. Seven-hr test period.

modified by the corticotropin of preparation D. It has been reported that pre-treatment of mice with cortisone enhances the fat mobilization induced by some growth hormone preparations(22). In the present study the subcutaneous injection of 0.5 mg cortisone acetate once daily for 3 days did not appreciably change the percentage of fat (7-hour assay) in the livers of intact mice injected with oxycel-unadsorbed material, an extract containing growth hormone, as compared with control animals not receiving cortisone.

Intermedin and adipokinin do not appear to be the same principle. A partial separation of the intermedin and adipokinetic activities of preparation D may be made by the use of chromatographic columns of charcoal and of cation-exchange resin(23,24). The non-identity of the two principles has been further suggested by the results obtained on assay of the intermedin and fat-mobilizing activity of preparation D which had been heated in a boiling water bath for 2 to 3 minutes at pH 13, and then cooled, neutralized, diluted appropriately and injected into frogs and mice. The intermedin potency of preparation D treated in this way was found to have been greatly enhanced, while adipokinetic potency had been uniformly decreased (Table II). In its instability under these conditions adipokinin resembles corticotropin(25).

Solutions of preparation D in 0.1 N HCl

TABLE II.
Effect of Heat and Alkali on Adipokinin. 3 exp.

Preparation D	Dose (μg)	No. of mice	Hepatic lipid (%) ± S.E.
Heated	70	2	8.3 ± .65
Unheated	70	2	11.1 ±1.9
Heated	250	2	9.7 ± .60
Unheated	250	2	14.5 ± .65
Heated	3	4	**9.1 ± .47**
Unheated	3	3	11.9 ± .60

Preparation D in 0.1 N NaOH heated in boiling water bath 2-3 min., cooled, neutralized, diluted, and injected in mice. Unheated material handled in same manner except not heated. In exp. 3, 0.15 μg doses of heated and unheated materials were injected in intact frogs; heated material caused maximal darkening, while unheated preparation at this dose caused minimal darkening.

kept at 5°C for several months have shown no change in adipokinetic potency during this period, and no loss of potency was detected in one week at room temperature, pH 8. Incubation of preparations A and D in 0.1 N HCl with pepsin at room temperature for 18 to 24 hours, the ratio of enzyme to extract being 1/30 to 1/80, led to marked loss of activity, as did incubation of preparation D with trypsin at pH 8 for 18 hours at room temperature. Large doses of preparation D (2-8 mg) administered to mice by stomach tube resulted in a marked increase in the hepatic lipid concentration at seven hours; smaller doses were only irregularly effective by the oral route. The oral administration of glucose (0.5 cc, 50% solution) immediately before injecting mice with preparation D usually lessened the quantity of lipid accumulating in the liver at 3 or 7 hours, but often the difference was small, compared with controls not receiving glucose, and in no instance was the accumulation of lipid in the liver entirely prevented.

Discussion. The results support the view that the fat-mobilizing agent of the pituitary is a distinct principle. The marked adipokinetic activity of preparation D, together with the fact that this preparation contains no detectable thyrotropin, luteotropin, or gonadotropin (21), would tend to exclude these principles as the fat-mobilizing factor and evidence suggesting that neither growth hormone nor intermedin is responsible for the adipokinetic effect has been indicated above. It is more difficult to determine whether adipokinin and corticotropin are not identical, particularly since these activities tend to accompany each other in the glacial acetic acid-oxycellulose fractionating procedure. Some pituitary preparations which have been reported to cause fat mobilization have had little corticotropic activity (10,13). Since the administration of cortisone does not reproduce the fat mobilization observed to result from preparation D injections, and since the adipokinetic activity of preparation D is independent of the presence of the adrenal glands, if the adipokinetic activity were inherent in corticotropin, it presumably would have to be by virtue of an extra-adrenal action of the latter. It would appear unlikely that 2 such diverse phenomena as mobilization of depot fat and stimulation of the adrenal cortex should be attributable to the same substance, but decision as to the nonidentity of adipokinin and corticotropin must await the complete separation of these activities from one another.

Summary. Purified corticotropin prepared from hog anterior pituitary by the glacial acetic acid-oxycellulose method was found to be potent in adipokinin. Doses of 3 to 15 μg produced an increase of 40% in the hepatic lipid concentration of mice within three hours after injection. Pituitary adipokinin appears to be a principle distinct from the gonadotropins, thyrotropin, corticotropin, growth hormone, and intermedin.

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Effects of Total Body X-Irradiation on Some Constituents of Liver and Kidney of the Rat. (20225)

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To characterize certain metabolic alterations in the liver and kidney due to x-irradiation, phosphate distribution, glycogen, sulfhydryl, sodium, potassium, and water content as well as cytochrome oxidase activity of these organs were studied in rats subjected to lethal total body x-irradiation.

Inasmuch as the content of some of these constituents is known to be influenced by the secretion of the adrenals, some of these studies were done on adrenalectomized animals. Since decreased food intake is part of the post-irradiation syndrome, the effect of fasting alone on the above tissue constituents was also studied.

Materials and methods. Male Sprague-Dawley rats, weighing 235-280 g and fed Purina chow diet, were used throughout. The adrenalectomized rats were maintained on 0.95% NaCl drinking solution and used about 10 days after operation. Radiation dosage was 1000 r in the experiments on phosphate distribution in the liver and kidney, and on cytochrome oxidase activity in the liver; 880 r in the other experiments. Radiation factors were: 200 kv, 6 ma, ½ mm Cu 1 mm Al filter, target distance approximately 29 cm and 40 r/min dosage rate measured in air.* The majority of deaths occurred within 6 to 14 days after irradiation with 880 r and 4 to 6 days after irradiation with 1000r. A. *Phosphate Distribution.* Phosphate determinations on the tissue were made according to the method of Umbreit(1) except that the animals were killed by decapitation and the tissues were quickly removed, frozen in a dry ice-acetone bath, and lyophilized. Trichloroacetic acid extracts of the tissues were prepared and the barium-insoluble fraction was analyzed for inorganic phosphorus and easily hydrolyzable phosphorus(15 min., 100°C, 0.2 N HCl). All results are reported as mg P/100 g dry tissue. The irradiated animals, which had access to food and water, were sacrificed 1 to 6 days after irradiation. Food, but not water, was withheld overnight prior to sacrifice. B. *Liver Glycogen and Blood Glucose.* Food was withheld for periods indicated in Table II, while water or 0.95% NaCl solution (adrenalectomized animals) was allowed *ad libitum.* The animals were sacrificed by decapitation, and samples weighing approximately 1 gram were removed from the median lobe of the liver and immediately immersed in cold (3° to 5°C) saline. The samples were then cut into 6 nearly equal parts under cold saline, blotted on filter

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