Response of Epididymal Adipose Tissue to Small Concentrations of Insulin: Effect of Cortisone.* (25641)

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Addition of insulin to rat epididymal adipose tissue *in vitro* causes increases in glucose uptake(1-4), in CO_2 evolution with glucose as substrate(2,5,6,7), in fat synthesis(2), and in incorporation of amino acids into protein (8). The stimulatory actions upon glucose uptake and CO_2 evolution have been proposed as the basis for insulin detection methods(5, 6,9). In attempting to assay insulin quantitatively it has been found, in confirmation of Ball *et al.*(6), that the response to insulin of adipose tissue from successive rats of a given colony may undergo sudden changes which negate the assay procedure. The causes of these changes are being studied. The present experiments show that sensitivity of adipose tissue to small concentrations of insulin added *in vitro* is significantly decreased by injection of cortisone acetate into the donor rat from which the adipose tissue is taken.

Methods. Non-fasting‡ Wistar rats weighing 124-168 g, were sacrificed by cervical separation attended by minimal previous excitement. The 2 epididymal fat pads were transferred to filter paper which had been well moistened with the medium to be used for incubation, *i.e.*, Krebs-Henseleit-bicarbonate solution containing 200 mg % each of glucose and gelatin(6). Each pad was quickly divided into 3 approximately equal pieces. Each piece was transferred to 1.9 ml of the above medium in the main compartment of a 15 ml Warburg flask; the pieces were added in a predetermined and known order. The flasks were equilibrated with 95% O_2 - 5% CO_2 and incubated 3 hours at 37°C at a shaking rate of 120 oscillations per minute and an amplitude of 6 cm; 0.1 ml insulin solution was tipped in from the side-arm at end of the first hour to give the final concentrations shown; after incubation, the fat pads were drained on filter paper and weighed. Before insulin addition, consumption of oxygen and evolution of CO_2 are usually such as to give only a small pressure change with time; after insulin, there is increased CO_2 evolution, presumably associated with increased fat synthesis(6). In Fig. 1, the gas exchange is arbitrarily recorded as μl CO_2 per g tissue, *i.e.*, mm pressure change x K_{CO_2}/tissue weight in g). In Table I, the results are expressed as net *increase* in CO_2 evolution after insulin (μl CO_2 per g tissue per hour); this is calculated by subtracting pressure change for the first hour (before insulin addition) from that for the third hour (period of maximal insulin response), multiplying by K_{CO_2}, and dividing by tissue weight. Values for glucose uptake were also measured (10) on the same samples and include uptake for the equilibration period of 15 minutes, plus one hour of incubation without insulin, plus 2 hours with insulin. Insulin was prepared by diluting Lilly U-80 insulin with the incubation medium. Identical responses were obtained in trials with Lilly crystalline insulin§ stated to contain approximately 0.4% glucagon (Lot No. 535664).

Results. The changes in CO_2 evolution and glucose uptake in response to insulin were found to vary according to the position in the fat pad from which the fragment was removed, the left distal portion being the most responsive of the 6 pieces to insulin, just as it is the most responsive of 4 pieces cut from 2 fat pads(7). Accordingly, each concentration of insulin was always tested in duplicate upon

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‡ Rats were provided *ad lib.* with diet containing the following as % by weight: milk, 64; bread, 20; wheat, 8; corn, 8; dried beer yeast, .2; cod liver oil, .08, and alfalfa were added to the ration during one day weekly.

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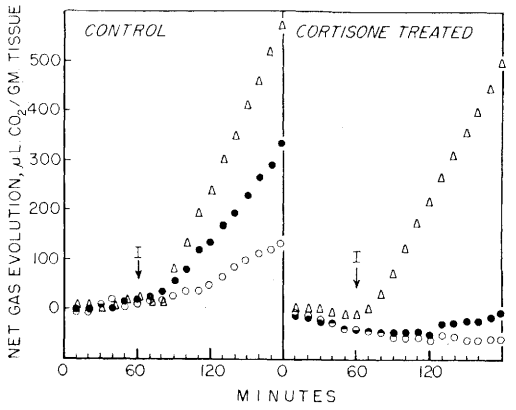

FIG. 1. Effect of prior injection of cortisone on response of rat epididymal adipose tissue to insulin added *in vitro*. Insulin concentrations, as microunits/ml, were: ○, 20; ●, 50; △, 10,000. Insulin was added at 60 min. For details, see *Methods*.

the same 2 segments of the fat pads from a single rat, *e.g.*, 20 μu/ml upon the right distal and left proximal portion, 50 μu upon the right and left middle portions, and 10^4 μu upon the right proximal and left distal portions; averages of the 2 duplicates are recorded. This procedure yields a log dose-response line with approximately the same slope as that reported for 4 pieces of fat from one rat(6).

Cortisone acetate,§ suspended in 0.9% NaCl, was given subcutaneously, 2 mg per rat per day (1 mg each at 8 a.m. and 5 p.m.) for 3 days plus a final dose of 1 mg 3 hours before sacrifice. Control rats of nearly the same weight received the saline solution concurrently.

The effects of insulin and cortisone upon gas exchange are illustrated in Fig. 1, which presents the results for a single experiment, and summarized in Table I. Results for the controls are qualitatively similar to those for normal rats(6). Prior injection of cortisone acetate did not significantly change the slope of the curve for CO_2 evolution during the first hour, prior to addition of insulin; however, it significantly reduced *in vitro* stimulation of CO_2 production resulting from addition of 20 or 50 μu insulin per ml and had a similar but less significant antagonistic action toward 10^4 μu per ml (Table I).

The increases in glucose uptake produced by the same concentrations of insulin were also smaller with adipose tissue from the cortisone-injected rats than with tissue from saline injected rats; in fact, no stimulation above the control level was obtained with 20 or 50 μu insulin per ml. A single dose of 1 mg cortisone acetate 3 hours before tissue removal did not prevent insulin stimulation.

Discussion. The response to insulin of adipose tissue from successive rats of a given strain and colony may spontaneously decrease, with no significant change in basal glucose uptake or net gas exchange without insulin. Or, basal glucose uptake and CO_2 evolution may spontaneously increase to such high values that addition of small doses of insulin *in vitro* has no further effect(6‖). The present experiments show that the first of these anomalies can be reproduced by injection of cortisone acetate, suggesting that the spontaneous decrease in sensitivity may stem in part from excess endogenous adrenal steroids.

The relation of cortisone to the effects of very small concentrations of insulin does not appear to have been previously studied in individual tissue. There is evidence that insulin in sufficiently high concentrations can partly or wholly counteract the inhibitory effects of

TABLE I. Effect of Cortisone Acetate on Glucose Uptake and CO_2 Production of Epididymal Adipose Tissue in Presence of Small Concentrations of Insulin. Cortisone acetate injected into donor rats as described in text; insulin added *in vitro*.

Insulin conc., μunits/ml	Adipose tissue from control rats	Adipose tissue from cortisone-inj. rats	Significance of difference, P, between control and cortisone samples
Net increase, after insulin, in gas evolution, calculated as mm^3 (μl) CO_2/g/hr			
20	80 ± 6.6*	22 ± 2.2	.001
50	125 ± 8.9	41 ± 4.7	.001
10,000	298 ±26.	220 ±23.	.04
Glucose uptake, mg/g/3 hr			
0	1.4 ±.21	1.4 ±.15	
20	1.9 ±.19	1.2 ±.29	.06
50	2.9 ±.33	1.1 ±.20	.001
10,000	4.9 ±.32	2.9 ±.50	.005

* Mean ± stand. error; means are obtained from duplicate tissue samples from each of 8 rats, except for the values for glucose uptake without added insulin; each of these is mean of 9 duplicate samples from 3 rats.

‖ Unpublished experiments.

cortical steroids on CO_2 evolution or fatty acid synthesis by mammary gland slices(11), on fatty acid synthesis by rat adipose tissue (12), or on glucose uptake by rat diaphragm (13,14,15).

Summary. The stimulation of glucose uptake or CO_2 evolution produced by 20, 50, or 10^4 μu insulin per ml *in vitro* to rat epididymal adipose tissue was markedly reduced by prior subcutaneous injection of the rat tissue donor with 7 mg cortisone acetate in divided doses over a period of 3½ days.

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1. Krahl, M. E., *Ann. N. Y. Acad. Sci.,* 1951, v54, 649.

2. Winegrad, A. I., Renold, A. E., *J. Biol. Chem.,* 1958, v233, 267.

3. Itzaki, S., Wertheimer, E., *Endocrinology,* 1957, v61, 72.

4. R—Candela, J. L., *Rev. Iberica de Endocrinol.,* 1959, v6, 171.

5. Martin, D. B., Renold, A. E., Dagenais, Y. J., *Lancet,* 1958, v2, 76.

6. Ball, E. G., Martin, D. B., Cooper, O., *J. Biol. Chem.,* 1959, v234, 774.

7. Hagen, J. M., Ball, E. G., Cooper, O., *ibid.,* 1959, v234, 781.

8. Krahl, M. E., *Biochim. Biophys. Acta,* 1959, v35, 556.

9. Humbel, R. E., *Experientia,* 1959, v15, 256.

10. Somogyi, M., *J. Biol. Chem.,* 1952, v195, 19.

11. Balmain, J. H., Folley, S. J., Glascock, R. F., *Nature,* 1952, v169, 447.

12. Hausberger, F. X., *Endocrinology,* 1958, v63, 14.

13. Manchester, K., Randle, P. J., Young, F. G., *J. Endocrinol.,* 1959, v18, 395.

14. Bartlett, G. R., Wick, A. M., MacKay, E. M., *J. Biol. Chem.,* 1949, v178, 1003.

15. Verzar, F., Wenner, V., *Biochem. J.,* 1948, v42, 35.

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Action of Mucoproteins *in vivo* and in Tissue Culture on Poliomyelitis Virus. (25642)

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The present report contains our findings on the protective effect of various mucoprotein fractions obtained from hog gastric mucosa in experimental poliomyelitis infection of mice (Lansing strain II) and the influence of the same proteins on development of the virus in monkey kidney cells.

Materials and methods. Three hog gastric mucosa fractions (A,B,C) received from Prof. V. Capraro (Milano, Italy) and 2 extracts (D,E) prepared in the Armour Laboratories by E. Samsa, V. Snider and P. Van Melle were used for study. The characteristics of these substances as determined by V. Capraro are: (A) A and H group specific substance, 95% pure by electrophoretic determination (free phase). Anti-agglutinating potency 10^7. (B) "B_{12} binding factor"(1) 100% pure. B_{12} binding titer, 22 μg/mg in the *E. coli* assay. (C) Acid aminopolysaccharide. Electrophoretically 95% pure, uronic acid content 35%. (D) Armour preparation KO-45-102-1. Uronic acid content 1.62%. (E) Armour preparation KO-45-124. B_{12} binding titer 228 mμg/mg, uronic acid content 1.6%. Doses of 1 and 2 μg showed A, respectively H substance activity, inhibiting 10 minimal agglutinating doses in the systems A red cells/anti A serum and O cells/anguilla anti H serum.

Infection. Swiss mice of 14-18 g, 4-5 weeks of age, were inoculated intracerebrally with 0.03 ml Lansing strain (Type II) virus, diluted 1:3000. Standard procedure was to infect mice Friday and begin treatment Monday; however on 4 groups of mice treatment was started prior to inoculation. Ten treatments were given, 2 daily for a period of 5 days, except when otherwise stated. Experimental animals were observed daily for a pe-