Influence of Anti-insulin Serum on Glucose Metabolism

I. In Isolated Adipose Tissue

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SUMMARY

1. A consistent in vitro effect of anti-insulin serum on glucose metabolism in isolated rat adipose tissue has been demonstrated.

2. Anti-insulin serum added in vitro to epididymal fat pads caused an average decrease in the C-14-O_2 formation from glucose-1-C-14 of over 50 per cent, whereas it reduced the incorporation of glucose-U-C-14 into lipids by about 20 per cent.

3. Epididymal fat pads pretreated in vitro with anti-insulin serum oxidized glucose-1-C-14 at a reduced rate compared to control fat pads preincubated in buffer; in the presence of added insulin, however, glucose oxidation was the same in the control and anti-insulin treated fat pads. DIABETES *16*:472-74, July, 1967.

The injection into rats of anti-insulin serum, prepared from guinea pigs, as described by Moloney and Coval,[1] was first introduced by Wright[2] and has since been used by a number of investigators to study the diabetic syndrome under conditions in which acute transitory insulin deficiency is obtained.[3-6] In the present paper the results of investigations on the in vitro effect of anti-insulin serum on glucose metabolism in the isolated epididymal fat pad are reported.

MATERIAL AND METHOD

Male albino rats of the Sprague-Dawley strain, weighing from 130-150 gm., which had been fed on a standard rat diet (known as Thompson's cubes) were used.

Radioactive substrates

Uniformly labeled C-14 glucose and glucose-1-C-14 were obtained from the Radiochemical Centre, Amersham, Bucks.

Crystalline glucagon-free insulin from Burroughs Wellcome was used.

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Anti-insulin serum

The anti-insulin serum was prepared as described by Mansford[7] and diluted 1 to 2.5 for all experiments.

Incubation procedure

In each experiment, pieces of epididymal fat pad weighing approximately 100 mg., taken from three or four rats, were pooled in a buffered solution, and weighed samples were transferred to small glass bottles containing 1.8 ml. of a Krebs-Ringer bicarbonate medium (*p*H 7.4) plus 0.2 per cent glucose.

Additions of 0.1 ml. of diluted anti-insulin serum, normal guinea pig serum or insulin (final concentration 20 mU./ml.) were made as indicated in table 1.

TABLE 1

Influence of anti-insulin serum, normal serum and insulin on glucose-1-C-14 oxidation and lipid formation from glucose-U-C-14 in epididymal fat pads*

Additions to incubating medium (0.1 ml.)	H_2O	Anti-insulin serum	Serum	Insulin (20 μU./ml.)
No. of experiments	7	7	3	3
C-14-O_2 from glucose-1-C-14 (0.1 μC)	1,996±226 (13)	825±116 (15)	2,260±369 (7)	7,524±1,254 (6)
No. of experiments	3	3	3	3
Lipid formation from glucose-U-C-14 (0.2 μC)	4,607±516 (8)	3,776±234 (8)	4,294±429 (8)	23,714±2,840 (8)

*Approximately 100 mg. tissue incubated in Krebs-Ringer bicarbonate medium containing 0.2 per cent glucose + C-14 glucose as indicated, for two hours at 37°.

Results expressed as $\mu C \times 10^{-6}$. Mean values given ± S.E.M. Number of estimations in parentheses.

In each experiment two or three samples of adipose tissue were incubated under identical experimental conditions.

In experiments in which C-14-O_2 was measured, approximately 0.1 μC of glucose-1-C-14 was added per bottle, and in those experiments in which the C-14 content of the lipids was measured, 0.2 μC of glucose-U-C-14 was added.

In another group of experiments the pooled adipose tissue samples were distributed into bottles containing 2 ml. of Krebs-Ringer bicarbonate medium with or without the addition of 0.1 ml. of anti-insulin serum. The tissue was incubated for 30 min. at 37° C, then removed, washed three times in 0.9 per cent w/v saline, and transferred to incubating bottles containing fresh medium plus 0.2 per cent glucose and 0.1 μC of glucose-1-C-14. One-tenth milliliter H_2O or 0.1 ml. insulin was added to these bottles as indicated in table 2.

TABLE 2

Influence of pre-incubation of tissue with anti-insulin serum on glucose I-C-14 oxidation in epididymal fat pad*

Preincubated in buffer		Preincubated in anti-insulin serum	
Control	Insulin	Control	Insulin
2,508±336	4,903±385	1,358±238	4,452±657

*Approximately 100 mg. tissue preincubated for thirty minutes in Krebs-Ringer bicarbonate medium without or with anti-insulin serum followed by two-hour incubation in medium containing 0.2 per cent glucose + 0.1 μC glucose-1-C-14 without or with insulin.

Results expressed as μC C-14-$O_2 \times 10^{-6}$. Mean values of six estimations (three experiments) given ± S.E.M.

In all experiments the tissue was incubated for two hours at 37° C after gassing the medium with a 95 per cent O_2:5 per cent CO_2 mixture.

Measurement of C-14-O_2 and C-14 content of lipids

C-14-O_2 was collected from a small glass center well inserted into the incubating bottles as described by Keen,[8] containing 0.1 ml. ethanolamine.[9] The total lipids were extracted from washed tissue by the method of Folch, Lees and Stanley.[10]

The ethanolamine carbonate and the extracted lipids were counted in a liquid scintillation counter in 10 ml. of modified Brays dioxan scintillant.[11]

RESULTS

The results given in table 1 show that the addition of anti-insulin serum to the isolated epididymal fat pad reduced the oxidation of glucose-1-C-14 to less than half the control value (about 40 per cent), and this was found in all experiments, whereas normal guinea pig serum showed no inhibitory effect.

The influence of anti-insulin serum on lipid formation from glucose-U-C-14 was very much less pronounced, giving approximately a 20 per cent average decrease. In the three experiments carried out the decrease in lipid synthesis was 36 per cent, 19 per cent and 9 per cent respectively. Insulin itself, as has been previously established,[12] greatly increased both glucose oxidation and lipid synthesis from glucose.

The results given in table 2 show that adipose tissue which had been preincubated in the presence of anti-insulin serum showed a significantly reduced rate of glucose oxidation as compared to tissue preincubated in a buffered medium without anti-insulin serum; in the presence of insulin, however, the anti-insulin treated tissue and the control tissue oxidized glucose to the same extent.

DISCUSSION

The results reported demonstrate for the first time a direct in vitro effect of anti-insulin serum on glucose metabolism in an isolated tissue of a normal rat in the absence of added exogenous insulin. This therefore provides a technic for depleting a tissue of its endogenous insulin and studying the glucose metabolism of the insulin depleted tissue without any preliminary pretreatment of the experimental animal. This effect can be obtained either by adding the anti-insulin serum and the glucose together or by first pretreating the tissue with the anti-insulin serum and then measuring its capacity to oxidize glucose. In the latter case glucose oxidation can be restored to normal by the addition of exogenous insulin. Wright[13] reported that the in vitro addition of anti-insulin serum to the isolated rat diaphragm did not reduce the basal glucose uptake, and in some preliminary experiments in this laboratory we were unable to find an in vitro effect of anti-insulin on glycogen synthesis from glucose in the isolated rat diaphragm.

The fact that the oxidation of glucose via the hexose monophosphate shunt can be considerably reduced whereas the incorporation of glucose into lipids is very much less affected would suggest that under the experimental conditions used, the rate of reduction of NADP to NADPH was not a primary factor in controlling the rate of lipid synthesis from glucose.

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Congenital Absence of Beta-Lipoproteins

M. Lamy et al. (Pediatrics *31*:277, 1963) have presented a study of two cases showing an interesting disease picture. The outstanding features of this condition are steatorrhea, growth retardation, neurologic ataxia, retinal changes, acanthocytosis, and low plasma lipids.

The first patient was a child, born to consanguineous parents, who developed symptoms of vomiting, steatorrhea, abdominal distention, and failure to thrive at the age of four months. Although he was considered to have celiac disease, a gluten free diet did not result in improvement in his clinical condition. During the second year of life, a low fat diet (4 gm. daily) was tried, with prompt gain in weight.

The patient was next seen at age five, having been given an uncontrolled diet in the interim. At this time there were evidences of marked growth failure (weight 9.2 kg., height 85 cm.), neurological abnormalities (unsteady gait, intelligence quotient 50), muscle wasting, and abdominal distention. Skeletal roentgenograms revealed osteomalacia. The stools were described as bulky and greasy; fat absorption was low. Glucose and xylose absorption studies were normal. Barium studies showed considerable fragmentation and clumping of the small bowel pattern.

Examination of the blood smear revealed almost all of the erythrocytes to have the striking spiculate, or prickly, appearance for which the term acanthocyte is used. These cells have a shortened life span.

Other tests included peroral duodenal biopsy, which showed the mucosa to be somewhat flattened, but not with the typical pattern of celiac disease.

The second patient was a thirty-four-year-old woman who had suffered episodes of fat intolerance since childhood, and who exhibited short stature (139 cm.), muscle wasting, abdominal distention, absence of the deep tendon reflexes, slight ataxia, and mental retardation. Acanthocytosis was present.

Studies of the serum lipids of these two patients revealed striking abnormalities. Total lipids were very low, 110 and 123 mg. per 100 ml. respectively, and cholesterol 30 and 36 mg. Only a trace of triglyceride could be detected. Gas chromatography showed a reduced proportion of linoleic acid and an increased proportion of oleic and palmitoleic acids in the esterified cholesterol, triglyceride, and phospholipid fractions.

Lipoproteins were studied by means of paper electrophoresis, immuno-electrophoresis, and ultra-centrifugation. Alpha-lipoprotein was present in only one-third the normal concentration (200 to 300 mg. per 100 ml.) and only trace amounts of beta-lipoproteins could be detected. Hence the name a-beta-lipoproteinemia given to this condition.

The authors attempt to relate the various manifestations of the disease to the biochemical abnormality, but without much success. The low serum content of linoleic acid may reflect an impairment of intestinal absorption, but this is probably not the cause of the lowered concentration of lipoproteins. The pathogenesis

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