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An Adrenergic Substance in the Blood of Alloxan Diabetic and Acetoacetate Injected Rabbits.* (31731)

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Sirek *et al* (1), making use of the observation of Ellis and coworkers(2) that dihydroergotamine can block the hyperglycemic effect of adrenaline but not of glucagon, have adduced experimental evidence to indicate that the hyperglycemic factor found in the pancreatico-duodenal vein of growth hormone treated animals(3,4) is an adrenaline-like substance. Osman Saka(5), while working on the hyperglycemic glycogenolytic factor of the pancreas, found that 4 ml of blood from an alloxan diabetic rabbit, when injected to a normal rabbit elicited a hyperglycemic response. This hyperglycemic response was attributed to the presence of glucagon in the blood of alloxan diabetic rabbits. In view of the observations of Sirek *et al,* it was thought desirable to determine whether the hyperglycemic factor, reported by Saka was an adrenergic substance.

Acetoacetate has been reported to cause hyperglycemia and associated disturbances in carbohydrate metabolism(6,7). Repeated daily injections of acetoacetate to normal and scorbutic guineapigs were found to increase the adrenaline content of the adrenals(8). Therefore, it was decided to investigate the possible presence and nature of a hyperglycemic substance in the blood of acetoacetate treated animals. Experiments were also performed to determine whether adrenergic mediation played a part in the immediate hyperglycemia following a single injection of acetoacetate to rabbits.

Materials and methods. Rabbits were made diabetic by intravenous injection of alloxan. Eight of these rabbits, having blood sugar values above 200 mg % a week after the alloxan injection, were used as blood donors.

Eight rabbits were injected with sodium acetoacetate, intraperitoneally every day for 2 months. The starting dose of acetoacetate was 100 mg/kg and it was increased by 50 mg/kg every 15 days. At the end of 2 months, those rabbits which had blood glucose values of about 170 mg % were used as blood donors, to test the presence of an adrenergic substance in their blood.

Four ml of blood from each of 8 alloxan diabetic rabbits along with 10 mg heparin was injected in the marginal ear vein of normal rabbits. The blood sugar of the recipient rabbits was determined by Nelson's method(9) every 10 minutes. Four ml of blood from the alloxan diabetic rabbits was similarly injected to other normal rabbits, which had been injected with dihydroergotamine (0.2 mg/kg) half an hour earlier, and the blood glucose content of the recipients determined every 10 minutes. The results are presented in Fig. 1.

Ten to sixteen mg of glucose (which was the amount present in 4 ml of alloxan diabetic blood injected to normal rabbits) along with 10 mg heparin was added to 4 ml of

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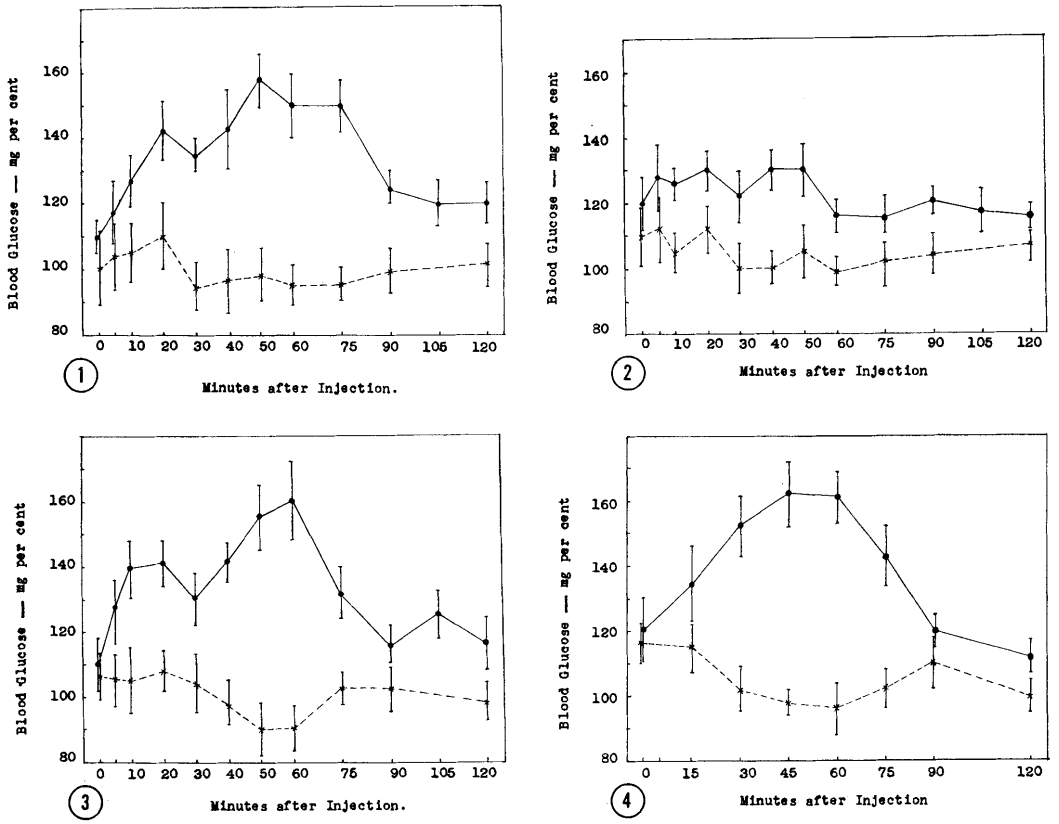

FIG. 1. Effect of alloxan diabetic blood on normal rabbits. ●—● Blood glucose of normal rabbits injected blood of alloxan diabetic rabbits. X—X Blood glucose of DHE treated rabbits injected blood of alloxan diabetic rabbits. Vertical bars represent standard deviation.

FIG. 2. Effect of normal rabbits blood on normal rabbits. ●—● Blood glucose of normal rabbits. X—X Blood glucose of DHE treated rabbits injected blood of normal rabbits. Vertical bars represent standard deviation.

FIG. 3. Effect of blood from acetoacetate injected rabbits on normal rabbits. ●—● Blood glucose of normal rabbits injected blood from acetoacetate injected rabbits. X—X Blood glucose of DHE treated rabbits injected blood acetoacetate injected rabbits. Vertical bars represent standard deviation.

FIG. 4. Effect of acetoacetate on normal rabbits. ●—● Blood glucose of normal rabbits injected acetoacetate intravenously. X—X Blood glucose of DHE treated rabbits injected acetoacetate intravenously. Vertical bars represent standard deviation.

blood from 8 normal rabbits and injected into the marginal ear vein of normal rabbits. The blood glucose levels of the recipient rabbits were determined every 10 minutes. The experiment was repeated using recipient rabbits which had been injected with dihydroergotamine (0.2 mg/kg) half an hour prior to the injection of normal blood. The blood sugar values of the recipients are shown in Fig. 2.

Cross-over tests were carried out. The animals which were injected blood from alloxan diabetic rabbits were after a few days injected with blood from normal animals and vice-versa. Similar experiments were carried out with blood from rabbits injected with acetoacetate for 2 months. The results are shown in Fig. 3.

To test whether the hyperglycemia immediately following a single injection of acetoacetate was due to adrenergic stimulation, sodium acetoacetate injected intravenously to normal rabbits, in a dose of 300 mg/kg and their blood sugar estimated every 15 minutes. Acetoacetate was similarly injected to dihydroergotamine treated rabbits and their blood sugar determined. The results are shown in Fig. 4.

Results and discussion. Blood from alloxan

diabetic rabbits induces hyperglycemia in normal rabbits (Fig. 1). These results are essentially in agreement with those of Saka (5). The hyperglycemia induced by alloxan diabetic blood can be effectively checked by dihydroergotamine (Fig. 1). The differences of blood glucose levels between the dihydroergotamine treated and untreated rabbits are statistically significant. Therefore, the hyperglycemic substance present in the blood of alloxan diabetic rabbits seems to be an adrenalinelike substance and not glucagon as suggested by Saka. The blood of acetoacetate-injected rabbits also elicits a hyperglycemic response in normal rabbits which can be blocked by dihydroergotamine (Fig. 3). The hyperglycemia is either due to an adrenergic substance or mediated through an adrenergic mechanism. According to Saka(5) the hyperglycemic factor is present in the blood of alloxan diabetic rabbits only when their blood sugar is above 200 mg %. In the case of acetoacetate injected rabbits, however, it is present even when the blood sugar is below 200 mg %.

Intravenous injection of a single massive dose of sodium acetoacetate causes hyperglycemia in normal rabbits (Fig. 4). Acetoacetate however, does not cause hyperglycemia in dihydroergotamine treated rabbits, indicating that the hyperglycemia following a single injection of acetoacetate is largely due to adrenergic stimulation. In fact there was an immediate fall in blood sugar of dihydroergotamine treated rabbits, which gradually rose to normal values after about 3 hours. This is perhaps due to the stimulation of insulin secretion by acetoacetate, reported earlier by Nath and Brahmachari(10). In the normal rabbit this hypoglycemia is perhaps masked by hyperglycemia resulting from the adrenergic breakdown of liver glycogen.

An initial hyperglycemia due to adrenergic mediation has also been reported following the injection of alloxan. Gaarenstroom and Siderius(11), who made a detailed study of the blood sugar changes during the first hour after alloxan injection, have concluded that an initial hypoglycemia is masked by the hyperglycemia due to the breakdown of liver glycogen. This hyperglycemic phase is absent in hepatectomized and eviscerated animals (12). It has also been reported to be abolished by adrenalectomy(13-15) or destruction of the adrenal medulla(14). Corkill *et al* (16) prevented the hyperglycemia by means of ergotamine. It is of interest to note that atropine, which reverses the sympathomimetic effect of dehydroascorbic acid(17), also prevents the diabetogenic effect of dehydroascorbic acid(18).

Summary. A hyperglycemic substance was found to be present in the blood of alloxan diabetic rabbits. The hyperglycemia due to this factor could be prevented by dihydroergotamine indicating that it is an adrenergic substance and not glucagon. A similar hyperglycemic substance was also found in the blood of acetoacetate injected rabbits. A single intravenous injection of acetoacetate causes an immediate hyperglycemia in normal rabbits which can be prevented by dihydroergotamine.

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Effect of Insulin on Blood Glucose and Corticosterone Levels in Sodium Fluoroacetate Induced Diabetes.* (31732)

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Sodium fluoroacetate (SFA) has been shown to produce hyperglycemia and ketonemia in the rat(1,2). Although this SFA-induced diabetes has been described as being partially insensitive to insulin by Engel *et al* (3), a significant insulin effect has been demonstrated(4). Karam and Grodskey(5) reported that greater than normal amounts of insulin were present in the pancreatic tissue of the SFA-treated animal. The present study was designed to investigate the effect of exogenous insulin in reducing the blood glucose levels in control and SFA-induced hyperglycemic rats. In addition, the effects of SFA and insulin on the levels of circulating corticosterone were determined.

Materials and methods. After fasting for 24 hours, 143 female Sprague-Dawley rats, weighing from 170 to 190 g, were divided into 4 groups. The first group (41 rats) received sodium fluoroacetate (SFA), 30 mg/kg, followed 150 minutes later by insulin, at a dosage of 0.2 U/kg. The second group (38 rats) received SFA, followed later by saline comparable in volume to the insulin administered. Group three (30 rats) received saline and insulin 150 minutes apart. The fourth group (34 rats) received 2 injections of saline over the same time interval as the preceding groups. All materials were administered intraperitoneally. Animals were decapitated at 30, 60, 90 and 120 minutes after the second injection and blood from the neck was collected in heparinized beakers. Aliquots of 50 μl of blood were immediately removed for glucose determination by the glucose oxidase method(6). Blood samples were then rapidly centrifuged in the cold to obtain plasma. Corticosterone was determined in the individual samples of plasma by the fluorometric method of Zenker and Bernstein(7).

Since the non-specific plasma fluorescence may have been greater in those animals given SFA or insulin and interpreted incorrectly as higher corticosterone values, equal volumes of plasma from each animal of each time group were pooled and from 2.0 to 3.0 ml were taken for determination of corticosterone by isotopic dilution. Each of the plasma pools was diluted with an equal volume of saline; 0.55 μg of rechromatographed corticosterone-1, 2, ^{3}H (6.9×10^6 d/m/μg) from New England Nuclear Corp. added, and the mixture extracted with 15 volumes of redistilled $CHCl_3$. Three additional solutions without added steroids and 3 containing radioactive corticosterone were run simultaneously to determine the blanks and initial specific activities, respectively. The $CHCl_3$ extracts were washed once with 0.05 volume of 0.1 N NaOH and twice with 0.05 volume of water. The solvent of the extract was removed *in vacuo* at 30°C with a rotary evaporator and the residue transferred to a conical tube with 3 ml methanol. To prevent the destruction of the steroids, 10 μg of ethylenediamine tetraacetic acid in methanol were added(8). The samples were then evaporated under nitrogen, the residues taken up with methanol and dichloromethane and applied to thick (0.014 in.) glass fibre paper (Carl Schleicher and Schuell Co.) impregnated with 0.1 M

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