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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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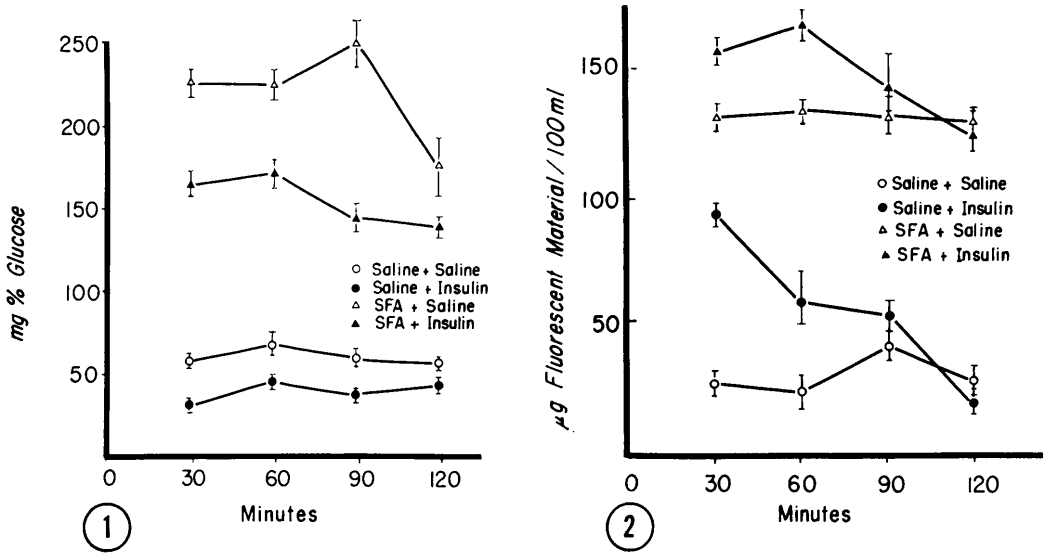

FIG. 1. Blood glucose levels after the administration of saline or insulin to animals pretreated with saline or sodium fluroacetate (SFA) 150 min earlier. Vertical bars represent one standard error of mean.

FIG. 2. Plasma fluorescent material levels obtained after administration of saline or insulin to animals pretreated with saline or sodium fluoroacetate (SFA) 150 min earlier. Vertical bars represent one standard error of mean.

phosphate for ascending chromatography(9). Suitable quantities of corticosterone standards were applied to each side of the paper. The solvent system employed, benzene:ethanol (800:5), separates cholesterol from corticosterone with Rf values of 1.0 and 0.5, respectively. After development, portions of the chromatogram containing the corticosterone standards were cut off and sprayed with sulfuric acid(9). The zones aligned with the standards were eluted with acetone and aliquots taken for measurement of radioactivity, using a toluene base scintillation solution, containing 5.0 g PPO and 0.5 g POPOP per liter, and a Packard liquid scintillation spectrometer. A sufficient number of counts was accumulated to give a counting error of 3% or less. The mass of the corticosterone, in another aliquot, was determined by the Zenker-Bernstein reagent(7). The recovery of labeled corticosterone varied from 50 to 70%. The amounts of corticosterone in the plasma samples were calculated by isotope dilution(10). Six 3.0 ml aliquots from a plasma pool were assayed and found to contain 33.2 $\pm$2.37 μg (SD) corticosterone per 100 ml plasma.

In an attempt to determine the factors responsible for the very high levels of corticosterone found in the SFA-treated animals, the contributions of the hypothalamus-pituitary-adrenal axis were eliminated. Fifty-five animals weighing from 187 to 312 g were subjected to bilateral adrenalectomies and maintained on 1% saline. Three days later they were divided into an experimental group which received SFA as previously described and a control group administered saline. One hour and fifty minutes following the initial injections, all animals received subcutaneous injections of an aqueous suspension of corticosterone (12.5 mg/kg)(11). One hour later, 6 control and 8 experimental animals were decapitated. These were followed at 2 hours by 6 control and 9 experimental animals. At the 3-hour interval, 6 control and 9 experimental animals were sacrificed. Plasma samples were obtained and equal volumes from each animal of each time group were pooled and treated in a manner similar to that previously described using corticosterone-4-^{14}C for isotope dilution.

Results and discussion. As shown in Fig. 1, significantly lower levels of blood glucose were found during the 30 to 90 minute period after the administration of insulin to the

TABLE I. Percent Corticosterone in Plasma Fluorescent Material of Pooled* Samples Obtained After Administration of Saline or Insulin to Animals Pretreated with Saline or Sodium Fluoroacetate (SFA) 150 Minutes Earlier.

	Min after last injection			
	30	60	90	120
Saline—Saline	75 (9)†	86 (9)	71 (8)	65 (8)
Saline—Insulin (.2 U/kg)	74 (7)	69 (7)	85 (8)	72 (8)
SFA—Saline (30 mg/kg)	92 (10)	94 (7)	97 (7)	88 (14)
SFA—Insulin	93 (9)	89 (10)	72 (8)	81 (14)

* Obtained from equal volumes of plasma from each animal in each group.
† No. of animals in each group.

SFA-induced hyperglycemic animals. These data agree with those previously reported(4) in which a different method of analysis was employed, but do not reveal any insulin insensitivity as reported by Engel *et al*(3). However, our data were obtained with one-half the dose of SFA and twice the amount of insulin administered by these investigators. That the insulin dosage resulted in a significant decrease in blood glucose levels in the control animals is also shown. The effect of exogenous insulin in decreasing the blood glucose levels of the SFA-treated rats suggests that the high levels of this hormone stored in the pancreas, as reported by Karam and Grodskey(5), are not being released into the general circulation.

The very high levels of plasma fluorescent material found in the SFA-treated animals are seen in Fig. 2. These levels are at least one-third higher than those previously reported(11) employing the same procedure for the determination of plasma fluorescent material in animals under acute stress. The physical stress of the intraperitoneal injections was presumbaly the same for all animals, thus indicating that the insulin must have increased the levels of the fluorescent material in the controls as well as in the SFA-treated animals for the 30 to 60 minute period.

Table I shows that the high levels of fluorescent material found in the plasma are primarily due to their corticosterone content and not to any non-specific fluorescence induced by SFA. Only a small variation was found in the osmolarity of selected plasma samples from each group, indicating that hemo-concentration was not responsible for the high glucose and corticosterone levels.

The elevated levels of corticosterone found in the SFA-treated animals suggested that the adrenals were producing more corticosterone or that the liver was inactivating it to a lesser extent than in the controls. The levels of this steroid found in the adrenalectomized animals administered saline or SFA and then administered corticosterone are shown in Fig. 3. The plasma corticosterone values of the control animals found in this experiment fall within the range previously reported during the one- to three-hour period following the injection(11). The higher plasma levels of this steroid obtained from the SFA-treated animals clearly indicate that the metabolism of corticosterone is impaired. The effect of SFA on the adrenal secretion of corticosterone remains unexplained.

As postulated by Engel *et al*(3) and later confirmed by Bowman(12) ATP concentration in heart muscle is reduced by half when

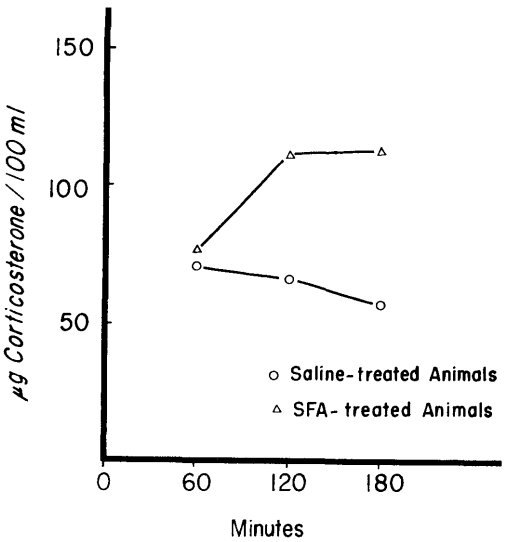

FIG. 3. Corticosterone levels of plasma pools obtained after administration of corticosterone to adrenalectomized animals pretreated with saline or sodium fluoracetate (SFA) 150 min earlier.

SFA was given. Any reduction in ATP would be expected to reduce the energy levels necessary for the oxidation, reduction and conjugation of corticosterone in the liver. This metabolic poison may possibly be of some value in reducing the very rapid rate of metabolism of steroid hormones in the liver, thus permitting a more detailed study of their metabolites.

Summary. The hyperglycemia produced by SFA can be significantly reduced by small amounts of insulin. The very high levels of plasma corticosterone found to occur following treatment with SFA can be transiently increased by the administration of insulin. These high levels are thought to be due to a SFA induced decrease in the hepatic inactivation of the adrenal hormone.

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Effect of Ethacrynic Acid on Calcium and Magnesium Excretion.* (31733)

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Methylenebutyryl phenoxyacetic acid (ethacrynic acid) has been reported to have a profound effect on the transport of sodium, chloride, potassium, and hydrogen ion by the kidney(1,2,3). The renal clearance of calcium has been shown to be closely related to that of sodium(4,5,6), and the renal clearance of magnesium is stated to be similar to that of calcium(7,8). The present study was designed to determine whether ethacrynic acid while effecting sodium excretion also modified the renal excretion of divalent cations, mainly calcium and magnesium, and to compare the magnitude of the effect with that of meralluride and chlorothiazide.

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Intravenous administration of ethacrynic acid, chlorothiazide, and meralluride was found to enhance the renal excretion of calcium and magnesium in man during water diuresis. The observed calciuresis in these studies was roughly proportional to the magnitude of the increase in sodium excretion produced. Oral administration of ethacrynic acid has also produced an increase in excretion of calcium when a natriuresis occurred. Phosphate excretion was slightly decreased after ethacrynic acid administration under these circumstances.

Methods. Renal clearance studies were performed on 3 healthy, paid, volunteer, adult males on the metabolism ward of the First (Columbia) Medical Division of Bellevue Hospital. In these studies a control renal clearance was compared to a clearance measured after intravenous administration of etha-