inhibit only the latter and by doing so prevents the increase of liver triglycerides by ethionine.

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Received June 11, 1962. P.S.E.B.M., 1962, v111.

## Amino Acid Incorporation into Protein of Diabetic Rat Liver Slices After Acute Insulin Deprivation.* (27829)

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These experiments bear on the question whether insulin can act upon isolated liver. Earlier(1,2) it was found that insulin could stimulate amino acid incorporation into protein of liver slices from one type of mildly diabetic rat, *i.e.*, animals partially pancreatectomized 2-4 months previously. In the present series the effect of insulin on liver slices has been tested, using another type of diabetic liver donors, namely, alloxan-diabetic rats from which insulin had been withdrawn for various periods(3). Incorporation of leucine-1-$C^{14}$ into protein was used as the test indicator of an insulin effect.

*Materials and methods.* Diabetes was produced in 24-hour fasting 150-250 g male Holtzman rats by intravenous injection of Eastman alloxan-monohydrate, 37 mg per kg; the rats were immediately returned to food and water. Insulin injection (subcutaneous) was started on the fourth day after alloxan. On 4th and 5th days the dose was 8 units regular insulin per rat in the morning and 8 units Lente Iletin (Insulin, Lilly, U-40) in the evening. On the 6th day and thereafter doses were reduced to 4 units each, morning and evening; regular insulin replaced Lente insulin on last 2 days before sacrifice, which was 15-18 days after alloxan. This regimen permitted the animals to maintain weight, with some nocturnal polyuria. All animals were fasted 24 hours before sacrifice, and used 3, 12, 24, or 48 hours after the last insulin injection. Blood sugars were determined at time of sacrifice(4).

Four liver slices (150-200 mg) were cut free-hand from the left lobe of each liver; one slice was placed in 1 ml Krebs-Henseleit bicarbonate buffer containing $1.6 \times 10^5$ cpm DL-leucine-1-$C^{14}$ (Nuclear Chicago) at the tracer concentrations shown in Table I; the 2nd, 3rd, and 4th slices were put in 1-ml aliquots of the same medium containing glucose, insulin, or insulin plus glucose respectively, as shown in Table I. All slices were then shaken for 2 hours in a Dubnoff incubator at 37°C(2). Crude protein was isolated(2) and its radioactivity estimated with a D-47 Nuclear Chicago counter; all radioactivity values are corrected for self-absorption, but not for counter efficiency (35-40%).

*Results.* Liver slices from fasting diabetic rat donors incubated in Krebs solution containing leucine-1-$C^{14}$ without added glucose

* This investigation was aided by grants from Nat. Inst. of Health and the Dr. Wallace C. and Clara A. Abbott Memorial Fund, Univ. of Chicago.

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TABLE I. Effects of Insulin and Glucose, Singly and Together, on Incorporation of Leucine-1-$C^{14}$ into Protein by Rat Liver Slices from Alloxan-Diabetic Rats at Various Times after Insulin Withdrawal from the Donor.

| Exp. No. | Type of rat | Hr after last insulin inj | Mean terminal fasting blood sugar, mg % | Incorporation of leucine-1-$C^{14}$ into liver protein, cpm mg protein after 2 hr in Krebs-Henseleit-bicarbonate solution containing: No addition | Glucose, 2 mg/ml | Insulin, .01 unit/ml | Glucose + insulin |
|---|---|---|---|---|---|---|---|
| 1* | Normal (5) | No inj | 88 | 228 ± 9† | 291 ± 12 | 239 ± 20 | 283 ± 24 |
| | Diabetic (6) | 3 | 54‡ | 220 ± 6 | 230 ± 19 | 232 ± 26 | 228 ± 18 |
| 2 | Normal (14) | No inj | 95 | 336 ± 8 | 369 ± 16 | 344 ± 17 | 397 ± 16 |
| | Diabetic (12) | 12 | 305 | 266 ± 9 | 281 ± 14 | 253 ± 13 | 308 ± 16 |
| | " (8) | 24 | 208 | 249 ±12 | 275 ± 21 | 285 ± 30 | 329 ± 17§ |
| | " (11) | 48 | 346 | 191 ±16 | 200 ± 23 | 190 ± 19 | 229 ± 18 |

* Values for incorporation cannot be compared between Exp. 1 and 2. In Exp. 1, .027 mg DL-leucine-1-$C^{14}$ with $1.6 \times 10^5$ cpm were added to each ml incubation medium; in Exp. 2, .0043 mg containing $1.6 \times 10^5$ cpm were added.

† Mean ± stand. error of mean. No. of rats used is given in parentheses.

‡ Mean fasting blood sugar for this group was 354 mg % at time of last insulin inj, 3 hr before sacrifice.

§ The difference between the "no addition" and "glucose + insulin" groups is significant to $P = .003$ in this diabetic group and to $P = .002$ in the concurrent normal series.

showed a progressive decrease in incorporation of radioactivity into protein as the time after insulin withdrawal from the liver donor was increased from 3 up to 48 hours; the value at 3 hours was not different from that for paired normal controls; values at 12, 24, and 48 hours were 21, 26, and 43% below normal, respectively (Table I). The per cent of the total DL-leucine-1-$C^{14}$ radioactivity in the medium which is incorporated into the liver protein may be calculated as follows: an average 36 mg dry protein was recovered from each 200 mg of liver slices incubated in 1 ml medium containing $1.6 \times 10^5$ cpm of DL-leucine-1-$C^{14}$. The percentages of the total tracer dose converted to protein may therefore be obtained by multiplying each incorporation value of Table I by $\frac{36 \times 100}{1.6 \times 10^5} = 0.0225$; representative percentages for the "no addition" groups were: Exp. 1, normal, 5.1, diabetic 3 hours after insulin, 4.9; Exp. 2, normal, 7.6, diabetic 24 hours after insulin, 5.6, diabetic 48 hours after insulin, 4.3.

Insulin and glucose, added together to the medium *in vitro* stimulated leucine incorporation into protein; the difference between the "no addition" and "glucose plus insulin" groups was statistically significant only for the diabetic series in which insulin had been withdrawn 24 hours, and for the concurrent normals. Neither insulin alone nor glucose alone gave a significant stimulation in any of the diabetic groups (Table I).

These results are similar to those obtained with liver slices from partially pancreatectomized donors in 3 respects: first, the capacity to incorporate amino acid into protein progressively decreases as time after insulin withdrawal (or partial pancreatectomy) increases; second, insulin added *in vitro* can stimulate amino acid incorporation, this stimulation being relatively greatest at some particular time after insulin withdrawal (or partial pancreatectomy); third, the insulin stimulation depends on presence of glucose in the medium. The role of ketosis was not studied in the present series; it is known that severe ketosis may affect the response of perfused liver to insulin(5) and incorporation of leucine into protein by liver microsomes (6).

*Summary.* Incorporation of leucine-1-$C^{14}$ into protein of liver slices decreased progressively up to 48 hours after insulin withdrawal from alloxan-diabetic rats. Insulin and glucose added together to the medium stimulated this incorporation at 24 hours after insulin withdrawal from the liver donors; smaller but statistically non-significant stimulation was obtained at 12 and 48 hours.

Neither insulin nor glucose alone gave a significant stimulation in any diabetic group.

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Received June 19, 1962. P.S.E.B.M., 1962, v111.

## Supersensitivity of the Cat Heart to Catecholamine-Induced Arrhythmias Following Reserpine Pretreatment.* (27830)

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It has been shown that reserpine pretreatment increases the sensitivity of the heart to the positive chronotropic(1) and positive inotropic(2) effects of norepinephrine. However, since the cardiac rate(3) and contractility(4) of the hearts of reserpinized animals are reduced below control values, changes in the responses to norepinephrine are difficult to interpret(3). The incidence of an all or none response of the heart, such as arrhythmia, in reserpinized animals therefore seems of value. Supersensitivity to the arrhythmia-inducing action of catecholamines is of further interest since this is a toxic rather than a physiological effect. This report compares the occurrence of ventricular arrhythmias produced by catecholamines in reserpinized cats as compared to untreated cats.

*Materials and methods.* Cats of both sexes, weighing 2 to 4 kg, were used. Twenty cats served as controls and received no reserpine. Twenty-six cats received reserpine, 0.1 mg/kg/day intraperitoneally, for periods ranging from 7 to 28 days preceding the experiment. This dose of reserpine has been shown to produce nearly maximal depletion (*ca.* 95%) of norepinephrine stores in the cat heart in 24 hours(5).

The cats were pithed under ether anesthesia using the spinal method of Burn(6). Following this operation the cats were maintained on artificial respiration and the etherization discontinued. The hearts were therefore free of effects from anesthetic, neural control, and reflexes resulting from the blood pressure responses to the injected catecholamines. Carotid blood pressure was measured with a Statham transducer and recorded on a Grass polygraph. Needle electrodes were placed subcutaneously on the forelegs and right hindleg and the lead I electrocardiogram recorded on the Grass polygraph.

Consecutive single injections of l-norepinephrine bitartrate (levarterenol) or l-epinephrine bitartrate were given into the femoral vein, beginning with a dose of 0.01 μg/kg (free base) and ending with a dose of 100 μg/kg. No injection was made until the heart and blood pressure effects of the preceding dose had completely subsided. Some cats received only the norepinephrine injections and some received both norepinephrine and epinephrine. When both drugs were used, alternate injections of norepinephrine and epinephrine were made, beginning with the lowest doses. This eliminated the possibility that large doses of one catecholamine might influence the response to a small dose of the other.

*Results.* The first arrhythmias produced by catecholamine injections were in the form of ventricular premature systoles and extrasystoles. In some of the reserpinized animals, large doses of norepinephrine and epinephrine produced ventricular tachycardia for

* Supported by grant from Nat. Inst. of Neurol. Dis. and Blindness, N.I.H.