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Received July 24, 1958. P.S.E.B.M., 1958, v99.

Mode of Action of Insulin, Carbutamide, and Tolbutamide.* (24379)

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Carbutamide and Tolbutamide may lower the concentration of blood glucose in several ways, one of which is release of insulin by the pancreas. Although based on strong experimental evidence(1-16), this mode of action cannot be accepted as the sole explanation for hypoglycemia unless it can be shown that other metabolic effects of insulin are also shared by the drugs. Results of experiments designed to study this point have not been uniform. One group of investigators believes that tolbutamide, like insulin, stimulates peripheral glucose utilization(7), while other groups could find no evidence for this stimulation(8,9). The action of sulfonylureas on muscle glycogen is also uncertain, as it has been reported that its synthesis *in vivo* is stimulated(10), inhibited(11) or not affected (12,13) by tolbutamide. Contrary to insulin, carbutamide and tolbutamide stimulate synthesis of liver glycogen in fasted animals(11-15), but tolbutamide does promote glycogen deposition into peripheral fat as does insulin (15). Blood pyruvate increases during insulin hypoglycemia(16,17), but it was found increased(18), unchanged(17,19) or decreased(16,20,21) during sulfonylurea hypoglycemia. Like insulin, tolbutamide depresses ketosis(15), but, unlike insulin, it does not affect the disappearance of D-xylose and L-arabinose from the blood(21). The reasons for these contradictory results are not clear: perhaps the sulfonylureas act on the liver as well as on the pancreas(13,22), or perhaps insulin, secreted directly into the portal system under sulfonylurea stimulation, acts differently from insulin injected into the periphery. Purpose of this work was to investigate this possibility.

Methods. Forty-seven mongrel dogs of both sexes were fasted for 16-18 hours and anesthetized with a mixture of sodium pentobarbital (35 mg/kg) and sodium barbital (100 mg/kg), injected intravenously. This anesthetic mixture provided the rapid induction and the long lasting, uniformly deep anesthesia with complete muscle relaxation, which is essential to prevent excessive fluctuations in blood pyruvate and lactate, unrelated

* Aided by Grant from Nat. Inst. of Arthritis and Metabolic Diseases, U.S.P.H.S.

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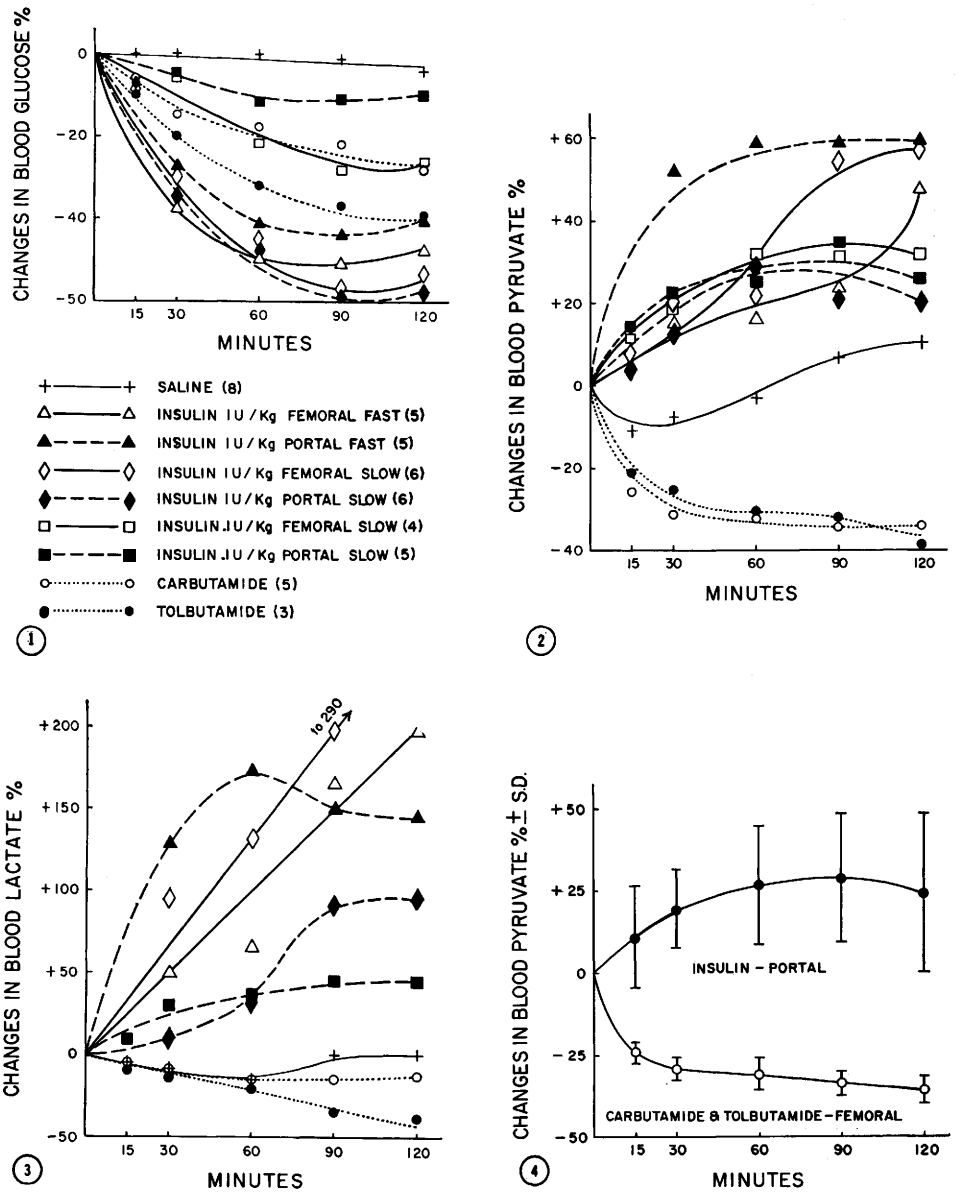

Fig. 1. Effect of insulin, carbutamide and tolbutamide on blood glucose. Figures in parentheses indicate no. of experiments.

FIG. 2. Effect of insulin, carbutamide and tolbutamide on blood pyruvate. Symbols as in Fig.1.

FIG. 3. Effect of insulin, carbutamide and tolbutamide on blood lactate. Symbols as in Fig. 1.

FIG. 4. Comparison of effect of slow intraportal injections of insulin (0.1 and 1 U/kg) and of intrafemoral injections of sulfonylureas on blood pyruvate. S.D. = Standard deviation.

to the experiment. Carbutamide‡ and tolbutamide§ (50 mg/kg) and saline were injected into the femoral vein, glucagon-free insulin‡ (0.1 or 1 U/0.1 ml of saline/kg) into the femoral or into the portal vein. Rapid injections were done with syringe and needle, slow injections (0.1 ml/5 min) with a polyethylene catheter introduced through a No. 17 hypodermic needle and attached to a continuous injection pump by means of a No. 24 needle. The abdomen was opened either for injection into the portal vein or for sham

‡ Gift of Eli Lilly and Co.

§ Gift of Upjohn Co.

manipulations. To check the general state of the animal, blood pressure was measured with a mercury manometer attached to the carotid artery. Blood samples were taken from an exposed femoral vein for duplicate analyses. Glucose was determined according to Nelson (23), pyruvate according to Friedmann and Haugen(24), lactate according to Barker and Summerson(25), and potassium by means of the Coleman flame photometer. Unless otherwise stated, the effects of the substances investigated were compared to those of saline. Statistical significance of the results was calculated according to Fisher(26).

Results. Average changes in blood *glucose* concentration are shown in Fig. 1. Saline injections had no significant effects. Insulin, in doses of 1 U/kg, caused comparable degrees of hypoglycemia whether injected intraportally or into the femoral vein. Slow injections appeared to have a more protracted effect than fast injection, but in all cases blood sugar decreased between 40 and 50%. The drop in blood sugar was less pronounced following slow injections of 0.1 U/kg and these were less effective in the portal than in the femoral vein (P values varied between 0.02 and 0.05). Carbutamide and tolbutamide caused an average hypoglycemia intermediate between those obtained with the 2 doses of insulin. Average changes in blood *pyruvate* and *lactate* are shown in Figs. 2 and 3. Saline had no significant effects on either pyruvate or lactate. In all cases insulin caused a significant increase in pyruvate and lactate concentration (P values varied between 0.02 and 0.001), which was more marked after large doses injected rapidly, than after small doses injected slowly. Carbutamide and tolbutamide caused a significant decrease in blood pyruvate ($P<0.01$). Both substances caused a decrease in blood lactate, but when compared with the effects of saline, the decrease was significant ($P<0.05$) for tolbutamide only. However, the effect of both drugs on pyruvate and lactate was significantly different from that of insulin in either dose and by either route of administration (P values varied between 0.02 and 0.001). Fig. 4 illustrates this difference when the effect of the 2 drugs on pyruvate is compared to that of insulin injected slowly into the portal vein. Average changes in plasma *potassium* concentration are shown in Fig. 5. Saline had no significant effects. Insulin, in doses of 1 U/kg, caused a significant ($P<0.001$) decrease in plasma potassium. This decrease was less pronounced and of shorter duration, but still significant ($P<0.05$), when the hormone was injected in doses of 0.1 U/kg. Carbutamide and tolbutamide caused a decrease comparable to that of small doses of insulin, although only the effect of carbutamide was statistically significant ($P<0.001$).

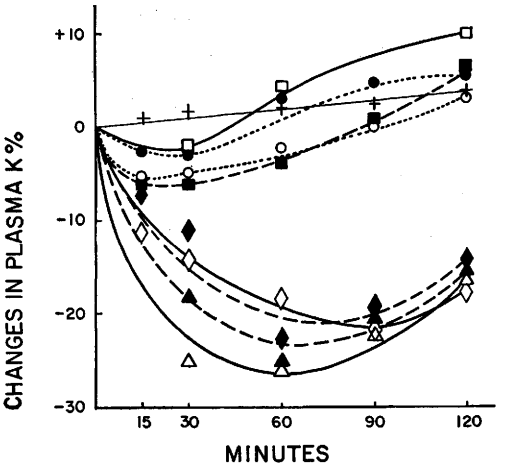

FIG. 5. Effect of insulin, carbutamide and tolbutamide on plasma potassium. Symbols as in Fig. 1.

Discussion. The results indicate that route of administration does not alter effects of insulin on blood glucose, pyruvate, lactate and potassium qualitatively, although, when small amounts of insulin are injected slowly into the portal vein, these effects may be less intense. Probably, this is due to the fact that the fraction of insulin destroyed by the liver under these conditions is larger than when insulin is injected peripherally or in large amounts. The experiments provide no evidence for the hypothesis that insulin secreted into the portal vein may act in an intrinsically different manner from insulin injected parenterally. A recent study of peripheral glucose utilization, based on determination of A-V differences, led the authors to the opposite conclusion(27), but the conditions of the experiments are not directly comparable to those here described. On the other hand, the effects of carbutamide and tolbutamide on py-

ruvate and lactate differ qualitatively from those of insulin. These results do not contradict the strong evidence for a pancreatropic action of the sulfonylureas, but suggest that the drugs may act also in other ways. It is possible that following administration of these substances insulin may be secreted at a very slow rate and that, at the same time, release of glucose may be depressed by a direct action of the drugs on the liver, resulting in hypoglycemia. On the other hand, the lowering of blood pyruvate and lactate, which precedes the maximum hypoglycemic effect, could be the result of a decreasing glucose supply to the muscle and/or an inhibition of its metabolic breakdown, in spite of an increased insulin secretion.

This hypothesis would explain why the drugs cause hypoglycemia in the absence of the liver(22), when glycogenolysis is absent and insulin destruction, probably, reduced and why they are not effective in the absence of functioning pancreatic tissue or of exogenous insulin(28), when their action is overwhelmed by glycogenolysis and gluconeogenesis proceeding at an accelerated rate. Thus carbutamide and tolbutamide may exert their hypoglycemic and glycostatic(29) effects only in the normal, partially alloxanized or partially depancreatized animal, or in mild diabetic patients where insulin may be provided by stimulation of the B cells in quantities sufficient to make the liver action of the drugs possible.

Summary and conclusions. Insulin was injected in different doses and at different rates into either the femoral or the portal vein of anesthetized dogs. No qualitative differences were noted between the 2 routes of administration: in all cases insulin caused a decrease in concentration of blood glucose and potassium and an increase in concentration of pyruvate and lactate. Intravenous carbutamide and tolbutamide caused a decrease not only in blood glucose, and potassium, but also pyruvate and lactate. It is suggested that the drugs may stimulate release of insulin from the pancreas, and that when a minimum (permissive?) amount of insulin is available and glycogenolysis is not accelerated, as in severe diabetes, they may also inhibit glucose production by the liver or its utilization by the muscle. If this hypothesis is correct, prolonged therapy with sulfonylurea-like drugs should be attempted with caution, for continued stimulation of the B cells with sulfonylurea may lead to their exhaustion(1,30) and suppression of hepatic glucose production may be a sign of tissue damage.

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Received July 28, 1958. P.S.E.B.M., 1958, v99.

Progestational Activity of Certain Steroid-17-Spirolactones. (24380)

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The structural requirements for estrogenic activity have long been known to be highly non-specific. Nevertheless, it has been previously considered that progestational activity depends upon highly specific structural relationships. More recently, a number of steroids which represent major structural deviations from the progesterone molecule have been shown to actually exceed progesterone itself in biological potency(1-4). Accordingly, in assessing the progestational potency of a wide variety of steroidal agents, we found that the synthetic steroid 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl) propionic acid γ-lactone (I) and its 19-nor analogue (II) possess substantial progestational activity as determined by a previously described modification of the Clauberg assay(1,2). In these assays we employed 2 samples of compound II and one sample of compound I. Each sample has been checked for chemical homogeneity by melting point determination and chromatographic analysis.*†

TABLE I. Progestational Activity of Steroid-17-Spirolactones.

Series	Total dose (mg)	Progestational proliferation* Compound II	Progesterone
A	5 †	+4, +4	
	1 †	+3, +1, +2	
	.5		+4, +3
B	1.0	+2, +2, +2	
	.5	+2, +3, +1	+4, +3, +1
	.25	+1, +1, +1	+4
C	1.0‡	+3, +2, +3	
	.5	+2, +2	
	.25	+1, +1	+2, +2, +1
		Compound I	
D	10	+3, +2, +3	
	5	+2, +2, +2	
	1	0, 0	
	.5		+3, +3, +4

* Each number represents one animal.

† Administered as a suspension. Sesame oil solution given in all other cases.

‡ Oral admin.: Compound II = 19-nor-3-(3-oxo-17β-hydroxy-4-androsten-17α-yl) propionic acid γ lactone. Compound I = 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl) propionic acid γ lactone.

Results. The data presented in Table I permit only an approximate comparison of the potency of these spirolactones with that of other parenterally or orally active progestogens. However, it may be estimated that the 19-nor-analogue (II) is about half as potent by subcutaneous administration as progesterone. Similarly, I is about 1/10th as active as progesterone when each is given subcutaneously. Moreover, II is approximately as active as 19-nor ethinyl testosterone when each is given orally(2).

It is of special interest that these steroid-17-spirolactones have been shown to antagonize the sodium-retaining effects of desoxycorticosterone and of aldosterone in rat and man(5,6). Desoxycorticosterone is itself a weak progestogen. In addition, progesterone has been shown to produce natriuresis in rat and man, a metabolic effect quite comparable to that seen with the steroid-17-spirolactones (5,7). Hence we are confronted with an apparent paradox in that progestational action

* We are indebted to Dr. M. B. Lipsett for these chromatographic analyses.

† We wish to thank Dr. Victor Drill, Searle & Co., for providing the steroid-17-spirolactones used.