Lack of Effect of Tolbutamide upon Certain Insulin-Responsive Sugars. (29073)

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The metabolic consequences characteristic of an insulin injection, including enhanced glucose uptake by peripheral tissues, have been difficult to demonstrate following administration of hypoglycemic sulfonylureas, to either man or experimental animals(1). Increased peripheral utilization of glucose resulting from insulin injection is thought to result largely from its ability to facilitate passage of glucose from the extracellular compartment to the intracellular compartment, particularly of skeletal muscle(2). Sugars other than glucose have been found to be susceptible to this action of insulin and are said to be insulin-responsive. Included in this category are D-galactose, D-mannose, several pentoses(3), and certain glucose analogues such as 2-deoxyglucose(4,5). The results of studies presented below show that certain of the insulin-responsive sugars are unaffected by the hypoglycemic sulfonylurea, tolbutamide, even when it is administered in dosage in excess of that necessary to demonstrate marked hypoglycemic effects.

Methods. Male Sprague-Dawley rats, weighing 180-250 g were used in all studies. The animals were fasted for 24 hours to reduce blood glucose levels and to minimize endogenous insulin secretion. Immediately prior to the experiment the rats were functionally nephrectomized by ligation of the renal vessels through a retroperitoneal incision, surgery being performed under ether anesthesia. The sugar under study was administered into a tail vein as a 20% solution in normal saline at a dose of 1 g/kg. Drugs were administered immediately following injection of the sugar. Blood samples were taken from retro-orbital vessels by means of heparinized capillary pipettes.

Pentose studies. Two hours after administration of the pentose a single blood sample was removed and the plasma pentose concentration determined by the method of Roe and Rice(6). The volume of distribution was calculated as the ratio of the amount of pentose injected to the plasma pentose concentration divided by the body weight expressed as a fraction of 100 g.

Other Sugars. Blood samples were removed at 15, 30, and 60 minutes following injection of the sugar. Determination of 2-deoxy-D-glucose was by the method described by Waravdekar and Saslaw(7). D-galactose was estimated by the anthrone procedure(8) after removal of glucose enzymatically.

Results. Following administration of L-arabinose or D-xylose these sugars are found to be distributed in a volume only slightly greater than extracellular water (Table I). Treatment with insulin increases the distribution in contrast to the lack of effect following treatment with tolbutamide. The lack of effect of tolbutamide is in agreement with the results of Segal *et al*(9) who found that insulin, but not tolbutamide, accelerated the disappearance of pentoses from the blood in human subjects, even though the degree of hypoglycemia produced by both agents was comparable.

In addition (Table II) there was no detectable potentiation by tolbutamide of the effects of a subthreshold dose (0.5 unit/kg) of insulin. This dose was selected as being just without discernible effects on pentose distribution; thus any increased elaboration of insulin or prevention of its destruction should in theory potentiate the effects of this dose.

In this study when insulin was given directly into the portal vein the effects on pentose distribution were unchanged from those noted when this agent was given by subcutaneous injection (Table III). It is evident that exogenous insulin administered by the intraportal route escapes modification or destruction by the liver to the extent that peripheral effects may be still detected.

Comparison of volumes of distribution is

TABLE I. Effect of Insulin and Tolbutamide on Volume Distribution of 2 Pentoses.

Treatment	Distribution of pentose: L(+) arabinose No. of rats	L(+) arabinose ml/100 g*	D(+) xylose No. of rats	D(+) xylose ml/100 g*
Saline control	8	48.6 ± 1.0	6	46.9 ± 1.2
Insulin, 2 units/kg S.C.	9	58.9 ± 3.0	6	55.2 ± 1.8
Tolbutamide, 200 mg/kg S.C.	7	51.6 ± 3.0	6	46.0 ± 2.0

* Mean ± S.E.

not as feasible in the intact animal in the case of 2-deoxy-D-glucose or D-galactose since both are metabolically modified in contrast to the pentoses. However both sugars are responsive to insulin(2,4,5) and rates of disappearance from the blood are measurably accelerated by this agent, particularly in the functionally nephrectomized animals in which urinary excretion is absent. Despite the fact that these sugars do respond to insulin (Fig. 1), administration of tolbutamide had no effect on the rate of disappearance of either.

Discussion. Comparison of the volume of distribution of the pentoses in treated and control animals gives an indication of whether the agent under study facilitates passage of these insulin-responsive sugars into intracellular water. This is possible since metabolism has been shown not to be a significant factor in the increased distribution of these pentoses after insulin(10), and no excretion by way of the kidneys is possible in the functionally nephrectomized animals used in the present study.

TABLE II. Effect of Combinations of Tolbutamide and Insulin on Volume Distribution of L(+) Arabinose.

Treatment	No. of rats	Distribution of L(+) arabinose ml/100 g*
Saline control	9	49.1 ± 2.0
Insulin, .5 unit/kg S.C.	6	50.3 ± 2.0
" 2.0 " " "	9	58.8 ± 3.0
Tolbutamide, 200 mg/kg S.C.	7	50.9 ± 1.8
" 200 mg/kg plus insulin, .5 unit/kg S.C.	5	48.8 ± 2.2
Tolbutamide,200 mg/kg plus insulin, 2.0 units/kg S.C.	5	59.1 ± 2.2

* Mean ± S.E.

TABLE III. Comparison of Insulin Effect on L(+) Arabinose Volume Distribution by Intraportal and Subcutaneous Routes.

Treatment	Route	No. of rats	Distribution of L(+) arabinose (ml/100 g)*
Saline control	—	9	49.1 ± 2.0
Insulin, 2 units/kg	Subcutaneous	9	58.9 ± 3.0
Idem	Intraportal	5	58.8 ± 3.2

* Mean ± S.E.

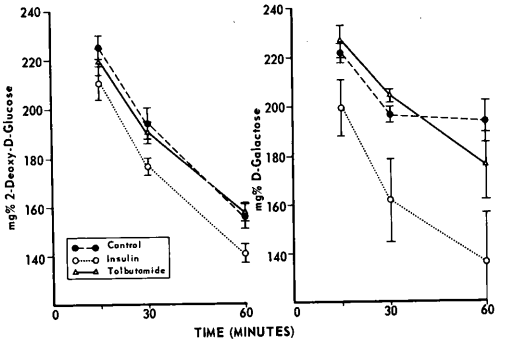

FIG. 1. Effect of tolbutamide and insulin on disappearance of 2-deoxy-D-glucose and D-galactose from peripheral blood. Insulin was given in a dose of 2 units/kg s.c.; tolbutamide 200 mg/kg s.c. Each point is the mean of 6 determinations; brackets indicate standard error of mean.

In view of the considerable evidence indicating that the hypoglycemic sulfonylureas release insulin or an insulin-like substance from the pancreas it is surprising that it should prove so difficult to duplicate the consequences of injected insulin. Other than hypoglycemia no clear parallelism between insulin effects and those resulting from sulfonylurea administration has been consistently noted(1). It is conceivable that the release of insulin by these agents could be small and sustained over such a period so as to preclude demonstration of peripheral effects characteristic of an acute insulin injection. However, many studies indicate the amount of insulin-like material released to be considerable, ris-

ing to a maximum soon after sulfonylurea administration(11,12,13).

It has been postulated that any insulin-like substance released from the pancreas would first reach the liver and exert its major effect at that point, not escaping in sufficient amount to exert any effect at the periphery(14). However, the present study has shown that the rapid injection of insulin intraportally produced effects equal to those of the same dose given subcutaneously. Moreover, despite a large number of investigations the existence or exact nature of a direct insulin effect on the liver remains controversial(15).

The hypothesis that these agents act by release of pancreatic insulin is rendered more attractive by the numerous studies showing them to be ineffective in depancreatized or fully alloxanized animals. However, it is possible to obtain hypoglycemia with sulfonylureas in the absence of the pancreas if the animals are given maintenance amounts of insulin(16,17) indicating that insulin apparently exerts a permissive effect. That is, by virtue of its effect on sugar transport as well as through its other metabolic effects it provides an environment in which these agents may exert their hypoglycemic action.

Summary. Insulin markedly increased the volume of distribution of L-arabinose and D-xylose and accelerated the disappearance of D-galactose and 2-deoxy-D-glucose from the blood in functionally nephrectomized rats. Tolbutamide even in a dose in excess of that necessary to produce marked hypoglycemia was without effect on these sugars. In addition, tolbutamide did not potentiate the effect of suboptimal nor optimal doses of insulin upon the volume distribution of L-arabinose.

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Cellular Damage by Soluble Antigen-Antibody Complexes in Tissue Culture.* (29074)

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Recently, biological action of certain soluble antigen-antibody complexes formed in antigen excess has received increasing attention(1-5); these observations have suggested that the anaphylactic injury not always requires the actual interaction of antigen and

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