No evidence of increased peripheral glucose utilization was found in man when the arteriovenous blood sugar difference was estimated after a glucose meal before and after sulfonylurea medication.

Clinically, generally satisfactory results were obtained in a group of twelve elderly diabetics treated over prolonged periods of time. It appeared that carbutamide was slightly more effective than tolbutamide when equal doses were employed. Only minor and transitory side effects of carbutamide were noted. In general, not more than 20 to 25 units of insulin per day could be replaced by the sulfonylurea derivatives.

# The Effect of Sulfonylureas on the Rates of Metabolic Degradation of Insulin-$I^{131}$ and Glucagon-$I^{131}$ in Vivo and in Vitro

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Recent studies on the hypoglycemic action of various sulfonylureas have not elucidated the mechanism by which their action is effected. However, the absent or diminished response of the blood sugar to these agents in the pancreatectomized or completely alloxan-diabetic animal has suggested that the hypoglycemic effect may be mediated through inhibition of insulin destruction or stimulation of insulin secretion. The possibility of an influence on glucagon secretion or glucagon degradation has also not been excluded. The present study was designed to evaluate the effect of the sulfonylureas on the rates of metabolic degradation of $I^{131}$ labeled insulin and glucagon.**

## METHODS

The preparation of the $I^{131}$ labeled hormones, and the evaluation of alterations induced during preparation have been discussed in detail in previous communications.[1, 2, 3] A small moiety of the labeled hormones is frequently damaged, by irradiation or through other causes, and binds to serum proteins so that it leaves the blood stream less rapidly than the unaltered labeled hormone.[1, 2, 3] Since the fraction altered varies with each preparation, experimental and control studies were performed simultaneously with every lot of labeled hormone, with a single exception noted below. In vivo studies were performed in rabbits fasted for about eighteen hours; in vitro studies were carried out with rat liver homogenates. Equivalent doses of insulin–$I^{131}$ or glucagon–$I^{131}$ were administered intravenously to control rabbits and to sulfonylurea-treated rabbits and its disappearance from the blood stream was followed by an assay of radioactivity in washed trichloracetic acid precipitates of the plasma. The labeled hormones were given three to four hours after oral administration of 1-butyl, 3-p-aminobenzene sulfonylurea (BZ-55, U6987)* or 1-butyl, 3-p-tolylsulfonylurea (U-2043, D860, Orinase) or one to two and one-half hours following the intravenous administration of the sodium salt of Orinase (U7064). Both BZ-55 and Orinase† (orally and intravenously) were used in the insulin–$I^{131}$ experiments but only Orinase sodium (intravenously) was employed in the glucagon $I^{-131}$ studies.

The effect of Orinase sodium was tested on insulinase glucagonase and adrenocorticotropinase activity of rat liver homogenates at various concentrations of

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** We are indebted to Dr. O. K. Behrens and Dr. C. W. Pettinga of Eli Lilly and Company for a generous supply of crystalline regular insulin lot No. 535664 and to Dr. O. K. Behrens for a generous supply of crystalline glucagon.

* We are indebted to Dr. F. B. Peck, Sr., and Dr. W. R. Kirtley of Eli Lilly and Company for a generous supply of BZ-55.

† We are indebted to Dr. Cornelius J. O'Donovan of the Upjohn Company for a generous supply of Orinase.

Orinase, hormone and liver enzyme preparations. Slices of fresh rat liver were minced with two volumes of 0.067 M phosphate buffer, *p*H7.4, in a Virtis homogenizer. The homogenates were centrifuged at 2,000 g. for five minutes and the supernatant portion was employed either without further dilution or diluted again with 0.067 M phosphate buffer. Substrate mixtures containing labeled hormone together with measured amounts of unlabeled hormone were incubated at 37° C. with or without Orinase until temperature equilibrium was obtained. Liver homogenate preparations at 37° C. were then rapidly mixed with substrate solutions. At intervals thereafter, 0.2 ml. aliquots of the mixtures were pipetted into test tubes containing 5 ml. 10 per cent cold trichloracetic acid. Filtrates and precipitates were assayed separately in a 5 ml. capacity well scintillation counter with a sensitivity of 1.00 x $10^6$ C./M./$\mu c$. $I^{131}$ above a background of 200 C./M. Appropriate corrections were made, when necessary, for different volumes employed. All solutions of $I^{131}$ labeled hormones were made up with added pooled human serum albumin to prevent absorption of significant amounts of the labeled hormones to the walls of glass containers.

Venous blood sugar concentrations were determined according to the method of Folin-Malmros.[4]

## RESULTS

*In vivo experiments.* The disappearance of precipitable radioactivity from the plasma of control and sulfonylurea-treated rabbits given insulin–$I^{131}$ is shown in figure 1. In all but two of the sulfonylurea-treated rabbits, the curves showed no distinct differences from those of the control rabbits. In one of the two exceptional cases, the rabbit died during the experiment in spite of the administration of intravenous glucose five minutes previously. In this case, the slow disappearance of precipitable $I^{131}$ is conceivably related to a lowered circulation rate due to shock. In the other exceptional case, a control rabbit was unfortunately not studied with the same lot of insulin–$I^{131}$. It may be of interest that these two animals were the first to be studied in this investigation. Although many subsequent experiments were performed under apparently identical conditions, a slower than normal disappearance was never again observed. It has previously been demonstrated that the disappearance of precipitable radioactivity from the plasma is not

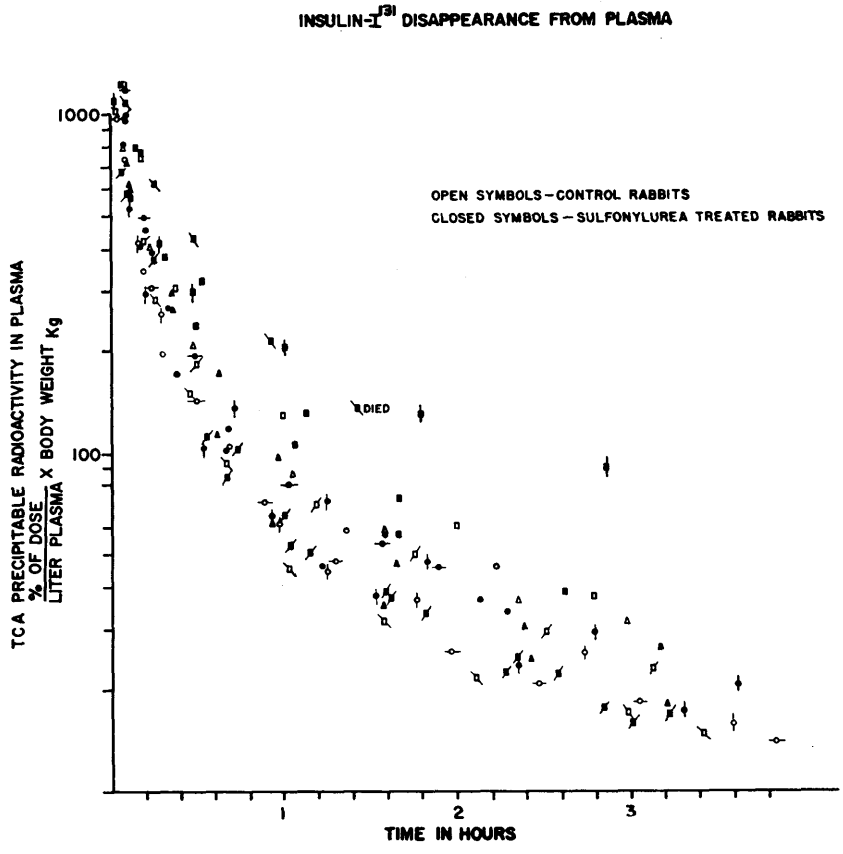



FIG. 1. Disappearance of precipitable radioactivity from plasma following intravenous administration of insulin-$I^{131}$ to control and sulfonylurea-treated rabbits. The top two curves were obtained from rabbits given Orinase. The remainder were given either Orinase or BZ-55. See text for details. Like symbols indicate same lot of insulin-$I^{131}$.

due simply to extravascular distribution or organ concentration but is primarily a manifestation of metabolic degradation[1] as evidenced by the concomitant appearance of labeled monoiodotyrosine and iodide in the plasma.[3]

The disappearance of glucagon–I$^{131}$ was not significantly different in Orinase-treated and control rabbits (figure 2a). The blood sugar responses to equal doses of glucagon (figure 2b) likewise showed no definite differences but it should be noted that the dose level of glucagon employed was apparently much above threshold level. In a previous study[3] significant hyperglycemia was observed with doses one-fourth as large.

*In vitro experiments.* In a variety of experiments in which different concentrations of liver, hormone and Orinase were employed, there was no detectable effect on liver insulinase (figure 3) or glucagonase (figure 4) activity at Orinase concentrations of 1 mg./ml. or less. At Orinase concentrations of 2.5 mg./ml. inhibition of liver insulinase appeared to be slight (figure 3) but became progressively more marked at higher concentrations. At 100 mg./ml. inhibition was virtually complete for both insulinase and glucagonase activities. At this concentration adrenocorticotropinase activity was also completely inhibited (figure 5).

## DISCUSSION

The inactivation of insulin by tissue extracts was demonstrated by Mirsky and Broh-Kahn;[5] and Mirsky and associates[6] subsequently made a thorough study of the kinetics of the rat liver insulinase system. Mirsky[7] reported a noncompetitive inhibition of this system with the sulfonylureas and Mirsky, Diengott and Dolger[8] had suggested that this might be the mode of action by which the sulfonylurea drugs produce a hypoglycemic effect. However, Vaughan[9] working with insulin concentrations of 200 μg. per ml., failed to find any inhibition of insulinase activity in whole liver homogenates or partially purified insulinase systems with Orinase at concentrations of about 0.13 to 0.8 mg. per ml. or with BZ-55 at the latter concentration.

The present studies confirm not only that Orinase

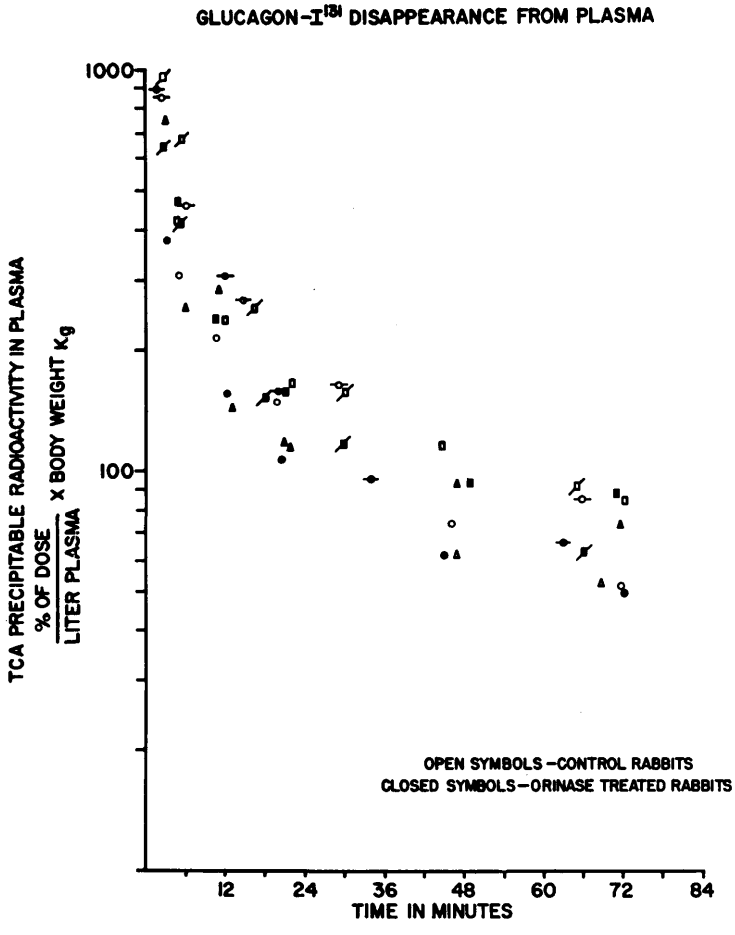



FIG. 2a. Disappearance of precipitable radioactivity from plasma following intravenous administration of glucagon-I$^{131}$ to control and Orinase-treated rabbits. Like symbols indicate same lot of glucagon-I$^{131}$. Spiked symbols = 125 mg. Orinase per kg. body weight. Unspiked symbols = 250 mg. Orinase per kg. body weight.

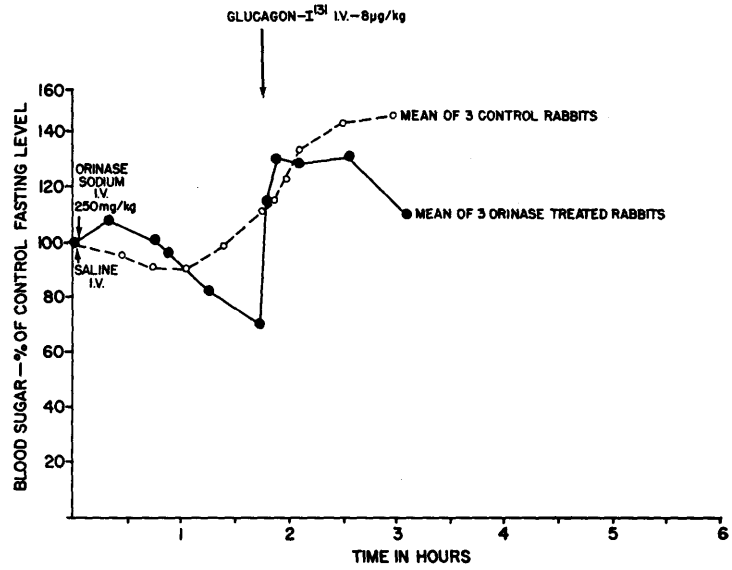
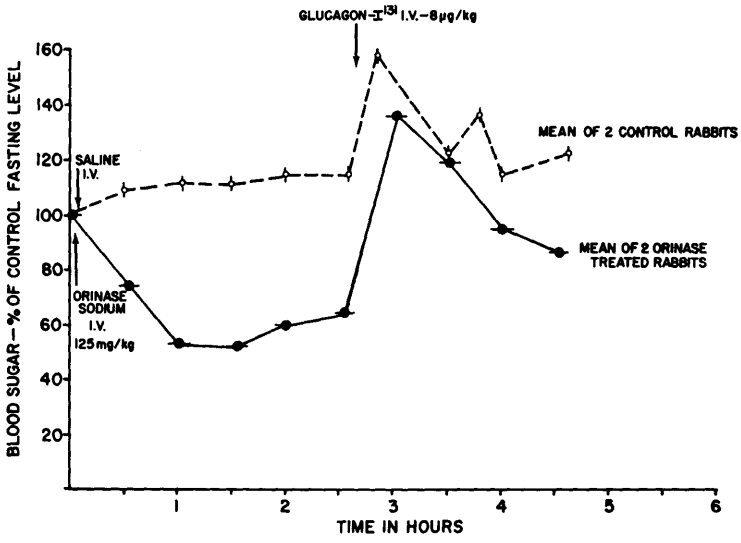



FIG. 2b. Blood sugar curves of same rabbits as in figure 2a.




in high concentrations is a strong inhibitor of liver insulinase but also indicate that at these concentrations it is also an effective inhibitor of liver glucagonase and adrenocorticotropinase activity as well. However, in agreement with Vaughan,[9] the observations reported here indicate that at concentrations which are sufficient to produce hypoglycemia, inhibition of insulinase or stimulation of glucagonase activity is not detectable. The blood levels of Orinase following oral administration of effective doses (25 to 100 mg. per kg. body weight) to dogs, generally do not exceed 0.1 to 0.25 mg. per ml. plasma,[10] but in the liver homogenate system it was not until concentrations of 2.5 mg. per ml. were reached that inhibition of insulin destruction was observed. The possibility that the sulfonylureas might be more concentrated in liver than in the plasma is not supported by the absence of concentration of $S^{35}$ labeled Orinase reported by Baender and Scholz.[11] Even if such hepatic concentrations were obtained, the in vivo experiments reported here indicate that doses as high as 250 mg. per ml. given intravenously are generally insufficient to inhibit the destruction of insulin or glucagon. It is possible that the two exceptional cases in the insulin–$I^{131}$ series represent instances in which there was such an inhibition, but the absence of inhibition in the majority of animals studied makes it seem unlikely that the

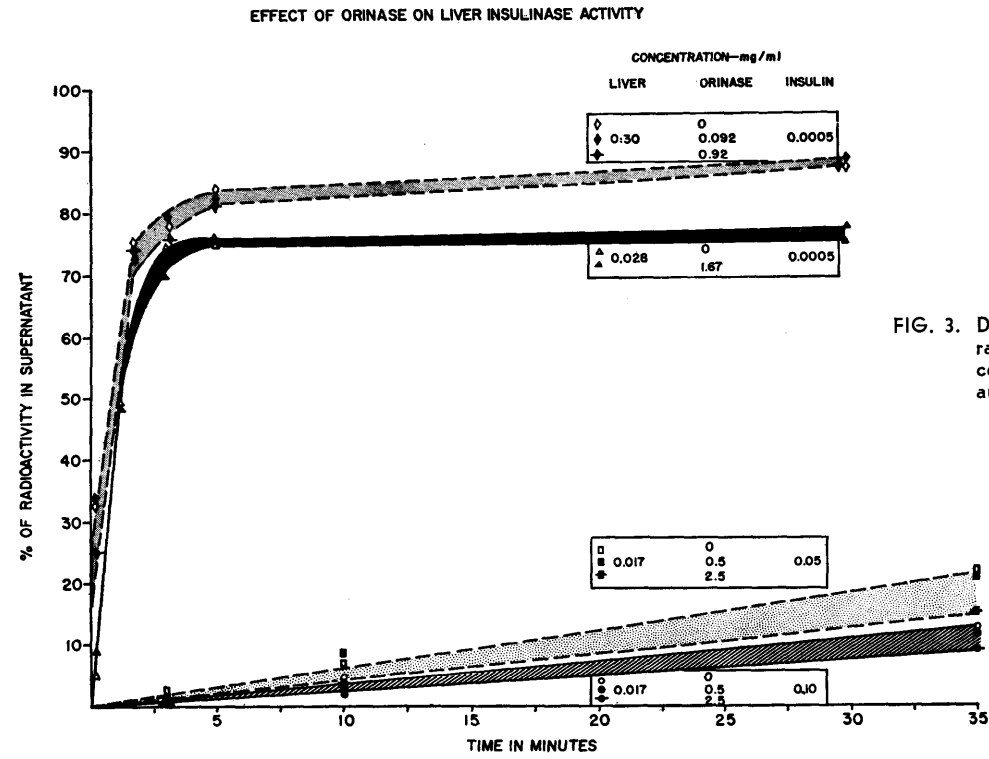



FIG. 3. Degradation of insulin-I$^{131}$ by rat liver homogenate at various concentrations of liver, insulin and Orinase.

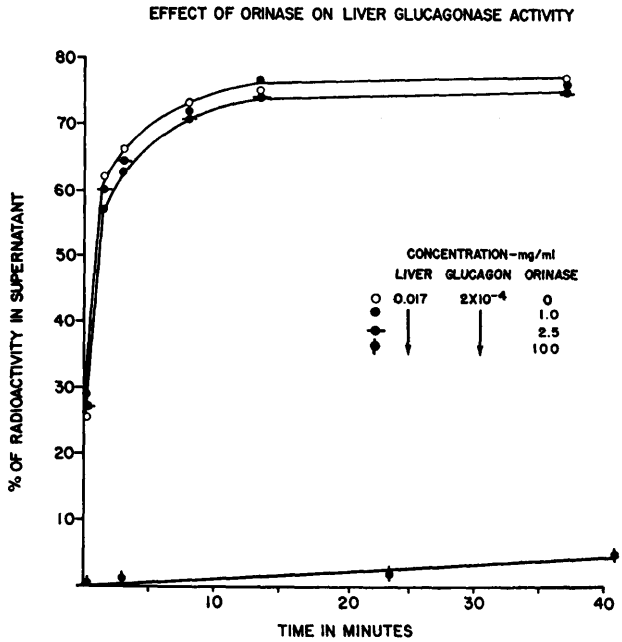



FIG. 4. Degradation of glucagon-I$^{131}$ by rat liver homogenate.

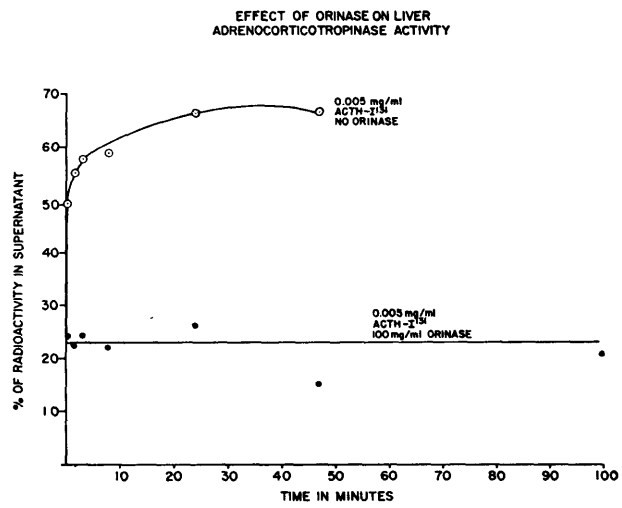



FIG. 5. Degradation of ACTH-I$^{131}$ by rat liver homogenate. Twenty per cent of this preparation of labeled hormone was not precipitable in control experiments without liver homogenate.

hypoglycemic action of the sulfonylureas is dependent on this mechanism.

The studies of Rall, Sutherland and Wosilait[12] have suggested that glucagon might act by stimulating some portion of the liver phosphokinase* system and

* This is the abbreviation employed by this group for dephosphophosphorylase kinase, a liver enzyme capable of reactivating liver phosphorylase after it has been inactivated (by removal of phosphate) by liver phosphorylase-inactivating enzyme. Both inactivating and reactivating enzymes have been identified and partly purified in their laboratory.

Vaughan's observations,[9] that in the presence of Orinase (0.8 mg. per ml.), glucagon fails to stimulate glucose formation in liver slices, led her to suggest that the effect of Orinase might be mediated through an inhibition of phosphokinase. The in vivo experiments of the present study do not support this action since the hyperglycemic response to glucagon was not any less marked in the sulfonylurea-treated rabbits than in the control group, although the possibility that a difference might be observed with smaller doses than were employed cannot be excluded.

Some suggestion of inhibition of glucose release by rabbit liver slices in the presence of BZ–55, 0.25 mg. per ml., was reported by Clarke and associates[13] although this effect was not observed by Vaughan[9] in the absence of glucagon or epinephrine even at higher concentrations of Orinase. Clarke and associates[13] observed a much more striking inhibition of cytochrome oxidase in liver slices at BZ-55 concentrations of 0.5 and 1.5 mg. per ml. Hawkins and coworkers[14] reported a significant depression of glucose-6-phosphatase activity, which Vaughan[9] had also suggested as an alternate explanation to the phosphokinase inhibition for the effects observed on release of glucose from the liver slice.

Both evidence and speculations about inhibition of a variety of hepatic enzymes are therefore not lacking from in vitro studies. Furthermore, the inhibiting effect on three apparently different hormone degrading enzyme systems observed in the present study questions the specificity of action of sulfonylurea on any enzyme system. Since it has been observed that insulin competes in both the glucagon and adrenocorticotropin systems and that glucagon competes in the insulin system,[15] the three hormone degrading activities may all be referable to a single hormonolytic enzyme. Nevertheless, the inhibitory effect of Orinase on this or these enzyme or enzymes, on cytochrome oxidase, and on one or more of the enzymes involved in the carbohydrate cycle, strongly suggests that the sulfonylureas may, in high concentrations, act as general enzymatic poisons. If so, the demonstration of any anti-enzyme effect in vitro is not sufficient to explain its hypoglycemic action unless this can be shown by experiments to result from the specific anti-enzyme effect in vivo at the lowest effective dose. By these criteria the action of sulfonylureas cannot be attributed to their anti-insulinase effect.

## SUMMARY AND CONCLUSIONS

1. The administration of BZ–55 (orally) and Orinase (orally and intravenously) to rabbits, in doses sufficient to induce significant hypoglycemia, does not alter the rate of metabolic degradation of $I^{131}$ labeled insulin or glucagon.

2. At Orinase concentrations of 1 mg. per ml. or less there is no detectable effect on rat liver insulinase or glucagonase activity. At significantly higher concentrations of Orinase there is inhibition of liver insulinase, glucagonase and adrenocorticotropinase.

3. It is concluded that the lowest concentrations of Orinase capable of inhibiting degradation of insulin and glucagon by rat liver homogenates are significantly in excess of those likely to be obtained by doses effective in producing a fall in blood sugar in the intact animal.

4. The hyperglycemic response to glucagon (8 μg. per kg. body weight) was not inhibited by the intravenous administration of Orinase.

## ACKNOWLEDGMENTS

We are indebted to Mrs. Katherina Newerly, Mrs. Eunice Kratzer and Miss Marguerita Pascullo for the blood sugar determinations. We also wish to thank Mrs. Melanie Knopf and the staff photographers of the Medical Illustration Department of the Veterans Administration Hospital for the illustrations. The authors also acknowledge with thanks the secretarial assistance of Mrs. Frieda Steiner and Miss Eve Spelke, and the technical assistance of Mr. Carl Bacot and Mr. Herbert Lew.

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# Intrapancreatic Infusions of the Sulfonylureas

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The influence of the sulfonylureas on the pancreas has been studied by introduction of these compounds into the pancreatic arterial circulation in twenty-seven experimental and control dogs. Small amounts of carbutamide or tolbutamide were injected into the pancreas, femoral vein or portal system. Injection was made into the pancreatico-duodenal artery through one of its branches, the right gastroepiploic artery, in such a manner that the pancreatic blood flow was not impaired. Peripheral venous blood glucose was estimated at intervals following infusion of amounts ranging from 3 to 71 mg. per kg. of body weight over periods of twenty or ninety minutes.

Hypoglycemic responses were obtained, the degree varying directly with the dose administered and the resulting blood sulfonamide level. Carbutamide in a dosage of 7 mg. per kg. of body weight given into the pancreas produced an average reduction in blood sugar of 20 per cent, even though the peripheral blood sulfonamide level (2 to 4 mg. per cent) was below the usual therapeutic range (10 to 15 mg. per cent). The same dose failed to lower the blood glucose when injected into the femoral vein in control animals subjected to sham pancreatic operations. Tolbutamide injected into the pancreatic artery in a dosage as low as 3 mg. per kg. of body weight also caused the blood sugar to fall. Hyperglycemia occurred when a blank solution lacking the compound was infused into the portal vein. The addition of a 7 mg. per kg. dose of carbutamide to this infusion produced no lowering of the blood sugar elevation.

Biopsies of the pancreas taken before and after infusion were examined by Professor W. Stanley Hartroft of Washington University Medical School. No significant histological changes in alpha or beta cells were reported. Insulin assays of pancreatic tissue performed by Dr. Carl Kuether of the Lilly Research Laboratories have not yet been completed.

These studies indicate that the sulfonylurea compounds cause hypoglycemia by some pancreatic mechanism, not as yet elucidated, and that the direct exposure of the liver to them causes no such effect.

From the Department of Medicine, Northwestern University Medical School, Chicago, Illinois.

Summary of paper presented at the Third Conference on Carbutamide in Indianapolis on Sept. 13, 1956. Much of this material has been published in *Metabolism*.