Studies on the Disposition of Blood Glucose

A Comparison of Insulin and Orinase

James Ashmore, Ph.D., George F. Cahill, Jr., M.D., A. Scott Earle, M.D., and S. Zottu, B.A., Boston

INTRODUCTION

The aromatic sulfonylureas elicit a hypoglycemic response similar to insulin when administered to normal animals. In order to learn more of the mechanism by which the blood glucose is lowered, the effects of Orinase and insulin on the disposition of blood glucose in normal dogs and rats have been compared.

PORTAL-HEPATIC SUGARS IN UNANESTHETIZED DOGS

Preparation of animals. Mongrel dogs weighing from 17 to 21 kg. were anesthetized with intravenous Nembutal, 30 mg./kilo. Respiration was maintained during the intrathoracic portion of the procedure using a positive pressure pump and endotracheal tube. Aseptic technic was maintained throughout the surgical procedure.

The operation has been described in some detail since it has proved a useful and successful technic in studying hepatic physiology. The upper abdomen was entered through a short midline incision, the spleen removed, and the splenic artery and vein isolated. Two cannulae (consisting of a tip of 205 gauge polyethylene tubing 12 cm. long joined to a 60 cm. length of nonkinking "Genflex" plastic tubing by a larger gauge polyethylene cuff) were threaded into the splenic artery and vein and guided respectively into the aorta and portal vein. The cannulae were sutured in place to the cut ends of the splenic vessels through a heat-flared polyethylene cuff. In this way the less reactive polyethylene tip lay within the vessel. The cannulae were filled with dilute heparin, closed at the distal ends with variously colored aluminum plugs, and passed subcutaneously over the right rib margin and beneath the skin of the right lateral thorax, where they were found during the subsequent thoracotomy. The abdominal wound was closed and the animal placed in the left lateral position.

During the second step the right chest was entered, excising the lowermost rib joining the sternum. After retracting the lung, the inferior vena cava was carefully dissected free where it pierces the diaphragm to enter the chest. The largest hepatic vein was identified at the entrance to the cava from the left. The cava, opposite the junction with this vein, was caught in a miniature caval clamp and incised. Fine cotton mattress sutures were placed on both sides of the venotomy. A cannula similar to those previously described but with a 6 cm. tip, and with a malleable copper-wire obturator inside, was threaded into the cava through the venotomy incision while the mattress sutures were held taut to prevent blood loss. The cannula was guided into the largest left hepatic vein and the mattress sutures tied. The tip of the cannula lay approximately 4 cm. within the liver so that the possibility of contamination with caval blood was minimized. The obturator was removed and the cannula filled with dilute heparin and plugged. The thoracotomy wound was closed and the three cannulae were threaded subcutaneously to the midback between the scapulae, through the skin, and into a plastic box held by wire sutures (figure 1).

Six hours later, and then twice daily, the cannulae were filled with heparin which gradually diffused into

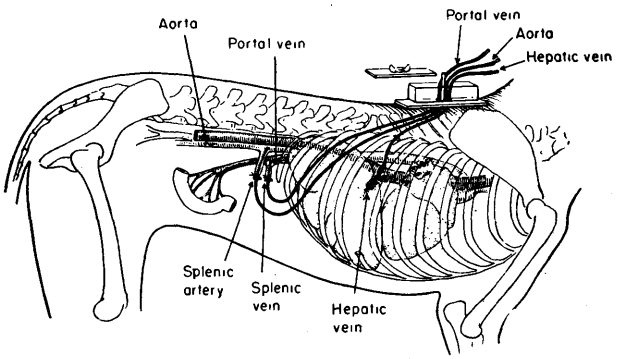

FIG. 1. Schematic drawing showing the technic used in arterial, portal and hepatic vein cannulae in dogs.

From the Departments of Biological Chemistry and Surgery, Harvard Medical School, Boston, Massachusetts.

Parts of this article are reprinted with permission from "Studies on the Disposition of Isotopic Glucose *in Vivo* and *in Vitro* Under the Influence of Sulfonylureas" by James Ashmore, George F. Cahill, Jr., and A. Scott Earle, which appeared in *The Effects of the Sulfonylureas and Related Compounds in Experimental and Clinical Diabetes, Annals of the New York Academy of Sciences,* Volume 71, Article 1, pages 131-40.

the circulation to provide local and systemic heparinization. The animals were given penicillin and streptomycin postoperatively. Blood samples could be collected from such cannulae at any time, intra- or postoperatively, to study whatever facet of liver physiology desired.

Portal-hepatic changes. Experiments were begun on the third to fourth day following operation and done in the following order: On the first experimental day a glucose load was given portally and the hepatic, portal and arterial samples drawn simultaneously at 5, 15, 30, 45 and 60 minutes. The cells were immediately separated and the plasma analyzed for glucose, potassium and inorganic phosphate. Glucose was determined by the method of Nelson[1] on Somogyi[2] filtrates of the plasma; potassium was measured on an internal standard flame photometer, and inorganic phosphate by the method of Fiske and Subbarow.[3] The glucose tolerance of these dogs was found to be normal in all cases.

On the second day, glucagon was injected via the portal catheter and the study repeated. These tests were performed to ascertain that the catheters were in place and operating satisfactorily. On the third day, 7 units of glucagon-free insulin (Lilly) were given intraportally and the experiment repeated. On the fourth day, 500 mg. of sodium Orinase (Upjohn) was injected and the experiment again repeated.

The effects of insulin and Orinase are compared in figure 2. Dog 3 represents a typical experiment in a series of four animals studied. Both substances caused a rapid fall in portal and hepatic glucose, and comparable degrees of hypoglycemia were obtained in all experiments. It was observed that in the case of hypoglycemia produced by insulin, hepatic glucose levels were always appreciably higher than portal values, but following Orinase there was a corresponding fall in both portal and hepatic glucose.

Portal-hepatic differences have been plotted in figure 3 as a function of time following insulin (top curves) and following Orinase (bottom curves). After insulin, the liver released glucose into the hepatic venous blood, and potassium and inorganic phosphate were initially taken up and then released as hypoglycemia persisted. No significant portal-hepatic differences in glucose, potassium or inorganic phosphate were observed with Orinase.

In figure 4, portal glucose concentration has been plotted as a function of hepatic glucose concentration. These points represent all analyses from a series of four dogs. The line at 45° indicates equal glucose in portal and hepatic plasma. All points above this line would represent concentrations of hepatic glucose greater than

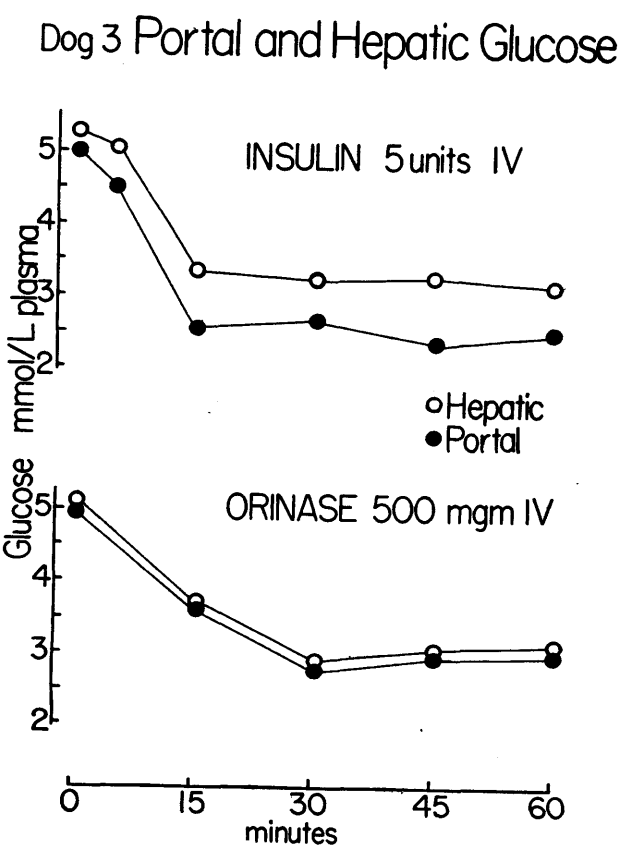

FIG. 2. Changes in hepatic and portal glucose are plotted as a function of time following injection of 5 units of glucagon-free insulin (top curves) and 500 gm. sodium Orinase (lower curves). Dog 3 represents a typical experiment in a series of four animals studied.

portal glucose; all points below the line represent higher portal than hepatic glucose. Insulin hypoglycemia (open circles) appears to result in hepatic glucose release; conversely, there was no evidence of hepatic glucose production after Orinase (closed circles) and it appears that the liver removes glucose from the circulation. Similar results on the effect of Orinase on portal and hepatic glucose have previously been reported by Purnell et al.[4]

Calculation of hepatic glucose production. Assuming that the hepatic blood flow does not change appreciably after insulin and Orinase administration, hepatic glucose production has been calculated on the basis of a maximal and a minimal hepatic blood flow of 3 and 1 liters/kilo./hour.[5] Such calculations have been summarized in tables 1 and 2. In a typical unanesthetized dog (table 1) blood samples drawn over the period of one hour indicate an average glucose production of 6.4 mg./kilo./min., varying between 4.4 and 8.7 mg./kilo./min. for mean values. Individual samples drawn from a series of five animals (table 2) show an average produc-

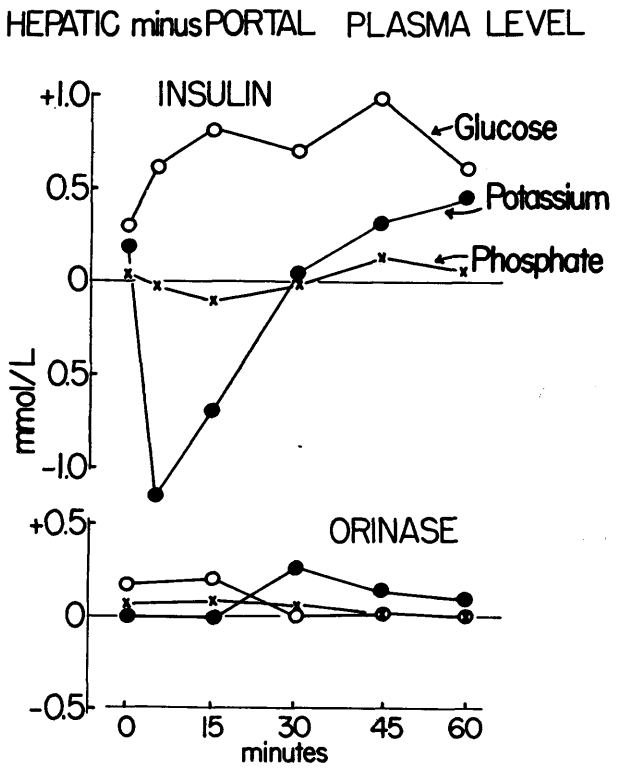

FIG. 3. Hepatic-portal plasma differences in glucose (o), potassium (●) and phosphate (x) are plotted following injection of 5 units of insulin (top curves) and 500 mg. sodium Orinase (lower curves). These data were obtained on Dog 3 and represent a typical experiment in a series of four animals studied.

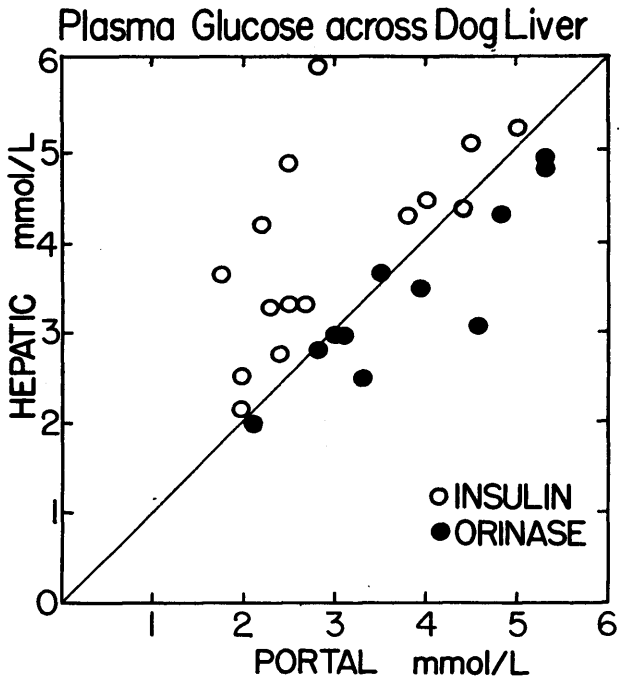

FIG. 4. Hepatic plasma glucose in mmoles/L. has been plotted against portal plasma glucose. These points were obtained by withdrawal of simultaneous portal and hepatic blood samples in a series of four dogs with portal and hepatic vein catheters. Samples obtained after insulin injection (o): after Orinase (●).

tion of 6.1 mg./kilo./min. and vary between 2.4 and 9.6 mg./kilo./min. After insulin injection, hepatic glucose production continues at a normal or slightly accelerated rate; while after Orinase, hepatic glucose production appears to be inhibited. Values for glucose production after glucagon (0.05 mg./kilo. Lilly) are presented for comparison. In this case there was a threefold increase in hepatic glucose production.

It should be pointed out that Orinase injection may be associated with an alteration in blood flow, as suggested by Purnell et al.[4] Even if the flow were to change, this would not alter the interpretation of our data since there was no evidence of any hepatic glucose production in the Orinase injected animals.

Hepatic glucose production in normal and depancreatized dogs has previously been determined by Lipscomb and Crandall[5,6] using London cannulae[7] for portal and hepatic sampling. Values for normal hepatic glucose production obtained by these investigators were lower (2 mg./kilo./min.) than those obtained in this study. One possible explanation for this difference might be that their animals were starved for several days while the dogs used in this study were without food for only twelve to fifteen hours prior to the experimental period.

DISTRIBUTION OF BLOOD GLUCOSE IN RATS

Preparation of animals. Normal male rats of the Wistar strain weighing 200 to 250 gm. were used in these studies. The animals were lightly anesthetized with ether, the femoral vein exposed, and 1 mg. (1.8μ Curies) of randomly labeled glucose-C^{14} injected in 0.5 ml. saline. When the effects of insulin and Orinase were studied, these compounds were injected in the same solution with the glucose. Only glucagon-free insulin (Lilly) was used and at a dose of 0.25 units/animal. Sodium Orinase (Upjohn) was given at a dose of 20 mg./animal.

Blood samples (0.2 ml.) were obtained from the tail at 5, 15, 30, 45 and 60 minutes after injection. A single Somogyi filtrate was prepared and 1 ml. of this filtrate analyzed for reducing sugar. Carrier glucose (10 mg.) was added to another aliquot of the filtrate and glucose isolated as the phenylosazone. This was recrystallized, plated and assayed for C^{14}.

Specific activity of blood glucose. When the logarithm of blood glucose specific activity was plotted as a func-

TABLE 1

Hepatic glucose production in a normal unanesthetized dog over a period of one hour

Time	Blood glucose Portal	Hepatic Arterial		Hepatic Glucose Output Max.	Min.	Mean
	(mg./100 ml.)			(mg./kg./min.)		
	No injection					
9:00	104	115	104	6.6	2.2	4.4
9:20	104	126	114	13.0	4.4	8.7
9:40	115	128	120	7.8	2.6	5.2
10:00	109	127	106	11.0	3.6	7.3
					Average	6.4
	After insulin intraportally					
9:00	90	96	92	3.6	1.2	2.4
9:05	81	91	87	6.0	2.0	4.0
9:15	45	60	47	9.0	3.0	6.0
9:30	47	59	48	7.2	2.4	4.8
9:45	42	60	48	11.0	3.6	7.3
10:00	44	56	47	7.2	2.4	4.8

TABLE 2

Hepatic glucose production in normal unanesthetized dogs after insulin, Orinase or glucagon

	Blood glucose Portal	Hepatic Arterial		Hepatic Glucose Output Max.	Min.	Mean
	(mg./100 ml.)			(mg./kg./min.)		
Uninjected						
	108	119	116	6.6	2.2	4.4
	90	96	92	3.6	1.2	2.4
	99	119	102	12.0	4.0	8.0
	80	104	83	14.0	4.8	9.6
	104	115	104	6.6	2.2	4.4
					Average	6.1
Sixty minutes after insulin						
	39	76	47	22.0	7.4	15.0
	68	78	76	6.0	2.0	4.0
	42	60	48	11.0	3.6	7.2
					Average	8.6
Sixty minutes after Orinase						
	70	63	68			0
	38	35	34			0
	55	55	54			0
					Average	0
Fifteen minutes after glucagon						
	216	252	218	22.0	7.2	14.0
	130	155	131	15.0	5.0	10.0
	200	282	270	49.0	16.0	33.0
					Average	19.0

tion of time in fed normal rats after injection with saline and glucose-C^{14}, a typical linear decay curve was obtained (figure 5). When insulin and glucose-C^{14} were injected, the decay curve was more rapid. The fall in specific activity resulted from a dilution with unlabeled glucose. When sodium Orinase was injected, the fall in specific activity was identical with that of the saline-injected controls. The same degree of hypoglycemia was produced by insulin and Orinase.

Cori and Cori[8] have demonstrated that insulin hypoglycemia causes epinephrine release from the adrenal medulla and glycogen breakdown. We had reasoned that if this were the case, dilution of blood glucose could be minimized by using fasted rats. The experiments were therefore repeated in animals that had been fasted for twenty-four hours prior to the injection. The results of such experiments are given in figure 6. Once again, insulin caused a greater dilution than that obtained with Orinase or saline. As in the previous experiments, the same degree of hypoglycemia was produced by both insulin and Orinase.

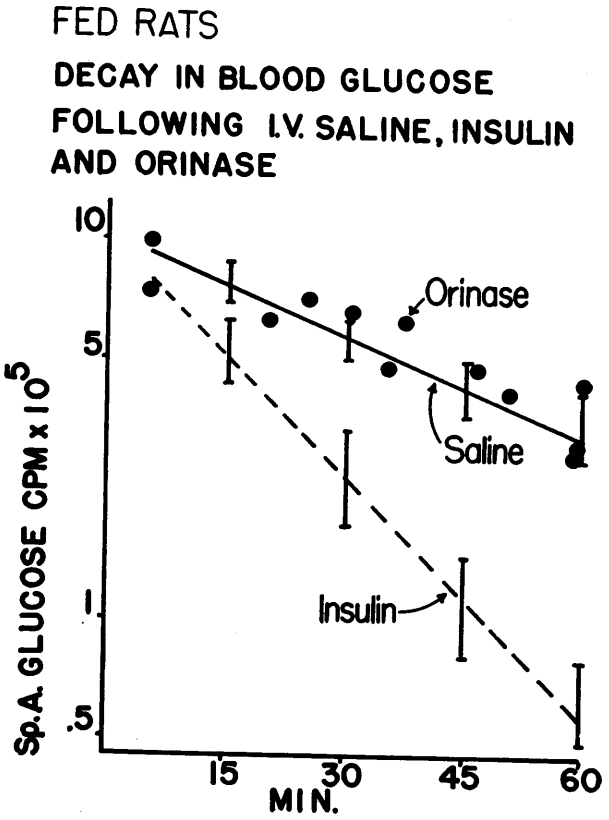

FIG. 5. Log. specific activity of blood glucose has been plotted as a function of time in variously treated fed normal rats after injection of 1 mg. 1.8 μC glucose-C^{14}. Each curve represents the mean of three experiments. Total spread of points is indicated (I). Individual points following Orinase injection (●) have been plotted.

The experiments were next repeated in animals that had been adrenalectomized four hours previous to the injection of labeled glucose. Such a time arrangement was selected in order to minimize any effect of diminished adrenocortical function. The results of these experiments are given in figure 7. Although insulin given to adrenalectomized animals still resulted in a more rapid fall in blood glucose specific activity than that observed in the saline-injected controls, the dilution effect

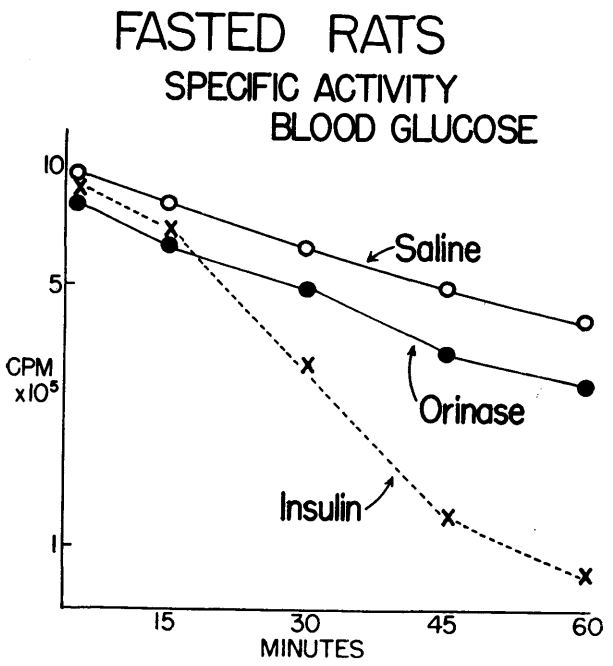

FIG. 6. Changes in specific activity of blood glucose after injection of saline (o), 20 mg. of Orinase (●) and 0.25 units of insulin (x) to fasted rats are shown. Conditions are identical with those given in figure 4.

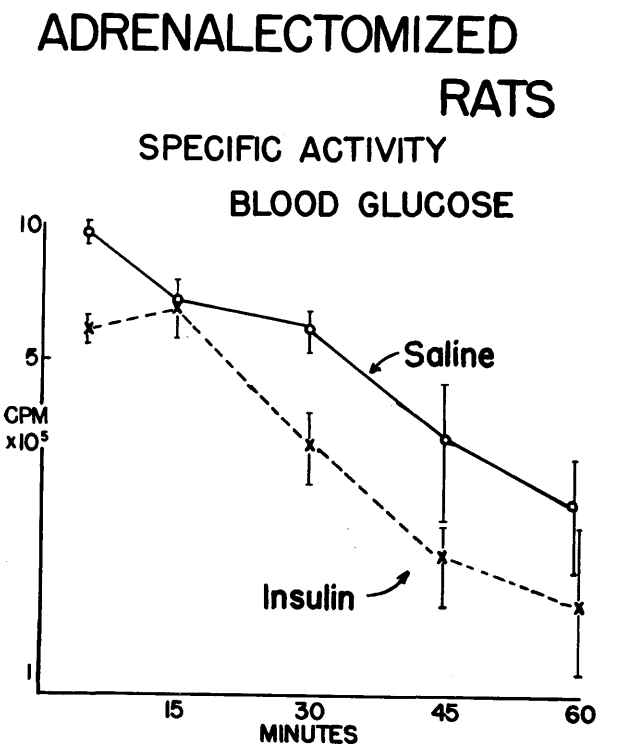

FIG. 7. Changes in specific activity of blood glucose after intravenous administration of saline (top curve) and insulin to adrenalectomized rats. Each curve represents the mean of three animals and total spread of points is indicated.

observed was not as marked as that in normal animals.

Effect of insulin and Orinase on liver and muscle glycogen. Male rats, 220 to 250 gm., were fasted for twenty-four hours and injected as described in the preceding section with glucose-C^{14}, plus either insulin, Orinase or saline. At the end of one hour the animals were stunned, exsanguinated, and liver and muscle samples removed and placed in hot 30 per cent potassium hydroxide. Tissue glycogen was isolated and hydrolysed to glucose[9] which was determined colorimetrically[1] and assayed for C^{14} as the phenylglucosazone.[10] The results of these experiments are given in table 3. The animals injected with insulin had deposited very little of the C^{14} in liver glycogen. Orinase-injected animals, however, deposited as much, if not significantly more C^{14} in liver glycogen than the saline-injected controls. In the case of muscle, insulin resulted in appreciably more C^{14} deposition into glycogen than did Orinase. Again both insulin and Orinase produced the same degree of hypoglycemia.

TABLE 3

Action of insulin and Orinase on the incorporation of C^{14} glucose into liver and muscle glycogen in fasted normal rats

Injection	Liver Glycogen		Muscle Glycogen	
	μM./gm.	cpm./gm.	μM./gm.	cpm./gm.
Saline	13	1,450	34	19
	3	585	18	19
Insulin	3	9	44	320
	9	25	11	242
Orinase	15	2,100	12	52
	6	2,200	16	43

Although the hypoglycemia produced in sixty minutes was the same, there was a marked difference in the rate of fall of blood glucose after insulin and Orinase. After intravenous insulin, there was an immediate drop in blood glucose with hypoglycemia continuing for the balance of the sixty-minute period. With Orinase, the glucose concentration fell slowly over the experimental period. In order to obviate effects that might result from this difference in rate of fall of blood glucose, we compared the effects of insulin administered subcutaneously with Orinase injected intravenously. Under these conditions the same rate of blood glucose change was produced by both compounds (figure 8). Changes in the specific activity of blood glucose in these animals are recorded in the lower curves of figure 8. As in previous experiments, the decay of glucose specific activity was quicker with insulin than with saline or Orinase, indicating again a more rapid dilu-

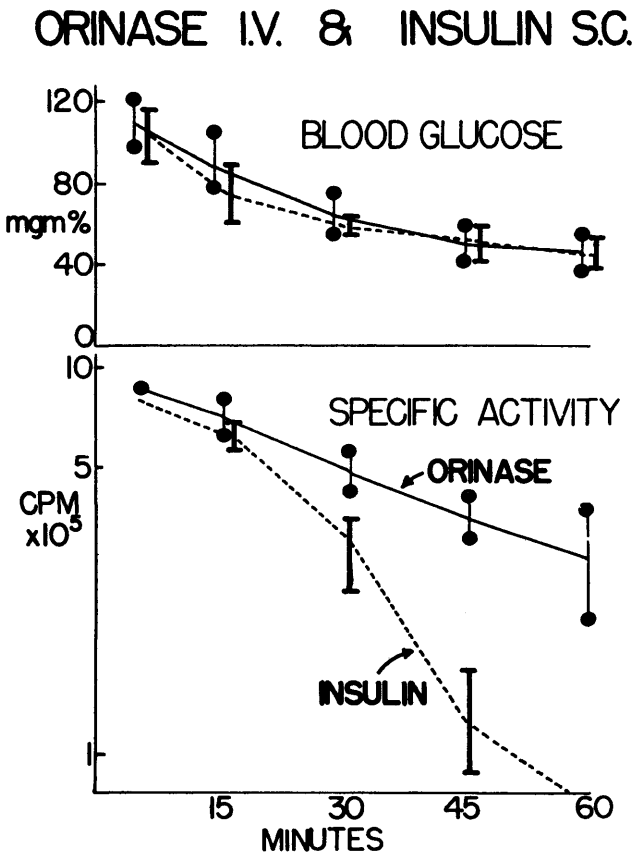

FIG. 8. Changes in concentration of blood glucose (top curves) and specific activity of blood glucose (lower curves) after Orinase (intravenous) and insulin (subcutaneous) administration to fed normal rats. Each curve represents the mean of three animals and total spread of points is indicated.

TABLE 4

Action of insulin and Orinase on the incorporation of C^{14} glucose into liver and muscle glycogen in fed normal rats

Injection	Liver Glycogen		Muscle Glycogen	
	μM./gm.	cpm./gm.*	μM./gm.	cpm./gm.*
Saline (4)	191	100±33	19	29±13
Insulin (5)	88	14±6.3	19	262±59
Orinase (4)	145	143±58	18	61±18

* ±S.E.

TABLE 5

Action of insulin and Orinase on the incorporation of C^{14} glucose into liver and peripheral fatty acids in fed normal rats. All values expressed as cpm./gm. fatty acid

	Liver	Peripheral
Saline (4)	300	375
Insulin (5)	940	1,770
Orinase (4)	1,770	475

TABLE 6

Action of insulin on the incorporation of C^{14} glucose into liver and muscle glycogen of adrenalectomized rats

Injection	Liver Glycogen		Muscle Glycogen	
	μM./gm.	cpm./gm.	μM./gm.	cpm./gm.
Saline	32	24	10	8
	78	42	25	50
	50	17	28	29
Insulin	93	13	20	480
	6	0	21	325
	38	11	33	545

tion of the labeled blood glucose.

Liver and muscle samples were removed at the end of the experimental period and total glycogen and C^{14} content determined. Insulin resulted in little of the C^{14} from the injected glucose being incorporated into liver glycogen, but increased C^{14} incorporation into muscle glycogen (table 4). Although blood glucose fell to the same level with Orinase as with insulin, Orinase did not significantly increase the incorporation of C^{14} from blood glucose into muscle glycogen.

In these animals liver and peripheral fatty acids were recovered after saponification and assayed for C^{14} content. Mean values, expressed as counts/min./gm. fatty acid, are given in table 5. Both insulin and Orinase increased the activity of liver fatty acids, but only insulin increased the activity of peripheral fatty acids.

Values for C^{14} content of liver and muscle glycogen of adrenalectomized rats are given in table 6. Administration of insulin with the labeled glucose did not cause an increase in the activity of liver glycogen, but appreciably increased incorporation of label into muscle glycogen.

DISCUSSION

The experiments with intact dogs have shown that the hypoglycemia resulting from Orinase administration is accompanied by a cessation of hepatic glucose output. The rat experiments corroborate these findings. A fall in specific activity in blood glucose reflects dilution by new unlabeled glucose added to the circulation by the liver. The hypoglycemia after Orinase administration to rats is associated with a fall in blood glucose specific activity which is equal in rate to that of the untreated rat. However, a reduction of the total circulating glucose in the hypoglycemic Orinase treated animal to approximately one-half that of the control would likewise necessitate a reduction in the rate of inflow of un-

labeled glucose from the liver if the activity of circulating glucose were to fall at a normal rate.

On the other hand, insulin administration to dogs resulted in a slightly increased rate of hepatic glucose production. Likewise, the insulin-treated rat shows a markedly accelerated decay of blood glucose specific activity.

A decrease in hepatic glucose production following Orinase administration to normal animals might have one of several meanings. Since the phenomenon of glycogenolysis following insulin hypoglycemia is believed to be mediated by the adrenal medulla,[8] the possibility exists that Orinase in some way prevents a normal hepatic response to epinephrine. Such a conclusion is further strengthened by observations that Orinase will reduce the rate of glycogenolysis in liver slices stimulated by epinephrine.[11, 12] The possibility also exists that Orinase may prevent glucose release from the liver by inhibiting glucose-6-phosphatase or some other enzyme system. In vitro experiments on glucose-6-phosphatase indicate that such a mechanism is most unlikely.[13, 14]

Berson and Yalow[15] have recently reported similar results. Following the disappearance of labeled glucose in rabbits injected with glucose-C^{14}, they concluded that the sulfonylureas produced hypoglycemia without increasing the rate of glucose removal, in contrast to insulin which produced hypoglycemia with an accelerated rate of glucose removal from the blood stream.

Apart from any hepatic mechanism, insulin and Orinase differ in their ability to stimulate peripheral glycogen deposition and lipogenesis. When quantities of insulin and Orinase which produce identical blood glucose changes were compared, insulin markedly stimulated incorporation of C^{14} from labeled glucose into muscle glycogen and peripheral fatty acids. Orinase did not cause a significant increase in C^{14} content of either peripheral glycogen or fatty acids.

SUMMARY

Insulin hypoglycemia in normal dogs is associated with an increased glucose production by the liver. Orinase hypoglycemia is associated with a diminished glucose production and frequently with an uptake of glucose by the liver.

In normal rats, insulin hypoglycemia causes a more rapid disappearance of radioactive glucose as measured by the decay in specific activity of blood glucose. Orinase hypoglycemia is accompanied by a decreased rate of decay in the total activity of circulating glucose.

Recovery of the radioactive carbons from glucose in glycogen and fat, both in the liver and periphery, suggests that insulin provokes incorporation of glucose into muscle glycogen and peripheral fat. Orinase treatment does not produce this effect and must lower blood sugar by other physiologic mechanisms.

SUMMARIO IN INTERLINGUA

Studios in Re le Disposition de Glucosa de Sanguine: Un Comparation de Insulina e Orinase

Hypoglycemia a insulina in canes normal es associate con un augmento del production de glucosa per le hepate. Hypoglycemia a orinase es associate con un diminuite production de glucosa e frequentemente con le acceptation de glucosa per le hepate.

In rattos normal, hypoglycemia a insulina causa un plus rapide disparition de glucosa radioactive, mesurate per le disintegration de activitate specific de glucosa del sanguine. Hypoglycemia a orinase es accompaniate per un relentation del degeneration del activitate total de glucosa circulante.

Le recovration de carbones radioactive ab glucosa in glycogeno e grassia, tanto in le hepate como etiam in le peripheria, suggere que insulina provoca le incorporation de glucosa in le glycogeno muscular e le grassia peripheric. Tractamento a orinase non produce iste effecto. On debe concluder que illo reduce le sucro de sanguine per altere mechanismos physiologic.

ADDENDUM

Since the preparation of this manuscript, "Studies on the Utilization of Uniformly Labeled-C^{14} Glucose by Rats Given Tolbutamide" has been published by W. L. Miller, Jr., J. J. Krake and M. J. Van der Brook (J. Pharm. Expt. Therap. [1957], *119*:513). These authors have compared the effects of insulin and Orinase on the disposition of labeled blood glucose over a six-hour period. They observed that after six hours both insulin and Orinase increased the incorporation of C^{14} from glucose into muscle glycogen. However, in agreement with these studies, only Orinase increased the incorporation of C^{14} into liver glycogen.

ACKNOWLEDGMENT

This work was supported in part by the United States Atomic Energy Commission and Swift and Company.

REFERENCES

[1] Nelson, N.: A photometric adaptation of the Somogyi method for determination of glucose. J. Biol. Chem. *153*:375, 1944.

[2] Somogyi, M.: J. Biol. Chem. *160*:69, 1945.

[3] Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. *66*:375, 1925.

[4] Purnell, R., Arai, Y., Pratt, E., Hlad, C., Jr., and Elrick, H.: Some observations on the mode of action of Orinase. Metabolism *5*:778, 1956.

[5] Lipscomb, A., and Crandall, L. A., Jr.: Hepatic blood flow and glucose output in normal unanesthetized dogs. Am. J. Physiol. *148*:302, 1947.

[6] Crandall, L. A., Jr., and Lipscomb, A.: A direct measurement of hepatic glucose production in experimental diabetes mellitus. Am. J. Physiol. *148*:312, 1947.

[7] London, I. S.: Angiostomie und Organstoffwechsel, Moscow, 1935.

[8] Cori, C. F., and Cori, G. T.: The influence of insulin and epinephrine injection on hexosephosphate content of muscle. J. Biol. Chem. *94*:581, 1931.

[9] Good, C. A., Kramer, H., and Somogyi, M.: The determination of glycogen. J. Biol. Chem. *100*:485, 1933.

[10] Hastings, A. B., Teng, C.-T., Nesbett, F. B., and Sinex, F. M.: Studies on carbohydrate metabolism in rat liver slices. J. Biol. Chem. *194*:69, 1952.

[11] Vaughan, M.: *In vitro* studies on the action of sulfonamide hypoglycemic agents. Science *123*:885, 1956.

[12] Berthet, J., Sutherland, E. W., and Makman, M. H.: Observations on the action of certain sulfonylurea derivatives. Metabolism *5*:768, 1956.

[13] Ashmore, J., Cahill, G. F., Jr., and Hastings, A. B.: Inhibition of glucose-6-phosphatase by hypoglycemic sulfonylureas. Metabolism *5*:774, 1956.

[14] Kuether, C. A., Scott, E. G., Martinez, C., Lee, H. M., and Pettinga, C. W.: Studies of the effect of carbutamide on glucose-6-phosphatase. Diabetes *6*:26, 1957.

[15] Berson, S. A., and Yalow, R. S.: Some remarks on the mechanism of action of the sulfonylureas. Diabetes *6*:274, 1957.

Mechanism of Weight Loss by Amphetamine

The use of sympathomimetic drugs as appetite depressants began with the observations of physicians who noted weight reduction as a side effect in patients undergoing amphetamine treatment for other illness.

In attempts to explain this appetite-depressing phenomenon, the following mechanisms have been suggested by various investigators: (1) increased basal metabolism, (2) increased general metabolism, (3) diuresis, (4) incomplete digestion or absorption of food, and (5) reduced food intake from appetite reduction.

In one experiment seven obese and two nonobese subjects were fed three meals a day ad libitum for two eight-week periods, separated by a four-week vacation, and were given either 5 mg. of D- or 10 mg. of DL-amphetamine one hour before meals, during the second half of each eight-week period. Both voluntary caloric intake and body weight were reduced. During the first eight weeks of the experiment, the subjects lost an average of 5.87 lb.—2.44 lb. during the four-week control period, 2.79 lb. during the first week of treatment, and 3.43 lb. over four weeks of medication. In the second eight-week experiment, the results were 2.01 lb. lost during the four control weeks, 2.74 lb. during the first week of medication, and 5.50 lb. over four weeks of treatment.

To assess any toxic or metabolic effects of amphetamine, ten nonobese men were given 3,000 calories per day for fourteen weeks. From day 27 to 56 inclusive, each man received 5 mg. of D- or 10 mg. of DL-amphetamine before each meal. An average weight loss of 0.7 lb. occurred during the control period. On medication, the group averaged, during the first week, a loss of 0.9 lb. but the average loss for the first fifty-six days of treatment was 1.8 lb., only 0.4 lb. less than if the control rate had continued. This is interpreted as indicating an increased total metabolism during the first week of treatment. Supporting this view were reports of wakefulness, during the first week, by eight of the ten subjects.

The mechanism of action of this drug on the central nervous system remains obscure. No significant changes in blood glucose tolerance were observed in human subjects, either immediately or weeks after amphetamine treatment. There was no indication of intoxication. Addition of barbiturates allayed the restlessness of certain patients without inhibiting the anorexia. Thus, the possibility that anorexia was a product of cerebral excitation seems to be eliminated.

The question of whether amphetamine has a specific inhibitory effect upon an "appetite center" or a specific stimulatory effect upon an "appetite inhibiting center" might be answered by testing the drug on carefully prepared subjects.

Tolerance to the anorexigenic drugs often occurs. Some clinicians seem to prefer to use the drugs until they are tolerated, hoping that the patient will have formed new eating habits by that time. Others feel that judicious reduction of the caloric intake of the patient (by as little as 10 per cent) remains the method of choice.

From "Mechanism of Weight Loss by Amphetamine" in *Nutrition Reviews* *14*:71-72, March 1956.