Action of Insulin and Tolbutamide on Blood Glucose Entry and Removal

Gerson Jacobs, M.D., George Reichard, B.A., Edward H. Goodman, Jr., Bernice Friedmann, M.A., and Sidney Weinhouse, Ph.D.
Philadelphia

Although it is generally recognized that the blood sugar of the fasting animal is maintained constant by a precise balance between entry and removal rates, it is only recently, with the advent of C^{14}-labeled glucose, that reliable estimations of the rates of these processes have become possible. The availability of tracer methods has also greatly facilitated studies of the mechanism of action of agents which affect the blood sugar level. Such studies, by Searle and Chaikoff,[1] Henderson et al.,[2] Wall et al.,[3] Berson et al.,[4] and Dunn et al.[5] have shed considerable light on the relationships between blood glucose level and its entry and removal rates, and the effects thereon of various hormones. In our own studies,[5] we have been struck by the marked and immediate action of insulin in suppressing the entry of new glucose into the blood of the fasting, normal dog. As a further step in the investigation of this presumably hepatic action of insulin, its effect on the normal and diabetic human has been studied, and its action on the entry and removal rates of blood glucose has been compared with that of the hypoglycemic drug, tolbutamide (Orinase).* These studies are described in the present report.

Though the methods used by different investigators differ in experimental details, they all are similar in principle. Briefly summarized, our method involves the intravenous injection of a "trace" dose of glucose-C^{14} to label the blood glucose. Samples of blood are then removed at intervals for estimation of blood glucose levels and specific activity. The changes in specific activity of the blood glucose, determined by a method involving the specific oxidation of glucose carbon to formic acid, allow calculation of entry and removal rates. Our experimental procedure, and the principles and calculations involved, have been described in our previous study with dogs.[5]

The subjects used in the present study were thirty middle-aged and elderly patients of a chronic disease home and clinic of a large community hospital.* Those classified as nondiabetic had no obvious endocrine disorder, and had a normal two-hour postprandial blood sugar concentration. The thirteen diabetic patients were non- or mildly obese, and were responsive to insulin and tolbutamide. All of the subjects were fasted for at least fourteen hours prior to an experiment, and the diabetics had not received insulin for forty-eight hours. During the course of the experiments they were comfortably at rest in a bed or chair; no food or medication was given and care was taken to avoid any apprehension.

The uniformly C^{14}-labeled glucose (glucose-U-C^{14}) was purchased from the Volk Radiochemical Company. An amount equivalent to 100 microcuries was dissolved in 10 ml. of sterile water and was injected into an arm vein. Samples of blood were removed from a vein in the other arm at regular, frequent intervals. A No. 20 spinal needle ground to a length of 1.5 inches, carrying a stylet moistened with a heparin solution was used. It was possible to keep the needle in the vein throughout the experiment without creating any difficulty in withdrawing blood samples of 2 ml. each.

Usually, three samples were collected at fifteen-minute intervals. After the forty-five-minute blood sample was removed, glucagon-free insulin† or Na-tolbutamide‡

* 1-Butyl-3-p-tolylsulfonylurea.

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From the Department of Radiology, The Albert Einstein Medical Center, Northern Division, The Department of Chemistry, Temple University, and The Institute for Cancer Research, Philadelphia, Pennsylvania.

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†Kindly supplied by Eli Lilly and Company, Indianapolis, Indiana.

‡Kindly supplied by Dr. C. J. O'Donovan of The Upjohn Company, Kalamazoo, Michigan.

was rapidly injected intravenously through the same indwelling needle. Details of the insulin and tolbutamide dosages are given in the legends of the individual figures. In some of the experiments the insulin was injected subcutaneously into an arm. Blood samples were then removed rapidly (usually at five-minute intervals) for twenty minutes and then every ten minutes for one-and-a-half hours. In the diabetics, urine sugars and acetone were determined at the end of the experiments.* Usually twelve to fourteen blood samples were collected through the same indwelling needle.

RESULTS

Data on the patients used in the six experiments to be reported are given in table 1.

To illustrate the procedure used and to provide a background for the type of information obtained from these experiments, a study of glucose turnover in a nondiabetic individual is described in detail. The patient, an elderly female (F.C.), suffering from posterior lateral sclerosis, was given an intravenous injection of 18 mg. (101 μC.) of glucose, with a specific activity of 12.5 $\times$ 10^6 c.p.m., and blood samples were withdrawn at regular intervals during the ensuing 130 minutes. Results are given in figure 1. Throughout the period the blood sugar remained essentially constant, at approximately 100 mg./100 ml., while the specific activity fell from an extrapolated initial value of 12,000 to 4,800 c.p.m. From the initial dilution of the glucose specific activity, the glucose pool size was estimated to be 12.5 $\times$ 10^6 $\times$ 18/12,000 = 18,600 mg. From the average glucose concentration of 100 mg. per 100 ml., we can calculate that the volume occupied by the 18.6 gm. glucose pool was 18.6 liters. This corresponds to 18.6 $\times$ 100/71 = 25 per cent of the body weight.

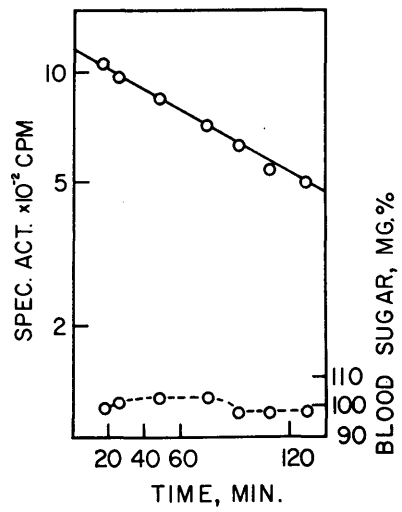

FIG. 1. Blood glucose turnover in a nondiabetic individual (F.C.). Blood sugar level, broken line, right ordinate; specific activity, solid line, left ordinate plotted on a logarithmic scale. Radioactive glucose was administered at time zero.

In figure 1 the logarithm of the specific activity is plotted against time. The linear fall in activity is indicative of a constant rate of replacement of glucose which follows the kinetics of a first order reaction and allows

*At no time was the loss of glucose from the body pool into the urine significant, and ketonuria was never observed.

TABLE 1

Data on patients used in experiments described in figures 1 to 6

Patient	Diagnosis	Sex	Age	Weight	Fasting Blood Sugar	Pool	Glucose Space	Turnover rate
			Years	Kg.	Mg./100 ml.	Gm.	Liters	Mg./100 ml./min.
F.C.	Posterior lateral sclerosis, minimal	F	61	71	100	19	19	0.7
Y.N.	Multiple sclerosis, minimal	F	60	55	100	12	12	1.2
M.R.	Parkinsonism, moderately advanced	M	57	50	90	12	13	0.8
S.I.	ASHD, class III Prostatectomy and orchiectomy for non-metastatic carcinoma of prostate	M	85	66	110	20	16	1.0
A.L.	Diabetes mellitus	M	70	60	250	42	17	1.9
M.W.	Diabetes mellitus	F	68	53	170	19	11	1.6

the calculation of a replacement rate of 0.71 mg. per 100 ml. per minute.[5]

The values of glucose pool size, volume of the pool, and replacement rate thus calculated for all of the experiments here described are listed in table 1. These agree well with those reported by us and others for various species,[6-11] and are typical of values obtained in forty experiments with thirty patients.

EFFECT OF INSULIN ON NONDIABETIC AND DIABETIC HUMANS

Figure 2 shows the results of an experiment in which insulin was administered intravenously to a nondiabetic patient (Y.N.). Again the postabsorptive state was characterized by a logarithmic fall of blood glucose specific activity while the blood sugar level remained constant at 102 mg. per 100 ml. At forty-six minutes, 20 units of glucagon-free insulin was rapidly injected intravenously. The blood sugar began to fall five minutes after injection, and in fifteen minutes it reached the low point of 40 mg. per 100 ml. It rose slowly thereafter, but seventy minutes later it was still depressed. Coinciding with the drop in blood sugar of 62 mg. per 100 ml., there was a clear "plateau" in the specific activity, signifying that glucose was not entering the blood. Coincident with the "leveling off" of the blood sugar concentration, the specific activity resumed its downward trend, and while the blood sugar gradually rose, its specific activity continued to drop at a gradually reducing rate.

Since glucose was not entering the blood during the initial fifteen-minute period of hypoglycemia, we can calculate that the glucose removal rate was (104—42)/15 or 4.1 mg. per 100 ml. per minute. On the basis of a glucose space of 21 per cent in this patient, this equals 8.6 mg. per kilo per minute, which is more than three times the original turnover rate before injection of insulin. The resumption of a downward trend in the specific activity of the blood glucose indicates that after the initial phase of hypoglycemia, glucose again enters the blood, and this is reflected in the return of the blood sugar toward normal. During the period following the plateau, in which the blood sugar is slowly rising and the specific activity is falling, the turnover was calculated by means of Equations Two and Three as described previously.[5] The rates thus calculated are presented graphically in the inset of figure 2. Though the values thus obtained are regarded as only rough approximations, they provide a clear insight into the immediate effects of insulin. One effect is the rise in removal of glucose to three times the original rate. The other immediate response was the complete inhibition of glucose entry. During the recovery period the entry rate was slightly higher than the removal rate; this small difference accounts for the gradual rise toward the normal glucose level. In all aspects these results are virtually identical with those reported previously with normal dogs.[5]

Figure 3 shows essentially the same picture in a diabetic patient (A.L.) given insulin intravenously. The

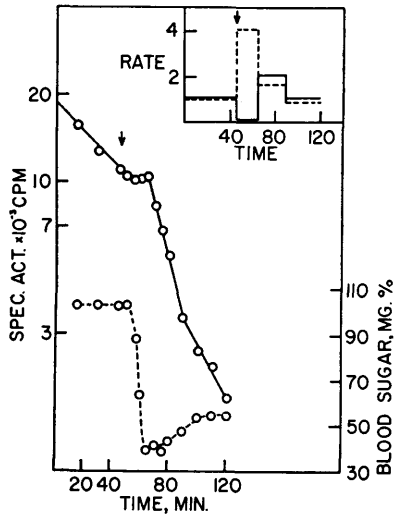

FIG. 2. Time course of blood glucose concentration and specific activity plotted as in figure 1. Arrow indicates time of intravenous administration of 20 units of insulin to a nondiabetic patient (Y.N.) weighing 55 kilos. Inset: Time course of blood glucose entry and removal rates as affected by insulin administration. Entry rate, solid line; removal rate, broken line. The values are given in mg. per 100 ml. per minute.

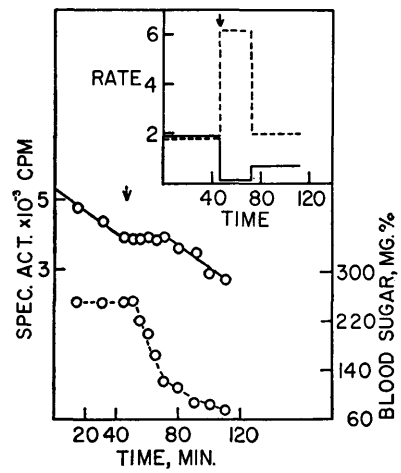

FIG. 3. Same experiment as figure 2, with a diabetic patient (A.L.), weighing 60 kilos and given 6 units of insulin at the arrow.

blood sugar was at a higher level initially, of course, but remained fairly constant, as did the rate of drop in specific activity prior to insulin injection. It is of interest that the postabsorptive turnover rate in this diabetic patient was over twice that of the normal patient of experiment 1. In general, the turnover rates were at least as high, and often higher in the diabetic than in the nondiabetic patients. Five minutes after insulin injection, the blood sugar dropped rapidly, and was still dropping at the termination of the experiment one hour later. During the first eighteen minutes, the blood sugar fell 126 mg. per 100 ml., and the specific activity "plateaued" indicating suppression of glucose entry. Hence the removal rate was $126/18 = 7.0$ mg. per 100 ml. per minute, or about three times the pre-insulin rate. Thereafter, the blood sugar continued to drop, falling to 72 mg. per 100 ml. in the next thirty-eight minutes. During this final period, the specific activity resumed its downtrend, indicating a resumption of glucose entry. The calculated approximate rates of entry and removal are shown in the inset.

EFFECTS OF TOLBUTAMIDE

Figure 4 demonstrates the effect of intravenous tolbutamide in a nondiabetic patient (M.R.). Qualitatively, the pattern of response is strikingly similar to that of insulin. After a ten-minute delay, and exactly coinciding with the period of blood sugar fall, there is a "plateauing" of specific activity. Thus, like insulin, tolbutamide suppresses the output of glucose. The rates calculated for this experiment, shown in the inset, however, demonstrate a remarkable difference between the two substances, in that removal of glucose was not enhanced by tolbutamide. Repeatedly, it has been observed in similar experiments that the entry rate drops to zero but the removal rate does not increase significantly on tolbutamide injection.

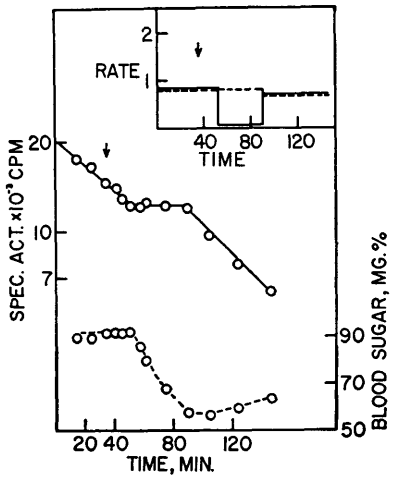

FIG. 4. Blood sugar changes in a nondiabetic patient (M.R.) weighing 50 kilos, given 1.2 gm. Orinase intravenously at the arrow.

Figure 5 demonstrates the effects of intravenous tolbutamide in a diabetic patient (M.W.). Despite the difference in initial glucose level the result is quite similar to the previous experiment. There was initially a logarithmic postabsorptive drop in specific activity, a plateau in specific activity during the hypoglycemic phase following tolbutamide injection, then a resumption of the logarithmic drop in specific activity. Again, the entry was suppressed and the removal rate did not increase.

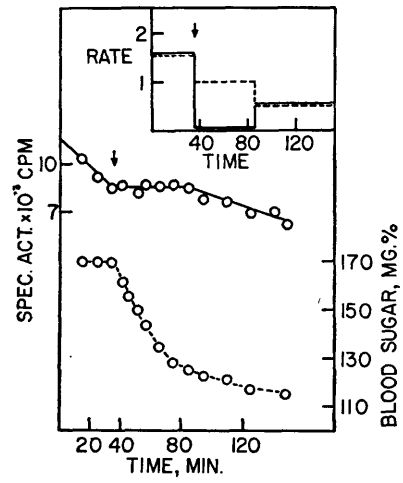

FIG. 5. Same as figure 4, but for a diabetic patient (M.W.) weighing 53 kilos, given 1.0 gm. Orinase intravenously at the arrow.

In every experiment with tolbutamide the same results were obtained. Even when a high dose of 45 mg. per kg. of tolbutamide was rapidly administered intravenously to a normal individual, leading to a mild hypoglycemic reaction, there was no increase in the glucose removal rate.

EFFECT OF SUBCUTANEOUS INSULIN

As discussed previously,[5] one may assume that during fasting essentially all of the glucose which enters the blood comes from the liver, and essentially all which leaves the blood enters peripheral tissues, e.g., brain, muscle, etc. Thus, on this basis, insulin simultaneously and rapidly suppresses hepatic glucose output and stimulates its utilization in peripheral tissues. In contrast tolbutamide, while exerting the former effect, does not appear to influence the latter. At first glance one might assume that this argues against the participation of in-

sulin in the hypoglycemic action of tolbutamide. Indeed, in experiments similar in principle to the present ones, Berson et al.[4] obtained identical results and assumed the Orinase was acting independently of insulin. However, the evidence that tolbutamide acts through insulin, either by eliciting its secretion[12-15] or by preventing its destruction,[16-18] seems hardly disputable. The possibility occurred to us that endogenous insulin, whether elicited physiologically such as during hyperglycemia, or with a drug such as tolbutamide, might exert an hepatic effect but not a peripheral effect.* To test the possibility that a slow, regular introduction of insulin to the bloodstream might give a response different from a rapid intravenous injection, experiments similar to those described above were carried out, but with subcutaneous insulin administration. As shown in figure 6, this hypothesis was clearly confirmed. The results are representative of four such experiments, each on a different individual, and one of whom was a diabetic. Following a normal, fasting blood sugar level and turnover rate, 10 units of insulin was given. Beginning ten minutes later, there was a drop in blood sugar, from the initial level of 105 mg. to a low point of 54 mg. per 100 ml. fifty-two minutes later. During this entire period, there was no decrease in specific activity, indicating a complete suppression of hepatic glucose output. The rate of blood sugar fall thus corresponds to a removal rate of 51 mg. per fifty-two minutes or 1 mg. per minute per 100 ml. This is almost exactly the same as the removal rate prior to insulin injection, and thus clearly demonstrates that insulin, when slowly released to the blood, exerts an hepatic, but not a peripheral action. Another distinguishing feature of subcutaneous insulin administration in these experiments was the unusual lengths of the "plateaus" of specific activity. With intravenous injection of even very large doses, entry rates were not suppressed for longer than twenty minutes, whereas with subcutaneous injection, suppression lasted from forty to sixty minutes.

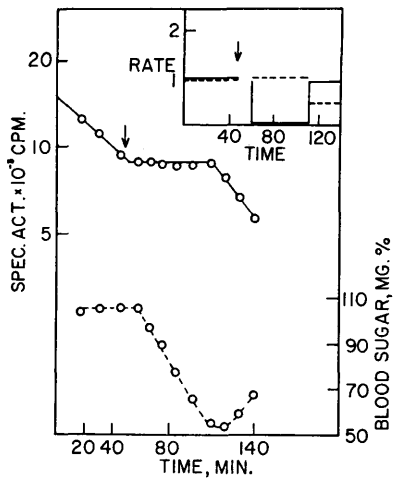

FIG. 6. Effect of 10 units of insulin given subcutaneously to a nondiabetic patient weighing 66 kilos, on the time course of blood glucose concentration and specific activity plotted as in figure 2. Arrow indicates time of insulin administration.

*This possibility has also been considered by Bressler and Engel[19] and has been suggested also by Elrick and Purnell.[20]

DISCUSSION

In agreement with previous work with normal dogs[5] the present study has shown that insulin exerts two immediate effects when given intravenously to diabetic and nondiabetic humans. It completely suppresses the entry of new glucose molecules into the blood and it increases their rate of removal. Since both manifestations have consistently coincided in time with the immediate hypoglycemic action of the hormone, there is little doubt that both are responsible for the hypoglycemia. The significance of these findings to the role of insulin and its mechanism in regulating hepatic glucose output, and to the part this aspect of insulin action may play in clinical diabetes, has been discussed.[5]

In demonstrating that insulin suppresses hepatic glucose output in the human, these results confirm those of Bearn, Billing and Sherlock.[21] By comparing the glucose levels in the blood entering and leaving the liver of normal and diabetic humans, these investigators found an immediate suppression of glucose output following intravenous insulin injection. Recently, Searle et al.[22] also reported, in a preliminary communication, a lowering of hepatic glucose output in the liver of humans immediately following insulin or tolbutamide injection.

On the other hand, Ashmore et al.[23] have recently reported results which are in conflict with the interpretation of the present study. Using an ingenious operative procedure designed to sample hepatic vein blood, these investigators found that tolbutamide diminished hepatic glucose production, but insulin actually increased glucose output. Further support for an increase in hepatic glucose production in response to insulin injection was obtained in C^{14}-glucose turnover studies in rats.

The reason for these discrepancies is not clear; however, in regard to the turnover studies in rats, the suggestion might be offered that, since samples were removed at fifteen-minute intervals, a "plateau" in blood glucose specific activity might have been missed. In some instances we observed a ten-minute "plateau" followed by an accelerated output in our experiments with normal dogs.[5] In a private communication Dr. Ashmore has in-

formed us that they have also observed "plateaus" in blood C^{14}-glucose specific activities of normal dogs following insulin injection.

The hepatic and peripheral effects of tolbutamide have recently been thoroughly reviewed by Stadie.[24] Whenever it has been tested for its action on liver, a suppressive effect on glucose output has been noted.[17,20,26] This action of tolbutamide has been confirmed in the present study, which has also shown that this suppressive effect coincides in time with the hypoglycemic effect and is principally or wholly responsible for the lowering of the blood sugar. The lack of influence of tolbutamide on peripheral glucose utilization noted also by others,[20,28,29] coupled with the virtual certainty that this drug requires insulin for its hypoglycemic action, suggested to us that the physiological action of insulin secreted by the pancreas may also be exerted principally or exclusively on hepatic glucose output. This theory gains plausibility when one considers that the pancreatic secretions drain into the portal vein and must traverse the liver before entering the general circulation. Attempts are being made to test this hypothesis directly by measuring the effect of intraportally administered insulin on the blood glucose turnover of experimental animals.

In the meantime, experiments of the type displayed in figure 6 have clearly shown that the slow release of insulin to the bloodstream following a subcutaneous injection of the hormone has little if any effect on the peripheral utilization of glucose, but causes a sustained suppression of hepatic glucose output.*

For many years the role of the liver in insulin action has been controversial.[cf.32-34] Though recent work from our laboratory has largely discounted a primary role of insulin in liver glycogen storage[35] the present results, coupled with our previous results[5] and those of Bearn et al.[21] suggest that its effect on hepatic glucose output may be more imporant in the physiological regulation of the blood sugar level than its effect on peripheral utilization.

SUMMARY

Diabetic and nondiabetic humans were given a "trace" dose of uniformly C^{14}-labeled glucose and blood samples were removed at frequent and regular intervals before and after administration of insulin or tolbutamide. Before insulin or tolbutamide injection, the logarithmic drop of specific radioactivity coincident with a constant blood glucose concentration indicated a constant rate of replacement of the blood glucose. The "turnover" rates, at 1 to 2 mg. per 100 ml. per min. were at least as high in the diabetic as in the normal subjects. On intravenous insulin injection, there was an immediate, transient suppression of glucose entry and an approximately threefold increased removal rate. With intravenous tolbutamide, glucose entry was suppressed as with insulin, but the removal rate was unaffected. This action of tolbutamide was similar to that of subcutaneously injected insulin, which also caused a suppression of blood glucose entry without affecting its removal. The data are in accord with an action of tolbutamide in stimulating insulin secretion, and are regarded as emphasizing the role of insulin on hepatic glucose output in the physiological action of the hormone.

SUMMARIO IN INTERLINGUA

Action De Insulina E De Tolbutamido Super Le Entrata De Glucosa In Le Sanguine

Humanos diabetic e nondiabetic recipeva doses "traciatori" de glucosa uniformemente marcate con C^{14}, e specimens de sanguine esseva prendite serialmente a intervallos regular, ante e post le administration de insulina o tolbutamido. Ante le injection de insulina o tolbutamido, le descendita logarithmic del radioactivitate specific, in le presentia de constante concentrationes de glucosa sanguinee, indicava un constante intensitate del reimplaciamento de glucosa sanguinee. Le magnitude del reimplaciamento—1 a 2 mg per 100 ml per minuta—esseva al minus tanto alte in diabeticos como in subjectos normal. Post le injection intravenose de insulina, il occurreva immediatemente un suppression transiente del entrata de glucosa insimul con un acceleration approximativemente triple in le processo eliminatori. Post le injection de tolbutamido, le entrata de glucosa esseva supprimite como in le caso del injection de insulina, sed le intensitate del elimination remaneva inalterate. Iste action de tolbutamido esseva simile al action de insulina in administrationes subcutanee, le qual etiam supprime le entrata de glucosa in le sanguine sin afficer le elimination de illo. Le datos se trova de accordo con le conception que tolbutamido stimula le secretion de insulina. Illos es interpretate como apte a relevar le importantia—in le functiones physiologic de insulina—del effecto de iste hormon super le rendimento hepatic de glucosa.

*Miller et al.[30] also noted that the effects of insulin injected subcutaneously on blood pyruvate and lactate levels more nearly resembled the effects of tolbutamide than they resembled those of intravenous insulin. Dulin and Johnston[31] reported that in contrast with rapidly intravenously injected insulin, neither a slow intravenous insulin injection nor tolbutamide increased the muscle glycogen of eviscerated rats. These results also may be explained on the basis of a primarily hepatic action of insulin in low dosage.

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REFERENCES

[1] Searle, G. L., and Chaikoff, I. L.: Inhibitory action of hyperglycemia on delivery of glucose to the blood stream by liver of the normal dog. Am. J. Physiol. *170*:456, 1952.

[2] Henderson, M. J., Wrenshall, G. A., and Odense, P.: Effects of insulin on rates of glucose transfer in the depancreatized dog. Canad. J. Biochem. and Physiol. *33*:926, 1955.

[3] Wall, J. S., Steele, R., deBodo, R. C., and Altszuler, N.: Effect of insulin on utilization and production of circulating glucose. Am. J. Physiol. *189*:43, 1957.

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

[5] Dunn, D. F., Friedmann, B., Maass, A. R., Reichard, G. A., and Weinhouse, S.: Effects of insulin on blood glucose entry and removal rates in normal dogs. J. Biol. Chem. *225*:225, 1957.

[6] Feller, D. D., Strisower, E. H., and Chaikoff, I. L.: Turnover and oxidation of body glucose in normal and alloxan-diabetic rats. J. Biol. Chem. *187*:571, 1950.

[7] Welt, I. O., Stetten, D., Jr., Ingle, D. J., and Morley, E. H.: Effect of cortisone upon the rates of glucose production and oxidation in the rat. J. Biol. Chem. *197*:57, 1952.

[8] Searle, G. L., Strisower, E. H., and Chaikoff, I. L.: Glucose pool and glucose space in the normal and diabetic dog. Am. J. Physiol. *176*:190, 1954.

[9] Feller, D. D., Chaikoff, I. L., Strisower, E. H., and Searle, G. L.: Glucose utilization in the diabetic dog, studied with C^{14}-glucose. J. Biol. Chem. *188*:865, 1951.

[10] Baker, N., Shreeve, W. W., Shipley, R. A., Incefy, G. E., and Miller, M.: C^{14} studies in carbohydrate metabolism. J. Biol. Chem. *211*:575, 1954.

[11] Miller, M., Shipley, R. A., Shreeve, W. W., Baker, N., and Craig, J. W.: The metabolism of C^{14}-glucose in normal and diabetic subjects. Trans. Assn. Am. Phys. *68*:199, 1955.

[12] Loubatieres, A.: Analyse du mecanisme de l'action hypoglycemiante du p-aminobenzenesulfamidothiodiazol (2254 R.P.). Compt. rend. soc. biol. *138*:766, 1944.

[13] Colwell, A. R., Jr., Colwell, J. A., and Colwell, A. R., Sr.: Intrapancreatic perfusion of the antidiabetic sulfonylureas. Metabolism 5:749, 1956.

[14] Ashworth, M. A., and Haist, R. E.: Some effects of BZ-55 (carbutamide) on the growth of the islets of langerhans. Can. Med. Assoc. J. *74*:975, 1956.

[15] Pozza, G., Galansino, G., and Foa, P. P.: Insulin secretion following carbutamide injections into normal dogs. Proc. Soc. Exp. Biol. & Med. *93*:539, 1956.

[16] Mirsky, I. A., Perisutti, G., and Diengott, D.: The inhibition of insulinase by hypoglycemic sulfonamides. Metabolism *5*:156, 1956.

[17] Tyberghein, J. M., Halsey, Y. D., and Williams, R. H.: Action of butyltolylsulfonylurea on liver glycogenolysis. Proc. Soc. Exp. Biol. & Med. *92*:322, 1956.

[18] Williams, R. H., and Tucker, B. W.: Hypoglycemic action of tolbutamide and carbutamide. Metabolism *5*:801, 1956.

[19] Bressler, R., and Engel, F. L.: Some metabolic actions of Orinase. Proc. Soc. Exp. Biol. & Med. *95*:738, 1957.

[20] Elrick, H., and Purnell, R.: The response of kidney, liver, and peripheral tissues to tolbutamide and insulin. Ann. N. Y. Acad. Sci. *71*:38, 1957.

[21] Bearn, A. G., Billing, B. H., and Sherlock, S.: Hepatic glucose output and hepatic insulin sensitivity in diabetes. Lancet *1*:698, 1951.

[22] Searle, G. L., Mortimore, G. E., Buckley, R., and Reilly, W. A.: Influence of insulin and Orinase on the turnover of plasma glucose as studied with C^{14} glucose in humans. Federation Proc. *17*:308, 1958.

[23] Ashmore, J., Cahill, G. F., Jr., Earle, A. S., and Zottu, S.: Studies on the disposition of blood glucose. Diabetes *1*:1, 1958.

[24] Stadie, W. C.: An Editorial: Is the metabolism of peripheral tissues affected by the arylsulfonylureas? Diabetes 7: 61, 1958.

[25] Renold, A. E., Winegrad, A. I., Froesch, E. R., and Thorn, G. W.: Studies on the site of action of the arylsulfonylureas in man. Metabolism *5*:757, 1956.

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

[27] Recant, L., and Fisher, G. L.: Studies on the mechanism of tolbutamide hypoglycemia in animal and human subjects. Ann. N. Y. Acad. Sci. *71*:62, 1957.

[28] Renold, A. E., Martin, D. B., Boshell, B. R., and Thorn, G. W.: Studies on the site of action of the arylsulfonylureas in man. II. Ann. N. Y. Acad. Sci. *71*:71, 1957.

[29] Bastenie, P. A., Frankson, J. R. M., Demanet, T. C., DeMeutter, R., and Conard, V.: Metabolic effects of carbutamide in selected diabetics. Lancet *1*:504, 1957.

[30] Miller, M., Craig, J. W., Mackenzie, M. S., Drucker, W. R., Cammarn, M., and Woodward, H., Jr.: Studies on the effect of intravenous tolbutamide on pyruvic and lactic acid concentrations in peripheral venous blood in normal and diabetic subjects, and on splanchnic metabolism of fructose and glucose. Ann. N. Y. Acad. Sci. *71*:51, 1957.

[31] Dulin, W. E., and Johnston, R. L.: Studies concerning the role of the liver in the hypoglycemic response of animals to tolbutamide. Ann. N. Y. Acad. Sci. *71*:177, 1957.

[32] Bouckaert, J. P., and deDuve, C.: The action of insulin. Physiol. Rev. *27*:39, 1947.

[33] deDuve, C., and Hers, H. G.: Carbohydrate metabolism. Ann. Rev. Biochem. *26*:149, 1957.

[34] Levine, R., and Fritz, I. B.: The relation of insulin to liver metabolism. Diabetes *5*:209, 1956.

[35] Levin, H., and Weinhouse, S.: J. Biol. Chem., in press.