Rates of Production, Utilization, Accumulation and Apparent Distribution Space of Glucose

Effects of Insulin in Dogs Using a Validated Tracer Method

G. Hetenyi, Jr., M.D., Ph.D., G. A. Wrenshall, Ph.D., and C. H. Best, M.D., D.Sc., F.R.S., with the technical assistance of W. G. Layng, Toronto

Various tracer methods have been proposed to determine the rate of appearance, frequently termed the turnover rate, of a selected substance in the blood plasma through application of the dilution principle. The accuracy and applicability of most of these methods depend on a number of assumptions regarding the dynamic state and the pattern of distribution in the body of the selected metabolite. These assumptions have frequently been left unstated in publications on the subject. With the exception of the method of successive tracer injections[1] none of the published tracer methods for calculating rates of production and utilization has been validated in vivo against absolute measurements.[2,3]

In this investigation the effects of insulin on the amount and the rates of appearance and disappearance of glucose in the central compartment, of which blood plasma is a representative part, have been studied using the method of successive tracer injections. As no chemical transformation of glucose takes place in the plasma the above rates represent rates of transfer of glucose into and out of the central distributing compartment. The rate of production (appearance) of glucose in this compartment probably represents the rate of glucose output by the liver under our experimental conditions and perhaps a very small contribution by other tissues. The rate of disappearance of glucose from this compartment represents the rate of glucose uptake (utilization) by all tissues outside the central mammillary compartment, and includes the rate of glucose excretion if such is present. The steps followed in calculating the intermixing amount and rate of appearance of glucose at each time of tracer injection are the same as those described and used in establishing the validity of the method.[2,3]

It is also possible to calculate the amount (N) of rapidly intermixing glucose (termed the *glucose mass*) and the *apparent glucose space* $V = N/C$ at each time of intravascular tracer injection, where C is the plasma glucose concentration. It is emphasized, however, that V is not required for the calculation of rates of transfer of glucose into and out of the central compartment if the method of successive tracer injections is used.

MATERIALS AND METHODS

a. *Animals*

The experiments were performed on normal and depancreatized dogs under Nembutal anesthesia. Removal of the pancreas was carried out six to twenty-one days prior to an experiment. The depancreatized animals were fed a diet containing raw pancreas. Crystalline Insulin was injected twice daily. The last injection of insulin was given forty-eight to sixty hours before the experiment.

b. *Experimental technics*

Glucose uniformly labeled with C-14 was injected intravenously via an indwelling plastic catheter inserted about three to six inches into the cephalic vein. When a constant infusion was administered, it was given through another plastic catheter inserted *via* the saphenous into the iliac vein. Samples of mixed venous blood were withdrawn through a third catheter from either the inferior vena cava or from the right auricle of the heart. The latter was catheterized via the left jugular vein. Immediately after being withdrawn the blood samples were mixed with sodium fluoride and dry heparin, cooled in an ice water bath and centrifuged within one hour.

For the infusion of solutions a standard infusion pump was used (Harvard Apparatus Co., Dover, Mass., U.S.A.). The amount of fluid delivered was 0.38 ml./min. In experiments 3, 4 and 7 the infusion was given from a calibrated Marriotte burette delivering 0.5 ml./min.

Presented at the Twenty-first Annual Meeting of the American Diabetes Association in New York City on June 24, 1961.

From The Department of Physiology, and the Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

c. *Chemical methods and determination of specific activity.*

Plasma was deproteinized according to Somogyi.[4] The concentration of glucose was determined in an aliquot of the filtrate by the method of Nelson.[5,6] Glucosazones were prepared by the standard procedure[7] from the rest of the filtrate after a measured amount of carrier glucose had been added. The glucosazones were transcrystallized from boiling 60 per cent ethanol, plated on preweighed filter paper circles and were counted in a windowless flow counter. Radioactive strengths of all samples, recorded as net counts per minute (c.p.m.), were corrected for self-absorption. The specific activities of plasma glucose samples were expressed as c.p.m./mg. of glucose carbon.

d. *Experimental design*

In most experiments five successive injections of glucose uniformly labeled with C-14 were given, with 15-30 μC of C-14 being administered per injection. The start of the infusion or the injection of insulin was made between the second and third injections. Thus two sets of calculated values for glucose production and utilization and the glucose mass were obtained before infusion of insulin and three afterward. A sixth value for the glucose mass was calculated at the time of the start of the insulin infusion as the product of the average apparent glucose space in the pre-insulin period and the concentration of plasma glucose at the start of the infusion of insulin.

The hepatic glucose output was also calculated immediately after insulin by extrapolating the specific activity versus time curve back to the time when the infusion of insulin was started, using the formulae given by Henderson et al.[7] In contrast to the rates calculated by the method of successive tracer injections, the validity of the rate of appearance calculated from the slope of this curve rests on the additional assumption that the apparent glucose space did not change abruptly in magnitude at this time. The validity of this assumption has been verified previously[1,8] and again in this study (table 2).

e. *Curve fitting*

The course of the specific activity of plasma glucose, plotted as a function of the time, appeared by inspection to follow a single exponential function when an initial intermixing time of fifteen minutes was allowed. This function was fitted to each set of data using the method of least squares.

A polynomial function was fitted to interrelate the calculated glucose masses and the times at which they were determined for the calculation of the rates of glucose utilization. Separate functions were fitted to pre-

TABLE 1

Spontaneous variation of the rates of glucose production (R_A) and utilization (R_D) in one normal and one depancreatized dog. Rates are calculated at tracer injection times and are expressed as mg. glucose/kg. body weight/min. t = 0 at the time of the first injection.

Tracer inj. time (min.)	0		60		120-125		165		180		210	
Rates:	R_A	R_D	R_A	R_D	R_A	R_D	R_A	R_D	R_A	R_D	R_A	R_D
Normal dog	2.87	3.16	2.41	2.68	3.39	3.64	4.83	5.07	—	—	3.60	3.83
Depancreatized dog	7.76	7.79	10.63	10.66	11.94	11.96	—	—	8.78	8.78	—	—

TABLE 2

Effect of insulin on plasma glucose level, C, (in mg. per 100 ml.), glucose mass, N, (gm.) and apparent glucose space, V, (in per cent of body weight) in normal dogs

No. of expt.	Wt. of dog kg.	Mode of administration of insulin	Before insulin: 100 min. N	V	C	40 min. N	V	C	Times of injection of tracer glucose after injection or start of infusion of insulin: 23-25 min. N	V	C	60-69 min. N	V	C	108-114 min. N	V	C
1*	12.0	Inject. 1.0 U/kg.	2.41	17.3	115.7	2.02	15.7	106.9	—	—	—	1.69	46.8	30.0	—	—	—
7A	23.6	Inject. 0.3 U/kg.	3.58	18.0	84.2	2.94	14.7	84.2	2.73	44.1	26.2	3.42	33.3	43.5	4.85	24.1	85.4
3	11.5	Infus. 8 mU/kg./min.	2.40	23.4	89.1	—	—	—	1.77	24.3	63.2	1.60	44.4	31.4	1.34	30.3	38.7
6	16.9	Infus. 3.8 mU/kg./min.	3.56	19.8	106.2	3.67	22.3	97.4	1.65	17.7	55.1	10.23†	182.3†	33.2	1.59	29.0	32.5
7	25.0	Infus. 1.9 mU/kg./min.	4.23	21.8	77.8	4.21	21.6	77.8	3.35	20.9	64.0	4.31	31.0	55.5	2.26	32.0	28.2

*In this experiment the first two injections of tracer were given 120 and 60 min. before insulin; the third tracer injection was given simultaneously with insulin. N was found to be 1.42 gm.; and V 12.5 per cent with this last injection of tracer.

†These determinations are based on a specific activity vs. time curve of a very poor fit. Therefore it may represent a gross overestimation of N and V. They are included in order to present unselected data.

insulin and post-insulin data. The glucose mass was determined by extrapolation of each specific activity versus time curve back to the time of tracer injection for each of the five injections of tracer. A sixth value was calculated as described in (d) above. The rates of changes of the glucose mass were calculated as the derivative of the above-mentioned polynomial function at times of tracer injections. All curve fittings were performed by an IBM 650 digital computer.

f. *Materials*

Glucose uniformly labeled with C-14 was used (Merck and Co., Montreal, Canada). The injected solution contained 5 mg. per ml. glucose corresponding to an activity of 5 μC. Glucose of analytical grade (Analar BDH) was used for infusion of nonlabeled glucose. Glucagon-free insulin (Lilly, Lot 499667) was used in all but two experiments. A fresh acid-aqueous solution of the insulin was prepared before each of these experiments. In experiments 1 and 4 Insulin-Toronto was used.

RESULTS AND DISCUSSION

1. *Control experiments*

The glucose mass, the apparent glucose space and the rates of output and utilization of glucose, were calculated in two experiments in which no insulin was given. Figure 1 shows the spontaneous variation of the glucose mass and the apparent glucose space in a normal dog. A similar experiment yielding essentially the same results for a depancreatized dog was reported earlier.[9]

The spontaneous variations of the rates of hepatic glucose output and utilization are shown in table 1 for these two dogs. Although rates of transfer vary within ± 41 per cent in the normal and ± 23 per cent in the depancreatized dog around their respective mean values, no particular trend is detectable in their variation.

2. *Effects of insulin in normal dogs*

In a preliminary report it was shown that there is a delayed increase, after either the injection or infusion of insulin, in the apparent glucose space.[8] This is illustrated in table 2. The rates of glucose transfer calculated at injection times and at the time of the start of the infusion of insulin are shown in tables 3 and 4. These data and calculations indicate that, after a short depression, the rate of hepatic glucose output increases during the infusion of insulin, at a time when the plasma glucose level is low. In each case a considerable increase in the rate of utilization was observed throughout the first seventy minutes of the infusion or after injection of insulin. Later during hypoglycemia both rates decreased.

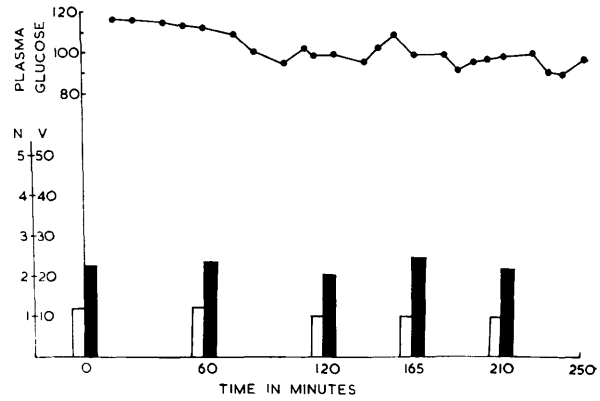

FIG. 1. Spontaneous variation with time of plasma glucose level (as mg. per 100 ml.), the amount of rapidly intermixing glucose (N, in grams) and apparent glucose space (V, as per cent of body weight) in a normal anesthetized dog. Upper line: plasma glucose level; white columns: the amount of rapidly intermixing glucose; black columns: apparent glucose space.

3. *Effects of simultaneous infusion of insulin and glucose in normal dogs*

Insulin caused severe hypoglycemia in the above-mentioned experiments. In order to separate the effects of insulin from those of the hypoglycemia caused by it glucose and insulin were infused simultaneously in three normal dogs in an effort to maintain the blood glucose at a constant level. In one of these three experiments the amount of glucose was insufficient to prevent hypoglycemia. As shown in table 5 the increase of the apparent glucose space after insulin was obtained even in the absence of hypoglycemia. Calculated rates of hepatic glucose production are shown in table 6. This rate was computed as the difference between the tracer-calculated rate of glucose appearance and the measured rate at which glucose was infused. It is evident that even in the absence of hypoglycemia the rate of hepatic glucose production may increase, but the increase was only transient in one of the two experiments. On the other hand, the rate of glucose utilization was consistently greater when hypoglycemia was avoided (compare tables 4 and 6).

4. *Effects of insulin in depancreatized dogs*

The effects of insulin infusion on the intermixing mass of glucose, apparent glucose space and the plasma concentration of glucose in three insulin-deprived depancreatized dogs are shown in table 7. In these diabetic animals it can be seen that, in contrast to the normals, insulin did not increase the apparent glucose space. The rate of hepatic glucose output either was not increased at all during the insulin infusion, or only transiently and slightly. In the second hour of the infusion the cal-

TABLE 3

The effect of insulin on the rate of glucose production in normal dogs. Rates are expressed as mg. glucose/kg. body weight/min. The magnitude of rates printed in italics are not calculated by the method of successive tracer injections but from the slope of the specific activity vs. time curve of plasma glucose at the indicated time. (See text.) The plasma glucose concentrations for each of the above experiments at the times indicated are shown in table 2.

No. of expt.	Time before insulin (min.) 120	100	60	40	Time after injection or infusion of insulin (min.) 0	23-25	60-69	108-114	Mode of administration of insulin
1	1.91	—	2.82	—	4.73*	—	5.34	—	INJ. 1 U/kg.
7A	—	1.71	—	2.70	*0.09*	9.08	4.05	3.28	INJ. 0.3 U/kg.
3	—	2.06	—	2.51	*0.94*	4.50	6.84	5.12	INF. 8.0 mU/kg./min.
6	—	2.36	—	4.13	*0*	6.72	20.76	4.44	INF. 3.8 mU/kg./min.
7	—	2.33	—	4.19	*1.00*	8.70	6.65	3.82	INF. 1.9 mU/kg./min.

*Determined by the extrapolation of ln specific activity vs. time curve to tracer injection time. Insulin and tracer glucose were injected simultaneously at t = 0; blood samples were collected between 15 and 60 min. after injection.

TABLE 4

Effect of insulin on the rate of the utilization of glucose in normal dogs. Rates are expressed as mg. glucose/kg. body weight/min. See legend to table 3 concerning figures in italics. The plasma glucose concentrations for each of the above experiments at the times indicated are shown in table 2.

No. of expt.	Time before insulin (min.) 100	40	Time after the injection or the start of the infusion of insulin (min.) 0	23-25	60-69	108-114	Mode of administration of insulin
7A	2.24	3.54	*6.29*	9.80	4.45	2.29	INJ. 0.3 U/kg.
3	2.47	2.20	*4.07*	4.96	7.30	5.58	INF. 8.0 mU/kg./min.
6	2.37	4.14	*5.70*	10.32	21.01	0.92	INF. 3.8 mU/kg./min.
7	3.05	4.14	*4.06*	8.78	7.28	5.54	INF. 1.9 mU/kg./min.

TABLE 5

Effect of the simultaneous infusion of insulin and glucose on the plasma glucose level C (mg. per cent), glucose mass N (gm.) and apparent glucose space V (in per cent of body weight) in normal dogs

No. of expt.	Infused amount of Glucose mg./kg./min.	Insulin mU/kg./min.	Before Infusion 100 min. N	V	C	40 min. N	V	C	During infusion 20-25 min. N	V	C	60-65 min. N	V	C	110 min. N	V	C
9	5.3	5.3	2.86	31.9	78.2	3.14	35.6	78.2	3.70	52.0	63.0	2.05	64.7	28.0	3.26	160.1	**18.0**
10	10.5	3.1	3.05	24.0	82.7	2.86	22.5	82.7	7.54	33.0	148.2	8.76	51.7	110.1	4.78	28.8	**108.0**
11	8.4	3.2	3.22	27.8	77.8	3.21	26.7	77.7	5.58	44.6	84.0	4.46	48.2	62.2	2.92	36.6	**53.6**

TABLE 6

The effect of the infusion of insulin plus glucose on the rates of glucose production (R_A) and utilization (R_D) in normal dogs. Rates are expressed as mg. glucose/kg. body weight/min. The plasma glucose concentrations for each of the above experiments at the times indicated are shown in table 5.

No. of expt.	Infusion rate of glucose mg./kg./min.	insulin mU/kg./min.	Before infusion 100 min. R_A	R_D	40 min. R_A	R_D	During infusion 20-25 min. R_A	R_D	60-65 min. R_A	R_D	110 min. R_A	R_D
9*	5.3	5.3	3.37	2.90	4.82	4.35	10.09	16.73	6.96	15.80	12.31	6.23
10	10.5	3.1	2.39	2.19	3.52	3.35	6.92	9.87	16.88	28.66	2.61	25.30
11	8.4	3.2	2.52	2.52	3.48	3.48	7.40	17.80	3.49	17.89	0.48	11.50

*In this experiment the amount of glucose infused was insufficient to prevent hypoglycemia. The plasma glucose level fell to 18 mg. per cent 110 min. after infusion had been started.

culated rate of glucose output dropped to the pre-insulin level. The rates of glucose utilization increased very considerably during insulin infusion (table 8).

Considering all the above results it appears that in *normal* dogs insulin increased the apparent distribution space of glucose. The initial reduction of the level of plasma glucose was due mainly to an early increase in the rate of utilization of glucose from the plasma compartment. The rate of output of glucose into this compartment was transiently depressed following insulin administration. Later it increased above the pre-insulin level. This increase, however, did not exceed the enhanced rate of peripheral utilization, and the plasma glucose level remained low. When hypoglycemia was prevented by the simultaneous infusion of glucose with insulin the rate of glucose utilization rose to very high levels while the increase in the rate of hepatic glucose output appeared to be less than when hypoglycemia was permitted. The increase in the apparent glucose space was still observed under this condition. In the *depancreatized* dogs the apparent glucose space was not increased by insulin and no compensatory elevation of the rate of appearance of glucose was observed during the infusion of insulin. In other respects the response of depancreatized dogs was similar to that of normal animals.

Changes in the apparent glucose space could arise from alterations in the relative concentrations of the glucose distributed between the different parts of the extra-cellular fluid volume. The possibility that the observed increase of the apparent glucose space after insulin is due to such a shift in glucose concentration is rendered unlikely by the increase in glucose utilization after insulin in the peripheral tissues. This increased utilization widens the difference between the concentrations of glucose in the blood plasma and in the interstitial fluid. Therefore the calculated value of the apparent glucose space, based on the concentration of glucose *in the blood plasma,* would tend to be reduced after insulin. In fact, however, the apparent glucose space in normal dogs is *increased* by insulin.

The increase of the apparent glucose space is thus due to the addition of glucose, probably that within cells, to the rapidly intermixing mass of glucose *without a corresponding increase in plasma glucose concentration.* The fact that the calculated amount of intermixing glucose exceeds the estimated amount of extracellular glucose even in the absence of excess insulin[10] indicates that some part of the rapidly intermixing glucose is intracellular. The difference between the calculated glucose mass and the amount of extracellular glucose is roughly the quantity of glucose which one would expect to find as free glucose in the liver.[11] It is postulated here that insulin acts by adding more *intracellular* glucose to the glucose mass. This may mean either an actual increase in the volume of fluid in which glucose is distributed — as predicted by the "permeability hypothesis" of insulin action[12] — or an increase in the concentration of glucose

TABLE 7

Effect of the infusion of insulin on the glucose mass, N (gm.), the apparent glucose space, V (in per cent of body weight) and the plasma glucose level, C (mg. per cent) in depancreatized dogs

No. of expt.	Amount of insulin infused mU/kg./min.	Before infusion 100 min. N	V	C	40 min. N	V	C	During infusion 25 min. N	V	C	65 min. N	V	C	110 min. N	V	C
12	6.3	14.90	24.7	398	16.28	26.9	398	11.15	20.9	350	8.33	18.6	294	7.76	26.0	196
13	6.3	12.00	25.5	389	12.67	29.6	353	7.41	25.2	243	5.62	23.4	198	4.22	25.1	139
14	6.1	9.10	24.0	283	8.40	22.5	279	7.51	25.4	221	6.79	23.4	217	3.25	17.1	142

TABLE 8

The effect of the intravenous infusion of insulin on the rates of glucose production (R_A) and utilization (R_D) in depancreatized dogs. Rates are expressed as mg. glucose/kg. body weight/min. See legend to table 3 concerning figures in italics. The plasma glucose concentrations for each of the above experiments at the times indicated are shown in table 7.

No. of expt.	Rate of infusion of insulin (mU/kg./min.)	Before infusion 100 min. R_A	R_D	40 min. R_A	R_D	During infusion 0 min. R_A	R_D	25 min. R_A	R_D	65 min. R_A	R_D	110 min. R_A	R_D
12	6.3	6.79	5.77	8.99	7.91	*≈0*	*18.90*	10.58	18.48	9.47	10.74	9.36	11.68
13	6.3	7.44	5.38	8.62	8.84	*1.91*	*24.96*	14.88	24.46	8.29	8.63	9.04	17.03
14	6.1	3.80	4.75	7.27	8.21	*0*	*1.50*	4.76	5.42	4.93	7.62	4.20	14.12

in those intracellular compartments in which intermixing glucose is distributed even in the absence of insulin or of an excess of insulin. If the first possibility should be the only mechanism of the action of insulin, it would be expected that the apparent glucose space would increase in both normal and depancreatized dogs, as both animals responded well to insulin. This increase, however, could not be demonstrated in depancreatized dogs. To account for the difference it is suggested that free hepatic glucose originating from glycogen may play a role. Hepatic glucose is presumably a part of the amount of rapidly intermixing glucose.[13] Insulin results in a breakdown of glycogen in the liver in normal animals[14,15] even when glucose is administered.[16,17] This glycogen may be converted at least partly to free intracellular glucose, which in terms of the preceding hypothesis would contribute to the calculated amount of rapidly intermixing glucose. In insulin-deprived depancreatized dogs the glycogen content of the liver is low, the effect of insulin being definitely glycogenic.[18] Therefore little or no increase in free glucose and thus in the apparent glucose space would be expected. There is no evidence at present to support this suggestion. On the other hand it is possible that the difference in the responses of normal and diabetic dogs is due to the fact that the plasma glucose did not reach as low levels in the latter group as in the former.

It must be recognized that, if the intracellular hepatic glucose is part of the rapidly intermixing glucose in the body, the calculated rate of glucose production will be higher than the actual hepatic glucose output whenever glycogen is breaking down at a faster rate to intracellular glucose than the latter is released from the liver. Further studies of the role of the liver in the mechanism of the increase of the apparent glucose space are in progress.

In most of the normal dogs tested, insulin was found to cause a sudden and transient decrease in the rate of production of glucose. As the evidence for this was based on the shape of the fitted specific activity versus time curves its validity and accuracy remain questionable (see "Methods" in this paper and references 1, 19). No such reservations are necessary when we consider the rates of glucose production calculated at the times of tracer injection.[1] Rates of hepatic glucose output calculated after the infusion of insulin had proceeded for about twenty-five minutes were markedly elevated. These later decreased slowly toward pre-insulin values. The output of glucose did not decrease during the infusion of insulin as described by de Bodo et al.[20] These workers, using the method of Wall et al.,[21] had to assume that the apparent glucose space remained constant, a premise which now seems to lack adequate support.[8] This assumption would lead to an underestimation of the rate of glucose production.

When hypoglycemia was prevented insulin still tended to increase the rate of hepatic glucose production. This increase, however, was confined to the first hour of the infusion, and was small in one of the two experiments. It is conceivable that this increase is due to a breakdown of glycogen to glucose in the liver, as pointed out previously. In depancreatized dogs insulin either did not increase the rate of hepatic glucose production, or the increase was confined to the first twenty-five minutes of the insulin infusion (table 8).

Insulin caused an abrupt increase in the rate of glucose utilization in both depancreatized and normal dogs. This finding is in agreement with the results of previous investigators.[7,21-23] In normal dogs the increase was transient, unless hypoglycemia was prevented by the simultaneous infusion of glucose. Where hypoglycemia was avoided the magnitude of the increase in the rate of glucose disappearance exceeded that found without infusing glucose, and the high rates were maintained throughout the experiment. These experiments support the hypothesis,[24] that the uptake of glucose by the tissues is a function of the concentration of glucose in the surrounding fluid (e.g. interstitial fluid), and thus that the hypoglycemic effect of insulin in normal dogs is self-limiting through *both* the rates of production and utilization of glucose. It is concluded that the hypoglycemia after insulin is mainly due to the accelerated uptake of glucose by the cells of the organism.

SUMMARY

1. The effects of both injection and infusion of insulin on the apparent distribution space and the rates of production and utilization of glucose have been studied in the fasting dog under Nembutal anesthesia, using the validated method of successive tracer injections.

2. There was a delayed increase in the apparent glucose space following the intravenous administration of a hypoglycemic dose of glucagon-free insulin to normal dogs.

3. This change was not seen in fasting depancreatized dogs receiving similar treatment after having been without insulin for forty-eight to sixty hours.

4. The intravenous injection of insulin caused a prompt and appreciable increase in the rate of utilization of glucose in both the normal and depancreatized dogs.

5. In the normal dog insulin administration caused an initial decrease in the rate of hepatic glucose output,

followed by an increase above the pre-insulin rate which at first was insufficient to prevent a fall in blood sugar. This increase in the rate of glucose output was transient or absent in the depancreatized dog following insulin injection.

6. When hypoglycemia caused by the infusion of insulin in the normal dog was prevented by the simultaneous administration of glucose, the rate of hepatic glucose output increased to a lesser extent, or more transiently, while the rate of glucose utilization was markedly increased.

7. Under these circumstances the administered insulin still caused an increase in the apparent glucose space of the normal dog. The working hypothesis that the effect of insulin on the apparent glucose space in the normal but not in the depancreatized dog results from an intracellular breakdown of liver glycogen to substances whose glucose moiety can interchange more rapidly with the circulating extrahepatic glucose is being investigated.

SUMMARIO IN INTERLINGUA

Proratas de Production, Utilisation, e Accumulation de Glucosa e le Apparente Spatio de Distribution de Illo: Effectos de Insulina in Canes, Determinate per Medio de un Provate Methodo a Traciatores

1. Le effectos del injection e etiam del infusion de insulina super le apparente spatio de distribution e super le proratas de production e de utilisation de glucosa esseva studiate in canes in stato jejun sub anesthesia per Nembutal, con le utilisation de un provate methodo de successive injectiones de traciatores.

2. Esseva constatate un retardate augmento in le apparente spatio de glucosa post le administration intravenose de un dose hypoglycemic de insulina sin glucagon a canes normal.

3. Iste alteration non esseva incontrate in dispancreatisate canes in stato jejun quando illos esseva similemente tractate post haber essite sin insulina durante quaranta-octo a sexanta horas.

4. Le injection intravenose de insulina causava un prompte e appreciabile augmento in le proratas del utilisation de glucosa tanto in le canes normal como etiam in le canes que habeva essite dispancreatisate.

5. In canes normal le administration de insulina causava initialmente un reduction del rendimento hepatic de glucosa. Isto esseva sequite de un augmento a nivellos supra illos constatate ante le administration de insulina. Le augmento in le rendimento de glucosa non esseva observate in canes dispancreatisate quando illos recipeva injectiones de insulina.

6. Quando le hypoglycemia causate in canes normal per le infusion de insulina esseva prevenite per le administration simultanee de glucosa, le rendimento hepatic de glucosa cresceva minus marcatemente o plus transientemente, durante que le utilisation de glucosa esseva marcatemente intensificate.

7. Sub iste circumstantias le insulina exogene causava nonobstante un augmento in le apparente spatio de glucosa in canes normal. Es investigate currentemente le hypothese preliminari que le effecto de insulina super le apparente spatio de glucosa in canes normal — un effecto que non occurre in canes dispancreatisate — resulta ab un decomposition intracellular de glycogeno hepatic con le formation de substantias que include un portion de glucosa capace a excambiar se plus rapidemente pro le circulante glucosa extrahepatic.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. L. C. Lax for the preparation of the program for fitting exponential curves by the IBM 650 digital computer, and also for many discussions; to Dr. R. J. S. Metz for some constructive remarks and comments; and to Mr. W. P. Kotorynski who prepared the program for fitting polynomial curves by the IBM 650 digital computer. Table 2 is reproduced here with the kind permission of the Editors of *Nature* (London, England). The generous gift of glucagon-free Crystalline Insulin by the Eli Lilly Company is gratefully acknowledged. The work was supported by grants from the National Research Council of Canada and the Banting Research Foundation.

REFERENCES

1 Wrenshall, G. A., and Hetenyi, Jr., G.: Successive measured injections of tracer as a method for determining characteristics of accumulation and turnover in higher animals with access limited to blood: Tests in hydrodynamic systems and initial observations on insulin action on dogs. Metabolism *8*:531, 1959.

2 Hetenyi, G., Jr., Rappaport, A. M., and Wrenshall, G. A.: The validity of rates of glucose appearance in the dog calculated by the method of successive tracer injections. I. Effects of surgical hepatectomy, evisceration and order of tracer injection. Canad. J. Biochem. Physiol. *39*:225, 1961.

3 Wrenshall, G. A., Hetenyi, G., Jr., and Best, C. H.: The validity of rates of glucose appearance in the dog calculated by the method of successive tracer injections. II. The influence of intermixing time following tracer injection. Canad. J. Biochem. Physiol. *39*:267, 1961.

4 Somogyi, M.: Determination of blood sugar. J. Biol. Chem. *160*:69, 1945.

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

6 Somogyi, M.: Notes on sugar determination. J. Biol. Chem. *195*:19, 1952.

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

[8] Hetenyi, G., Jr., and Wrenshall, G. A.: Abrupt and delayed effects of insulin on the distribution space of glucose in the dog. Nature *186*:808, 1960.

[9] Wrenshall, G. A.: Discussion of presented papers. Ann. New York Acad Sc. *82*:449, 1959.

[10] Hetenyi, G., Jr.: The measurement of the accumulation and transfer rates of glucose in the intact dog. The effect of insulin. Ph.D. thesis. Toronto 1960.

[11] Applebloom, J. W., Brodsky, W. A., and Dekker, E.: Free glucose content of liver. Am. J. Physiol. *187*:583, 1956.

[12] Levine, R., Goldstein, M., Klein, S. and Huddlestun, B.: The action of insulin on the distribution of galactose in eviscerated, nephrectomized dogs. J. Biol. Chem. *179*:985, 1949.

[13] Cahill, G. F., Jr., Ashmore, J., Scott Earle, A., Zottu, S.: Glucose penetration into liver. Am. J. Physiol. *192*:491, 1958.

[14] Cori, C. F.: Insulin and liver glycogen. J. Pharmacol. & Exper. Therap. *25*:1, 1925.

[15] de Bodo, R. C., and Neuwirth, I.: Relation of insulin to liver glycogen. Am. J. Physiol. *103*:5, 1933.

[16] Bridge, E. M.: The action of insulin on glycogen reserves. Bull. Johns Hopkins Hosp. *64*:408, 1938.

[17] Arensmeyer, E., and Molinsky, H.: Über den Gehalt an Glykogen und Kalium in Blut, Leber, Muskel und Niere von Ratten nach Hunger, Glukosemast, Insulin- und Adrenalineinwirkung. Ztschr. f. d. ges. exper. Med. *122*:564, 1954.

[18] Banting, F. G., Best, C. H., Collip, J. B., and Noble, E. C.: The effect of insulin on the percentage amounts of fat and glycogen in the liver and other organs of diabetic animals. Tr. Royal Soc. Canada *16*:39, 1922.

[19] Landau, B. R., and Leonards, J. R.: Significance of changes in blood glucose specific activity following insulin administration. Am. J. Physiol. *198*:793, 1960.

[20] de Bodo, R. C., Altszuler, N., Dunn, A., Steele, R., Armstrong, D. T., and Bishop, S. J.: Effects of exogenous and endogenous insulin on glucose utilization and production. Ann. New York Acad. Sc. *82*:431, 1959.

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

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

[23] Searle, G. L., Mostinose, G. E., Buckley, R., and Reilly, W. A.: Plasma glucose turnover in humans as studied with C-14 glucose. Diabetes *8*:167, 1959.

[24] Issekutz, B., Jr., Hetenyi, G., Jr., and Winter, M.: Beiträge zum Wirkungsmechanismus des Insulins II. Acta Physiol. Acad. Sc. Hung. *7*:273, 1955.

On Teaching Diabetes

What Is Needed in the Medical Schools to Improve the Teaching of Diabetes?

We discussed preclinical teaching of diabetes and the vertical versus the horizontal approach. Someone asked what these symbols might mean. In the first an attempt is made to co-ordinate the special interests of each department into teaching at every level. In the second each discipline is taught separately and its application to a disease is made apparent during the teaching of the subject at hand. The problem of utilizing clinical faculty in the teaching of basic sciences and the problem of utilizing basic science personnel in clinical teaching were discussed, and it was felt that the latter was the more important. There was discussion concerning whether integration should be encouraged by small group teaching or whether this should be applied to large classes. It was felt that this depended upon the availability of faculty in any particular institution.

We then directed our attention to the use of the teaching clinic. It was stressed that the teaching clinic (in this case the diabetes clinic) frequently deteriorates to a service function and that exposure to or experience with patients is not actually training. Adequate supervision is required at both the student and house staff level. With an indigent patient population the students and house staff would get a distorted view of the practice of diabetes. Wherever possible the student and house staff should have an opportunity to treat private patients and to learn the mechanisms of taking care of private patients with diabetes. The problem of continuity in the entire training program was stressed. It was felt that the block system, in which a student and house staff are in the clinic for only six to eight weeks, is not the preferable method. It is preferable to have a student in the clinic one afternoon or one morning a week for a period of several months, in order to learn something of the natural history of diabetes. It was felt, also, that for continuity it was essential to utilize the services of a nurse, social worker, dietitian, and perhaps also a psychiatrist and ophthalmologist wherever possible. A large number of patients is not essential for proper training of students and house staff. Exposure to a few patients with adequate supervision is preferable to exposure to a large number of patients with poor supervision.

By Benjamin I. Heller, M.D., in
Teaching and Research in Diabetes,
Charles C Thomas, Springfield,
Illinois, pp. 28-29, 1960.