Action of a Supramaximal Insulin Load on the Glucose Hepatic Balance of Anaesthetized Dogs under Strict Normoglycaemic Conditions*

By

W. MALAISSE, Y. ARNOULD, E. RASIO, V. CONARD and J. R. M. FRANCKSON

Departments of Experimental Medicine and General Pathology, University of Brussels

Received April 13, 1965

Summary. The action of insulin upon the liver glucose balance has been studied by hepatic catheterisation and by isotopic dilution in 7 healthy anaesthetised dogs. Insulin was infused in very large amount (100 U/kg/h) during several hours to obtain a homogeneous distribution of the hormone in its diffusion space; to avoid the disturbing action of hypoglycaemia, insulin action was compensated by constantly adapted glucose infusions.

Under strict normoglycaemic conditions, insulin has a slow and progressive effect upon the hepatic glucose balance; one hour is needed to produce a definite decrease in the release of ^{12}C -glucose.

From the onset of insulin infusion, the blood ^{14}C -glucose disappearance curve ceases to be expressed by a simple exponential and becomes a fall-off curve which tends to an equilibrium level. The mathematical analysis shows that this curve is the resultant of 2 antagonist exponential phenomena; the first one, expressed by a steeper ^{14}C -decay, represents the increased tissue uptake induced by insulin; the second one, expressing an influx of ^{14}C -glucose into the blood, reveals an early recycling of the labelled glycogen previously stored in the liver.

As normoglycaemic conditions allow the exclusion of interference by glycogenolytic hormones, the comparison of the ^{12}C - and ^{14}C -data suggests a two-fold action of insulin on the synthesis and breakdown of the hepatic glycogen, accelerating the turn-over rate of this compound.

Résumé. L'action de l'insuline sur le débit glucosé hépatique a été étudiée chez sept chiens normaux anesthésiés par le cathétérisme sus-hépatique et la dilution isotopique du glucose- ^{14}C . L'insuline a été perfusée à raison de 100 U/kg/h durant plusieurs heures de manière à obtenir une distribution homogène de l'hormone dans son espace de diffusion. Des conditions de stricte normoglycémie ont été maintenues par une perfusion glucosée constamment adaptée au taux de la glycémie.

Dans ces conditions, l'insuline a un effet modéré et progressif sur le bilan glucosé hépatique; il faut une heure de perfusion insulinaire pour observer une réduction nette du débit hépatique du glucose ^{12}C .

Dès le début de la perfusion d'insuline, la courbe de disparition sanguine du glucose- ^{14}C cesse d'être une exponentielle simple mais devient une courbe à saturation tendant vers un état d'équilibre. L'analyse mathématique de cette courbe indique qu'elle est la résultante de deux

phénomènes exponentiels antagonistes: le premier, exprimé par une vitesse de disparition accrue, traduit l'augmentation de la captation tissulaire du radioglucose sous l'influence de l'insuline; le second phénomène, correspondant à un influx de glucose- ^{14}C dans le sang, témoigne d'une recirculation précoce du glycogène marqué préalablement stocké dans le foie.

Les conditions de normoglycémie permettant d'exclure l'intervention des hormones glycogénolytiques; la comparaison des données des glucose- ^{12}C et ^{14}C suggère que l'insuline agit simultanément sur la synthèse et le catabolisme du glycogène, accélérant ainsi la vitesse de renouvellement de ce métabolite.

Zusammenfassung. Bei 7 gesunden narkotisierten Hunden wurde mittels Leberkatheter und Isotopenverdünnung die Insulinwirkung auf den Glucosehaushalt der Leber untersucht. Mehrere Stunden wurden sehr große Dosen Insulin (100 E/kg/Std) infundiert, um eine gleichmäßige Verteilung des Hormons innerhalb seines Verteilungsraumes zu erreichen. Um die störenden Auswirkungen der Hypoglykämie zu vermeiden, wurde die Insulinwirkung durch eine dauernd angepaßte Glucoseinfusion kompensiert.

Unter streng normoglykämischen Bedingungen hat Insulin eine langsame und sich steigernde Wirkung auf den Glucosehaushalt der Leber; eine Stunde ist erforderlich, um ein deutliches Absinken der Freisetzung von ^{12}C -Glucose zu erreichen.

Mit Beginn der Insulininfusion kann die Abfallkurve der Blut- ^{14}C -Glucose nicht mehr durch eine einfache Exponentialfunktion ausgedrückt werden. Es resultiert eine abfallende Kurve, die einem gleichbleibendem Niveau zustrebt. Die mathematische Analyse zeigt, daß diese Kurve die Resultante zweier antagonistischer Exponentialerscheinungen ist. Die erste, die durch einen steileren ^{14}C -Abfall ausgedrückt ist, repräsentiert die gesteigerte durch Insulin induzierte Gewebsaufnahme; die zweite, die einen Zufluß von ^{14}C -Glucose in das Blut ausdrückt, läßt ein frühzeitiges Wiedererscheinen des markierten vorher in der Leber gespeicherten Glykogen erkennen.

Da bei den normoglykämischen Bedingungen der Einfluß glycogenolytischer Hormone ausgeschlossen werden kann, läßt der Vergleich der ^{12}C - und ^{14}C -Ergebnisse auf eine zweifache Wirkung des Insulins auf die Synthese und den Abbau des Leberglykogens schließen, wodurch die Umsatzrate des Glykogens beschleunigt wird.

Introduction

In previous experiments carried out on normal anaesthetised dogs injected successively with guinea pig anti-insulin serum and insulin¹³, we have shown that the onset of the insulin-deficient state is characterised by a decrease in the rate of utilisation of glucose-

^{14}C and an increase in hepatic glucose output. Insulin injection then rapidly restores to normal both hepatic glucose production and tissue utilisation of glucose. This latter observation contrasts with the effects produced by portal infusions of insulin into normal anaesthetised dogs. Under these conditions insulin fails to have any inhibitory effect upon hepatic glucose output^{27,12}. Two hypotheses could be put forward to explain these contrasting effects of insulin upon hepatic glucose metabolism. First, it could be

* Work performed under the association contract Euratom-Universities of Pisa and Brussels (n° 026-63-04 BIAC).

postulated that the normal liver is saturated with insulin and that supplementary injections would therefore have no effect; only when it is deprived of its normal supply would the effects of exogenous insulin become apparent. Secondly, the part played by insulin in the regulation of hepatic glucose metabolism is secondary to the influence of glucose concentration in the blood; it cannot prevent the normal increase in hepatic glucose output which accompanies the lowering of the blood glucose level.

The aim of the present work was to study the influence of insulin upon liver glucose production under strictly normoglycemic conditions achieved by constant glucose regulation. Glucose production was estimated by multiple hepatic catheterisations and by isotopic dilution. Insulin was infused in very large amounts for several hours to obtain a complete distribution of the hormone in its diffusion space^{20, 21}; indeed, following a single intravenous injection, insulin may be fixed by some tissues before equilibration and thereby induce heterogeneity of the glucose pool²⁴. The plasma levels of insulin reached by the infusion technique approached the concentrations generally used in *in vitro* studies.

Materials and Methods

Dogs. Seven male Alsatian dogs (22 to 37 kg) were treated for intestinal worms and vaccinated against canine distemper one to two months before use. They were fed for seven days on a diet rich in carbohydrates (about 80 per cent).

Insulin. The preparation used was a glucagon-free pork insulin, soluble at pH 7 in saline and containing 40 U/ml*. For each experiment, to about 300 ml containing 12,000 Units, was added 0.5 mC of insulin-¹³¹I, (specific activity, 10 mC/mg**). The specific activity of the injected solution was 1 µC/mg insulin or 1.65 µC/ml.

Chemical Determinations. Blood sugar concentrations were determined with the autanalyser*** by a modification of the Hoffman method¹⁵. Heparinized blood samples containing fluoride were analysed immediately after collection. BRADLEY's method⁴ for the determination of *bromsulphalein* was adapted for use with the autoanalyser and the results obtained with portal and hepatic venous plasma used to estimate hepatic blood flow.

The *hepatic glucose balance* was calculated from these measurements and the differences in glucose content of simultaneously drawn samples of hepatic and portal venous blood. This type of calculation entails an over-estimation of the glucose output since the total splanchnic blood flow is used instead of the unknown portal flow. Separate calculations were made for the data obtained from the left and right hepatic veins.

The *glucose-1-¹⁴C* content of the arterial whole blood was estimated after fermentation with *Leuconostoc mesenteroides* using the methods of BERNSTEIN³ and DEMOSS⁸; the ¹⁴CO₂ was counted as barium carbonate in thin layer in a proportional methane flow-counter*. This method of fermentation is highly specific since the leuconostoc produces CO₂ only from the first carbon atom of the d-glucose molecule.

The slope of the semilogarithmic straight line relating labeled glucose content of the whole blood (ordinate) with time (abscissa) expresses the rate of utilization of such glucose by the tissues, when measured after it had equilibrated in its diffusion space (after approximately 40 minutes). It may be easily calculated using the following formula:

$$K^* = \frac{\log_{10} R_1 - \log_{10} R_2}{t_2 - t_1} \times \log_{10} 10,$$

where R_1 and R_2 are the radioactivities (cpm/ml) measured on the semi-logarithmic straight line at times t_1 and t_2 . Multiplying by $\lg_{10} 10$ is necessary to convert decimal logarithms into Napierian ones (⁹, ¹⁸).

Plasma potassium was determined by flame photometry. *Oxygen saturation* of the blood was measured by reflection oxymetry²⁸ using a Brinkman haemore-flector**. ¹³¹I insulin concentration was estimated in plasma using a crystal scintillation counter after precipitation with trichloroacetic acid***. *Thiocyanate space*, determined by the technique of CACHERA et al.⁵ was used to assess variations in the volume of the glucose diffusion space⁶.

Surgical Preparation. Following withdrawal of food for sixteen hours, anaesthesia was induced by intravenous injections of pentobarbitone (15 mg/kg) and phenoperidin (1 to 2 mg) and maintained by further smaller doses of these two drugs according to requirements. During the whole experiment, the dogs were connected by an intratracheal tube to an automatic respirator providing a gaseous mixture of oxygen (60%) and nitrogen (40%). All losses and withdrawals of blood were compensated by transfusion of fresh heparinised compatible blood. Diuresis was ensured by saline infusion, the urines being collected by bladder catheterization. The surgical manoeuvres comprised the insertion of polyethylene catheters (1 mm diameter) into five peripheral veins and into the aorta, via a branch of the femoral artery. In the abdomen, a 3 mm catheter was inserted into the splenic vein and the tip passed under manual control to the portal vein at the porta hepatis. The right external and internal maxillary veins were opened close to their confluence into the external jugular vein and two radio-opaque catheters**** passed under fluoroscopic control into the left common and right hepatic veins. The animals were then placed under a heating tent and closely watched for one to

* FH 407 Frieseke & Hoepfner, Erlangen-Brück, Germany.

** Kipp & Sons, Delft, the Netherlands.

*** Well type, 2 inch., Philips, Belgium.

* Novo Therapeutisk Laboratorium, Copenhagen, Denmark.

** Farbwerke Hoechst AG, Frankfurt, Germany.

*** Technicon Instruments Co., Chauncey, N.Y.

**** Ödman-Ledin, Kifa, Solna E, Sweden.

two hours: body temperature, arterial and portal blood pressures, diuresis, O_2 saturation and glucose content of the blood were checked. This whole procedure usually took about four hours and therefore ended approximately twenty hours after the last food had been consumed.

The catheters in the hepatic and portal veins and in the artery were used for simultaneous sampling of blood at regular intervals by four operators. Peripheral veins were used to infuse bromsulphalein (BSP), glucose, insulin, potassium chloride, blood and drugs (Figure 1).

Fig. 1. Canine preparation. The arrows indicate the blood sampling areas. BSP and insulin are delivered by slow injectors; glucose and potassium by finger pumps.

Experimental Procedure. All times reported for these experiments have been referred to that at which the insulin infusion was started (zero time) and, for convenience, each experiment can be divided into four periods.

a. *Preliminary period (-80 to -40 min).* A tracer dose of $60 \mu\text{C}$ of glucose- $1\text{-}^{14}\text{C}$ (2 mC/mM) was injected 80 minutes before commencement of the insulin infusion (time stated as -80 min). Twenty minutes later (-60 min), a priming dose of BSP (60 mg) was given and constant infusion of this compound started at a rate ranging from 1.64 to 2.65 mg/min according to the weight of the animal.

b. *Basal period (-40 min to zero).* Blood samples were simultaneously withdrawn from the four vessels: every 10 minutes for glucose, hematocrit and BSP esti-

mations, and once for determination of oxygen saturation. In arterial blood, the activity of glucose- $1\text{-}^{14}\text{C}$ was determined every 10 minutes and the potassium concentration twice.

c) *Compensation period of blood-sugar stabilisation (zero to +120 min).* Immediately following the last sampling (time 0), insulin infusion was started at a rate of 100 U/kg/hr and the infusions of glucose and potassium begun. Glucose was delivered as a 10% solution in normal saline and potassium as Darrow's solution (35 mEq/l). The rates of these infusions were constantly adapted according to the results of blood-sugar estimations which took less than 12 minutes with the autoanalyser. During two hours glucose- ^{12}C and ^{14}C were determined every 15 minutes, BSP, potassium and organic ^{131}I , every 30 minutes, and oxygen saturations every hour.

d) *Period of blood-sugar changes (+120 to +210 min)* — At 120 minutes, the glucose infusion was stopped but the insulin infusion was continued at the same rate. During the next 90 minutes blood sampling was performed according to the time schedule used in the preceding period.

Results

Blood-Sugar Level. As shown in Figure 2, no marked change in blood-sugar concentration occurred during the period of 120 minutes in which both glucose and

Fig. 2. Mean arterial concentrations ($\pm$ S.E.M.) of insulin and glucose during insulin and glucose infusions, and the mean rate of glucose infusions.

insulin were infused intravenously; the data for individual dogs are shown in Table 1. During the second hour of infusion a steady state was nearly reached. Thus the

Table 1. Arterial blood sugar concentration (mg/100 ml) in the basal period and during the 1st and 2nd hours of insulin infusion (compensation period). Figures given are the means in the time and their standard error

Exp. no.	Blood Sugar Level		
	Basal	1 st hour	2 nd hour
132	78 ± 1.9	67 ± 7.9	59 ± 5.6
133	68 ± 3.9	74 ± 3.8	75 ± 0.8
135	54 ± 1.6	56 ± 4.2	54 ± 4.2
136	54 ± 2.1	52 ± 3.7	64 ± 9.8
142	63 ± 1.4	63 ± 2.5	61 ± 4.8
143	59 ± 2.2	53 ± 6.7	61 ± 1.5
147	58 ± 2.8	70 ± 6.7	63 ± 5.0

plasma insulin concentration exceeded 0.5 U/ml and increased only slightly; glucose infusion was constant, ranging from 200 to 550 mg/min according to the animal and the blood-sugar concentration was stable.

When the glucose infusion was stopped there was an immediate and sharp drop in the blood-sugar concentration. The lowest values were reached within 15 to 45 minutes, the rate of disappearance from the

blood averaging 5.2×10^{-2} range 3.6 to 6.9×10^{-2} . This dramatic fall was followed in every case by a small transient increase of about 10 mg/100 ml, the blood-sugar level then resuming its decline to values around 20 mg/100 ml.

BSP Excretion and Circulation Data. The infusions of bromsulphalein (BSP) were kept at a constant rate during the whole experiment. As shown in Figure 3, the portal plasma-level of BSP remained practically unchanged, thus illustrating the constancy of the drug's excretion, the rate of which varied from 97 to 104% of the infused amount and did not show any significant variation with time. By contrast, the differences between portal and hepatic venous concentrations diminished progressively during the insulin and glucose infusions and so reflected an increase in the estimated splanchnic blood flow. Values reached at the ends of the first and second hours of insulin infusion represent 156 and 179% (respectively) of the mean basal value (22 ml/kg/min).

Table 2. Hepatic release of glucose measured from the left (L) or right (R) hepatic vein during the basal period and during the 1st and 2nd hours of the insulin infusion (compensation period). Figures given are the means in the time followed by their standard errors; they are expressed in mg/min

Exp. no.	Side	Liver Glucose Releases		
		Basal	1 st hour	2 nd hour
132	L	41 ± 13.8	52 ± 6.3	24 ± 22.3
	R	116 ± 8.6	76 ± 11.9	45 ± 6.2
133	L	46 ± 4.3	37 ± 4.6	47 ± 3.7
	R	52 ± 2.2	58 ± 6.7	35 ± 19.1
135	L	64 ± 13.1	26 ± 13.4	16 ± 4.2
	R	50 ± 9.8	37 ± 19.7	19 ± 8.5
136	L	102 ± 5.6	83 ± 8.1	74 ± 7.6
	R	113 ± 7.8	115 ± 10.4	105 ± 10.5
142	L	148 ± 14.1	183 ± 17.4	130 ± 7.5
	R	148 ± 22.9	87 ± 10.9	66 ± 7.7
143	L	79 ± 13.3	36 ± 6.2	14 ± 3.7
	R	102 ± 20.5	49 ± 8.0	28 ± 4.3
147	L	107 ± 7.1	100 ± 6.4	98 ± 8.5
	R	114 ± 9.3	94 ± 11.5	87 ± 23.5
Means	L	84 ± 7.7	75 ± 9.2	53 ± 9.8
	R	100 ± 8.4	74 ± 7.3	57 ± 8.3
	L+R	92 ± 5.8	75 ± 5.8	55 ± 6.2

Fig. 3. Evolution of the BSP concentration in portal plasma, of the estimated splanchnic blood flows and of the oxygen saturation in arterial (A), hepatic (LHV & RHV) and portal (PV) bloods. Figures given are the mean values and include their standard error

Blood oxygen saturations measured in the differing vessels are also reported in Figure 3. The values observed in the portal and hepatic veins increased consistently during the insulin infusion. There is a close correlation between the increase in estimated splanchnic blood flow on one hand and the decrease of the difference in oxygen saturation between both the arterial and hepatic bloods ($r=0.536$; $df=51$; $P<0.001$) and the arterial and portal bloods ($r=0.811$; $df=25$; $P<0.001$) on the other.

Mean arterial pressure was not affected during combined glucose and insulin infusion (132 mm of mercury). One hour after glucose infusion was stopped, however, a significant fall (averaging 23 mm Hg) was noticed. Portal venous pressure, measured at the porta

hepatis, decreased progressively at a rate of 10 mm of water per hour following the start of insulin administration.

Fig. 4. Evolution of the blood sugar level in portal and hepatic veins, and of the liver glucose release. Figures given are the mean values and their standard errors; they are calculated from data pooled from left and right hepatic veins

As illustrated by Figure 4, an equilibrium was reached during the second hour of this period; glucose outflows then averaged 60% of basal values. The decrease in outflow recorded during this second hour, as shown in Table 3, is statistically significant. This change in glucose balance may be related to insulin's own action, as there is no significant correlation between minor changes in the hourly level of arterial blood glucose (Table 1) and the mean glucose outflows observed during insulin and glucose infusions (Table 2): $r = 0.530$; $df = 12$; $P > 0.05$.

Cessation of the glucose infusion rapidly induced a systematic increase in hepatic glucose release from both explored lobes and within one hour all values had returned to the basal level or above it.

Glucose $1\text{-}^{14}\text{C}$ content of the blood. The activities of the glucose- $1\text{-}^{14}\text{C}$ in blood are recorded in table 4. In the basal period, the labeled glucose content of the blood decreases exponentially: plotted on a semi-logarithmic graph (blood radioactivities in logarithmic scale as ordinates, times as abscissae), the successive values observed in each experiment yield a straight line, the slope of which averages $1.5 \times 10^{-2} \pm 0.13 \times 10^{-2}$ for the whole group (figure 5). As the glucose pool is constant (blood glucose and thiocyanate space stable), the individual glucose- ^{14}C utilisation rates may easily be used to calculate the turn-over rates. The mean

Table 3. Significance of the changes in liver glucose release observed under the influence of insulin during the 2 hours of glucose compensation in the left (L) and right (R) lobes, and on the averaged results (L+R)

type of test	Comparison	Side	Statistical data		
			df	t	P
t with pairing on hourly means	Basal — 1 hour	L	6	0.975	>0.05
		R	6	2.565	$0.05 > P > 0.02$
		L+R	13	2.483	$0.05 > P > 0.02$
t on pooled experimental values	Basal — 2 nd hour	L	6	3.337	$0.02 > P > 0.01$
		R	6	3.880	<0.01
		L+R	13	5.165	<0.001
	Basal — 1 hour	L	35	0.725	>0.05
		R	35	2.266	$0.05 > P > 0.02$
		L+R	70	2.077	$0.05 > P > 0.02$
	Basal — 2 nd hour	L	34	2.560	$0.02 > P > 0.01$
		R	34	3.576	<0.001
		L+R	68	4.333	<0.001

Liver Glucose Balance. During the basal period the liver released more glucose than it took up from the portal blood. The mean net rate of hepatic glucose release for the whole period ranged from 41 to 148 mg/min according to the dog and for the group of animals as a whole was 92 ± 6 mg/min (Table 2). When estimated from the arterio-hepatic differences in blood-sugar concentration, glucose was released at a mean rate of 77 ± 13 mg/min.

During the period of the combined insulin and glucose infusions, a progressive decrease of the glucose outflow was observed in both catheterised liver lobes.

value obtained is 0.94 ± 0.13 mg/min when expressed per 100 ml; when referred to the thiocyanate space taken as reference for the glucose diffusion space, the mean value for endogenous glucose- ^{12}C influx is 70 ± 9 mg/min. This value stands close to the mean figures obtained by the catheterisation technique.

From the start of insulin infusion, the variations of the blood glucose- ^{14}C in respect to time are no longer ruled by a simple exponential law. On the semi-logarithmic graph they yield a curve with concavity upwards which seems to tend to an asymptote differing from zero; moreover the shape of the curve is not modi-

Table 4. Glucose 1-¹⁴C content of the arterial blood expressed in cpm/ml in basal period and during insulin infusion. The slopes (K) of the exponential decreases observed in basal period are also reported in the table

Exp. n°	Basal-Period					K	During Insulin Infusion						
	-40	-30	-20	-10	-0		15	30	45	60	75	90	105
132	4,050	3,270	2,590	2,180	1,660	2.1×10^{-2}	1,180	570	420	360	280	190	130
133	7,440	7,080	5,980	5,710	4,770	1.1×10^{-2}	3,100	2,200	1,200	750	680	580	460
135	5,820	5,000	4,390	3,860	3,330	1.4×10^{-2}	1,700	1,200	850	700	620	530	480
136	6,120	5,380	4,650	4,190	3,780	1.3×10^{-2}	1,750	1,500	1,020	780	620	470	400
142	3,430	2,850	2,340	2,120	1,700	1.7×10^{-2}	1,100	520	410	300	230	190	160
143	2,210	1,880	1,600	1,360	1,150	1.7×10^{-2}	800	500	340	260	210	160	120
147	2,790	2,470	2,200	1,950	1,720	1.2×10^{-2}	1,000	680	430	350	270	200	160
Means	4,590	3,990	3,390	3,050	2,590	1.5×10^{-2}	1,520	1,025	670	500	415	330	272
st. error	730	710	610	590	520	0.13×10^{-2}	295	244	132	87	81	70	62

fied by the arrest of the glucose compensation and by the glycogenolytic response induced by hypoglycemia (figure 5).

Fig. 5. Evolution of the arterial concentrations in glucose-¹²C and -¹⁴C on a semi-logarithmic graph. Figures given are the mean values surrounded by their standard error

To analyse mathematically this curve (16) the successive concentrations in blood glucose-¹⁴C (y) have been plotted against their respective time of sampling (t) in Cartesian coordinates and the value of the tangent ($-dy/dt$) has been estimated for each experimental point (figure 6.I). When the different values of $-dy/dt$ are plotted against the corresponding values of y they give a straight line which does not pass through the origin but intersects the y -axis at a positive value (y_0) (figure 6.II). The equation which applies to this straight line is

$$y = -\frac{1}{k'} \frac{dy}{dt} + y_0 \quad (\text{I})$$

where $\frac{1}{k'}$ is the slope; on rearrangement it gives

$$\frac{dy}{dt} = -k'(y - y_0), \quad (\text{II})$$

which may be integrated as

$$\log(y - y_0) = \log A - k't \quad (\text{III})$$

where A is the ordinate of the semilogarithmic straight line for $t=0$ and k' , the slope (figure 6, III).

In its exponential form, equation (III) becomes

$$y = Ae^{-k't} + y_0 \quad (\text{IV})$$

showing clearly that during the insulin infusion, the ¹⁴C-glucose is not completely cleared from the blood but tends to an equilibrium (y_0). This analysis has been applied successfully to each individual curve.

The tissue uptake of glucose-¹²C induced by insulin is ruled by a simple exponential law¹⁰: the utilization rate is related to the amount of insulin rapidly injected by the venous route up to about 0.3 U/kg, dose for which the maximal speed is reached¹⁰. Therefore, the equations (III) and (IV) which fit the blood disappearance curves of glucose-¹⁴C from the onset of insulin infusion express the fact that during this period the radioisotope is pouring into the blood as well as being taken up by the tissues at an accelerated rate: the

Fig. 6. Mathematical analysis of the mean curve of blood glucose-¹⁴C disappearance during insulin and glucose infusions (compensation period)

- I. mean blood glucose-¹⁴C content (y) plotted against time in Cartesian coordinates
 II. Evolution of the slopes ($-\frac{dy}{dt}$) against the respective values of blood glucose-¹⁴C (y)
 III. Exponential function drawn from curve I: ($y - y_0$) in logarithmic scale against time

second phenomenon is dominant during the first hour, equilibrium being practically reached after two hours.

If the disappearance rate of glucose- ^{14}C from blood is represented by K , its entry into the blood by F , and the initial concentration in blood by C , the resulting change in blood level will be in the derivative form

$$-\frac{dy}{dt} = Ky - F(C - y). \quad (\text{V})$$

At equilibrium, when $-\frac{dy}{dt} = 0$,

$$Ky_{eq} = F(C - y_{eq}) \text{ or } \frac{F}{K} = \frac{y_{eq}}{C - y_{eq}}, \quad (\text{VI})$$

equations in which y_{eq} is the value of y at equilibrium.

Combining equations (V) and (VI), we obtain by integrating:

$$\log(y - y_{eq}) = \log \text{Const.} - (K + F)t \quad (\text{VII})$$

Under its exponential form, it becomes

$$y = B \cdot e^{-(K+F)t} + y_{eq}, \quad (\text{VIII})$$

where $B = C - y_{eq}$.

It is obvious that equation (VIII) expressing the glucose- ^{14}C moves by reference to the influx and efflux rates F and K , is identical to equation (IV) drawn from the analysis of the experimental curve, when symbols B and y_{eq} are replaced by the corresponding ones used for the experimental data.

Therefore $k' = K + F$.

On figure 7 are reported the curves of differing ^{14}C -glucose movements which can be constructed from the analytical data.

Fig. 7. Semi-logarithmic representation of the observed curve of blood glucose- ^{14}C disappearance and of the constructed curves expressing the isolated efflux and influx of glucose- ^{14}C

Curve (1), observed evolution of the glucose radioactivity in the blood compartment:

$$y = Ae^{-k't} + y_0 \text{ or } y = 2100 e^{-0.033t} + 210;$$

Curve (2), theoretical blood disappearance according to the sole ^{14}C efflux:

$$y = Ce^{-kt} \text{ or } y = 2310 e^{-0.033t};$$

Curve (3), ^{14}C -influx in blood derived from (1) by subtraction of (2):

$$y = [Ae^{-k't} + y_0] - [Ce^{-kt}] \text{ or}$$

$$y = (2100 e^{-0.033t} + 210) - (2370 e^{-0.033t});$$

Curve (4), maximal ^{14}C influx compatible with the values obtained for K' ,

$$y = C(1 - e^{-Ft}) \text{ or } y = 2310(1 - e^{-0.004t}).$$

Discussion

Glucose Balance. Under strict normoglycemic conditions, continuous insulin infusion at a very high rate has a slow and progressive effect upon hepatic glucose

balance in the normal anaesthetised dog. About one hour is needed to produce a definite decrease in glucose release from both lobes in all animals. The reduction in glucose outflow is less marked than the reduction of the portohepatic difference in glucose concentration, due to the increase in estimated splanchnic blood flow recorded under the influence of glucose and insulin infusions. This latter change does not appear to be related to changes in the metabolism of bromsulphalein. There is no change in the rate of drug excretion and the decrease in the porto-hepatic differences of BSP concentration closely correlates with simultaneous increases in the oxygen content of portal and hepatic venous bloods. Moreover a moderate and progressive lowering of the portal pressure coincides with the maintenance of an unchanged arterial blood pressure. As the fasted and anaesthetized animals are kept at constant levels of oxygenation and hydration, in a state of muscular relaxation and at a constant body temperature, these changes must reflect true circulatory changes in the splanchnic area.

The present findings concerning hepatic glucose balance are in agreement with preceding experiments performed on the same canine preparation with insulin anti-bodies¹³. On the other hand, they do not support the idea that the absence of inhibitory effect observed in the normal animal after insulin administration and without compensatory glucose infusion, could be due to saturation of the liver by its normal insulin supply^{1, 12, 11}. This lack of effect in the latter instance is more probably related to the slowness of the liver to respond to insulin administration. Without simultaneous glucose administration, insulin's own action is masked by the influence of hypoglycemia. When the concentration of glucose in the blood lies outside the normal range, the disturbing effects of these abnormal concentrations may well dominate the action of insulin itself. In hyperglycemia the action of insulin enhances the stimulus of the glucose load upon glucose uptake by the liver¹⁴ whilst even mild hypoglycemia induced by portal infusions of insulin overcomes the hepatic actions of the hormone¹⁸.

Glucose- ^{14}C Studies. The exponential rate at which glucose- ^{14}C disappears from the blood makes it possible to calculate the turnover rate of glucose which is very close to that determined from the results obtained by liver catheterisation¹⁹. Striking changes in glucose- ^{14}C metabolism are observed following the onset of insulin and glucose infusions. Intravenously injected insulin without glucose compensation does not change the shape of the blood glucose- ^{14}C disappearance curve, but produces an increase of the slope which is only observable during the first 20 to 30 minutes and which is later on interrupted by the glycogenolytic response to hypoglycemia¹². In the present experiments, however, we found a more complex relationship which may be defined by a single equation covering the complete period and which does not have to be modified to cover the later period after the cessation of the

glucose infusion when there is a glycogenolytic response to hypoglycemia. According to this equation the glucose- ^{14}C content of the blood, following insulin administration, is at any time the resultant of two antagonistic exponential phenomena, the averaged rates of efflux and influx being respectively 2.9×10^{-2} and 0.4×10^{-2} . Such an influx rate is too marked to be attributed to redistribution of radioactivity between the differing glucose pools inside the extracellular space. Since it is not modified by the glycogenolytic response of the liver to hypoglycemia, this recycling phenomenon must originate in the liver for this organ is the only one possessing significant quantities of glucose-6-phosphatase.

BAKER and coworkers², using a digital computer to analyse the changes in blood glucose— ^{14}C specific activity in the anaesthetised rat, concluded that, under basal condition and following a single intravenous injection of the labeled glucose, the simple exponential equation proposed by the group of CHAIKOFF⁹ was only an approximation. They suggest a system of equations corresponding to a mamillary model of three compartments in which the glucose- ^{14}C would equilibrate between the plasma, the interstitial compartment and a third non-glucose metabolic compartment in connection with the first two. The present experiments are in agreement with that concept.

Comparison between Glucose- ^{12}C and ^{14}C Data. The strict normoglycemic conditions maintained during the infusions of glucagon-free insulin exclude the possibility of interference by a glycogenolytic hormone. One is forced to conclude, therefore, that under these experimental conditions insulin is capable of simultaneously reducing the outflow of glucose- ^{12}C and increasing that of glucose- ^{14}C previously stored into the liver. A direct action of insulin upon glucokinase^{23, 28} and upon liver glycogen synthesis⁷ has been established. In particular, DE DUVE⁷ showed that the incorporation of ^{14}C from glucose- ^{14}C into glycogen in normal rabbit-liver slices is stimulated by insulin; but this effect, highly significant after an hour's incubation, disappears completely after two hours.

The discrepancy between the results obtained with glucose- ^{12}C and ^{14}C may be explained by the structure and the growth of the glycogen molecule. In the first hour following administration of glucose- ^{14}C to a normal animal, radioactivity is fixed at the periphery of the molecule^{25, 26}. As phosphorylase begins at the periphery and progresses towards the centre of the molecule^{25, 26}, a moderate degradation process will first remove the labeled glucose radicals preferentially. This effect has recently been demonstrated by GOLDMAN and CAHILL¹⁵; analyses of liver 15 minutes after injection of labeled glucose, with and without insulin, to differing groups of normal rats showed a marked decrease of labeled glycogen specific activity without changes in glycogen content in the insulin treated groups. The following calculations made on the basis of their data for fasting animals illustrate this fact:

	Control	Insulin Treated
μM glycogen/g liver	75	94
DPM/ μM glycogen	9.80	1.31

One hour after insulin administration, the depletion of hepatic unlabeled glycogen secondary to hypoglycemia is obvious.

A working hypothesis concerning the twofold action of insulin is that this hormone acts both on the synthesis and breakdown of the hepatic glycogen thus accelerating the turnover rate of this compound. The resultant effect upon the balance between glucose and glycogen in the liver will depend upon the relative influences of these two processes. In insulin deficiency, for example, the stimulant effect on glycogen synthesis would cease. The hypothesis needs further experimental support but, if confirmed, might account for the discrepancies recorded in the literature concerning the action of insulin upon hepatic glucose metabolism.

Acknowledgment

We wish to thank the Novo Therapeutisk Laboratories (Copenhagen, Denmark) and the Radiochemical Laboratories of Farbwerke Hoechst A.G. (Griesheim, Germany) for the generous supply of the insulin and insulin- ^{131}I used in the present study.

References. ¹ASHMORE, J., G.F. JR. CAHILL, A.S. EARLE and S. ZOTTU: Studies on the disposition of blood glucose. A comparison of insulin and orinase. *Diabetes*, **7**, 1–8 (1958). — ²BAKER, N., R.A. SHIPLEY, R.E. CLARK and G.E. INCEFFY: ^{14}C studies in carbohydrate metabolism glucose pool size and rate of turnover in the normal rat. *Amer. J. Physiol.* **196**, 245–252 (1959). — ³BERNSTEIN, I.A., K. LEUTZ, M. MALM, P. SCHAMBYE and H.G. WOOD: Degradation of glucose- ^{14}C with *Leuconostoc mesenteroides*; alternate pathways and tracer patterns. *J. biol. Chem.* **215**, 137–152 (1955). — ⁴BRADLEY, S.E., F.J. INGELFINGER, G.P. BRADLEY and J.J. CURRY: The Estimation of hepatic blood flow in man. *J. clin. Invest.* **24**, 890–897 (1945). — ⁵CACHERA, R. and M. LAMOTTE: La détermination du volume des liquides extracellulaires par la méthode au thiocyanate de sodium. *Sem. Hôp. Paris* **26**, 500–516 (1950). — ⁶CONRAD, V., J.R.M. FRANCKSON, H.-A. OOMS, W. MALAISSE and E. RASIO: Comparaison des espaces de diffusion mesurés par le glucose ordinaire et par le radioglucose (^{14}C) in *Radioaktive Isotope in Klinik und Forschung*, p. 325–336, 1 vol. Urban Ed. München 1965. — ⁷DE DUVE, C., F. G. YOUNG, W. A. BROOM and F.W. WOLFF: The mechanism of action of insulin, p. 85–91, Blackwell Scientific publications, Oxford 1960. — ⁸DEMOS, R.S., R.C. BARD and J.C. GUNSALES: The mechanism of the heterolactic fermentation; a new route of ethanol formation. *J. Bact.* **62**, 499–511 (1951). — ⁹FELLER, D.D., E.H. STRISOWER and I.L.: CHAIKOFF: Turnover and oxidation of body glucose in normal and alloxan diabetic rats. *J. biol. Chem.* **187**, 571–588 (1950). — ¹⁰FRANCKSON, J.R.M.: Mesure de l'activité de l'insuline chez l'homme. 1 vol. Acta med. belg. éd. Bruxelles 1958. — ¹¹FRANCKSON, J.R.M., V. CONRAD and H.-A. OOMS: Modification des glycémies systémique, splanchnique et du débit glucosé du foie sous l'influence du glucose et de l'insuline. *Arch. internat. Pharmacodyn.* **136**, 26–46 (1962). — ¹²FRANCKSON, J.R.M., H.-A. OOMS, R. BELLENS, Y. ARNOULD and V. CONRAD: Action d'une perfusion intraportale d'insuline sur la glycémie artérielle et le débit glucosé hépatique du chien. *Arch. internat. Pharmacodyn.* **142**, 293–311 (1963).

- ¹³ FRANCKSON, J. R. M., Y. ARNOULD, W. MALAISSE and V. CONARD: Glucose metabolism in the normal anesthetized dog injected successively with anti-insulin serum and insulin. *Diabetes*, **13**, 532–541 (1964). — ¹⁴ FRANCKSON, J. R. M. and V. CONARD: Analysis of the action of insulin on glucose metabolism in the liver, in: Second international Congress of Endocrinology, 1 vol. Excerpta Medica ed. (in the press). — ¹⁵ GOLDMANN, J. K. and G. F. JR. CAHILL: Insulin effect on glucose-¹⁴C incorporation into rat liver and adipose tissue in vivo. *Metabolism*, **13**, 572–578 (1964). — ¹⁶ GREVILLE, G. D.: The Intravenous glucose tolerance equation. *Biochem. J.* **37**, 17–24 (1943). — ¹⁷ HOFFMAN, W. S.: A rapid photoelectric method for the determination of glucose in blood and urine. *J. biol. Chem.* **120**, 51–55 (1937). — ¹⁸ MADISON, L. L., D. MEBANE, F. LECOCQ and B. COMBES: Physiological significance of the secretion of endogenous insulin into the portal circulation. V. The quantitative importance of the liver in the disposition of glucose loads. *Diabetes*, **12**, 8–15 (1963). — ¹⁹ OOMS, H. A., J. R. M. FRANCKSON and V. CONARD: Comparaison des mesures de la sécrétion glucosée du foie par le cathétérisme et par le radioglucose. *Arch. intern. Pharmacodyn.* **134**, 343–359 (1961). — ²⁰ PROUT, T. E. and I. E. EVANS: Determination of the rate of insulin destruction in vivo. *Ann. N.Y. Acad. Sc.* **74**, 530–536 (1959). — ²¹ SCOTT, G. W., T. PROUT, J. WEAVER and S. ASPER: A comparison of the behavior of insulin and insulin labelled with ¹³¹I in serum. *Diabetes* **7**, 38–45 (1958). — ²² SHOEMAKER, W., R. MAHLER and J. ASHMORE: The effect of insulin on hepatic glucose metabolism in the unaesthetized dog. *Metabolism* **8**, 494–511 (1959). — ²³ SOLS, A.: Glucokinase, hexokinase and the regulation of glucose phosphorylation. Communication at the 6th international congress of Biochemistry. New York 1964 (in press). — ²⁴ STEELE, R., J. S. WALL, R. C. DE BODO and N. ALTSZULER: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Amer. J. Physiol.* **187**, 15–24 (1956). — ²⁵ STETTEN, M. R. and D. JR. STETTEN: A study of the nature of glycogen regeneration in the intact animal. *J. biol. Chem.* **207**, 331–340 (1954). — ²⁶ STETTEN, M. R., and D. JR. STETTEN: Glycogen regeneration in vivo. *J. biol. Chem.* **213**, 723–732 (1955). — ²⁷ TARDING, F. and P. SCHAMBYE: The action of sulphonylureas and insulin on the glucose output from the liver of normal dogs. *Endocrinology*, **36**, 222–228 (1958). — ²⁸ VESTER, J. W.: Insulin effect on hepatic glucokinase: response to variation in substrate level. in International Congress series, **74**, p. 57, Excerpta Medica ed. Amsterdam 1964. — ²⁹ ZIJLTRA, W. G. and G. A. MOOK: Medical reflection photometry. 1 vol. Van Gorkum ed. Assen, The Netherlands, 1962.

W. MALAISSE
Departments of Experimental
Medicine and General Pathology
University of Brussels/Belgium