The Role of the Liver in the Disposal of Orally Administered ^{14}C -Glucose in the Normal Rat*

S. L. JEFFCOATE and A. J. MOODY

Novo Research Institute, Copenhagen, Denmark

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Summary. 6- ^{14}C -glucose (specific activity 6.0 $\mu\text{Ci/g}$) was administered by oesophageal tube to fasted normal rats; the load was 1.5 g/kg. Rats were killed, in groups, at 0, 10, 20, 30, 60, 120 and 180 min after administration of the load, and the blood and liver sampled. Serum insulin and serum glucose reached a peak at 20 min. The radioactive serum glucose measured by the radioactivity in the dimedone derivatives of 6-C of glucose reached a peak at 60 min. At this time, 92% of the serum glucose originated from the load. Liver glycogen started to rise after 10–20 min, reaching a maximum at 120 min. The incorporation of ^{14}C -glucose into liver glycogen paralleled the changes in total glycogen. It is suggested that during the first hour following an oral glucose load there is a reduction in the hepatic release of glucose, accompanied by an increase in hepatic glycogen synthesis. During the second hour there is a further marked increase in hepatic glycogen. Between 120 and 180 min there is a decrease in hepatic glycogen, and hepatic glucose release increases. The results emphasize the important role of the liver in the disposal of an oral glucose load.

Le rôle du foie dans l'utilisation de ^{14}C -glucose administré par voie orale au rat normal

Résumé. Le 6- ^{14}C -glucose (activité spécifique 6.0 $\mu\text{Ci/g}$) a été administré à l'aide d'une sonde oesophagienne à des rats normaux à jeun; le quantité administrée fut de 1.5 g/kg. Les rats furent sacrifiés par groupes, 0, 10, 20, 30, 60, 120 et 180 min après l'administration du glucose, afin d'en prélever le sang et le foie. L'insuline et le glucose du sérum plafonnèrent après 20 min. Le glucose radioactif contenu dans le sérum et mesuré par la radioactivité des dérivés du dimédon du 6-C du glucose, plafona après 60 min. A ce moment 92% du glucose sérique provenaient du glucose radioactif administré. Le glycogène du foie commença à augmenter après 10–20 min, pour atteindre son maximum à 120 min. L'incorporation du ^{14}C -glucose dans le glycogène du foie augmenta parallèlement au glycogène total. On suppose que pendant la première heure qui suit une administration orale de glucose, il y a réduction de la libération hépatique du glucose accompagnée

d'une augmentation de la synthèse du glycogène hépatique. Au cours de la deuxième heure, il y a augmentation ultérieure du glycogène hépatique. Entre 120 et 180 min, la quantité de glycogène hépatique décroît tandis que la libération du glucose hépatique s'accroît. Les résultats mettent en évidence le rôle important du foie dans l'utilisation du glucose administré par voie orale.

Die Rolle der Leber im Hinblick auf die Verwertung oral verabreichter ^{14}C -Glucose bei der normalen Ratte

Zusammenfassung. Normalen Ratten im Hungerzustand wurde 6- ^{14}C -Glucose (spezifische Aktivität 6.0 $\mu\text{Ci/g}$) mittels einer Schlundsonde zugeführt. Die verabreichte Menge betrug 1.5 g/kg. Die Ratten wurden gruppenweise getötet und zwar 0, 10, 20, 30, 60, 120 und 180 Min. nach der Glucosebelastung. Proben wurden sowohl dem Blut als auch der Leber entnommen. Das Serum-Insulin und die Serum-Glucose erreichten ihre Höchstwerte nach 20 Min. Die radioaktive Serum-Glucose, die als Radioaktivität der Dimedone-Derivate der 6-C-Glucose gemessen wurde, erreichte ihr Maximum nach 60 Min. Zu diesem Zeitpunkt entstammten 92% der Serum-Glucose der verabreichten Belastung. Der Glykogen-Gehalt der Leber begann nach 10–20 Min. zu steigen und erreichte sein Maximum nach 120 Min. Der Einbau der ^{14}C -Glucose verlief parallel zu den Änderungen der Gesamt-Glykogenmenge. Es wird vermutet, daß im Laufe der ersten Stunde nach einer oralen Glucose-Belastung die Glucose-Abgabe aus der Leber reduziert wird bei gleichzeitig gesteigerter Glykogen-Synthese der Leber. Im Laufe der darauffolgenden Stunde steigt der Glykogen-Gehalt in der Leber weiter deutlich an. Im Zeitraum zwischen 120 und 180 Min. nach der Glucoseabgabe verringert sich der Gehalt an Leber-Glykogen, während sich die Glucose-Abgabe aus der Leber erhöht. Diese Ergebnisse unterstreichen die wichtige Rolle der Leber im Hinblick auf die Verwertung oral zugeführter Glucose im Organismus.

Key-words: Glucose, ^{14}C -glucose, oral load, rat, liver, serum insulin, glycogen.

Introduction

The liver has been known to have an important role in glucose homeostasis since the work of Claude Bernard, but there is little information on its quantitative significance in the disposal of oral glucose.

The liver could enhance tolerance to glucose loads by reducing its glucose output and/or by increasing its glucose uptake. Both processes occur following the intravenous administration of glucose. This has been shown by a variety of techniques *in vivo*: transhepatic catheterization in dogs (COMBES et al., 1961; LANDAU et al., 1961); ^{14}C -glucose injection or infusion in dogs

and man (reviewed by STEELE, 1966). The hepatic disposal of an oral glucose load in unoperated animals has been little studied.

The experiments reported here were performed to elucidate the role of the liver in glucose homeostasis following an oral glucose load in fasted, unoperated rats.

Methods

Rats. 250 g male Wistar rats were fasted overnight.

Glucose load. 1.5 g glucose/kg body weight was administered to each rat as a 25 per cent solution of 6- ^{14}C glucose, 6.0 $\mu\text{Ci/g}$. A diluted aliquot of this stock solution was used as reference for the determination of ^{14}C in the samples.

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Administration of load. The rats were sedated with light ether anaesthesia, and an oesophageal tube passed until the tip was in the stomach. The glucose load was injected and the tube removed. Recovery from the anaesthetic took 2–3 min.

Experiments. Three experiments were carried out on different days. Rats were killed in groups of 4 by stunning and decapitation 10, 20, 30, 60, 120 and 180 min after administration of the glucose. Control animals were killed after handling and passage of the tube. Neck blood (about 2 ml) was drained into a cooled test-tube, which was placed in ice-water. In a control experiment, the glucose concentration of neck blood did not differ from that of arterial blood taken at the same time ($p > 0.3$). A lobe of liver (about 1 g) was rapidly excised and frozen in acetone-solid CO₂ within one minute of death. This method of freezing was compared with the freeze-clamping technique in one experiment. The differences were not significant ($p = 0.5$) for glycogen ($n = 22$) nor for ¹⁴C ($n = 16$). The remainder of the liver was removed for weighing.

The rate of absorption of the administered glucose from the gut was measured in a separate experiment. Rats were given a glucose load, and killed in groups of four at the same time intervals as above. The gastrointestinal tract from the oesophagus to the colon was transferred to a beaker containing about 50 ml of water. The gut was cut open and washed out, and an aliquot taken for counting of ¹⁴C.

Analysis of samples

a) Blood. The blood was allowed to clot on ice for 60 min, and the serum prepared by centrifugation at 4°C. The blood glucose of freshly-drawn, rat arterial blood does not alter during 90 min in ice-water. The serum immunoreactive insulin (IRI) was determined by radioimmunoassay (HEDING, 1966), using standards of rat insulin. The serum was deproteinised (SOMOGYI, 1945). Aliquots of the protein-free supernatant solution were assayed for glucose (glucose oxidase kit from Boehringer) and glucose 6-¹⁴C. This was measured following the formation of the dimedone derivative of formaldehyde after oxidation of glucose (10 mg carrier glucose was added to each sample) with periodic acid (DUNN et al., 1957). The dimedones were recrystallized once from acetone. The ¹⁴C in each sample was corrected for the recovery, measured by weight, of the added carrier glucose.

b) Liver. The frozen lobes of liver were weighed and transferred while frozen to 4 ml of hot (60–70°C) 15% KOH in 30% ethanol. The liver was then dissolved by heating at 100°C for 20–30 min. Aliquots of this solution were dried on Whatman 3 mm paper inside liquid scintillation vials. Twenty ml of toluene-based liquid scintillator (GLIEMANN, 1967) was added for the determination of total liver ¹⁴C. In one experiment, aliquots were taken in duplicate and counted with and without an internal ¹⁴C-glucose standard. The

mean quenching in 30 samples was 3.8 ± 2.6 per cent (SD). In the subsequent two experiments quenching was estimated by automatic external standardization.

Glycogen was precipitated from the remainder of the extract by adding two volumes of ethanol (8 ml) and saturated sodium sulphate (50 µl) (VAN HANDEL, 1965). After leaving at –20°C overnight, the precipitate was separated by centrifugation at 4°C, at 2000 x g for 40 min. The precipitate was dissolved in water (4 ml) and reprecipitated with ethanol (8 ml) at –20°C for 35 min, centrifuged and dried. The precipitate was then dissolved in 0.1 M phosphate buffer pH 6.0 (5 ml). Two ml was taken for ¹⁴C counting in 15 ml scintillator (BRAY, 1960), and aliquots (5–50 µl) taken for glycogen assay. The glycogen was determined using a FERMOCOZYME (Hughes and Hughes, England) plus peroxidase (Boehringer, Germany) reagent similar to that proposed by RERUP and LUNDQUIST (1967). The enzymes were dissolved in 0.1 M phosphate buffer (pH 6.0), and the chromogen was o-dianisidine. The glycogen samples were incubated with the reagent for 60 min at 37°C. The reaction was stopped by the addition of 25 per cent sulphuric acid, and the final colour read at 520 mµ.

The total liver fatty acids were isolated from the supernatant fluid of the first precipitation of the liver glycogen. The total ¹⁴C in fatty acids was estimated. The results are not reported, for the counts incorporated into fatty acids never rose higher than 0.1 per cent of the total load.

Calculations. The data from the groups of 4 rats at each time interval in the three experiments were pooled, and the means and standard errors of the means calculated.

1. Blood. Serum insulin was measured in µU/ml and serum glucose in mg/100 ml. The ¹⁴C in carbon 6 of serum glucose was expressed as per cent of the administered ¹⁴C/ml serum. From this the serum glucose derived from glucose in the oral load was calculated:

$$\text{load component of serum glucose (mg/100 ml)} = \\ (\text{per cent of administered } ^{14}\text{C in carbon 6 of glu-} \\ \text{cose/ml serum}) \times \text{total load (mg)} \times 100.$$

The serum glucose due to non-load glucose was then calculated by subtracting the load component from the total serum glucose.

2. Liver. The total ¹⁴C in the liver and that in the glycogen fraction were calculated for each sample by correcting the measured ¹⁴C by the appropriate aliquot factors, and expressed as per cent of administered ¹⁴C/liver. Total liver glycogen was measured as mg glucose/liver. The glycogen derived from glucose in the oral load was then calculated:

$$\text{load component of liver glycogen (mg glucose/liver)} \\ = (\text{per cent of administered } ^{14}\text{C in liver glycogen/} \\ \text{liver}) \times \text{total load (mg)}.$$

The non-load component was then calculated by subtraction as above.

Results

1. *Blood.* The concentrations of insulin and glucose in serum are shown in Fig. 1. The rise in insulin paralleled that of glucose; both rose to a maximum at 20 min and thereafter fell to reach fasting levels by 180 min.

Fig. 2 shows the rate of disappearance of the ad-

SERUM GLUCOSE $\text{---}\square\text{---}$ AND SERUM INSULIN $\text{---}\circ\text{---}$
MEAN AND S.E.M.

Fig. 1. Serum glucose (mean $\pm$ S.E.M.) and serum insulin (mean $\pm$ S.E.M.) after an oral glucose load (1.5 g/kg body weight) in normal rats

component fell until, at 60 min, 92 per cent of the total serum glucose consisted of load glucose. Between 120 and 180 min, the non-load component rose as the load glucose fell.

2. *Liver.* The total ^{14}C in the liver and that in liver glycogen are shown in Fig. 4. The appearance of total ^{14}C was almost linear from 0–120 min, but the incorporation of ^{14}C into glycogen was delayed for 20–30 min. Both reached a maximum at 120 min, when 16 per cent of the administered ^{14}C was in liver glycogen and a further 2.7 per cent in other liver constituents (which will include blood and interstitial fluid).

Fig. 5 shows the total liver glycogen (as mg glucose/liver) at each time and the amounts originating from load and non-load glucose. The total liver glycogen rose after 10–20 min and reached a peak at 120 min. At this time, the mean glycogen level was 102 mg/liver above the fasting level; this is equivalent to 27 per cent of the load. Fifty per cent or more of the rise in liver glycogen at 20, 30 and 60 min cannot be accounted for by incorporation of glucose from the load.

A calculation of the disposition of the load at various times after administration is shown in Table 1. The fraction in the glucose pool was calculated from the ^{14}C in carbon 6 of serum glucose, assuming a total glucose pool volume of 50 ml (20 per cent of body weight) (BAKER et al., 1959) and assuming the serum glucose ^{14}C to be representative of the ^{14}C in the whole glucose pool at any time. This will only be an approximation, since the ^{14}C in the serum was changing

Table 1. The disposal of an oral load of ^{14}C -glucose by the normal rat. The per cent of the load in the glucose pool was calculated assuming a pool volume of 50 ml

Per cent of total load	Time after load (minutes)					
	10	20	30	60	120	180
1. Absorbed	14.0	33.0	37.0	58.0	94.5	96.4
2. Glucose pool (as C 6 of glucose)	7.3	12.0	12.3	16.2	11.4	4.7
3. Liver	1.2	2.8	4.9	9.9	18.7	17.5
4. Oxidized ^a	—	—	3.5	10.0	30.0	40.0
5. Unaccounted for ^b 1 — (2 + 3 + 4)	5.5	18.2	16.3	21.9	34.4	34.2

^a data of SHIPLEY et al. (1967) and ZILVERSMIT et al. (1948).

^b includes disposal of ^{14}C by tissues other than the liver and ^{14}C in metabolites other than glucose (carbon 6) in the glucose pool.

ministered ^{14}C -glucose from the gut. The mean rate was 0.9 per cent of the load/minute or 3.4 mg/minute during the first 30 min, and 0.64 per cent of the load/minute or 2.5 mg/minute between 30 and 120 min. By 120 min, 94.5 per cent of the load had been absorbed.

Fig. 3 shows the amounts of the total serum glucose that originated from the ingested glucose and from endogenous non-load glucose. The rise in serum glucose during the first 10 min was due entirely to ingested glucose, the non-load component being unchanged. Thereafter the load component rose and the non-load

throughout the experiments. The data on the per cent of an injected load oxidized to CO_2 by normal rats at various times are taken from SHIPLEY et al. (1967) and ZILVERSMIT et al. (1948). The fraction not accounted for, represents glucose that has been taken up by tissues other than the liver, but it also includes ^{14}C in metabolites other than glucose (carbon 6) in the glucose pool.

Discussion

Serum insulin and serum glucose. During the first 10 min after administration of the load there was a

rise in serum glucose due to ingested glucose. By 20 min, both serum glucose and serum insulin had reached a maximum, and the non-load component of the serum

glucose was being removed from the glucose pool at a much faster rate than the supply of endogenous glucose by the liver.

Fig. 2. ^{14}C remaining in the intestine at various times after a ^{14}C glucose load (means $\pm$ S.E.M.)

Fig. 3. Load component (hatched areas) and non-load component (white areas) of serum glucose after an oral glucose load

glucose had begun to fall. At 60 min, when the load component was maximal, only 8 per cent of the serum glucose was composed of endogenous, unlabelled glucose. This means that during this period, endogenous

Fig. 4. Appearance of ^{14}C in the liver and in liver glycogen (mean $\pm$ S.E.M.)

Fig. 5. Load component (hatched areas) and non-load component (white areas) of liver glycogen glucose after an oral glucose load

A variety of experimental techniques have been used to show that glucose administration reduces hepatic glucose output. Experiments using transhepatic catheterization, which only measure *net* hepatic glucose

output (that is, the balance between output and uptake) have shown this effect of administered glucose in dogs (CHERRY and CRANDALL, 1937; SOSKIN et al., 1938; COMBES et al., 1961; LANDAU et al., 1961), in normal human subjects (BONDY et al., 1949; BEARN et al., 1953; HULTMAN and CASTENFORS, 1961) and in the perfused rat liver (McCRAW et al., 1967).

Using the technique of a single injection of tracer glucose, SEARLE and CHAIKOFF (1952) also demonstrated a decrease in the release of unlabelled glucose following intravenous glucose loading. The single-injection technique was shown to be unreliable (STEELE et al., 1959; STEELE, 1966). Later, STEELE et al. (1965) confirmed the conclusions of previous workers using the priming dose and constant infusion technique.

Despite earlier conflicting results, it has now also been shown that insulin administration reduces hepatic glucose output, provided hypoglycaemia is avoided (for refs., see STEELE, 1966).

In the present study, hepatic glucose output at any time is uncalculable. The marked fall in the non-load component of serum glucose between 10 and 60 min shows that the release of unlabelled glucose by the liver is slower than its uptake.

Liver uptake of glucose. The incorporation of ^{14}C -glucose into liver glycogen was maximal 2 h after oral administration of the load. At this time, 16 per cent of the administered glucose was present in glycogen, which corresponds to about 60 mg of the 375 mg load. The rise in total liver glycogen of glucose at this time was 102 mg/liver; thus about 40 mg of the increase was derived from non-load glucose.

In Fig. 6, the time-course of changes of specific activity of serum glucose and liver glycogen glucose are compared. The rise in specific activity of glycogen glucose is both delayed and less steep relative to that of serum glucose. This indicates that the glucose from the blood incorporated into liver glycogen, particularly in the first 60 min, is diluted with endogenous gluconeogenic precursors.

On the basis of experiments in which percutaneous liver biopsy samples were taken during intravenous ^{14}C -glucose infusion in dogs, BISHOP et al. (1965) reached conclusions that are similar to ours. They showed that during the early periods of insulin infusion the predominant hepatic effects were decreases in glycogen loss and unlabelled glucose release. Later, the outstanding effect was a great increase in the use of plasma glucose for the synthesis of liver glycogen. BISHOP et al. (1965) suggested two possible mechanisms to explain the observed early effects of insulin on the liver: either hepatic glycogenolysis was being inhibited or hepatic glycogen synthesis from endogenous (unlabelled) glucosyl residues was stimulated in a way other than through causing an increase in the uptake of plasma glucose by the liver. Our findings regarding the relative specific activities of glucose in the serum and in liver glycogen are in accordance with these postulates.

The increases in liver glycogen, both unlabelled and labelled, in these experiments were delayed for 10–20 min. BISHOP and LARNER (1967) have recently shown, in dogs, that an intravenous infusion of insulin activates liver uridine diphosphate glucose-glycogen transferase (E.C. 2.4.1.11) within 7 to 13 min and that it also inactivates liver phosphorylase. The observed delay of 10–20 min between the rise in serum insulin and the increase of hepatic glycogen in our experiments is compatible with this.

Fig. 6. Specific activity (expressed as per cent of administered ^{14}C /mg glucose) of serum glucose and liver glycogen glucose

Quantitative significance of the liver in disposal of oral glucose. The relative importance of the liver in the disposal of the ^{14}C -glucose can be seen in Table 1. During the first 20 min after the glucose load, 33 per cent of the load was absorbed from the intestine but only 2.8 per cent was in the liver. The liver thus contained only 9 per cent of the glucose that had been absorbed at that time. Hepatic disposal of the load increases in the later stages: 23 per cent of the glucose absorbed between 30 and 60 min and 25 per cent of that absorbed between 60 and 120 min were retained by the liver. These figures show the significance of the liver in the disposal of the administered ^{14}C -glucose, but will underestimate the total role of the liver in glucose tolerance since data on hepatic uptake and out-

put of unlabelled glucose are not included and also net flux cannot be estimated.

Several other attempts have been made to assess the quantitative role of the liver in the tolerance of glucose loads.

COMBES et al. (1961), measuring *net* hepatic glucose balance in dogs by transhepatic catheterization, and making assumptions regarding the glucose pool size, calculated that the hepatic component was almost as great as the peripheral uptake component following *intravenous* glucose.

Two studies have suggested that the liver may be responsible for the tolerance of 2/3 of an oral glucose load. SCOW and CORNFIELD (1954) gave oral glucose to rats, and from the observed rate of disappearance of glucose from the intestine and the observed rate of disappearance of intravenously injected glucose (in a separate experiment), they calculated a *theoretical* glucose tolerance curve. The area circumscribed by the *measured* oral glucose tolerance curve was about 1/3 the area of the theoretical curve. They concluded that the liver had accounted for the remaining 2/3.

Using a different approach in normal human subjects, PERLEY and KIPNIS (1967) came to similar conclusions. They found that the amount of glucose that was required to be infused intravenously to match the glucose curve produced by 100 g given orally (using each subject as his own control) was only 31 g.

STEELE et al. (1968) have calculated, using the ^{14}C -glucose tracer technique, that, following oral glucose loads in dogs, a reduction of the hepatic glucose output accounted for 42% of the glucose tolerance, the remainder being through glucose uptake by the liver and extrahepatic tissues.

These studies underline the important role of the liver in glucose tolerance in normal animals and suggest that any abnormality of liver function will have marked effects on glucose tolerance.

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S.L. JEFFCOATE M.D.
Dept. of Metabolic Diseases
St. Thomas's Hospital
London S.E. 1
England