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# The Contribution of Different Organs and Tissues of the Rat to Assimilation of Glucose

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*Summary.* After intravenous injection of glucose, 750 mg/kg, together with a tracer dose of [U-$^{14}$C] glucose, into fasted, adult white rats, the following percentages of the administered dose were found in whole organs and tissues: 1. after five minutes: skeletal muscle 30.3, skin 28.1, blood 13.1, adipose tissue 10.7, liver 8.9. 2. Forty minutes after injection the corresponding values were: 35.0, 11.1, 5.0, 4.6 and 9.4%. Expired $^{14}CO_2$ was negligible after five minutes: after 40 min it comprised 8% of the total dose administered. — After intragastric administration of 1500 mg/kg of glucose given with a tracer dose of [U-$^{14}$C] glucose under the same experimental conditions, the alimentary tract contained, after 15, 90 and 180 min, 60.5, 14.8 and 8.4% respectively of the total $^{14}$C dose given. At these times the liver contained 2.9, 10.7 and 15.0%; skin contained 7.5, 7.1 and 5.4%; adipose tissue 2.0, 3.8 and 3.5%, and expired $^{14}CO_2$ 0.4, 11.8 and 31.3% respectively. Details of the uptake of $^{14}$C glucose by other organs and tissues are given, and a balance sheet for the injected material is attempted.

*Contribution de différents organes et tissus du rat à l'assimilation du glucose*

*Résumé.* Après l'injection intraveineuse de glucose (750 mg/kg) en même temps qu'une dose traceuse de U-$^{14}$C-glucose, à des rats blancs adultes à jeun, les pourcentages suivants de la dose administrée ont été retrouvés dans tous les organes et tissus: 1. après cinq minutes: muscle squelettique 30.3, peau 28.1, sang 13.1, tissu adipeux 10.7, foie 8.9. 2. Quarante minutes après l'injection, les valeurs correspondantes étaient les suivantes: 35.0, 11.1, 5.0, 4.6 et 9.4%. Le $^{14}CO_2$ dégagé était négligeable après 5 min: après 40 min il représentait 8% de la dose totale administrée. — Après l'administration intragastrique de 1500 mg/kg de glucose, donné en même temps qu'une dose traceuse de U-$^{14}$C-glucose dans les mêmes conditions expérimentales, le tube digestif contenait au bout de 15, 90 et 180 min, 60.5, 14.8 et 8.4% respectivement de la dose totale de $^{14}$C administrée. A ces moments là le foie contenait respectivement 2.9, 10.7 et 15.0%; la peau contenait 7.5, 7.1 et 5.4%; le tissu adipeux 2.0, 3.8 et 3.5%, et le $^{14}CO_2$ dégagé était de 0.4, 11.8 et 31.3%. On donne des détails sur la captation du $^{14}$C-glucose par d'autres organes et tissus, et on essaye de dresser un bilan du produit injecté.

*Der Anteil verschiedener Organe und Gewebe der Ratte an der Glucoseassimilation*

*Zusammenfassung.* Nach i.v. Injektion von 750 mg Glucose/kg mit einer Sprüdosis U-$^{14}$C-Glucose fanden sich bei fastenden, erwachsenen weißen Ratten folgende Prozentsätze der verabreichten Menge in Gesamt-Organen und -Geweben: 1. Nach 5 min: Skeletmuskel 30.3, Haut 28.1, Blut 13.1, Fettgewebe 10.7, Leber 8.9. 2. 40 min nach der Injektion lauteten die entsprechenden Werte: 35.0, 11.1, 5.0, 4.6, 9.4%. Nach 5 min ließen sich in der Ausatmungsluft nur Spuren von $^{14}CO_2$ nachweisen, nach 40 min lagen 8% der verabreichten Dosis in dieser Form vor. — Nach intragastrischer Zufuhr von 1500 mg Glucose/kg mit einer Spürdosis von U-$^{14}$C-Glucose unter den gleichen Versuchsbedingungen enthielt der Verdauungstrakt nach 15, 90 und 180 min jeweils 60.5, 14.8 bzw. 8.4% der zugeführten Radioaktivität. In der Leber fanden sich zu diesen Zeiten 2.9, 10.7 und 15.0%, in der Haut 7.5, 7.1 und 5.4, im Fettgewebe 2.0, 3.8 und 3.5%. Die Ausatmungsluft enthielt 0.4, 11.8 und 31.3% als $^{14}CO_2$. Es folgen weitere Einzelheiten zur Aufnahme von Radioglucose durch andere Organe und Gewebe sowie der Versuch einer Bilanz für das injizierte Material.

*Key-words:* Glucose, assimilation, tissues, organs, rat, fasting.

## *Introduction*

Although the glucose tolerance test has been widely used in clinical medicine and in animal experiments for many years there is remarkably little information on the quantitative roles of various organs and tissues in assimilation of a glucose load, and it was this dearth of information which stimulated the present study on rats.

## *Methods and Materials*

### *Animals*

The experiments were made on male Wistar rats of an inbred strain maintained by the Bio-Assay Laboratories of the Wellcome Foundation. They weighed from 175 to 200 g and were fasted for 18 h before treatment.

### *Procedure*

The general plan of the oral and intravenous dosing experiments was the same — in each case a "physiological" dose of non-radioactive glucose was given, together with a tracer dose of [U-$^{14}$C] glucose. The animals were then killed by asphyxiation with $CO_2$ at times considered appropriate from preliminary studies of the blood glucose levels attained. Various organs and tissues were then rapidly dissected out and after homogenisation and extraction their $^{14}$C content was measured.

### *Selection of glucose dose and times for tissue analysis*

Our chief interest lay in the tissue distribution of $^{14}$C at the point in time when the blood glucose concentration returned to the baseline level, i.e. when the

administered glucose load had just been fully assimilated. We also chose either one or two earlier times for tissue analysis to give some idea of the rapidity of assimilation by different tissues.

*a) Intravenous dosing.* The dose given was 0.75 ml of a 20% w/v glucose solution, i.e. a total of 150 mg or 0.75 to 0.86 g/kg of non-radioactive glucose, together with 5 microcuries of [U-$^{14}$C] D-glucose (obtained from the Radiochemical Centre, Amersham, Bucks., England; specific activity 2.9 mCi/mM).

The tissue analysis times for the intravenous experiment were chosen from the results of a preliminary experiment in which 5 rats received 0.75 g/kg of glucose intravenously. The blood glucose concentrations at 0, 5, 10, 20, 40 and 80 min after injection were: $77\pm5$, $336\pm15$, $286\pm12$, $143\pm2$, $96\pm4$ and $89\pm3$ mg/100ml. Although the 40-min value had not quite reached the baseline level, we chose this as the most suitable interval for tissue analysis, since the fall of blood glucose in the succeeding 40 min was relatively small. In the actual experiment the blood glucose concentration was $241\pm13$ mg/100 ml 5 min after injection and $87\pm8$ after 40 min.

*b) Oral dosing experiment.* Where the glucose dose was given into the stomach we had considerably more difficulty in choosing a suitable dose and times for tissue analysis. Since surprisingly little information is available about the oral glucose tolerance curve of the rat, we made a preliminary study in which (non-radioactive) glucose doses of 3.0, 1.5 and 0.75 g/kg were given to 3 groups of 9 or 10 male Wistar rats. The blood glucose concentrations found at various times after dosing are shown in Table 1. Unexpectedly small rises of blood glucose resulted from all 3 doses, and no clear-cut dose-response effect was found. However, on the basis of the differences of blood glucose values between the 0.75 and 1.5 g/kg doses, we chose a glucose dose of 1.5 g/kg and selected 15, 90 and 180 min after glucose administration as the times for tissue analysis.

The oral dose was given through a long needle with a protected tip into the stomach, as 1.5 ml of a 20%w/v solution of non-radioactive glucose, containing approximately 5 microcuries of [U-$^{14}$C] D-glucose (specific activity 3.3 mCi/mM). The oral dose of non-radioactive glucose was thus twice the intravenous dose and amounted to 1.5 — 1.7 g/kg.

*Organs and tissues examined*

Dissection began immediately the animal was dead. Within 4 min the following organs and tissues had been dissected out, with times accurately recorded, in this order: blood (heart sample), snips from the pectoralis major and rectus abdominis muscles, heart, lungs, groin and epididymal fat pads, bladder, spleen, kidneys, the interscapular fat pads, the alimentary tract minus the oesophagus, the testes, skin and brain. Immediately after its removal each organ was dropped into liquid nitrogen, then kept deep-frozen until analysed for radioactivity.

Individual organs were weighed wet immediately before extraction. The weights of two important, diffusely distributed tissues — skeletal muscle and adipose tissue — were calculated as follows. For muscle, 2 untreated animals were first skinned and eviscerated completely and any visible adipose tissue was trimmed away. All the visible skeletal muscle was then dissected away and weighed; this amounted to 30% of the body weight in both animals. The remaining carcass was weighed, then heated in N KOH until the remaining muscles were just detached. The residual skeleton was picked out and weighed and the loss of carcass weight after this treatment was presumed to be due to the remaining muscle; it amounted to 7% and 8% of the body weight. This, together with the 30% originally dissected out, made the total skeletal muscle approximately 38% of body weight. The method is obviously rather crude, but seems adequate to indicate the muscle mass within $\pm5\%$. The total adipose tissue mass was estimated by a total lipid extraction of a 50 g aliquot of the minced animal, and dividing the total body lipid value so obtained by 0.82 [2] (the triglyceride fractional weight of adipose tissue).

*Collection of expired $^{14}CO_2$*

This was measured in separate experiments carried out under conditions identical with those described above for studying the tissue distribution of $^{14}$C after with intravenous or oral dosing. Each animal was kept singly in a 'Metabowl' (Jencon) chamber. A flow of 500 ml/min of $CO_2$-free, warm, dry air was maintained through the cage, and the $CO_2$ in the expired air was absorbed, via a bubbler, in 500 ml of N sodium hydroxide. The collection periods were 0-15, 15-90, and 90-180 min after dosing for the oral experiment, and 0-5, and 5-40 min for the intravenous. Three animals were used for each collection period. For measurement of $^{14}CO_2$, 1 ml of the absorbent solution was acidified and the $CO_2$ evolved was displaced by an air current into two successive wash bottles containing 20 ml of phenylethylamine absorbent mixture (see 'Reagents'). This was then mixed with 16 ml of toluene-based phosphor and the $^{14}$C content of a 15 ml aliquot was measured.

*Homogenization and extraction of tissue*

After the tissue had been thawed, about 1 g was homogenized and extracted. The tissues from the intravenous dosing study were extracted into 10 ml of heptane/isopropanol/N sulphuric acid, in a volume ratio of 10:40:1[5]. After vigorous mechanical shaking for 5 min, 6 ml of heptane and 4 ml of water were added and well mixed to bring the final volume to 20 ml. Centrifugation separated the mixture into heptane and isopropanol/water phases, and left the tissue debris as a basal protein pellet. 200 µl of the heptane and the isopropanol/water phases were counted separately in 8 ml of Bray's [3] solution.

In the oral dosing experiment, the tissues were extracted in 5 ml of chloroform/methanol (2:1 v/v),

using a Potter-Elvehjem glass homogenzier. A further 15 ml of chloroform/methanol was added; the mixture was then thoroughly shaken and filtered. The solid residue from this stage was retained for $^{14}C$ measurement by the oxygen flask combustion method, and the filtrate treated according to the method of FOLCH [7] being finally split into chloroform and isopropanol/water phases by the addition of 20% by volume of water.

The entire (lipid containing) chloroform phase was aspirated into a counting vial, evaporated off on a water bath at 70°C and the lipid residues were taken up into 8 ml of a toluene-based phosphor (see 'Reagents') for counting. In some instances these lipid solutions were brownish, and a quenching correction was necessary when counted for $^{14}C$. This was done by spiking with an internal [$^{14}C$]-hexadecane standard and recounting. A 200 µl aliquot of the methanol/water phase was then counted in 8 ml of Bray's [3] solution.

*Oxygen combustion of tissue residues*

The solid residue after extraction as above was dried in warm air to constant weight. An aliquot of about 50 mg was then burnt in a vessel of 1 l capacity by the oxygen flask method [9]. The $CO_2$ evolved was absorbed in 10 ml of phenylethylamine solution (see 'Reagents') mixed with 8 ml of toluene phosphor and a 15 ml aliquot was counted.

*Radioactivity measurement*

Counting was done in a Packard Tri-Carb liquid scintillation spectrometer, model 3003.

*Glucose concentration of blood*

For whole blood this was measured on duplicate samples of 0.05 ml, using the automated glucose oxidase method of FAULKNER [6].

*Reagents*

1. Toluene-based scintillator: this contained 100 mg of dimethyl POPOP, 5 g of PPO and 270 ml of methanol ('ANALAR'), brought to 1 l with toluene. 2. Phenylethylamine absorbent solution: 1 l contained 200 ml of 2-phenylethylamine (British Drug Houses) redistilled *in vacuo* until colourless, 200 ml of redistilled methyl oxitol, and 600 ml of toluene.

## *Results*

*Preliminary studies to determine dose and killing times*

After intragastric dosing with 3.0, 1.5 and 0.75 g of glucose/kg, the rats' venous blood glucose concentration showed surprisingly small rises and there was no clear-cut relation between the dose and the change of blood glucose concentration until 90 min after dosing (Table 1). We eventually selected 1.5 g/kg as the best dose. Since with none of these doses had the blood glucose concentration fallen to the baseline value by 120 min, we extended the final time for tissue analysis to 180 min after administration of the glucose. The blood glucose concentrations in the actual oral $^{14}C$ tracer experiment were $83\pm9$, $146\pm12$, $108\pm2$ and $78\pm1$ mg/100 ml at 0, 15, 90 and 180 min respectively. All these values were higher than those of the preliminary study at a dose of 1.5 g/kg (cf. Table 1). The differences were probably accounted for by the fact that the blood samples of Table 1 were obtained from the tail veins of unanaesthetised rats, whereas those of the actual experiment were obtained by heart puncture immediately after death by $CO_2$ asphyxiation, which can induce breakdown of liver glycogen [1].

*Distribution of $^{14}C$ in different tissues*

Details of the concentration of $^{14}C$ in all tissues and organs examined are shown in Table 2. Using the tissue concentrations and the organ weights given in Table 2, we have calculated the total $^{14}C$ uptake of various organs and of the two diffusely distributed tissues, skeletal muscle and adipose tissue: the results are shown in Table 3, which also gives values for $^{14}CO_2$ excretion.

Table 2 shows that, in general, the relative amounts of $^{14}C$ found in the alcohol/water and the solid residue phases were very much higher in the intravenous study

Table 1. *Preliminary study of blood glucose concentrations of rats after intra-gastric dosing with various glucose doses*

*Blood glucose (mg/100 ml)*

| Dose (g/kg) | | 0 | 15 | 30 | 45 | 60 | 90 | 130 | (min after glucose dosing) |
|---|---|---|---|---|---|---|---|---|---|
| (a) | 3.0 | 58.7 | 100.3 | 96.0 | 98.1 | 94.9 | 96.2 | 87.0 | |
| (b) | 1.5 | 55.6 | 91.3 | 92.3 | 93.5 | 95.4 | 84.6 | 74.0 | |
| (c) | 0.75 | 53.8 | 96.6 | 88.2 | 83.7 | 80.7 | 71.1 | 65.6 | |
| *Probability levels for significance of blood glucose differences* | | | | | | | | | |
| (a) vs. (b) | | | 0.2 | N.S. | N.S. | N.S. | 0.01 | 0.001 | |
| (a) vs. (c) | | | N.S. | 0.1 | 0.01 | 0.01 | 0.001 | 0.001 | |
| (b) vs. (c) | | | N.S. | N.S. | 0.05 | 0.01 | 0.001 | 0.010 | |

Notes: 1. Blood glucose values given are adjusted for initial levels by co-variance analysis and are the means of 9 or 10 animals.
2. N.S. = not significant ($P>0.2$)

Table 2. *Radioactivity concentrations in various tissues and organs of rats after administration of [U-$^{14}$C] D-Glucose*
Details of dosing of animals and methods of extraction of tissues are given in text. The 'non-polar' phase was heptane in the intravenous experiment (extraction with heptane/acid/isopropranol mixture), and was chloroform in the oral dosing experiment (extraction with chloroform/methanol, 2:1, according to Folch (1957)). Except where otherwise shown by an asterisk, the results are mean ± S.E.M. of 3 animals

*A. After intravenous injection of [U-$^{14}$C] D-glucose*

| Tissue or organ | Weight (g) | Time interval after dosing (min) | hundreds of counts/min. g wet weight | | | |
|---|---|---|---|---|---|---|
| | | | (a) alcohol/water phase | (b) non-polar phase | (c) solid residue | Total (a)+(b)+(c) |
| Skeletal muscle (rectus abdominis) | 73.8 | 5 | 272± 8 | 0.5± 0.2 | 18.0± 5.3 | 291± 9 |
| | 72.5 | 40 | 194±33 | 3.1± 0.3 | 32.2± 9.5 | 229±42 |
| Skeletal muscle (pectoralis major) | 73.8 | 5 | 250±16 | 2.2± 0.3 | 13.9± 1.1 | 266±15 |
| | 72.5 | 40 | 381±82 | 7.7± 1.5 | 32.7±11.2 | 421±87 |
| Skin | 38.5 | 5 | 407±18 | 0.5± 0.3 | 38.2± 7.8 | 446± 6 |
| | 39.0 | 40 | 153± 3 | 4.9± 0.1 | 23.0±11.1 | 181± 7 |
| Blood | 14.0 | 5 | 605±62 | 1.3± 0.6 | 26.9±11.4 | 633±72 |
| | 13.8 | 40 | 214±11 | 2.6± 0.8 | 21.7± 1.5 | 239±13 |
| Liver | 6.4 | 5 | 878±24 | 1.7± 0.4 | 61.4±15.1 | 941±39 |
| | 6.3 | 40 | 892±37 | 7.4± 0.1 | 101.0±15.2 | 1000±50 |
| Adipose tissues: Interscapular fat pad | 32.2 | 5 | 300± 6 | 7.9± 1.8 | 10.7± 2.3 | 318± 5 |
| | 31.8 | 40 | 278±21 | 40.0±10.3 | 14.3± 2.0 | 332±33 |
| Adipose tissues: Subcutaneous (groin) | 32.2 | 5 | 265±67 | 4.1± 1.9 | 9.9± 1.6 | 278±70 |
| | 31.8 | 40 | 90± 9 | 24.0± 6.1 | 5.3± 1.2 | 120±14 |
| Adipose tissues: Epididymal fat pad | 32.2 | 5 | 164±27 | 2.6± 1.0 | 2.6± 0.7 | 169±25 |
| | 31.8 | 40 | 46± 5 | 20.7± 5.1 | 1.0± 0.1 | 68± 9 |
| Alimentary tract (plus contents) | 11.0 | 5 | 303± 9 | 1.8± 0.4 | 24.4± 4.6 | 329±13 |
| | 11.6 | 40 | 139± 8 | 8.8± 1.7 | 48.2±17.7 | 196±15 |
| Kidneys | 1.4 | 5 | 1001±30 | 2.2± 0.3 | 94.0±12.9 | 1097±22 |
| | 1.4 | 40 | 259±39 | 2.7± 0.4 | 27.8± 5.3 | 290±25 |
| Lungs | 0.97 | 5 | 433± 3 | 1.6± 0.4 | 42.0* | 537* |
| | 0.97 | 40 | 186± 3 | 7.1± 2.0 | 21.6± 0.5 | 215± 4 |
| Brain | 1.3 | 5 | 337±12 | 7.4± 0.1 | 80.4±50.9 | 425±62 |
| | 1.4 | 40 | 435± 5 | 12.3± 0.7 | 46.5±13.7 | 494±18 |
| Heart | 0.58 | 5 | 614±52 | 4.1± 1.4 | 29.4± 9.9 | 647±59 |
| | 0.55 | 40 | 204±26 | 8.0± 0.4 | 13.8± 0.5 | 226±26 |
| Testes | 3.2 | 5 | 162± 5 | 1.5± 0.1 | 12.4± 1.3 | 175± 6 |
| | 3.4 | 40 | 119± 2 | 6.5± 0.1 | 12.8± 1.9 | 131± 3 |
| Spleen | 0.33 | 5 | 245±12 | 1.8± 0.1 | 6.4± 1.5 | 253±12 |
| | 0.43 | 40 | 124± 3 | 1.1± 0.2 | 18.1± 4.0 | 144± 5 |
| Bladder | 0.07 | 5 | 1097± 530 | 8.2± 3.8 | 8.5* | 1113* |
| | 0.08 | 40 | 2494±1086 | 10.4± 1.1 | 25.1± 3.6 | 2529±1089 |

*B. After oral (intragastric) administration of [U-$^{14}$C] D-glucose*

| Tissue or organ | Weight (g) | Time interval after dosing (min) | hundreds of counts/min. g wet weight | | | |
|---|---|---|---|---|---|---|
| | | | (a) alcohol/water phase | (b) non-polar phase | (c) solid residue | Total (a)+(b)+(c) |
| Skeletal muscle (rectus abdominis) | 73.8 | 15 | 28±21 | 7.2 ± 4.3 | 4.8± 4.3 | 40± 17 |
| | 72.5 | 90 | 56±13 | 2.6 ± 0.5 | 38.8±10.5 | 97± 18 |
| | 73.8 | 180 | 54± 6 | 1.7 ± 0.4 | 29.9± 3.0 | 86± 7 |
| Skeletal muscle (pectoralis major) | 73.8 | 15 | 16± 2 | 0.2 ± 0.04 | 6.0± 1.6 | 23± 4 |
| | 72.5 | 90 | 71± 4 | 2.2 ± 0.4 | 32.2± 3.2 | 105± 8 |
| | 73.8 | 180 | 111± 4 | 2.4 ± 0.6 | 22.1± 1.9 | 136± 4 |
| Skin | 41.4 | 15 | 29± 7 | 0.5 ± 0.3 | 43.7± 9.0 | 74± 15 |
| | 33.4 | 90 | 38± 5 | 3.6 ± 0.4 | 48.6± 7.1 | 91± 12 |
| | 36.9 | 180 | 16± 3 | 3.6 ± 0.5 | 37.6± 3.8 | 57± 6 |
| Blood | 14.0 | 15 | 27± 8 | 0.03± 0.00 | 19.1± 4.1 | 46± 10 |
| | 13.7 | 90 | 51±13 | 0.6 ± 0.1 | 40.6± 1.2 | 93± 14 |
| | 14.0 | 180 | 39± 1 | 0.5 ± 0.1 | 35.4± 5.8 | 75± 5 |
| Liver | 5.8 | 15 | 146±28 | 2.3 ± 0.6 | 86.8±25.3 | 235± 53 |
| | 5.4 | 90 | 495±40 | 11.6 ± 1.3 | 379.8±37.9 | 851± 75 |
| | 6.5 | 180 | 493±28 | 16.2 ± 1.2 | 486.1±53.2 | 995± 80 |
| Adipose tissues: Interscapular fad pad | 32.2 | 15 | 54±20 | 3.3 ± 1.0 | 14.3± 3.2 | 72± 23 |
| | 31.8 | 90 | 106± 5 | 26.8 ± 3.1 | 53.3± 6.6 | 186± 10 |
| | 32.2 | 180 | 112±26 | 41.4 ±13.4 | 39.5± 3.2 | 193± 42 |
| Adipose tissues: Subcutaneous (groin) | 32.2 | 15 | 26± 9 | 0.8 ± 0.01 | 15.7± 1.6 | 36± 8 |
| | 31.8 | 90 | 32± 7 | 11.0* | 25.1* | 68* |
| | 32.2 | 180 | 14± 5 | 13.8 ± 2.6 | 25.4±11.1 | 53± 9 |
| Adipose tissues: Epididymal fat pad | 32.2 | 15 | 14± 3 | 1.1 ± 0.2 | 5.3± 0.9 | 21± 4 |
| | 31.8 | 90 | 16± 2 | 6.6 ± 1.6 | 13.9± 1.2 | 37± 3 |
| | 32.2 | 180 | 12± 3 | 9.0 ± 0.5 | 21.4± 5.2 | 42± 6 |
| Alimentary tract (plus contents) | 15.2 | 15 | 1237±54 | 2.2 ± 0.6 | 552.3±65.1 | 1791± 56 |
| | 14.2 | 90 | 268±77 | 3.9 ± 0.2 | 177.0±42.1 | 449±119 |
| | 17.1 | 180 | 84±21 | 4.1 ± 0.6 | 115.2±13.0 | 203± 26 |
| Kidneys | 1.3 | 15 | 75±15 | 0.8 ± 0.1 | 32.5± 6.3 | 108± 20 |
| | 1.2 | 90 | 92±11 | 4.7 ± 0.3 | 57.8±10.5 | 154± 22 |
| | 1.4 | 180 | 53± 4 | 6.3 ± 0.6 | 57.3± 5.5 | 116± 8 |
| Lungs | 1.4 | 15 | 42± 3 | 0.6 ± 0.1 | 18.9± 1.3 | 62± 3 |
| | 1.1 | 90 | 94± 8 | 7.9 ± 0.5 | 33.2±13.5 | 135± 15 |
| | 1.1 | 180 | 50± 3 | 7.9 ± 0.9 | 28.2± 0.3 | 84± 2 |
| Brain | 1.6 | 15 | 47± 6 | 0.6 ± 0.1 | 27.0± 5.1 | 75± 11 |
| | 1.5 | 90 | 122± 2 | 14.9 ± 0.6 | 164.7* | 302* |
| | 1.6 | 180 | 73± 2 | 17.2 ± 3.2 | 117.6± 7.9 | 208± 9 |
| Heart | 0.6 | 15 | 64±10 | 0.6 ± 0.2 | 18.8± 5.3 | 83± 16 |
| | 0.5 | 90 | 97± 6 | 2.7 ± 0.2 | 39.3± 3.3 | 139± 7 |
| | 0.6 | 180 | 81± 8 | 2.2 ± 0.4 | 35.2± 0.6 | 118± 8 |
| Testes | 2.4 | 15 | 13± 3 | 0.2 ± 0.02 | 4.2± 1.3 | 17± 5 |
| | 2.4 | 90 | 31± 5 | 3.9 ± 1.2 | 27.3± 2.4 | 62± 3 |
| | 2.1 | 180 | 24± 1 | 7.2 ± 1.0 | 39.9± 3.6 | 71± 4 |
| Spleen | 0.33 | 15 | 91±47 | 0.4 ± 0.1 | 63.3±31.9 | 155± 79 |
| | 0.37 | 90 | 52± 2 | 2.0 ± 0.3 | 61.1± 5.6 | 115± 8 |
| | 0.37 | 180 | 34± 1 | 2.4 ± 0.4 | 60.2± 8.5 | 96± 8 |
| Bladder | 0.02 | 15 | 117±54 | 2.8 ± 0.3 | | 120* |
| | 0.06 | 90 | 88±55 | 2.8 ± 0.1 | | 91* |
| | 0.07 | 180 | 80±35 | 2.5 ± 0.9 | | 82* |

Table 3. *The radioactivity present in various whole organs or tissues of rats after administration of [U-$^{14}$C] glucose*
Calculated from the radioactive concentrations and organ weights given in Table 2. Results are expressed as the % of the total radioactivity administered to the animal which was found in the organs or tissues at the times indicated. The percentage contribution of whole organs or tissues to uptake of $^{14}$C was calculated from:

$$\frac{\text{Counts/min. g wet weight} \times \text{organ weight} \times 100.}{\text{Total cpm administered}}$$

In the oral study, the 3 fractions 'alcohol/water phase', 'non-polar phase' and 'solid residue' were counted under different conditions (see 'Methods'). The denominator for each fraction in the equation above was therefore different, as follows: Alcohol/water phase $4.98 \times 10^6$ cpm, non-polar phase $5.05 \times 10^6$ cpm, solid residue $3.65 \times 10^6$ cpm. In the intravenous study the denominator for all three fractions was $6.75 \times 10^6$ cpm. The calculation of total weights of diffusely distributed tissues (Skeletal muscle and adipose tissue) is described in 'Methods'

*A. After intravenous injection of [U-$^{14}$C]D-glucose*

| Tissue or organ | Alcohol/water (a) | | Heptane Phase (b) | | Solid Residue (c) | | Total (a)+(b)+(c) | |
|---|---|---|---|---|---|---|---|---|
| | 5 min | 40 min | 5 min | 40 min | 5 min | 40 min | 5 min | 40 min |
| Skeletal Muscle | 28.4 | 30.9 | 0.1 | 0.6 | 1.8 | 3.5 | 30.3 | 35.0 |
| Skin | 23.2 | 8.8 | <0.1 | 0.3 | 4.9 | 2.0 | 28.1 | 11.1 |
| Blood | 12.5 | 4.4 | <0.1 | 0.1 | 0.6 | 0.5 | 13.1 | 5.0 |
| Liver | 8.3 | 8.3 | <0.1 | 0.1 | 0.6 | 1.0 | 8.9 | 9.4 |
| Adipose Tissue (excluding I.S.) | 10.4 | 3.3 | 0.1 | 1.2 | 0.2 | 0.1 | 10.7 | 4.6 |
| Alimentary Tract (+ contents) | 4.9 | 2.4 | <0.1 | 0.2 | 0.4 | 0.8 | 5.3 | 3.4 |
| Kidneys | 2.0 | 0.6 | <0.1 | <0.1 | 0.1 | <0.1 | 2.1 | 0.6 |
| Lungs | 0.7 | 0.3 | <0.1 | <0.1 | <0.1 | <0.1 | 0.7 | 0.3 |
| Brain | 0.6 | 0.9 | <0.1 | <0.1 | 0.1 | 0.1 | 0.7 | 1.0 |
| Heart | 0.5 | 0.2 | <0.1 | <0.1 | <0.1 | <0.1 | 0.5 | 0.2 |
| Testes | 0.8 | 0.6 | <0.1 | <0.1 | <0.1 | <0.1 | 0.8 | 0.6 |
| Spleen | 0.1 | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.1 | 0.1 |
| Bladder | 0.1 | 0.3 | <0.1 | <0.1 | <0.1 | <0.1 | 0.1 | 0.3 |
| | 90.5 | 61.1 | 0.2 | 2.5 | 8.7 | 8.0 | | |
| | | | % recovered in tissues examined | | | | 101.4 | 71.6 |
| | | | % recovered in voided faeces | | | | <0.1 | 0.8 |
| | | | % recovered from expired $^{14}CO_2$ | | | | <0.1 | 8.0 |
| | | | Total % of injected dose accounted for | | | | 101.4 | 80.4 |

*B. After oral (intragastric) administration of [U-$^{14}$C]D-glucose*

| Tissue or organ | Alcohol/water phase (a) | | | Chloroform Phase (b) | | | Solid Residue (c) | | | Total (a)+(b)+(c) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 min | 90 min | 180 min | 15 min | 90 min | 180 min | 15 min | 90 min | 180 min | 15 min | 90 min | 180 min |
| Skeletal Muscle | 1.8 | 9.2 | 12.2 | 0.6 | 0.3 | 0.3 | 1.1 | 6.9 | 5.3 | 3.5 | 16.4 | 17.8 |
| Skin | 2.4 | 2.5 | 1.2 | <0.1 | 0.2 | 0.3 | 5.1 | 4.4 | 3.9 | 7.5 | 7.1 | 5.4 |
| Blood | 0.8 | 1.4 | 1.1 | <0.1 | <0.1 | <0.1 | 0.7 | 1.5 | 1.4 | 1.5 | 2.9 | 2.5 |
| Liver | 1.6 | 5.0 | 6.3 | <0.1 | 0.1 | 0.2 | 1.3 | 5.6 | 8.5 | 2.9 | 10.7 | 15.0 |
| Adipose Tissue (excluding I.S.) | 1.2 | 1.5 | 0.7 | 0.1 | 0.6 | 0.7 | 0.7 | 1.7 | 2.1 | 2.0 | 3.8 | 3.5 |
| Alimentary Tract (+ contents) | 37.8 | 7.7 | 2.8 | 0.1 | 0.1 | 0.1 | 22.6 | 7.0 | 5.5 | 60.5 | 14.8 | 8.4 |
| Kidneys | 0.2 | 0.2 | 0.1 | <0.1 | <0.1 | <0.1 | 0.1 | 0.2 | 0.2 | 0.3 | 0.4 | 0.3 |
| Lungs | 0.1 | 0.2 | 0.1 | <0.1 | <0.1 | <0.1 | 0.1 | 0.2 | 0.1 | 0.2 | 0.4 | 0.2 |
| Brain | 0.2 | 0.4 | 0.2 | <0.1 | <0.1 | 0.1 | 0.1 | 0.7 | 0.5 | 0.3 | 1.1 | 0.8 |
| Heart | 0.1 | 0.1 | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 |
| Testes | 0.1 | 0.1 | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.2 | 0.2 | 0.1 | 0.3 | 0.3 |
| Spleen | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 |
| Bladder | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| | | | | % recovered in tissues examined | | | | | | 79.1 | 58.2 | 54.5 |
| | | | | % recovered from expired $^{14}CO_2$ | | | | | | 0.4 | 11.8 | 31.3 |
| | | | | Total % of injected dose accounted for | | | | | | 79.5 | 70.0 | 85.8 |
| | 46.4 | 28.3 | 24.9 | 0.8 | 1.3 | 1.7 | 31.9 | 28.6 | 27.9 | | | |

I.S. = Interscapular fat pad

than in the oral. This is due in a small measure to the different methods of extraction used. The extraction techniques were changed because of the surprisingly small fraction of $^{14}C$ found in the lipid fraction in the oral experiment which was done first: we therefore changed to the more laborious but better validated Folch technique[7]. In extraction of tissues from the oral dose study we employed a chloroform/methanol extraction medium according to FOLCH [7]. The solid residue was obtained by filtration immediately after this extraction, at which time the only water present was that originally in the tissue. Although glucose and other highly water soluble substances may have remained in the solution, glycogen and other unidentified components would precipitate from this methanolic solution and be measured as part of the solid residue $^{14}C$. In the intravenous experiment, on the other hand, the solid residue was removed by centrifugation from an extraction medium containing approximately 25 times the original tissue water, with an isopropanol concentration of approximately 61%.

The possible differences caused by the two extraction procedures were investigated in a separate experiment, in which we injected, intravenously into 6 rats, 0.75 ml of a 200 mg/ml solution of "cold" glucose, containing 5 microcuries of $^{14}C$ glucose. The animals were killed by $CO_2$ asphyxiation 40 min later, and the whole carcass was minced. A 30 g aliquot was then extracted by the Dole and Folch procedures, and also by a modification of the Folch technique, in which the solid residue was filtered off *after* addition of 20% of water. The results are shown in Table 4. They indicate

Table 4. *Comparison of $^{14}C$ distribution between non-polar and alcohol/water phases of the Dole and Folch extraction methods, in rat tissues 40 min after intravenous injection of [U-$^{14}C$] glucose*

| Extraction method | % of injected $^{14}C$ dose | |
|---|---|---|
| | Alcohol/water phase | Non-polar phase |
| Dole[2] | 56.2 | 1.1 |
| Folch[4] | 40.3 | 1.7 |
| Modified Folch (see text) | 53.9 | 1.9 |

good agreement of the extraction into the alcohol/water phases by the Dole and the modified Folch methods. Extraction by the classical Folch technique was about 70% of that achieved by the Dole technique. Clearly this difference could account for only a trivial part of the great differences in the relative extraction into alcohol/water and solid residue phases, when the oral and intravenous studies are compared.

*Efficiency of recovery of $^{14}C$*

Details are given in Table 2. The recovery of $^{14}C$ in the intravenous experiment, 5 min after dosing, amounted to a nominal 101% of the total $^{14}C$ administered. At 40 min after injection the recovery was 80% (72% in tissues, plus 8%excreted as $CO_2$). The 20% of the $^{14}C$ dose not accounted for after 40 min may have been excreted in the urine or may have been present in the tissues not examined.

In the oral-dosing experiment, recovery was less satisfactory. At 15, 90 and 180 min after $^{14}C$ glucose dosing, the percentage of the dose recovered was 80, 70 and 86% respectively. It is unlikely that a significant amount was lost in the urine (not examined), since the blood glucose concentration remained relatively low throughout. Incomplete absorption of the administered glucose could have been a factor. PATKIN and MASORO [10] gave 1 g of glucose to male rats of about 250 g body weight and found that after 30 min 29% had been assimilated from the gut and after 1 hour only 40% was absorbed. Yet, since we measured the whole alimentary tract together with its contents, any glucose not absorbed from the gut should have been included in the final tally. We are, therefore, unable to account for the $^{14}C$ not recovered, unless it was present in those tissues we did not examine, or unless there was a gross error in the calculated mean value of $^{14}C$ uptake by one or more major tissues. This last seems unlikely, since the within-group variations were quite small: e.g. the individual values for total $^{14}C$ uptake 90 min after glucose administration were 69.9, 76.7 and 65.5% of the injected dose.

## *Discussion*

The greatest single tissue contribution to glucose assimilation was made by skeletal muscle, and skin and liver also took up important amounts. The large contribution of skeletal muscle to glucose disposal is not surprising if the great bulk of this tissue — about 38% of body weight — is taken into account. Similarly, the pelt of the rats comprised about 20% of the body weight, and the considerable $^{14}C$ uptake by skin is therefore not surprising. The $^{14}C$ uptake by the liver, approximately 9% of the intravenous dosing experiment, and 15% at the end of the intragastric dosing experiment is, on the other hand, large in relation to the weight of this organ: this is reflected in the high specific activity values (counts/min. g wet weight) of Table 2, where the figure for liver was several times larger than that of skeletal muscle in both the intravenous and oral experiment. Although the liver clearly played an important role in the disposal of both the intravenously and orally administered glucose load, its contribution is probably somewhat underestimated due to glycogenolysis induced by $CO_2$- asphyxiation at the time of killing.

The $^{14}C$ present in the lipid fraction of the summed tissues was small, amounting to only 1.7% of the injected amount at 180 minutes after oral administration and 2.5% after 40 min in the intravenous dosing experiment (Table 3). The $^{14}C$ fraction presumably mainly represents glucose incorporated into triglyceride, both as labelled fatty acids and as labelled glycerol, together

with $^{14}C$ labelled "free" fatty acids. The low fraction of the injected $^{14}C$ which we found in total body lipids (cf. Table 4 also) is compatible with the low rate of fat synthesis to be expected in rats starved for as long as 20 h. The results are compatible with those of other workers [10, 8, 4].

We have been unable to find any earlier work with which to compare our results in detail. VRBA [11] described the distribution of $^{14}C$ after intraperitoneal injection of [U-$^{14}C$] D-glucose into mice fed *ad lib.* This work was, however, designed to study the $^{14}C$ distribution into different chemical fractions of the body as a whole, rather than $^{14}C$ assimilation by individual tissues. Although the experimental procedures and methods were quite different, the present results seem to be in broad general agreement with VRBA's findings [11] except for the larger incorporation of $^{14}C$ into lipid (6.2% one hour after injection) in his study. The two approaches are complementary, and a complete pattern of glucose assimilation could be obtained by applying Vrba's detailed chemical separation to individual organs and tissues.

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