The Distribution of Glucose and [^{14}C]Glucose between Erythrocytes and Plasma in the Rat

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1. Of the glucose in rat blood $79.8 \pm 3.3\%$ (S.D.) was in the plasma. The variance was mostly due to differences between rats. 2. The concentration of glucose in erythrocyte water was $51 \pm 8\%$ (S.D.) of that in plasma water. 3. The ratio (specific radioactivity in plasma)/(specific radioactivity in whole blood), i.e. the P/B ratio, was estimated for glucose at intervals after intravenous injection of [^{14}C]glucose and [^{14}C]fructose. The ratio differed from unity by more than the standard error of a single determination of the specific radioactivity of blood or plasma glucose except from 10 to 17 min. after injection of [^{14}C]glucose and from 22 to 30 min. after injection of [^{14}C]fructose. At all other times specific radioactivities in blood had to be corrected to give specific radioactivities in plasma. How to do so is described. 4. The P/B ratios were accounted for by a turnover of glucose in erythrocytes of $0.14 \mu\text{mole/min./ml.}$ of erythrocytes. 5. Metabolism of glucose in rat erythrocytes is unlikely to be a major source of lactate.

Information on carbohydrate metabolism *in vivo* can be obtained from the specific-radioactivity-time curves for [^{14}C]glucose in plasma after the intravenous injection of [^{14}C]glucose (e.g. Baker, Shipley, Clark & Incefy, 1959; Ashby, Heath & Stoner, 1965; Shipley, Chudzik, Gibbons, Jongedyk & Drummond, 1967) or of glucose precursors, such as [^{14}C]pyruvate and [^{14}C]fructose (Ashby *et al.* 1965; Heath & Threlfall, 1968; Threlfall & Heath, 1968). To obtain the required specific-radioactivity-time curves several blood samples must be taken from each animal. It is usually impracticable to separate plasma from samples taken sequentially at intervals of a few minutes, so the specific radioactivity in plasma must be calculated from that in whole blood. It has always been assumed that the specific radioactivities are the same, i.e. that there is very rapid equilibration between plasma and erythrocyte glucose as is the case with human erythrocytes (Murphy, 1960; LeFevre & McGinniss, 1960), although some evidence to the contrary has been published (Baker *et al.* 1959). We show here that equilibration of label is quite slow, so that considerable corrections are needed to convert specific radioactivities in whole blood into specific radioactivities in plasma; and we have also measured the distribution of glucose between erythrocytes and plasma. The investigation has also shown that the turnover of glucose in rat erythrocytes *in vivo* agrees fairly well with the results obtained in experiments *in vitro* by other workers.

MATERIALS AND METHODS

Radioactive chemicals. [^{14}C]Glucose and [^{14}C]fructose (specific radioactivities about $25 \mu\text{C/mg.}$) were obtained from The Radiochemical Centre, Amersham, Bucks. For injection they were diluted with 0.9% NaCl containing sufficient heparin for each rat to receive 400 i.u.

Animal techniques. Male albino rats of the Porton strain (body wt. 189–225 g., age 8–10 weeks) were fed on M.R.C. diet 41b (Bruce & Parkes, 1956). Food was withdrawn at 9.00 hr. on the day of experiment, and the rats were injected during the period 9.15–12.00 hr. Each rat received $5\text{--}10 \mu\text{C}/100\text{g.}$ body wt. via the tail vein, except for a few that received $40\text{--}50 \mu\text{C}/100\text{g.}$ body wt. The environmental temperature was $18\text{--}22^\circ$.

Blood sampling and plasma separation. At the end of each experiment the rat was guillotined and 3–4 ml. of blood was collected in a cooled receiver ($5\text{--}10^\circ$). A portion (2.0–3.0 ml.) was rapidly transferred to a cooled polythene centrifuge tube and was immediately centrifuged for 7 min. at 4° and 2600 g (bottom of the tube). Meanwhile from the remaining blood two to four whole blood specimens were taken in 0.1 ml. capillary pipettes (Ashby *et al.* 1965; standardized by weighing, with S.D. 0.0002 ml.) for glucose determinations and two or three samples for the determination of haematocrit values. Haematocrit values were corrected for entrapped plasma as described by Chaplin & Mollison (1952). From the plasma separated by centrifugation two to four specimens were taken in 0.2 ml. syringes standardized by weighing with S.D. 0.0005 ml. The whole process from decapitation to precipitation of protein from the last plasma sample took about 15 min. In trial runs it was shown that the glucose loss on storing whole blood for 30 min. at 4° was $3.3 \pm 1.5\%$ (S.D., $n=4$). As the sampling

of whole blood and plasma was separated only by about 10 min. it was assumed that errors due to breakdown during centrifugation would be negligible.

Glucose in plasma and whole blood. Samples were deproteinized (Somogyi, 1930) and centrifuged. Of each supernatant 0.5 ml. was deionized by passage through a column (110 mm. $\times$ 4 mm.) of equal parts of Permutit Zeo-Karb 225 (H^+ form; 204–295 μ m. diam., –14+52 B.S. units) and Isopor De-Acidite M-IP (Cl^- form; 204–295 μ m. diam.) (bought from the Permutit Co. Ltd., London W.4). Each column was washed with deionized water, and the eluate (about 4 ml.) was weighed.

Glucose was determined on 1 ml. portions by using a Boehringer Blood Sugar Colorimetric method (TC-M-11 kit). The manufacturer's instructions were slightly modified by using 2.5 ml. of reagent, incubating for 55 min. at 25°, adding 0.4 ml. of 0.3 M- H_2SO_4 , and measuring the extinction at 430 nm. (Unicam SP.500 spectrophotometer). The standard error of a single determination was 2.5% of the mean, as estimated from the replicates in this investigation (160 degrees of freedom).

Specific radioactivity of glucose. One drop of acetic acid was added to each eluate after removal of the 1 ml. portion for glucose analysis, and the water was removed by a stream of compressed air blown on to the surface of the eluate, which was held at 37°. (Omission of acetic acid resulted in a big loss of glucose.) The glucose was redissolved in 0.02 ml. of deionized water, spotted on to Whatman no. 540 chromatography paper fenestrated into 2 cm. strips (Gey, 1956), with 20 μ g. of glucose and 20 μ g. of fructose markers on three fenestrations. The chromatogram was developed at 23° for 16–18 hr. for specimens when [^{14}C]glucose had been injected, with water-formic acid–2-methylpropan-2-ol–butan-2-one (15:15:42:48, by vol.) (after Fink, Cline & Fink, 1963), and for 36 hr. when [^{14}C]fructose had been injected, with the same solvents but in the proportions 10:15:42:48 (by vol.). The paper was dried, the markers were developed and the glucose was eluted as described by Threlfall & Heath (1968). From each eluate (about 2.5 ml.) 1 ml. was used for glucose estimation, as above, and 1 ml. was counted in a Packard automatic scintillation counter (series 4000) after addition of 15 ml. of scintillator fluid (700 ml. of toluene; 2100 ml. of dioxan; 35 g. of 2,5-diphenyl-oxazole, scintillator grade; 280 g. of naphthalene). [^{14}C]-Toluene was used as an internal standard. The standard errors of single determinations varied with the time after injection of [^{14}C]glucose. Expressed as percentages of the mean (degrees of freedom in parentheses) they were: 5–30 min., 3.7 (110); 60 min., 5.0 (23); 90–120 min., 9 (24). The high errors at later times were much greater than could be accounted for by counting errors (<3%).

Methods of computation. Calculations were carried out on an electronic desk calculator. Curves were fitted to data by trial and error, substantially in the way described by Siri (1949).

RESULTS

The haematocrit values were 42.5 ± 1.7 (s.d., $n = 38$). The concentration of glucose in plasma was 1.52 ± 0.18 mg./ml. (s.d., $n = 38$). The percentages of total blood glucose in the plasma at various times after injection of [^{14}C]glucose are shown in Table 1. In obtaining mean values three very low per-

centages (<70%) were omitted. Up to 90 min. after injection the percentages were very similar, mean value 79.8%. It follows that immediately after injection the specific radioactivity of glucose in plasma was 1.25 times (1/0.798) that in whole blood.

The variance was greater ($P < 0.01$, F test; Snedecor, 1960) than could be accounted for by errors in the glucose estimations; and the mean percentage at 120 min. was lower ($P < 0.05$) than the mean of the rest. Thus there were significant variations from rat to rat, and some indications of a fall in percentage with time after injection, perhaps due to an action of heparin.

The ratio of the specific radioactivity of glucose in plasma to that of glucose in whole blood, i.e. the P/B ratio, after injection of [^{14}C]glucose is shown in Fig. 1 and after the injection of [^{14}C]fructose in Fig. 2. The P/B ratio deviated significantly from unity over much of each experimental period, i.e. the specific radioactivities could not be taken as equal.

The specific-radioactivity–time curve for [^{14}C]-

Table 1. Percentages of total blood glucose in the plasma of rats at various times after the injection of [^{14}C]glucose

Means $\pm$ s.e.m. are given, with numbers of estimations in parentheses. From each set marked with an asterisk one very low percentage was omitted in estimating the mean (see the text).

Time after injection (min.)	Glucose in plasma (% of glucose in blood)
5	80.4 ± 0.6 (5)*
10	80.7 ± 1.9 (6)
20	79.5 ± 0.5 (5)
30	81.6 ± 2.1 (4)*
60	78.6 ± 1.4 (5)*
90	78.4 ± 2.1 (5)
120	73.7 ± 1.9 (4)
5–90	79.8 ± 0.6 (30)

Fig. 1. Relative specific radioactivities in plasma and whole blood (P/B ratio) at intervals after injection of [^{14}C]glucose. s.e.m. values are shown, with the numbers of rats in parentheses. The curve is theoretical (eqn. 7 *et seq.*).

Fig. 2. Relative specific radioactivities in plasma and whole blood (P/B ratio) after the injection of [^{14}C]fructose. Each point represents a single rat except at 0 min., where the value 1.25 was calculated from results on many rats (see the text). The curve is theoretical.

Fig. 4. Specific radioactivity of plasma glucose after injection of [^{14}C]fructose. Each point represents a single rat. The curve is the semi-empirical fit (eqn. 8).

Fig. 3. Specific radioactivity of plasma glucose after injection of [^{14}C]glucose. s.e.m. values are shown. Numbers of rats are as in Fig. 1. The curve is the semi-empirical fit (eqn. 4).

glucose in plasma after [^{14}C]glucose is shown in Fig. 3 and after [^{14}C]fructose in Fig. 4. The specific radioactivities were calculated as fractions of an initial specific radioactivity, which was that if the label was distributed initially throughout the plasma glucose pool. The plasma glucose pool was calculated from the haematocrit values, assuming that the erythrocyte volume was 2.8 ml./100 g. body wt. of rat (Stoner, 1958; Constable, 1963).

DISCUSSION

Baker *et al.* (1959) found (concn. of glucose in plasma)/(concn. of glucose in whole blood) to be

1.41, against 1.39 calculated from our results (79.8% blood glucose in plasma, haematocrit value 42.5%).

Concentration of glucose in rat erythrocytes. For rats 10 weeks old Hatai (1918) found the serum water content to be 92.7% and the whole blood water content to be 81% (interpolated). Combining these results with the haematocrit value (42.5; see the Results section) the water content of erythrocytes was calculated to be 67%. Laris (1958) gives a value of 65% for the erythrocytes of the rat. As the erythrocytes contained $20.2 \pm 3.4\%$ (s.d.) of the blood glucose the concentration of glucose in the water of rat erythrocytes was $51 \pm 8\%$ of the concentration in plasma, and was on the average 0.77 mg./ml. or 0.52 mg./ml. of erythrocytes (67%, w/v, water). Laris (1958) found 68% at rather higher plasma glucose concentrations than in our experiments. In mg./ml. of plasma water the concentration in his experiments was 2.04, and in ours was 1.63 ± 0.19 (s.d.). The percentage in the erythrocytes rose with concentration (Laris, 1958), but not rapidly enough to explain the difference.

Interpretation of the variation of P/B ratios with time after injection. For full use to be made of the data a method of interpolation is needed. The following method is self-checking, and is easily applied to similar data.

Denote by x the specific radioactivity in erythrocytes as a fraction of the specific radioactivity in plasma. Then, as erythrocytes contained 20% of the glucose, it can be shown that:

$$\text{P/B} = 1/(0.8 + 0.2x) \quad (1)$$

whence x can be calculated from the experimental values of the P/B ratio (Figs. 1 and 2).

Assume that erythrocyte glucose is a single pool, with turnover constant k /min. Represent the specific radioactivity of plasma glucose by $f(t)$, and the specific radioactivity of erythrocyte glucose by S_r . Then:

$$x = S_r/f(t) \quad (2)$$

$$\text{and} \quad dS_r/dt = k[f(t) - S_r] \quad (3)$$

When [^{14}C]glucose was injected the experimental specific radioactivities in plasma were adequately represented by (Fig. 3):

$$\text{Specific radioactivity of plasma glucose} = 0.088e^{-0.08t} + 0.025e^{-0.024t} \quad (4)$$

The equation gives the specific radioactivity relative to a presumed initial specific radioactivity of unity, assuming instantaneous mixing of label with the plasma glucose pool, and therefore does not represent the specific radioactivity at times shorter than 5 min. after injection. In fact the initial specific radioactivity must have exceeded unity, as some erythrocytes were exposed to almost undiluted [^{14}C]glucose at the time of injection. An arbitrary first term was therefore added to eqn. (4), giving:

$$\text{Specific radioactivity of plasma glucose} = f(t) = Ae^{-4t} + 0.088e^{-0.08t} + 0.025e^{-0.024t} \quad (5)$$

The arbitrary first term was made an exponential to ease computation. The big exponent, 4/min., was chosen so that the first term would contribute negligibly to $f(t)$ by 5 min. after injection. Provided that the exponent is big enough for this to be true it can be varied at will, and variations can be compensated for by adjusting A ; so eqn. (5) contains one unknown, A , to be chosen to fit the data (Fig. 1). Substitution of eqn. (5) in eqn. (3) and integration, with $S_r = 0$ at $t = 0$, gives:

$$S_r = k \left(\frac{Ae^{-4kt}}{k-4} + \frac{0.088e^{-0.08t}}{k-0.08} + \frac{0.025e^{-0.024t}}{k-0.024} + Be^{-kt} \right) \quad (6)$$

$$\text{where:} \quad -B = A/(k-4) + 0.088/(k-0.08) + 0.025/(k-0.024) \quad (7)$$

To fit the results k and A were chosen by trial and error to fit the P/B ratio exactly at 5 min., and k was chosen to give the best average fit to all the data. The curve obtained, with $k = 0.048$ /min. and $A = 2.4$, is shown in Fig. 1. Variations greater than 0.005/min. in k greatly worsened the fit.

The results with [^{14}C]fructose (Fig. 2) were analysed similarly. The specific radioactivity of glucose could be represented by (Fig. 4):

$$f(t) = 0.08(e^{-0.038t} + e^{-0.3t}) \quad (8)$$

Substitution of eqn. (8) in eqn. (3) and integration with $k = 0.048$ /min. gave S_r , and hence x and P/B by

eqns. (2) and (1). The calculated values of the P/B ratio are shown (Fig. 2). Their calculation required no new variables. The calculated values of the P/B ratio differed significantly ($P < 0.05$) in only one instance (the lowest value at 45 min.) from the experimental ones, the errors on which were calculated from the variance in the replicated determinations of specific radioactivity.

The values of the P/B ratio deviated from unity by more than the standard error of a single determination of specific radioactivity over nearly the whole time range. The exceptions are from 10 to 17 min. after injection of [^{14}C]glucose and from 22 to 30 min. after injection of [^{14}C]fructose.

Rate of metabolism of glucose by erythrocytes. The rate of turnover is given by kQ_r , where Q_r is the quantity of glucose/ml. of erythrocytes, i.e. 0.52 mg./ml. (see under 'Concentration of glucose in rat erythrocytes' above). Hence the turnover rate is 0.048×0.52 mg./min./ml. of erythrocytes, or $0.139 \mu\text{mole/min./ml.}$ of erythrocytes. This is similar to the maximum hexokinase activity found by Christensen, Plimpton & Ball (1949), i.e. $0.17 \mu\text{mole/min./ml.}$ of erythrocytes. It is rather more than the average rate of glucose utilization over a period of 1 hr. found *in vitro* by Laris (1958), i.e. $0.093 \mu\text{mole/min./ml.}$ of erythrocytes, but the rate in his experiments fell with time, and the initial rate may well have been as high as in our experiments. It follows that the turnover can mostly be accounted for by net utilization, and leaves little to be explained by exchange across the plasma membrane, unlike in human erythrocytes, in which exchange is 50–100 times the rate of utilization (Murphy, 1960; LeFevre & McGinniss, 1960).

The erythrocytes produce only a small fraction of the lactate and pyruvate produced in the whole

rat. This is shown by considering that lactate and pyruvate formation cannot exceed that equivalent to the total glucose turning over, as measured by a labelling experiment, but is likely to exceed that equivalent to the glucose oxidized to carbon dioxide. It is not certain to exceed the latter, as glucose can give carbon dioxide via the pentose shunt without forming lactate or pyruvate; but some lactate and pyruvate is not oxidized (see, e.g., Ashby *et al.* 1965) and the second factor is likely to predominate. The erythrocytes turned over 0.07 mg. of glucose/min./100 g. body wt. (assuming 2.8 ml. of erythrocytes/100 g. body wt., as above). The oxidation rate of glucose in post-absorptive rats is about 0.7 mg./min./100 g. body wt. (Ashby *et al.* 1965), ten times greater. Whether most of the

pyruvate + lactate in blood comes from the erythrocytes depends on the extent to which lactate and pyruvate are metabolized in the cells in which they are produced. If the Cori cycle (Cori, 1931) is to recover an appreciable fraction of lactate and pyruvate as glucose then most of the blood lactate and pyruvate also cannot be supposed to originate in the erythrocytes.

Calculation of correction factors. Ideally for rats in any nutritional or pathological state the experiments should be repeated, P/B-time curves obtained, and the correction factors, i.e. P/B ratios, read from the curves, e.g. those in Figs. 1 and 2, for the relevant times after injection. The following simpler method appears to be adequate in many instances.

Calculate the erythrocyte pool of glucose, Q_r , from the haematocrit value assuming that the concentration in erythrocyte water is half that in plasma, and that the erythrocytes contain 67% water (see under 'Concentration of glucose in rat erythrocytes' above). If the glucose concentration in whole blood is C , the equation is:

$$Q_r = 0.67C/(2 - 1.33H) \quad (9)$$

where H is the haematocrit value divided by 100. Assume that the turnover rate of glucose in erythrocytes is $0.14 \mu\text{mole/min./g.}$ of erythrocytes (see under 'Rate of metabolism of glucose by erythrocytes' above), whence:

$$k = 0.14/Q_r \quad (10)$$

(Q_r must be expressed in $\mu\text{moles.}$) When a [^{14}C]-glucose precursor is injected, e.g. labelled fructose or pyruvate, the empirical specific-radioactivity-time curve for [^{14}C]-glucose in blood (not plasma) should be expressed as the sum of exponentials, as in eqn. (8). When [^{14}C]-glucose is injected intravenously the experimental specific-radioactivity-time curve from a few minutes after injection should be expressed as the sum of exponential terms as in eqn. (4) and added to the function $2.4e^{-4t}$ to allow for the initial rapid falls in specific radioactivity. From k from eqn. (10) and the specific-radioactivity-time curves the P/B ratios should be calculated as described above. If desired these values of the P/B ratio can be used to correct the original data, new exponential functions found to fit the corrected data and the process repeated, but this is not usually necessary.

The procedure was checked by using the data given by Baker *et al.* (1959). There was fairly good agreement between their experimental values of the P/B ratio and those calculated as above without the iteration step (Table 2), except that a marked fall in rates from 1 to 6 min. after injection was

Table 2. *Comparison between calculated values of the P/B ratio and those found by Baker et al. (1959)*

Baker *et al.* (1959) give values for starved and post-absorptive rats given [^{14}C]-glucose intravenously over the time-ranges shown. For calculation the equations equivalent to eqn. (4) were taken from Baker *et al.* (1959):

$$\text{specific radioactivity of glucose} = 0.107e^{-0.074t} + 0.072e^{-0.017t}$$

(starved; plasma glucose = 1.1 mg./ml.), and:

$$\text{specific radioactivity of glucose} = 0.122e^{-0.15t} + 0.073e^{-0.018t}$$

(post-absorptive; plasma glucose = 1.4 mg./ml.). Assuming a haematocrit value of 42.5, the plasma glucose concentrations give $k = 0.069/\text{min.}$ for starved rats and $0.054/\text{min.}$ for post-absorptive rats, whence values of the P/B ratio were calculated as in the text.

Time after injection (min.) ...	1	6	14	18	31	40
Calc. starved	1.18	1.09	1.00	0.98	0.92	0.91
Calc. post-absorptive	1.18	1.10	1.00	0.97	0.92	0.92
Experimental	1.09		1.00		0.88	

calculated to take place, and Baker *et al.* (1959) say they did not observe one.

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