Rates of Glucose Utilization and Glucogenesis in Rats in the Basal State Induced by Halothane Anaesthesia

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1. Rates and rate coefficients of glucose utilization and replacement were determined with [$5\text{-}^3\text{H}$]- and [$\text{U-}^{14}\text{C}$]-glucose in rats starved for 24 h, either conscious or under halothane anaesthesia, in a thermoneutral environment. Plasma insulin concentrations were also measured. 2. Halothane anaesthesia decreased the turnover rate by 20%, which was similar to previously reported decreases in metabolic rates caused by natural sleep. 3. Fractional recycling of glucose carbon was little affected by halothane. 4. Comparison of values in one rat with those in another, among both conscious rats and those under halothane anaesthesia, showed that rate coefficients were inversely correlated with plasma glucose concentrations. 5. These findings indicated that halothane, in the concentration used (1.25%, v/v), had little specific effect on glucose metabolism. 6. Although equilibrium plasma glucose concentrations in different rats under halothane were widely different (4–8 mmol/l) the rates of utilization were very similar (2.5–3.1 $\mu\text{mol}/\text{min}$ per 100 g), indicating that these rates were determined by the production of glucose from gluconeogenic precursors released by basal metabolism, the rate of which is necessarily similar in different rats. 7. Among rats under halothane anaesthesia plasma insulin concentrations were negatively correlated with rate coefficients, showing that the differences between rate coefficients were mostly accounted for by differences between rats in tissue sensitivities to insulin. Thus in each 24 h-starved rat, sleeping or resting, the main regulators of the plasma glucose concentration were the rate of supply of gluconeogenic substrates from energy metabolism and the intrinsic sensitivity of the tissues to insulin. 8. We found that a commonly used deionization method of purifying glucose for determination of its specific radioactivity was inadequate.

Our present understanding of the homeostatic mechanisms controlling whole-body glucose metabolism is largely concerned with their response to a perturbation. We felt that important features might emerge from a study of glucose utilization and replacement in the basal metabolic state, and the present paper presents the results of an attempt at such a study.

The basal state is that in which total body oxygen consumption is minimal. It is achieved in the rat after starvation for 24 h when it is asleep in a thermoneutral environment (about 30°C). Unfortunately, starved rats cannot be trained to sleep naturally during turnover experiments. However, Denckla (1970) has shown that controlled pentobarbital anaesthesia induces sleep indistinguishable from natural sleep with respect to O_2 consumption. Halothane is similar to pentobarbital in its effects on heat production and, by implication, O_2 consumption (Heath & Rose, 1976), and as it is more easily controlled during long experiments we used it instead.

Our study is thus of glucose turnover in starved rats under halothane anaesthesia. The results, however, demonstrate that the rate of glucose turnover is just that expected during natural sleep, and we shall show that some of the homeostatic relationships that emerged from this study are almost certainly operative in the true basal state.

Rates were determined from the disappearance of intravenously injected [$5\text{-}^3\text{H}$]- and [$\text{U-}^{14}\text{C}$]-glucose from the blood. $5\text{-}^3\text{H}$ -labelling was chosen because it gives a rate of utilization closer to the rate of glucogenesis (i.e. gluconeogenesis plus hepatic glycogenolysis) than that given by other glucose labelling. The rate obtained is that at which label is sequestered in glycogen for the duration of the experiment plus the rate at which glucose moieties are converted into triose phosphates (Bloxham *et al.*, 1973; Clark *et al.*, 1974; Hue & Hers, 1974), and therefore includes the rates of all the processes (deposition of glycogen, glycolysis and pentose-shunt operation) that are balanced in the steady state by glucogenesis. It overestimates the rate of hepatic glucose output by the extent to which glucose is converted into triose phosphates in the liver against the net glucogenic

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flux owing to the reversibility of the glucose $\rightleftharpoons$ triose phosphate chain, but this rate is probably very low in the starved rat (Clark *et al.*, 1975). Labelling with [5-³H]glucose was chosen rather than with [2-³H]-glucose because the latter gives a rate that includes that of the conversion of glucose into fructose 6-phosphate in the liver, which is probably much faster than its conversion into triose phosphates (Katz & Dunn, 1967; Clark *et al.*, 1974). Labelling with [U-¹⁴C]glucose was used because the difference between the rates obtained with it and with [5-³H]-glucose gives an indication of the rate of recycling of carbon atoms (Katz *et al.*, 1974). It also allows comparison with earlier work (Heath & Corney, 1973).

Experimental

The design of the experiments was controlled by findings on the effects of halothane on core temperature and arterial P_{CO_2} and pH (Heath & Rose, 1976). The salient points were as follows.

(a) Since halothane decreased heat production to the basal rate rats under halothane anaesthesia had to be kept in a thermoneutral environment to maintain their body temperatures at normal values (Heath & Rose, 1976). Since glucose utilization in conscious rats depends on the environmental temperature (Heath & Corney, 1973), conscious controls also had to be at thermoneutrality, i.e. at 28–32°C ambient (Stoner & Marshall, 1971).

(b) Although for each rat under halothane the ambient temperature required to maintain the core temperature at 37°C was remarkably constant for periods up to 4h, it was not the same for all rats. Consequently, to maintain comparability with controls, the ambient temperature of each anaesthetized rat had to be adjusted to keep its core temperature normal.

Animal techniques

Rats. Male Wistar rats of the Porton albino strain were housed from weaning in a room at 18–22°C, lit from 07:00 to 19:00h. Diet 41B (Bruce & Parkes, 1956) was removed 25–30h before injection. Experiments on control rats were carried out in a room at 30°C, to which rats were moved at least 1h before injection. In order that rates of glucose utilization should be comparable with those in other nutritional states it is necessary to give body weights in some standard nutritional state, so that the fraction of lean body mass is the same. The post-absorptive state was chosen, as this corresponds to overnight starvation in man (see Heath & Corney, 1973, for references). In this state the weights were 219–266g and these weights were used in all calculations. Weight loss by the time of study was determined on a separate group and averaged $4.4 \pm 1.0\%$ (S.D., $n = 14$).

Anaesthesia and core-temperature regulation. These were carried out in prone rats as described by Heath & Rose (1976). As they found, maintenance of stage III anaesthesia required a halothane/air/oxygen mixture containing 1.25% halothane (v/v), and maintenance of normal P_{O_2} values a total O_2 concn. of 25–26% (v/v).

Procedure for rats under halothane. Serial blood sampling was used. Immediately after induction of anaesthesia each rat was cannulated in the ventral caudal artery (Agrelo & Dawson, 1968) for blood sampling and in a lateral tail vein for injection. About 0.5h after induction, and after checking that the core temperature was in the normal range, each rat was given, via its caudal venous cannula, 5 μ Ci of [U-¹⁴C]-glucose alone or with 25 μ Ci of [5-³H]glucose (The Radiochemical Centre, Amersham, Bucks., U.K.) in 0.2ml of 0.9% NaCl containing 300–400 i.u. of heparin, followed by 0.2ml of 0.9% NaCl to clear the cannula of label. Four blood samples (0.2ml) were taken via the arterial cannula for the measurement of glucose and its specific radioactivity at times after injection close to the optimum times calculated (Heath & Cunningham, 1975) from the results of trial experiments, namely 6, 27, 60 and 140 min. Additional blood was taken after the first and last samples for microhaematocrit determinations (about 0.06ml), after the last sample for insulin measurement, and between the third and fourth samples for arterial P_{CO_2} and pH measurement (0.15ml, by pH/blood gas analyser, model 313, Instrumentation Laboratory Inc., Altrincham, Cheshire, U.K.). Blood taken was replaced by an equal volume of 0.9% NaCl containing about 150 i.u. of heparin/ml. Haematocrit values fell by about 2% during the experiment and intermediate values were calculated by linear interpolation.

Procedure for conscious control rats. The single-sampling method described by Heath & Corney (1973) was used to minimize disturbance of glucose metabolism. Rats were decapitated to obtain blood samples. Blood gases were not measured.

Analytical procedures

Microhaematocrit values were corrected for entrained plasma (Heath, 1973).

Blood glucose concentrations and specific radioactivities. All concentrations and most specific radioactivities were determined as described by Frayn (1976). Some specific radioactivities at the first two sampling times were determined substantially as described by Issekutz *et al.* (1973). This very much shorter method, which omits paper chromatography, usually gives incorrect results at later sampling times (see the Appendix). The coefficient of error of a single determination of specific radioactivity was about

2.5% by the first method and 3.4% by the second (see the Appendix).

The coefficient of error of a single determination of blood glucose concentration was measured by taking duplicate or triplicate samples (0.2 ml) with the same pipette for each sample in a replicate, and was 1.28% (19 degrees of freedom). Six of the duplicates were taken direct from a rat via its arterial cannula. During serial sampling experiments only one pipette was used for each rat, so that any change greater than 2.8% in the blood glucose concentration in any one rat was statistically significant ($P < 0.05$, Student's t test). Comparison of glucose concentrations in different rats includes an additional error from the differences in delivery (range 2%) from different pipettes. The pipettes used were not recorded.

The total label injected (dose) was determined by delivering two specimens of the solution, injected from the same syringe used for the injection, into known volumes of dilute glucose solution (about 50 µg/ml) and counting portions for radioactivity. Comparative efficiencies of counting these specimens, and those used in the specific radioactivity determinations, were measured with internal standards, and corrections to the same efficiency were applied.

Plasma insulin. Plasma insulin concentrations were determined in duplicate 50 µl samples by a double-antibody radioimmunoassay (Frayn, 1976). Plasma was stored at -20°C after collection and all analyses reported in the present paper were performed in one batch. Since bovine insulin was used as a standard absolute insulin concentrations could not be calculated. Dilution curves of bovine insulin were, however, similar to those of rat plasma insulin, so that relative values were still correct. The mean within-samples coefficient of deviation over the range studied was 16%, and the coefficient of error of the mean of duplicates therefore $16/\sqrt{2}$, i.e. 11%.

Relative concentrations of glucose in erythrocytes ($[\text{glucose}]_r$) and plasma ($[\text{glucose}]_p$). The values of the ratio $[\text{glucose}]_r/[\text{glucose}]_p$ needed to calculate plasma from blood glucose concentrations were determined on conscious and anaesthetized rats. They agreed within experimental error with previous measurements in post-absorptive rats (Heath, 1973). The earlier value (0.306), which had been determined on larger numbers of blood samples, was used in calculations.

Calculation of rates and rate coefficients of irreversible disposal (utilization)

When, as during most of these experiments, glucose concentrations change in each rat, only rate coefficients and not rates can be calculated directly from the data (Heath & Barton, 1973). The coefficients (k) are inversely related to the areas under the (quantity of label) versus time (q/t) curves. These areas were calculated from the four values of q as described by

Corney & Heath (1970) and the errors of them as described by Heath & Cunningham (1975). The rate (R) is given by:

$$R = kQ \quad (1)$$

where Q is the quantity of glucose in which q is determined. For calculation q was taken as the quantity of glucose label/ml of blood and Q as the blood glucose concentration ($[\text{glucose}]_b$). Values of $[\text{glucose}]_p$ were calculated from those of $[\text{glucose}]_b$ by using the experimental $[\text{glucose}]_r/[\text{glucose}]_p$ ratio and haematocrit values (Heath, 1973), and 'plasma' rate coefficients were obtained by the relationship:

$$k(\text{plasma}) = k(\text{blood}) \cdot [\text{glucose}]_b/[\text{glucose}]_p \quad (2)$$

(Heath & Corney, 1973).

Calculation of k when $[\text{glucose}]_p$ varies during an experiment requires that a relationship between k and $[\text{glucose}]_p$ be assumed (Heath & Barton, 1973), although in practice the value of k is not very sensitive to the relationship chosen. The slowness with which $[\text{glucose}]_p$ changed (see Table 1) suggested that k was either independent of $[\text{glucose}]_p$ or inversely related to it (Heath & Corney, 1973). Calculations were carried out on both assumptions. The pattern of results was almost identical, but mean values were about 4% higher on the assumption that k and $[\text{glucose}]_p$ were independent. Since there seems no plausible mechanism for an inverse relationship within an individual rat, especially under halothane anaesthesia when sympathetic activity is very low (Roizen *et al.*, 1974), only results on the assumption of independence are shown. These results were calculated by using eqn. (4.7) of Heath & Barton (1973) with $M = 1$.

Ratios of rates and rate coefficients with ^3H - and ^{14}C -glucose in the same animals. As the same measurements of glucose concentration were used for both radioisotopes, in serial-sampling experiments the only random errors in the determination of the ratio in each rat were those of scintillation counting. In single-sampling experiments errors in measurement of concentration were eliminated by expressing for each rat the value of q obtained with ^3H as a fraction of that obtained with ^{14}C , and calculating the mean fraction and its coefficient of deviation at each sampling time. Calculation then proceeded as follows. Let $q(1) \dots q(4)$ be the mean value of q after ^{14}C glucose injection, and $f(1) \pm c(1)f(1) \dots f(4) \pm c(4)f(4)$ be the mean values of the ratio: $q(^3\text{H})/q(^{14}\text{C})$ at each sampling time $\pm$ s.e.m. of the fractions expressed as coefficients of error $\times$ mean values. Then at any sampling time (i) the value of q for ^3H -labelling was taken to be $f(i)q(i)$ with a coefficient of error of $c(i)$. These values were used to calculate a rate coefficient for ^3H and its coefficient of error in the usual way. The latter was the coefficient of error of the ratio of the rate coefficients. The ratio of the rate coefficients (k

values) was obtained by dividing the ^3H rate coefficient calculated in this way by the ^{14}C coefficient calculated from the original data.

Results

Variation of plasma glucose concentrations with time after injection

The mean values in groups of rats either fell slowly or remained substantially constant during rate determinations (Table 1). In individual rats serially sampled, i.e. those under halothane anaesthesia, concentrations fell slowly in 14, but in four remained constant and in one rose slowly.

No consistent hyperglycaemia was observed in rats under halothane (Table 1), although a few were mildly hyperglycaemic (Figs. 1 and 2).

Mean plasma glucose concentrations, rate coefficients and rates of glucose utilization

Mean rate coefficients and plasma glucose concentrations are shown in Table 2.

Since in all rate experiments plasma glucose concentrations were very similar at the last two sampling times, by the end of each experiment a steady state had been reached in which the rate of glucose utilization equalled its rate of replacement. These equilibrium rates were therefore calculated by eqn. (1) from the rate coefficients in Table 2 and the plasma glucose concentrations at the last sampling time. Mean values are shown in Table 3.

Halothane decreased significantly both mean rate

coefficients and mean rates ($P < 0.01$; Tables 2 and 3), and also slightly decreased the proportion of ^{14}C recycled, as shown by a decrease in the ratio rate from $[5\text{-}^3\text{H}]\text{glucose}$ data/rate from $[\text{U-}^{14}\text{C}]\text{glucose}$ data ($P < 0.01$; Table 2).

Plasma insulin concentrations were determined on terminal blood samples in the dual-labelling experiments under halothane and in a group of starved controls that had not been injected. The mean concentration in the rats under halothane was about one-third of that in the controls, a significant decrease ($P < 0.01$, Student's t test).

Dependence of rate coefficients and rates on plasma glucose concentration

Under halothane rate coefficients for glucose utilization were inversely correlated with mean plasma glucose concentrations (Fig. 1). To save space only values obtained with $[\text{U-}^{14}\text{C}]\text{glucose}$ are shown, but the pattern with $[5\text{-}^3\text{H}]\text{glucose}$ was almost identical except for a scaling factor.

Rates from dual-labelling experiments were independent of glucose concentration, but when combined with those from single-labelling experiments a small significant rise with concentration was found (Fig. 2). This correlation, however, depended entirely on the three high rates in rats in which plasma glucose concentrations exceeded 6.7 mmol/l.

An inverse relationship between rate coefficient and plasma glucose concentration similar to that in rats under halothane was also demonstrated in the controls by two different methods of statistical analysis. Rigorous statistical evaluation of this relationship from the q/t (glucose label/ml of blood

Table 1. *Plasma glucose concentrations during rate determinations*

Each rat was injected at zero time. Controls were killed at the times stated and blood was analysed. Mean values $\pm$ S.E.M. are given, with numbers of rats in parentheses, for each sampling time. Controls (1) are re-calculated from the data of Heath & Corney (1973), controls (2) from the present experiments. Blood from rats under halothane was taken at each of the stated sampling times (serial sampling) and analysed. Mean values $\pm$ S.E.M. are given for each sampling time with numbers of rats after the last value. Halothane (1): rats were given $^{14}\text{C}[\text{glucose}]$ only; halothane (2): rats were given ^{14}C - and ^3H -glucose.

Control					
(1)	Time (min)	6	30	70	160
	Concn. (mmol/l)	7.08 ± 0.10	6.17 ± 0.18	5.57 ± 0.29	5.86 ± 0.19
		(4)	(4)	(4)	(4)
(2)	Time (min)	6	27	60	140
	Concn. (mmol/l)	6.32 ± 0.48	6.33 ± 0.25	6.48 ± 0.13	6.11 ± 0.16
		(3)	(4)	(6)	(11)
Halothane					
(1)	Time (min)	7	26	55	120
	Concn. (mmol/l)	6.53 ± 0.39	6.61 ± 0.37	6.52 ± 0.33	6.54 ± 0.32
					(8)
(2)	Time (min)	6	27	60	140
	Concn. (mmol/l)	5.79 ± 0.66	5.53 ± 0.14	5.26 ± 0.17	5.06 ± 0.16
					(11)

Table 2. Mean rate coefficients for glucose utilization and the ratios of the coefficients determined with [5-³H]glucose and [U-¹⁴C]glucose

Values are means $\pm$ S.E.M. with numbers of rats in parentheses at the end of each line. Control values were obtained in single-sampling experiments and values in rats under halothane in serial-sampling experiments. In the latter the plasma glucose concentration in each rat was taken to be the mean of the concentrations at the four sampling times, and the overall mean of these mean values is given. For controls the mean of all the concentrations determined in each experiment is given. The designation of experiments is as in the legend to Table 1.

State of rat	Label used	Rate coefficient (ml/min per 100 g)		Ratio [5- ³ H]/[U- ¹⁴ C]	Plasma [glucose] (mmol/l)
		[U- ¹⁴ C]Glucose	[5- ³ H]Glucose		
Control					
(1)	¹⁴ C	0.51 ± 0.02	—	—	6.17 ± 0.17 (16)
(2)	¹⁴ C, ³ H	0.57 ± 0.01	0.77 ± 0.02	1.354 ± 0.007	6.27 ± 0.10 (26)
Halothane					
(1)	¹⁴ C	0.43 ± 0.01	—	—	6.55 ± 0.34 (8)
(2)	¹⁴ C, ³ H	0.52 ± 0.02	0.68 ± 0.02	1.311 ± 0.007	5.41 ± 0.14 (11)

Table 3. Mean rates of glucose utilization and replacement

The rates are those at the end of each experiment when utilization equalled replacement (see the main text). Mean values $\pm$ S.E.M. are given, and also those of the plasma glucose concentration at the last sampling time with the numbers of rats in parentheses. Other details are as in the legend to Table 1.

State of rat	Rate (μ mol/min per 100 g)		Final plasma [glucose] (mmol/l)
	[U- 14 C]Glucose	[5- 3 H]Glucose	
Control			
(1)	3.00 $\pm$ 0.14	—	5.86 $\pm$ 0.19 (4)
(2)	3.48 $\pm$ 0.12	4.73 $\pm$ 0.16	6.11 $\pm$ 0.16 (11)
Halothane			
(1)	2.81 $\pm$ 0.07	—	6.54 $\pm$ 0.32 (8)
(2)	2.61 $\pm$ 0.02	3.42 $\pm$ 0.04	5.06 $\pm$ 0.16 (11)

versus time) relationships at the four sampling times (Heath & Corney, 1973) was limited by the small number and spread of values of q found at early times after injection. Nevertheless this treatment showed a significant inverse relationship with both ¹⁴C- and ³H-labelling ($P < 0.05$, Student's t test). Rates did not rise significantly with increasing plasma glucose concentration. The inverse relationship was shown more clearly by the values of q in control group (2) at the last two sampling times, at which more rats were used (Table 1), and the concentration ranges were greater, about 1 mmol/l. At the first sampling time values of q were very similar in different rats, as in all previous determinations of rate coefficients in these laboratories. By the last two sampling times values of q had diverged, and in all four groups (two with each label) were positively correlated with plasma glucose concentration, with $P < 0.01$ in two groups, $P < 0.05$ in one group, and $P < 0.2$ in the last (Student's t test, linear correlation). Since the rate coefficient is inversely proportional to the area under the q/t curve, which is smaller the lower the terminal values of q , the rate coefficients were negatively correlated with plasma glucose concentration.

Correlations with plasma insulin concentrations and arterial P_{CO_2}

In rats under halothane anaesthesia at the end of dual-labelling experiments plasma insulin concentrations were negatively correlated with rate coefficients ($P < 0.02$, linear correlation) and positively correlated with terminal plasma glucose concentrations ($P < 0.05$, linear correlation). There was no correlation with rate and there were no significant correlations in control rats.

Arterial P_{CO_2} values were determined in all but one rat under halothane. The mean value, corrected to 37°C as for human blood (Severinghaus, 1965; for applicability to rat blood, see Little & Threlfall, 1974), was 57.9 $\pm$ 8 kPa (43.5 $\pm$ 6.0 Torr; $\pm$ S.D., 18 rats), higher than in normal rats [about 36 kPa (27 Torr); Little & Threlfall, 1974; Heath & Rose, 1976]. Although the range of P_{CO_2} values was large there was no significant correlation with rate coefficients, rates or terminal plasma glucose concentrations. The observed decreases in arterial pH values were substantially accounted for by the increase in P_{CO_2} values, as found previously (Heath & Rose, 1976).

Fig. 1. Dependence on plasma glucose concentration of rate coefficients for glucose disposal in rats under halothane

Values in rats under halothane were obtained by serial sampling in two series of experiments separated by several months. The rate coefficients shown are those determined in both groups with $[U-^{14}C]$ -glucose and are plotted against the mean plasma glucose concentration in each rat. ■, Single-labelling experiments; ●, dual-labelling experiments. Control values (1 and 2) are taken from Table 2 and are means $\pm$ s.e.m. of the rate coefficients (determined with $[U-^{14}C]$ -glucose) and the plasma glucose concentrations at which they were obtained.

Discussion

Throughout the Discussion section values of rates, rate coefficients and concentrations in each rat are compared with those in the other rats. The aim is to explain how these differences between rats arise, i.e. what sets the levels in each rat. Very little is stated or implied about relationships between these quantities within individual rats, which may be quite different from those among rats.

Effects of halothane on glucose utilization

Care was taken in this study to ensure that rats under halothane were normoxic and normothermic and that the control rats were also at thermoneutrality. We do not consider that valid comparisons between rats under halothane anaesthesia and controls can be made unless these conditions are observed.

One effect of halothane was to raise arterial P_{CO_2} values. The increases, however, varied from only about 9 kPa (7 Torr) to about 39 kPa (29 Torr), so that, since glucose turnover was not correlated with P_{CO_2}

Fig. 2. Dependence on plasma glucose concentration of rates of utilization and replacement of glucose in rats under halothane

Values in rats under halothane were obtained by serial sampling in two series of experiments separated by several months. The rates are plotted against final plasma glucose concentrations. ■, $[U-^{14}C]$ -glucose labelling in single-labelling experiments; ●, $[U-^{14}C]$ -glucose labelling in dual-labelling experiments; ○, $[5-^3H]$ -glucose labelling in dual-labelling experiments. The lines are least-mean-square lines of best fit. The slope of the top line is not significantly different from zero, but that of the bottom line is ($P < 0.01$). The control value is for set (1) ($[U-^{14}C]$ -glucose only) taken from Table 3 and is the mean $\pm$ s.e.m. ranges of rate and terminal plasma glucose concentration; the value for control group (2) has been omitted for clarity.

values, it seems unlikely that they could have affected the differences in turnover rates between controls and rats under halothane.

Halothane decreased the rate of glucose utilization by 10–27%. Training rats to sleep naturally brings about similar decreases in rates, of 22% in the rate of glucose utilization in post-absorptive rats (Heath & Corney, 1973), and of 15–20% in total body O_2 consumption in starved rats (Denckla, 1970). Since the rate of glucose utilization closely parallels the rate of heat production and O_2 consumption in both starved and post-absorptive rats (Heath & Corney, 1973), the decrease in the rate of glucose utilization caused by halothane anaesthesia in starved rats was close to that expected from inducing them to sleep naturally.

Halothane had only a slight effect on the fraction of ^{14}C recycled (Table 2). This suggests that all the rates controlling glucose turnover were decreased almost proportionately by halothane, confirming its lack of any specific action. Similarly Biebuyck & Lund (1974) found that halothane, in a concentration of 1.5%

(v/v), had very little effect on hepatic glucose metabolism in starved rats. Large effects have been observed in perfused livers, but at much higher concentrations (Biebuyck *et al.*, 1972).

Another effect of halothane in the present experiments was to produce a wider range of steady-state plasma glucose concentrations than those found in conscious rats. This made obvious certain unexpected relationships which were then also found between conscious control rats.

Homeostatic relationships

Comparison of one rat with another in each group showed two striking findings. First, the strong inverse relationship between $[\text{glucose}]_p$ and k among the anaesthetized rats (Fig. 1) was also found among control group (2), confirming previous observations by Dr. V. J. Cunningham (personal communication) and our own preliminary experiments. Secondly, within each group, rates of glucose utilization and replacement (which were necessarily equal in the steady state reached by the end of each experiment) were very similar in all rats although their equilibrium plasma glucose concentrations covered a fairly wide range (see the Results section and Fig. 2).

Control of glucose turnover rate. In this context the rates are those determined with $[5\text{-}^3\text{H}]\text{glucose}$ and are best regarded as rates of glucose production. Rates were similar within each group and differed between groups roughly in proportion to expected total body O_2 consumption and heat production. These findings seem only to be explicable if the major factor controlling glucose production was the total body fuel consumption. This is not implausible in the starved rat, in which nearly all the glucose is produced by gluconeogenesis, from glycerol release concomitant with fat oxidation, from amino acids from proteolysis and from C_3 units arising from glycolysis. Glycogen is not an important source of glucose in the rat starved for 24 h; liver glycogen is not falling at this time (Higgins *et al.*, 1932) and the rate of fall of muscle glycogen is only enough to provide about 5% of the glucose if it were all recycled via lactate etc. (Cori & Cori, 1928).

Control of plasma glucose concentration. Values of R , k and $[\text{glucose}]_p$ are related by:

$$R = k[\text{glucose}]_p \quad (3)$$

where the rate coefficient k is a measure of the capacity of the rat to take up glucose into its cells. Since within a group R was constant, $[\text{glucose}]_p$ and k must be inversely related, and the question is whether the plasma glucose concentration in any rat determined its value of k , or whether values of k were determined in some other way so that k controlled the plasma glucose concentration. There is no known

mechanism whereby a high plasma glucose concentration could cause a low capacity for cellular glucose uptake, especially in view of the low sympathetic activity in anaesthetized rats (Roizen *et al.*, 1974). Differences in capacity for uptake (i.e. in values of k), however, would automatically account for the observed relationship, since in a rat in which the capacity was low the plasma glucose concentration would have to be high for the glucose disposal rate to match the fixed rate of production.

What is the physiological meaning of variability of k ? The starved rat is very unlike starved man in that glucose uptake is mainly into insulin-sensitive tissues, whereas in starved man it is almost wholly into brain (Cahill *et al.*, 1966; Garber *et al.*, 1974). Rat brain accounts for only about 12% of the lowest whole-body glucose utilization found in our experiments (calculated from Hawkins *et al.*, 1974). The rate of uptake into insulin-sensitive tissues is proportional to the glucose and insulin concentrations at the cell surfaces (Cherrington *et al.*, 1972; Daniel *et al.*, 1975; Insel *et al.*, 1975; Sherwin *et al.*, 1974), which in the nearly steady state, as in these experiments, are closely proportional to their concentrations in plasma (Insel *et al.*, 1975; Sherwin *et al.*, 1974). Under these circumstances, eqn. (3) can be rewritten without much error in the form:

$$R = k[\text{glucose}]_p = k'[\text{insulin}]_p[\text{glucose}]_p \quad (4)$$

i.e. k consists of the product of the plasma insulin concentration, $[\text{insulin}]_p$, and a factor (k') that is a measure of the overall sensitivity of the tissues to insulin. Differences in plasma insulin concentrations cannot account for differences in values of k , since among the anaesthetized rats they were inversely related. The differences in values of k between the rats in each group must therefore be attributed to intrinsic differences, covering about a twofold range, in the sensitivities of their tissues to insulin. Even larger differences have been found in normal non-obese man, and are correlated with small differences in total body fat (Insel *et al.*, 1975).

We therefore conclude that in the basal or near-basal state the rate of glucose production is determined by the supply of gluconeogenic precursors arising as by-products of the basal metabolism, which is necessarily similar in different rats. The rate of glucose utilization must, in these near steady-state conditions, equal the rate of production, and the plasma glucose and insulin concentrations then adjust themselves according to the intrinsic sensitivity of the tissues to insulin, which is surprisingly variable between individual animals.

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APPENDIX

A Comparison of a Chromatographic and an Ion-Exchange Method of Determining the Specific Radioactivities of Blood Glucose Labelled with ^3H and ^{14}C

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The widely used ion-exchange methods of purifying blood glucose for the determination of its specific radioactivity are much faster than the paper-chromatographic method used as a routine in these laboratories. We investigated the validity of a typical deionization method and found it inadequate for measurements of the specific radioactivity of glucose in the blood of rats given labelled glucose, except in samples taken shortly after injection.

The preliminary clean-up required by the chro-

matographic method was carried out on all blood specimens: deproteinization (Somogyi, 1930), followed by passage of the supernatant with water washings through mixed-bed resin [equal volumes of Zerolit 325 (H^+ form) and Zerolit MIP (Cl^- form), both with granule diam. 0.3-1.2 mm (bought from BDH Chemicals, Poole, Dorset, U.K.); 5 ml of resin was required for extract equivalent to 1 ml of blood]. The specific radioactivities of glucose were determined by both methods on the eluates (Table 1).

The coefficient of error of a single determination by the chromatographic method was 2.5% by the analysis of closely spaced duplicated blood samples

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Table 1. *Relative specific radioactivities of glucose separated by two methods, chromatography and ion exchange, from rat blood at various times after injection of [U-¹⁴C]- and [5-³H]-glucose*

Unspecified experimental details are given in the main text. Rats were post-absorptive, of about 250 g body wt., with or without dorsal scald injury (Heath & Corney, 1973). Labelled glucose was injected intravenously. Blood specimens, obtained by decapitation or via tail arteries, were cleaned up before analysis as described in the text. The chromatographic method is described by Frayn (1976). For the ion-exchange method the blood extract was free of ³H₂O by evaporation twice to a volume of 0.5 ml from 5 ml at 40–45°C in a stream of N₂. Residues were passed with water washings through Dowex AG1 resin (1.5 ml of X8, 200–400 mesh, acetate form, pre-washed with 8 ml of water). Portions of the eluate were analysed for label (scintillation counting in Instagel with a Tri-Carb series 4000 spectrometer from Packard Instrument Co., Wembley, Middx., U.K.) and glucose. For each blood specimen the ratio of specific radioactivities (by chromatography/by ion exchange) was determined for each radioactive compound. Results are mean values at each time after injection $\pm$ S.E.M. with the numbers of rats in parentheses. Asterisks indicate values differing significantly from unity ($P < 0.01$, Student's *t* test).

Uninjured rats			Injured rats		
Time after injection (min)	Specific radioactivity ratio		Time after injection (min)	Specific radioactivity ratio	
	¹⁴ C	³ H		¹⁴ C	³ H
3	1.021 $\pm$ 0.022 (4)	1.020 $\pm$ 0.015 (4)	6	0.989 $\pm$ 0.019 (7)	0.978 $\pm$ 0.018 (7)
14	1.025 $\pm$ 0.007 (3)	0.993 $\pm$ 0.007 (3)	30	1.008 $\pm$ 0.011 (5)	0.973 $\pm$ 0.024 (5)
30	0.987 $\pm$ 0.014 (6)	0.966 $\pm$ 0.007* (6)	65	0.929 $\pm$ 0.011* (7)	0.934 $\pm$ 0.012* (7)
60	0.920 $\pm$ 0.020* (8)	0.910 $\pm$ 0.013* (8)	140	0.884 $\pm$ 0.019* (7)	0.858 $\pm$ 0.025* (7)

(0.2 ml) taken, via a tail artery 140 min after injection of [U-¹⁴C]glucose, from 24 h-starved rats under halothane anaesthesia. In these experiments specific radioactivities were as low as any in the series and the chances of interferences by metabolites probably as high.

In Table 1 a value of unity indicates agreement between the two methods, and a lower value that the ion-exchange method gave high results because the columns had let through non-glucose label. Both methods agreed when used on blood samples taken shortly after injection. Even the preliminary clean-up on mixed-bed resin was adequate on blood from 24 h-starved rats taken up to 30 min after injection (ratio = 1.002 $\pm$ 0.009; S.E.M., $n = 8$). On later samples, however, the ion-exchange method significantly overestimated the specific radioactivity.

[U-¹⁴C]Glucose and [5-³H]glucose gave similar results (Table 1). This seems to rule out the possibility that the labelled contaminant was in the original injected material, since the two radioactive isotopes were incorporated in different ways: ¹⁴C via ¹⁴CO₂ into sucrose in *Canna* leaves and ³H via a complex synthesis (data sheets, The Radiochemical Centre,

Amersham, Bucks., U.K.). The contaminant appears to have been a metabolite (or metabolites). The paper-chromatographic procedure provides evidence of non-glucose label (Heath, 1973; Frayn, 1976).

The underestimation of rate coefficients by using data from the deionization method could be as great as 3.5 times the standard error on the rate coefficient (³H data for injured rats), and is thus not always negligible.

The deionization method could be used on blood samples at short times after injection. The coefficient of error of a single determination was 3.4%, calculated by analysis of variance of the data at the first two sampling times in each group (Table 1) using 2.5% for the coefficient of error of the chromatographic method.

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