Glucose Turnover in the Post-Absorptive Rat and the Effects of Halothane Anaesthesia

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1. Rates and rate coefficients of glucose utilization and replacement in post-absorptive rats, either conscious or under halothane anaesthesia, were determined in a thermoneutral environment by using [5-³H]- and [U-¹⁴C]glucose. Label was not injected into rats under halothane until about 0.5 h after anaesthesia was initiated. 2. Comparison with the results for 24 h-starved rats in the preceding paper [Heath *et al.* (1977) *Biochem. J.* 162, 643–651] showed that insulin concentrations were considerably higher but rate coefficients for glucose utilization were little altered in post-absorptive rats. Sensitivity to insulin was thus considerably increased by a 24 h period of starvation in the rat. 3. Fractional recycling of glucose carbon in post-absorptive rats was under one-half of that in starved rats, reflecting the larger contribution of liver glycogenolysis to glucose production in the former. 4. In post-absorptive rats halothane decreased the mean rate of glucose utilization by about 17%. This decrease was associated with an increase in mean plasma insulin concentration, showing that halothane decreased sensitivity to insulin. 5. Recycling was slightly increased by halothane, indicating that the contribution of liver glycogen to the total glucogenic rate was decreased, probably because liver glycogen concentrations were about 40% lower throughout the rate determinations in halothane. 6. Comparison of our results with earlier work shows that during and shortly after induction of halothane anaesthesia glucose turnover must have been greatly increased whereas from about 0.5 h after induction it was decreased.

In the preceding paper (Heath *et al.*, 1977) it was shown that halothane anaesthesia decreased the rate of glucose turnover in the 24 h-starved rat to that appropriate for the basal metabolic state. This rate was similar in all rats, despite considerable differences in their equilibrium plasma glucose concentrations. It was also shown that there were differences between rats in the mean sensitivities of their tissues to insulin, i.e. in their values of k' as defined by the equation:

$$R = k'[\text{insulin}]_p[\text{glucose}]_p \quad (1)$$

where R is the rate of glucose turnover determined with [5-³H]glucose, and the concentrations are those in plasma.

Glucose metabolism in post-absorptive rats, i.e. those in a similar nutritional state to man after an overnight fast (Heath & Corney, 1973), differs in two important respects from that in starved rats: liver glycogen is a major source of glucose; and resting plasma insulin concentrations are considerably higher (Aynsley-Green *et al.*, 1973; Bosboom *et al.*, 1973; McGarry *et al.*, 1973; Kim &

Pi-Sunyer, 1974). We therefore decided to see whether the same relationships held between rates, rate coefficients and plasma glucose concentrations.

Experimental

Materials and methods were as in the preceding paper (Heath *et al.*, 1977) except as follows. (1) The weight range of the rats used was 233–266 g. (2) Food was withdrawn 4–6 h before the start of each experiment. (3) Blood samples in rate determinations were taken 4, 25, 50 and 140 min after injection from rats under halothane anaesthesia and 3, 14, 30 and 60 min after injection from conscious controls. (4) For the experiments described in Table 3 liver glycogen was measured on duplicate specimens of about 1 g each by ethanol precipitation and acid hydrolysis (Good *et al.*, 1933) and determination of the resulting glucose with hexokinase (EC 2.7.1.1) [Boehringer Corp. (London) Ltd., Lewes, East Sussex, U.K.]. (5) Terminal plasma samples from rate determinations under halothane anaesthesia were assayed for plasma insulin concentration in the same batch as those in the preceding paper (bovine insulin standard; Heath *et al.*, 1977) to provide a comparison between post-absorptive and starved rats; these values were also used in looking for

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correlations with rates, rate coefficients and plasma glucose concentrations.

Other plasma insulin measurements described in the present paper were made by using a rat insulin standard (kindly provided by Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen, Denmark). This assay had a mean within-samples coefficient of deviation over the range studied of 4.0%; all samples were assayed in one batch. (6) In conscious controls, unlike in rats under halothane anaesthesia, there was evidence that within each rat the rate coefficient (k) varied with blood glucose concentration ($[\text{glucose}]_b$) (see the Results section). Rate coefficients for these rats were therefore calculated from eqns. (4) and (5) (with $n = 1$) of Heath & Corney (1973). This procedure gives the equilibrium steady-state value of k achieved by the end of the experiment. It follows that the values in anaesthetized rats taken for purposes of comparison must be the end values also.

Results

Variation of plasma glucose concentration with time after injection

Mean values are shown in Table 1. In conscious controls mean plasma glucose concentrations fell fairly rapidly up to the third sampling time and then remained constant. As discussed by Heath & Corney (1973), this pattern is that expected if in each rat handling at injection caused mild hyperglycaemia and insulin release. Then k , which is the equivalent of $k'[\text{insulin}]_b$ in eqn. (1), will be proportional to plasma glucose concentration, and will only reach a steady value towards the end of the experiment when the glucose concentration stabilizes. Thus 'end' values of rate coefficient and rate are the best estimates available of those in the resting state.

Within rats under halothane, which were serially sampled, the concentrations fell slowly to steady values in 11 rats and altered irregularly and barely significantly in the other two. This pattern suggests that within these rats the rate coefficients were independent of plasma glucose concentrations, as in starved rats (Heath *et al.*, 1977), and were calculated accordingly. Mean terminal concentrations were significantly higher than in either group of controls ($P < 0.01$, Student's t test).

Mean values of the parameters

Table 2 shows that halothane decreased the mean rate of glucose utilization by about 17% but increased the fraction of carbon recycled, as shown by the significant increase ($P < 0.01$, Student's t test) in the ratio of the rates obtained with $[5\text{-}^3\text{H}]$ - and $[\text{U-}^{14}\text{C}]$ -glucose. The mean glucose concentration was raised by halothane ($P < 0.01$, Student's t test) and the mean rate coefficient (k) was decreased by about 25%.

It follows from eqn. (1) that halothane had decreased either the plasma insulin concentrations or the sensitivities of the tissues to insulin. These possibilities were investigated in a separate experiment (Table 3). Halothane anaesthesia raised insulin concentrations, and this finding is particularly striking since in this experiment the plasma glucose concentrations in the controls were unusually high (cf. Table 2). Thus halothane decreased the sensitivities of the tissues to insulin.

Liver glycogen concentrations were also determined in this experiment and were decreased under halothane by $39.3 \pm \text{s.e.m. } 5.6\%$ (Table 3).

Within neither group of rats were plasma insulin or liver glycogen concentrations correlated significantly with plasma glucose concentrations.

Table 1. *Plasma glucose concentrations during rate determinations*

Times were measured from injection. For controls, each of which provided only one blood sample, mean concentrations $\pm \text{s.e.m. } (n)$ are given. Controls (1) were calculated from the data of Heath & Corney (1973), controls (2) from those of the present experiments. The 13 rats under halothane were serially sampled, and the means of the values at each sampling time are given $\pm \text{s.e.m.}$

State of rat					
Controls	(1) Time (min)	4	20	45	90
	Concn. (mmol/l)	9.89 ± 0.29	8.94 ± 0.24	7.95 ± 0.08	8.23 ± 0.13
		(6)	(6)	(4)	(4)
	(2) Time (min)	3	14	30	60
Rats under halothane	Concn. (mmol/l)	10.32 ± 0.20	10.16 ± 0.31	8.64 ± 0.07	8.47 ± 0.16
		(4)	(4)	(6)	(7)
	Time (min)	4	25	50	140
	Concn. (mmol/l)	10.59 ± 0.22	10.04 ± 0.22	9.69 ± 0.20	9.43 ± 0.24
					(13)

Table 2. Mean values of rate coefficients and rates of glucose utilization and of the ratios of the values determined by using [^3H]glucose and [^{14}C]glucose in post-absorptive rats at thermoneutrality

Mean values $\pm$ S.E.M. are given. The plasma glucose concentrations given are those at the last sampling time, as only near these times were the rate coefficients for control rats constant. Rates and rate coefficients in anaesthetized rats were determined in serial-sampling experiments, those in controls in single-sampling experiments. Control values are numbered as in the legend to Table 1.

State of rat	Label used	Rate coefficient (ml/min per 100g)		Rate ($\mu\text{mol/min per 100g}$)		Ratio [^3H]/[^{14}C]	Plasma glucose concn. (mmol/l)
		[^{14}C]Glucose	[^3H]Glucose	[^{14}C]Glucose	[^3H]Glucose		
Control	(1) ^{14}C	0.75 $\pm$ 0.03	—	6.21 $\pm$ 0.25	—	—	8.23 $\pm$ 0.13 (4)
	(2) ^{14}C , ^3H	0.80 $\pm$ 0.03	0.91 $\pm$ 0.03	6.79 $\pm$ 0.24	7.69 $\pm$ 0.26	1.131 $\pm$ 0.006	8.47 $\pm$ 0.16 (7)
Halothane	^{14}C , ^3H	0.58 $\pm$ 0.01	0.68 $\pm$ 0.01	5.48 $\pm$ 0.12	6.41 $\pm$ 0.12	1.171 $\pm$ 0.010	9.43 $\pm$ 0.24 (13)

Table 3. Mean values of plasma insulin and glucose concentrations and liver glycogen concentrations

Control rats were treated exactly as in rate determinations except that labelled glucose was omitted from the injection. In anaesthetized rats cannulation of the tail artery and serial sampling were also omitted. All rats were killed by decapitation and blood and liver samples taken within 1 min, at the same times after injection as the last sampling times in rate experiments (60 min for controls, 140 min for rats under halothane) and at the same time of day within a few minutes. Values are means $\pm$ S.E.M. and there were 16 rats in each group.

	Plasma insulin ($\mu\text{units/ml}$)	Plasma glucose (mmol/l)	Liver glycogen (mg/g)
Controls	42.3 $\pm$ 1.5	8.84 $\pm$ 0.13	51.9 $\pm$ 2.1
Halothane	53.5 $\pm$ 2.3	9.24 $\pm$ 0.16	31.5 $\pm$ 2.1
P (Student's <i>t</i> test)	<0.001	<0.1	<0.001

Plasma insulin concentrations in the terminal samples from rate determinations under halothane were compared with those in anaesthetized starved rats (bovine standard; see the Experimental section); in the post-absorptive rats they were about eightfold greater ($P < 0.001$, Student's *t* test).

Dependence of rate coefficients and rates on terminal plasma glucose concentrations

Comparison of rats under halothane one with another showed that a high terminal glucose concentration tended to be associated with a low rate coefficient but a high rate of glucose utilization (Figs. 1 and 2). There was, however, considerable scatter. In these rats plasma insulin concentrations were not significantly correlated with rates or rate coefficients.

Statistical analysis of the data from conscious rats by the methods described in the preceding paper (Heath *et al.*, 1977) did not reveal any significant correlation between rate or rate coefficient and terminal plasma glucose concen-

Fig. 1. Dependence on terminal plasma glucose concentration of rate coefficients for glucose utilization in post-absorptive rats under halothane at thermoneutrality

The values obtained from each rat with [^{14}C]glucose ($\bullet$) and [^3H]glucose ($\circ$) are shown. The lines are those of least-mean-squares best fit. The slopes are significantly different from zero (top line, $P < 0.01$; bottom line, $P < 0.05$; Student's *t* test). Control values from [^{14}C]glucose experiments only are taken from Table 2 with S.E. ranges of rate coefficients and of terminal plasma glucose concentrations.

tration. The number of rats used was, however, too small to be likely to reveal such weak correlations as those found in rats under halothane anaesthesia.

Arterial P_{CO_2} values were determined in rats under halothane during 'rate' experiments. They were 59.3 ± 6.3 kPa (44.5 ± 4.7 Torr; S.D., 13 rats), about 70% higher than in earlier controls (Little & Threlfall, 1974; Heath & Rose, 1976), but very like those in starved rats under halothane. Within the group of rats under halothane P_{CO_2} values were not correlated with rates or rate coefficients, but were weakly and positively correlated with terminal plasma glucose concentrations.

Fig. 2. Dependence on terminal plasma glucose concentration of rates of glucose utilization in post-absorptive rats under halothane at thermoneutrality

Conventions are as in the legend to Fig. 1. The slopes of the lines are significantly different from zero (top line, $P < 0.02$; bottom line, $P < 0.05$; Student's t test).

Discussion

Throughout, comparisons with starved rats refer to the results reported in the preceding paper (Heath *et al.*, 1977) unless stated otherwise. Rates of gluconeogenesis and irreversible uptake into tissues will be taken as before to be the rates measured with $[5\text{-}^3\text{H}]\text{glucose}$.

The rates and rate coefficients used for comparisons are those in the steady state of glucose metabolism reached within each rat by the end of each experiment as shown by the similarity between the blood glucose concentrations at the last two sampling times. Except in conscious post-absorptive rats the rate coefficient within each rat was assumed to be independent of glucose concentration. Within each conscious post-absorptive rat it was assumed to be proportional to concentration. Although the experimental evidence was consistent with these assumptions, there was no proof that they held exactly. Even serious errors in them, however, would have caused errors of only about 4% in the mean values of the rates and rate coefficients in each group (an example is given in the preceding paper), too small to alter any conclusions. Patterns within groups were even less sensitive to the assumptions made.

Our most important conclusion follows from a comparison of the rate coefficients with those in starved rats (Tables 2 in the present paper and Heath *et al.*, 1977). Under halothane anaesthesia the mean rate coefficient was the same in both groups, and in conscious rats was decreased by only $15 \pm 4\%$ by starvation. In view of the well-known

severalfold decrease in plasma insulin brought about by 24h starvation in the rat (Aynsley-Green *et al.*, 1973; Bosboom *et al.*, 1973; McGarry *et al.*, 1973; Kim & Pi-Sunyer, 1974) and confirmed in our experiments, it follows from eqn. (1) that starvation greatly increased the mean sensitivity of the tissues to insulin. In man sensitivity to insulin is inversely related both to body fat (Insel *et al.*, 1975) and to plasma insulin concentration (Olefsky *et al.*, 1973), and these and our findings may be related to the increase in insulin receptor concentration, and hence in sensitivity to insulin, caused by lowering the ambient insulin concentration either *in vitro* over a few hours (Gavin *et al.*, 1974) or *in vivo* by food restriction (Soll *et al.*, 1975). It is not often realized that a 24h period of starvation in the rat establishes a metabolic pattern achieved only after about 4 days' starvation in man (Cahill *et al.*, 1966): steady low plasma glucose concentrations (Foster, 1967), a non-protein respiratory quotient of about 0.7 (Cori & Cori, 1926, 1928) and a 10% loss in body weight.

The $5\text{-}^3\text{H}/\text{U}\text{-}^{14}\text{C}$ rate ratios (Tables 2 in the present paper and Heath *et al.*, 1977) show that the fraction of glucose carbon recycled in post-absorptive rats was under half that in starved rats. This was to be expected, since in post-absorptive rats glycogen is a major source of glucose, and a smaller proportion is formed by recycling of C_3 units, the process that differentiates between the two labels.

As in starved rats, halothane decreased the rate of utilization of glucose by about 20%, and under halothane the rate coefficients were inversely correlated with plasma glucose concentrations (Fig. 1), indicating again differences in mean sensitivities to insulin.

The effects of halothane were, however, in some ways different in post-absorptive rats. It raised plasma insulin concentrations (Table 3) and increased carbon recycling (Table 2).

The rise in insulin concentrations is in contrast with the findings of Aynsley-Green *et al.* (1973), but there were considerable differences both in the pretreatment of their rats and in the duration of anaesthesia. Our results are consistent with the decrease in sympathetic activity in the rat under halothane (Roizen *et al.*, 1974), if, as in other species (baboon, Werrbach *et al.*, 1970; man, Robertson & Porte, 1973; dog, Bloom & Edwards, 1975; Kaneto *et al.*, 1975), insulin secretion is under tonic sympathetic inhibitory control. Since rate coefficients were decreased our results show that halothane decreased considerably the mean sensitivity of the tissues to insulin, i.e. decreased k' in eqn. (1). Why this should be so we do not know. It might be related to the finding that halothane interferes with Ca^{2+} mobility (Porsius & Van Zwieten, 1975), since there is now considerable evidence that many effects of

insulin are mediated via Ca^{2+} ion movements (Fraser, 1975).

The increase in carbon recycling suggests that liver glycogen contributed less to glucogenesis. By the end of the rate experiments liver glycogen concentrations were $20.4 \pm 3.0 \text{ mg/g}$ less than in controls. Since Biebuyck & Lund (1974) found a similar decrease after exposure for 1 h, and our rats were not injected until after about 35 min exposure, we conclude that liver glycogen concentrations under halothane were much lower throughout the rate experiments, and therefore contributed correspondingly less to glucogenesis.

A comparison of our results and those of Biebuyck & Lund (1974) demonstrates two phases in the effects of halothane on glucose metabolism in the post-absorptive rat. During and shortly after induction liver glycogenolysis was greatly accelerated (Biebuyck & Lund, 1974), whereas later it was somewhat decreased (present results). The initial glycogenolysis must have been accompanied by accelerated glucose uptake, otherwise the later hyperglycaemia would have greatly exceeded that observed. Thus an early increase in glucose turnover was followed by a later decrease.

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References

- Aynsley-Green, A., Biebuyck, J. F. & Alberti, K. G. M. M. (1973) *Diabetologia* **9**, 274–281
- Biebuyck, J. F. & Lund, P. (1974) *Mol. Pharmacol.* **10**, 474–483
- Bloom, S. R. & Edwards, A. V. (1975) *J. Physiol. (London)* **253**, 157–173
- Bosboom, R. S., Zweek, J. & Bouman, P. R. (1973) *Diabetologia* **9**, 243–250
- Cahill, G. F., Jr., Herrera, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A., Jr. & Kipnis, D. M. (1966) *J. Clin. Invest.* **45**, 1751–1769
- Cori, C. F. & Cori, G. T. (1926) *J. Biol. Chem.* **70**, 557–576
- Cori, C. F. & Cori, G. T. (1928) *J. Biol. Chem.* **79**, 309–319
- Foster, D. W. (1967) *J. Clin. Invest.* **46**, 1283–1296
- Fraser, T. R. (1975) *Proc. R. Soc. Med.* **68**, 785–791
- Gavin, J. R., Roth, J., Neville, D. M., De Meyts, P. & Buell, D. N. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 84–88
- Good, C. A., Kramer, H. & Somogyi, M. (1933) *J. Biol. Chem.* **100**, 485–491
- Heath, D. F. & Corney, P. L. (1973) *Biochem. J.* **136**, 519–530
- Heath, D. F. & Rose, J. G. (1976) *J. Pharm. Pharmacol.* **28**, 151–153
- Heath, D. F., Frayn, K. N. & Rose, J. G. (1977) *Biochem. J.* **162**, 643–651
- Insel, P. A., Liljenquist, J. E., Tobin, J. D., Sherwin, R. S., Watkins, P., Andres, R. & Berman, M. (1975) *J. Clin. Invest.* **55**, 1057–1066
- Kaneto, A., Kajinuma, H. & Kosaka, K. (1975) *Endocrinology* **96**, 143–150
- Kim, S. & Pi-Sunyer, F. X. (1974) *Diabetes* **23**, 605–609
- Little, R. A. & Threlfall, C. J. (1974) *Circulatory Shock* **1**, 209–216
- McGarry, J. D., Meier, J. M. & Foster, D. W. (1973) *J. Biol. Chem.* **248**, 270–278
- Olefsky, J., Farquhar, J. W. & Reaven, G. (1973) *Diabetes* **22**, 507–513
- Porsius, A. J. & Van Zwieten, P. A. (1975) *Arch. Intern. Pharmacodyn.* **218**, 29–39
- Robertson, R. P. & Porte, D. (1973) *Diabetes* **22**, 1–8
- Roizen, M. F., Moss, J., Henry, D. P. & Kopin, I. J. (1974) *Anaesthesiology* **41**, 432–439
- Soll, A. H., Kahn, C. R., Neville, D. M. & Roth, J. (1975) *J. Clin. Invest.* **56**, 769–780
- Werrbach, J. H., Gale, C. C., Goodner, C. J. & Conway, M. J. (1970) *Endocrinology* **86**, 77–82