The Effects of Starvation, Environmental Temperature and Injury on the Rate of Disposal of Glucose by the Rat

By DENNIS F. HEATH and PATRICIA L. CORNEY

*Experimental Pathology of Trauma Section, Medical Research Council Toxicology Unit,
Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, U.K.*

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1. The disposal rate of glucose, R , given by $R = k_p Q$, where Q is the quantity of plasma glucose and k_p is a rate coefficient, was determined from the disappearance of $[U-^{14}C]$ -glucose from blood after single intravenous injection. Values of R should be close to the carbohydrate oxidation rate in the states investigated. 2. Normal rats (i) experimental methodology was studied. The best (single sampling) method gave the following results. (ii) The plasma glucose concentration (C_p) and R were temporarily increased by the stress of handling and injection. (iii) R was increased by decreasing the environmental temperature from 29°C to 20°C in line with previously published (Stoner & Marshall, 1971) changes in total body O_2 consumption. (iv) Starvation decreased R such that $R = \text{constant} \times C_p^2$. (v) The results suggested some central control of cell permeability to glucose. 3. Injured post-absorptive rats were studied in the ebb phase after three severe injuries: scalding at 20° and 29°C (non-lethal) and bilateral hind-limb ischaemia at 20°C (85% mortality). (i) Handling and injection did not affect C_p . (ii) The rise in C_p after injury was not closely correlated with its severity. (iii) The value of R was nearly independent of severity. (iv) Unlike in normal rats R varied little with ambient temperature (in line with O_2 consumption) or with C_p . Values of k_p varied inversely as C_p . (v) The results were explained in terms of a centrally integrated response to injury involving the hypothalamus which over-rode the controls operating in normal rats. Hormonal factors are discussed.

This study of the rate of irreversible disposal of glucose in rats answers several questions. The answers are indicated in parentheses.

(1) The total body O_2 consumption of post-absorptive rats in our laboratory increases 43% when their environmental temperature is lowered from 28–30°C (thermoneutrality) to 20°C (Stoner & Marshall, 1971). Does the disposal rate of glucose rise correspondingly? (Yes.)

(2) How does the disposal rate vary with plasma glucose concentration in the normal range? (From a comparison of rates in post-absorptive and starved rats, as the square of concentration.)

(3) In an animal severely injured, whether fatally or not, energy metabolism quickly takes up a new steady state which may persist for several hours and which is abnormally unresponsive to changes in environmental temperature. This is the ebb phase of metabolism (Cuthbertson, 1942; Stoner, 1970). In this state total body O_2 consumption is independent of ambient temperature over the range 20–30°C (Stoner, 1969). Is glucose disposal also independent? (Yes.)

(4) In this state at these temperatures the O_2 consumption is the same as that in normal rats at an ambient temperature of 28–30°C (Stoner, 1969). Do the glucose disposal rates also match? (Approximately.)

(5) In post-absorptive rats shock causes hyper-

glycaemia, as in other species (Stoner, 1958). Is the increase in glucose concentration related to the severity of injury? (Not closely.)

(6) How does the disposal rate vary with plasma glucose concentration after injury? (Very little; the rate is nearly independent of plasma glucose concentration.)

(7) Disposal rates can be determined either by serial sampling or by taking only one sample from each rat. Several arguments favour single sampling (Heath & Barton, 1973). Which is in fact better? (Both are practicable, but single sampling takes much less time to achieve the same precision.) Only uninjured rats could be used to answer this question as blood loss from serial sampling increases the severity of an injury.

The results are presented so as to illustrate the procedures for calculating rates described by Heath & Barton (1973).

Rates were estimated from the disappearance from the blood of intravenously injected $[U-^{14}C]$ glucose. This tracer was chosen partly to help forward another investigation, but also because it gives in post-absorptive animals rates of disposal of glucose close to rates of carbohydrate oxidation (see the section Results and Discussion: 'Resting Disposal Rates and Whole Body O_2 Consumption'), and was therefore likely to give relevant answers to questions (1), (3) and (4).

Materials and Methods

Animal experiments

Experimental design and theory. The experiments were designed for ready applications of the equations given by Heath & Barton (1973). The nomenclature is as defined by Heath & Barton (1973) and equations given in that paper are indicated here by square brackets. Values referring specifically to whole blood or plasma are denoted by subscripts 'b' and 'p' respectively; but when the same equations apply to both, subscript '1' is used instead.

The basic parameter determined was the area from $t = 0$ to $t = \infty$ under the q_1 - t curve, A_{q1} , i.e. $\int_0^\infty q_1 dt$, where q_1 is the quantity of glucose label in whole blood or plasma. In a typical experiment each rat was given the same dose, D , of label as $[U-^{14}C]$ glucose by single intravenous injection and was decapitated at one of four set times afterwards. A single blood sample was taken from each rat, analysis of which gave the glucose concentration in it (C_1), the specific radioactivity of glucose (S_1) and the haematocrit value. From these were calculated the blood volume (V_1) (see below), the total glucose in blood (Q_1 , i.e. $C_1 V_1$) and q_1 (i.e. $S_1 Q_1$ or $S_1 C_1 V_1$). The values of q_1 at the four set times, chosen as described by Corney & Heath (1970), gave by their method the area A'_{q1} . (See also text near eqns. [6.5]–[6.8].) The total area, A_{q1} , is given by: $A_{q1} = A'_{q1} + \Delta A_{q1}$, in which $\Delta A_{q1} < 0.03 A'_{q1}$, and is the area not already accounted for under the q_1 - t curve at very short times after injection (see text near eqn. [6.3]). It was measured on a few rats (see below) and added as a standard correction to every value of A'_{q1} .

In serial-sampling experiments the results were similar, but were obtained on at least four successive samples from each rat via a cannula.

The value of A_{q1} gave a mean rate-coefficient defined by:

$$\bar{k}_v = D/A_{q1} = \int_0^\infty k_v q_1 dt / A_{q1} \quad (1), [4.9]$$

where k_v is the instantaneous rate-coefficient such that at any time, t , after injection:

$$R(t) = k_v(t) \cdot Q_1(t) \quad (2)$$

where (t) denotes values at time t , and R is the rate of disposal. There is a time, t_c , at which $\bar{k}_v$ and k_v coincide, when:

$$R(t_c) = \bar{k}_v \cdot Q_1(t_c) \quad (3)$$

As Q_1 varied only slowly with time only a rough value of t_c was required. To calculate t_c $\bar{k}_v$ was assumed to vary as a power function of C_1 , whence the time-dependence of k_v could be simulated by the experimental time-dependence of C_1 and thus introduced into eqn. (1). In injured rats C_1 varied linearly with time after injury, as in many serial

sampling experiments (Ashby *et al.*, 1965), and the procedure leads to eqns. [6.17] and [6.20], by which t_c was evaluated. In normal rats the C_1 - t -dependence was more complex, and is described in the Results and Discussion section.

In post-absorptive rats another rate coefficient, $k_v(e)$, was calculated, that towards which k_v was tending by the end of the experiment. Denoting by (4) the values at the fourth (last) sampling time, $k_v(e)$ is defined by:

$$k_v(e) = D[C_1(4)]^n / \int_0^\infty [C_1(t)]^n q_1 dt \quad (4)$$

when k_v varies as C_1^n . The present work confirms the assumption that n was near unity, the value which was used. The $C_1 q_1$ integral in eqn. (4) was calculated from $C_1 q_1$ in the same way as A'_{q1} was calculated from q_1 . The rate calculated by:

$$R(e) = k_v(e) \cdot Q_1(4) \quad (5)$$

was the best estimate which could be made of the resting rates in normal rats. In injured rats the shock state is transient, so $k_v(e)$ and $R(e)$ are meaningless.

Values of $\bar{k}_v$ and $k_v(e)$ could be determined from analyses of whole blood or plasma. For comparison the two values could be interconverted, as both methods give the same values of R , by the equations:

$$R = k_v(\text{plasma}) \cdot Q_p = k_v(\text{blood}) \cdot Q_b \quad (6)$$

whence:

$$k_v(\text{plasma}) = k_v(\text{blood}) \cdot Q_b / Q_p \quad (7)$$

Random errors in A'_{q1} were calculated in single-sampling experiments from the experimental errors on q_1 at each sampling time by eqn. [6.2] and in serial-sampling experiments by eqn. [6.9] by using the experimental mean coefficient of deviation of 5% on all values of q_1 . The systematic error in A'_{q1} should be under 2% (Corney & Heath, 1970); and in the $C_1 q_1$ - t integral of eqn. (5) was shown by a more exact calculation to be -2.6% in the most extreme case provided by the data.

To calculate the variation of $\bar{k}_v$ and $R(t_c)$ with plasma glucose concentration (C_p) in injured rats, use was made of the considerable variation of C_p caused by a standard injury. The additional information required was: the regressions of C_p on Q_1 , of Q_1 on time after injury and, at each of the four set times after injection, of q_1 on Q_1 . The regressions were assumed to be linear. By standard statistical techniques the most probable values of $\bar{k}_v$ and $R(t_c)$ were then calculated for chosen values of C_p , and, from the standard errors on C_p , Q_1 and q_1 , the standard errors on $\bar{k}_v$ and $R(t_c)$.

Details of experimental procedures

Environmental temperatures. Two rooms were used, one at 18–22°C, the other at 28–30°C. Rats,

values obtained etc. in these rooms are described as 'at 20°C' and 'at 29°C' respectively.

Rats. These were male, Porton-Wistar albinos, fed on M.R.C. diet 41B, and kept from the time of weaning at 20°C under artificial light from 07:00–19:00h. Food was removed from experimental rats at 09:00h. Those designated 'post-absorptive' were injected in the afternoon of the same day; those designated 'starved' during the morning of the next day. For experiments at 29°C normal rats were moved to the room at 29°C 2–5h before injection. All body weights were corrected to the values at 14:30h on the day food was withdrawn.

Injuries. All injured rats were post-absorptive. Two types of injury were induced, both under ether: 4h of bilateral hind-limb ischaemia (Rosenthal, 1943); and a full-thickness dorsal scald covering 20% of the body surface by partial immersion of the rat for 30s in water at 83°C (Arturson, 1961). Ischaemic rats were kept at 20°C, scalded rats at either 20°C or 29°C. Ischaemic injury was timed from the removal of the tourniquets. Rats for use at 29°C were moved to the room at 29°C immediately after injury. Ischaemic rats were not injected until at least 1h after injury or scalded rats until at least 1.4h after injury, by which time the ebb phase was fully developed.

Cannulations. Injured rats were cannulated in a lateral tail vein while anaesthetized for injuring. Of normal rats, only those used for serial-sampling experiments were cannulated (under ether), 5–8 days before use, most in a jugular vein (Ginsburg & Heller, 1953; Ashby *et al.*, 1965), but some in a carotid artery (Popovic & Popovic, 1961).

Solutions for injection. [^{14}C]Glucose was bought from The Radiochemical Centre (Amersham, Bucks., U.K.). It was injected at about 2 $\mu\text{Ci}/\text{rat}$ (less than 1 μmol of glucose/rat) in 0.20ml of 0.9% NaCl containing 300–400 i.u. of heparin. This dose of heparin combined with the handling the rats received has no effect on plasma free fatty acid concentrations in injured rats but causes a slight increase in normal rats (Heath & Stoner, 1968), in which it may affect 'mean' but not 'end' rates. Injections through cannulae were washed in with 0.15ml of 0.9% NaCl.

Analytical procedures. Microhaematocrit values were found for most blood samples, and were corrected for entrained plasma (Heath, 1973b). They gave, combined with erythrocyte volumes and F_{cells} (ratio of whole body haematocrit value to great vessel haematocrit), values of blood and plasma volumes. Heath (1973b) gives values of F_{cells} and erythrocyte volumes in normal and scalded rats. Stoner's (1961) results show that the values in ischaemic rats are the same as in normal rats.

In single-sampling experiments blood from the neck was analysed for glucose and glucose label as described by Heath (1973b).

In serial-sampling experiments 0.1ml samples,

taken as described by Ashby *et al.* (1965), were analysed as described by Heath & Rose (1969), except that a labelled contaminant that affected their results was excluded by cutting out the glucose spot after chromatography just within its leading edge.

Radioactivity was measured in a Packard Tri-Carb series 4000 liquid-scintillation spectrometer on specimens dissolved in Bray's (1960) scintillant. All count rates were normalized to an injected dose of $2 \times 10^6 \text{ c.p.m.}/100 \text{ g body wt.}$ Standard errors were less than $\pm 2\%$ after subtraction of background.

Computations. The most advanced machine used was a desk-top computer Model 9810A (Hewlett-Packard, Loveland, Colorado, U.S.A.). No procedure would have been accelerated (allowing for programming time) by using a more advanced computer.

Estimation of ΔA_{q1} . Estimation was carried out on normal post-absorptive rats with carotid cannulae.

Fig. 1. [^{14}C]Glucose in blood taken from a carotid cannula shortly after injection of $2 \times 10^6 \text{ c.p.m. per } 100 \text{ g body wt.}$

The shaded area is ΔA_{q1} . Each specimen took about 5s to draw from the cannula.

Each rat was given [$U\text{-}^{14}\text{C}$]glucose via a tail vein and blood samples ($\sim 30\mu\text{l}$) were taken every few s for a few min, ether anaesthesia being maintained throughout. The blood was centrifuged to give a haematocrit value and a plasma specimen, which was weighed and counted for radioactivity. The plasma c.p.m./100g body wt. were very close to q_1 at such short times (calculated from data in Heath, 1973b), and were taken to be identical. A typical q_1 - t curve is shown (Fig. 1). The value of ΔA_{q_1} was $293\,000 \pm 19\,000$ counts/100g body wt. (S.E.M., $n = 4$).

This value is controlled by rates of loss of label to extravascular space, which were assumed to be similar in all the conditions studied. They are in fact similar for [^{14}C]fructose in normal and ischaemic rats (Ashby *et al.*, 1965). Consequently ΔA_{q_1} was assumed to be the same in all rats studied, and the value shown was added as a constant correction to all values of A_{q_1} to give A_{q_1} . The contribution to the $C_1 q_1$ - t integral was taken to be $C_1(t_1) \cdot \Delta A_{q_1}$, where $C_1(t_1)$ was the experimental value of C_1 at the first sampling time.

Results and Discussion

Rates of disposal of glucose in normal rats

Basic data and calculations. Data for single-sampling experiments and the rate coefficients, k_v and $k_v(e)$, calculated from them are in Table 1. Values of the resting disposal rate, $R(e)$, are in Table 2.

The estimation of the mean rate, $R(t_c)$ (Table 2), from k_v by eqn. (3) required the coincidence time, t_c , to be calculated from the dependence of blood glucose concentration, C_1 , on time after injection. There were two types of C_1 - t curve.

1. In all but starved rats at 20°C excitation caused a rise in C_1 followed by a decrease, probably through a minimum at the third sampling time (Table 1). The minimum is a well-known phenomenon, and is caused by the insulin released by the initial rise in C_1 disappearing more slowly than the excess of glucose (Atkins, 1971). Consequently, k_v was probably still falling at the third sampling time, and its time-dependence could be represented sufficiently well by fitting the first three values of C_1 to an exponential, i.e. to:

$$C_1(t) = C_1(\infty) + \beta e^{-mt} \quad (8), [6.11]$$

The value of t_c was then calculated from m and q_1 by eqn. [6.16], which, in eqn. (8), gave $C_1(t_c)$, and hence, by multiplication by V_1 , $Q_1(t_c)$. The pool volume, V_1 , was time-invariant in these experiments.

2. In starved rats at 20°C , C_1 fell from one roughly constant value to another between the second and third sampling times, and $Q(t_c)$ was calculated by eqn. [6.21].

In serial-sampling experiments values of the rate coefficients and rates were obtained for each rat

(Table 2). The results are not shown in detail. The rats were in two groups, 'tamed' and 'untamed'. Each in the tamed group had been handled and petted for a few min every day for at least a week before injection, and, during and after it, was kept within sight and touch of another rat. Loud noises and sudden movements were avoided. The untamed animals were not petted, and, as they were kept in separate cages between cannulation and injection, were very excitable. In the tamed rats at 20°C initial blood glucose concentrations were lower (t test) and less variable (F test) than in the untamed [1.28 ± 0.027 mg/ml (12 rats) against 1.49 ± 0.064 mg/ml (14 rats), $\pm$ S.E.M.]. After injection the glucose concentration varied like in starved rats at 20°C (Table 3), and $Q_8(t_c)$ was calculated as for them. The tamed rats at 29°C were, in fact, very excitable, and in four out of five the blood glucose concentration rose slightly during the experiment; these were the only rats in which this happened.

Sampling of plasma versus sampling of whole blood. The same blood samples were used to obtain values for whole blood and plasma for post-absorptive rats at 20°C (Table 1) and hence two sets of values of R (Table 2, first two sets). The values were very similar. Ideally they should be identical (Heath & Barton, 1973), but differences in analytical methods, errors in interpolation etc., are expected to cause the values to be a little different. The values of k_v should not be similar, being related by eqn. (7).

Effects of excitation upon rates. Handling and other mild stress causes hyperglycaemia, and, via subsequent release of insulin, a temporary increase in k_v . Thus the mean rate, $R(t_c)$, should exceed $R(e)$. Except in serially sampled rats at 29°C this was the pattern observed (Table 2); but the small difference between the mean and resting rates showed that the rats were only very mildly stressed.

Rats in the same nominal state, however, gave different resting rates (Table 2). These were higher the more active the response of the rats to the laboratory environment after they had settled down. Thus of the post-absorptive rats at 20°C those which had been tamed were mostly asleep by the end of the experiment, those single sampled were awake but quiet except for head movements, and those untamed were jumpy and irritable. Plainly the 'resting state' must be referred to a specific environment and response to it; and even then minor variations of environment may have significant effects.

Single versus serial sampling. As shown above rates could be estimated reliably from serial-sampling experiments. To achieve comparable precision, however, such experiments took 2-4 times longer than single-sampling experiments, and did not save rats as half of those cannulated could not be used because of blocked cannulae or excitability. In general, therefore, single sampling is preferable.

Table 1. Determination of rate coefficients for irreversible disposal of glucose from normal rats in single-sampling experiments

For each treatment rats were killed after injection of [U-¹⁴C]glucose at the four times shown. The concentrations of glucose in blood (C_b), and, in post-absorptive rats at 20°C only, in plasma (C_p) were determined, and the quantities in the blood or plasma pool of glucose (Q_b or Q_p) and of glucose label (q_b or q_p)/100 g body weight. The last are as means $\pm$ S.E.M. (n) given in the units: $10^{-3} \times$ counts for a dose of 2×10^6 c.p.m./100g body wt. Values of k_e were calculated from D/A_4 , i.e. 2×10^6 /(area under the q_1 - t curve), and $k_a(e)$ from $DC_1(e)/\int_0^\infty C_1(t)q_1 dt$, i.e. $2 \times 10^6 \times$ final concentration of glucose/(area under the C_1q_1 - t curve). Coincidence times, t_c , were calculated where appropriate as described in the text.

Treatment	t (min)	...	4	16	35	70	k_e (per min)	$k_a(e)$ (per min)	t_c (min)
Post-absorptive at 20°C	C_p (mg/ml)	...	1.90 $\pm$ 0.08 (6)	1.65 $\pm$ 0.05 (6)	1.52 $\pm$ 0.03 (9)	1.54 $\pm$ 0.05 (6)	—	—	—
	Q_p (mg)	...	7.48 $\pm$ 0.31	6.84 $\pm$ 0.20	6.21 $\pm$ 0.12	6.40 $\pm$ 0.27	—	—	—
	q_p	...	243.4 $\pm$ 5.7	138.4 $\pm$ 6.8	61.2 $\pm$ 3.0	24.56 $\pm$ 2.41	0.258 $\pm$ 0.007	—	15
	C_pq_p	...	462.2 $\pm$ 22.1	227.0 $\pm$ 8.6	93.1 $\pm$ 5.4	37.7 $\pm$ 4.0	—	0.242 $\pm$ 0.012	—
	C_b (mg/ml)	...	1.32 $\pm$ 0.05 (6)	1.16 $\pm$ 0.02 (6)	1.07 $\pm$ 0.03 (9)	1.09 $\pm$ 0.03 (6)	—	—	—
	Q_b (mg)	...	8.45 $\pm$ 0.34	7.69 $\pm$ 0.08	7.19 $\pm$ 0.13	7.20 $\pm$ 0.29	—	—	—
Post-absorptive at 29°C	q_b	...	247.2 $\pm$ 5.6	153.3 $\pm$ 7.8	76.8 $\pm$ 3.7	31.6 $\pm$ 2.5	0.231 $\pm$ 0.006	—	17
	C_bq_b	...	326.8 $\pm$ 15.8	177.6 $\pm$ 7.4	82.1 $\pm$ 4.8	34.4 $\pm$ 3.1	—	0.209 $\pm$ 0.009	—
	t (min)	...	4	20	45	90	—	—	—
	C_b (mg/ml)	...	1.27 $\pm$ 0.04 (4)	1.12 $\pm$ 0.02 (4)	1.00 $\pm$ 0.02 (4)	1.05 $\pm$ 0.02 (4)	—	—	—
	Q_b (mg)	...	8.53 $\pm$ 0.31	7.15 $\pm$ 0.04	6.35 $\pm$ 0.29	6.83 $\pm$ 0.09	—	—	—
	q_b	...	276.2 $\pm$ 6.5	141.4 $\pm$ 3.7	71.4 $\pm$ 4.5	35.5 $\pm$ 2.6	0.176 $\pm$ 0.005	—	27
Starved at 20°C	C_bq_b	...	351.1 $\pm$ 17.0	158.8 $\pm$ 6.9	71.3 $\pm$ 5.6	33.6 $\pm$ 2.5	—	0.168 $\pm$ 0.006	—
	t (min)	...	6	23	50	120	—	—	—
	C_b (mg/ml)	...	0.761 $\pm$ 0.015 (5)	0.785 $\pm$ 0.014 (5)	0.701 $\pm$ 0.020 (6)	0.681 $\pm$ 0.019 (8)	—	—	—
	Q_b (mg)	...	4.68 $\pm$ 0.12	4.96 $\pm$ 0.15	4.38 $\pm$ 0.12	4.18 $\pm$ 0.09	—	—	—
	q_b	...	240.3 $\pm$ 12.3	149.1 $\pm$ 8.1	83.3 $\pm$ 1.6	23.56 $\pm$ 0.91	0.157 $\pm$ 0.003	—	—
	C_bq_b	...	182.6 $\pm$ 9.2	119.4 $\pm$ 8.0	58.5 $\pm$ 2.3	16.09 $\pm$ 0.90	—	0.145 $\pm$ 0.006	—
Starved at 29°C	t (min)	...	6	30	70	160	—	—	—
	C_b (mg/ml)	...	0.903 $\pm$ 0.017 (4)	0.783 $\pm$ 0.022 (4)	0.697 $\pm$ 0.035 (4)	0.732 $\pm$ 0.018 (4)	—	—	—
	Q_b (mg)	...	6.00 $\pm$ 0.18	5.17 $\pm$ 0.32	4.44 $\pm$ 0.24	4.65 $\pm$ 0.09	—	—	—
	q_b	...	278.2 $\pm$ 0.9	164.4 $\pm$ 6.9	73.9 $\pm$ 2.9	21.94 $\pm$ 1.51	0.116 $\pm$ 0.003	—	35
	C_bq_b	...	251.1 $\pm$ 4.2	128.6 $\pm$ 5.1	51.5 $\pm$ 3.4	16.11 $\pm$ 1.33	—	0.107 $\pm$ 0.004	—

Table 2. Mean and resting rates of disposal of glucose, $R(t_c)$ and $R(e)$, in post-absorptive and starved rats at 20° and 29°C

Means $\pm$ S.E.M. are shown with the number of rats in parentheses. Possible systematic errors are discussed in the text.

Treatment	Rates (mg/min per 100 g body wt.)	
	$R(t_c)$	$R(e)$
Post-absorptive at 20°C		
Single sampled, plasma	1.78 ± 0.07	1.55 ± 0.10
Single sampled, blood	1.76 ± 0.05	1.51 ± 0.09
Serial sampled, 'tamed'	1.36 ± 0.10 (12)	1.20 ± 0.10 (12)
Serial sampled, 'untamed'	2.10 ± 0.11 (14)	1.88 ± 0.11 (14)
Post-absorptive at 29°C		
Single sampled	1.20 ± 0.03	1.15 ± 0.04
Serial sampled, 'tamed'	1.41 ± 0.09 (5)	1.40 ± 0.12 (5)
Starved at 20°C		
Single sampled	0.68 ± 0.02	0.57 ± 0.03
Starved at 29°C		
Single sampled	0.55 ± 0.04	0.47 ± 0.02

Table 3. Concentrations of glucose in the blood of rats from which serial samples were taken, expressed as fractions of the terminal value

The terminal value was obtained at 70 or 90 min after injection. The ratios: (value at time t)/(terminal value) were calculated for each rat. Means $\pm$ S.E.M. (n , number of rats) are given. Specimens were taken within 1.5 min of the first two stated times or 3 min of the rest.

t (min)	...	4	8.5	12.5	17	30	60
Ratio		1.121	1.107	1.087	1.082	0.978	0.960
S.E.M.		0.017	0.016	0.015	0.016	0.018	0.010
n		12	8	8	12	8	7

Table 4. Comparison of rate coefficients and glucose concentrations in post-absorptive and starved rats

All values are ratios $\pm$ S.E.M.; post-absorptive/starved. Values were taken from those results from single-sampling experiments in which analyses were carried out on whole blood (Tables 1 and 2).

	k_v	$k_v(e)$	$C_1(t_c)$	$C_1(e)$
Rats at 20°C	1.48 ± 0.05	1.45 ± 0.08	1.57*	1.60 ± 0.07
Rats at 29°C	1.52 ± 0.05	1.57 ± 0.08	1.42 ± 0.06	1.43 ± 0.04

* No S.E.M. can be given, as the S.E.M. for $C_1(t_c)$ in starved rats at 20°C is not calculable.

In the present experiments comparison between singly sampled rats is more likely to be valid than between serially sampled ones, because their environments at the two temperatures were more closely matched and because the experiments were carried out much closer together in time. Only results from single-sampled rats are considered from now on.

Comparison of resting rates with earlier values. Those in rats starved 24 h (Table 2) were similar to those found by other workers [units are mg/min per 100g; only Depocas (1962) gives the ambient tem-

perature]: 0.65 and 0.58 by Baker *et al.* (1959; 1961), 0.52 by von Holt *et al.* (1961), and 0.51 by Depocas (1962), the last at 30°C in rats acclimatized at 30°C, and corrected by the author's formula to the weight of rat we used. Rates for post-absorptive rats agree less well. Baker *et al.* (1959; 1961) give 1.05 and 0.76, and Ashby *et al.* (1965) give 1.17, both groups from specific radioactivity-time curves continued for 2 h or more after injection. In our experience there is appreciable recycling of label at such long times, which increases the q_1-t integral and decreases the

estimate of *R. Depocas* (1962) in rats at 30°C as before found 0.61 (corrected for weight difference). Perhaps his very big rats are not comparable with ours. *von Holt et al.* (1961) give a value of 1.81 by using essentially a 1-pool model for glucose, which is expected to give a high value (*Skinner et al.*, 1959). The error from the method is greater the higher the rate, and may have been small in their experiments with starved rats which gave a value of *R* close to those we obtained (see above).

Dependence of rate coefficients and rates upon glucose concentration. At each temperature the post-absorptive/starved ratio of values of k_v and C_b were very similar (Table 4). The variations in k_v brought about in each rat by handling have been represented by $k_v = \text{constant} \times C_1^n$, as already stated. If starvation did not change the value of the constant then analysis of variance of the results in Table 4 gives $n = 1.06 \pm 0.11$ ($\pm$ S.E.M.).

This value of the S.E.M. corresponds to a systematic error of only about 1% in $k_v(e)$. The value of the constant may, of course, have been affected by starvation. If so the true value of *n* in each rat was probably further from unity, but it is unlikely that it would be so far different as to cause systematic errors in the estimate of $k_v(e)$ as big as the random errors.

The proportionality constant was, however, certainly lower at 29°C than at 20°C i.e. raising the temperature increased k_v rather than C_1 . In part this may have been because the room at 29°C was quieter than the room at 20°C. We shall assume that any such effect was negligible; but this remains an assumption not open to investigation.

The proportionality of disposal rates at each temperature to the square of the blood glucose concentration is, of course, only an approximation to a relationship known to be more complex (see e.g. *Atkins*, 1971). Rates of uptake may not follow the same relationship. These exceed rates of disposal by rates of recycling, which are uncertain; compare, e.g., *von Holt et al.* (1961) with *Ashmore et al.* (1961).

Resting disposal rates and whole body O_2 consumption. The significance of any comparison depends upon the extent to which the rate of glucose disposal can be equated with the rate of glucose or carbohydrate oxidation. The last can be measured by the non-protein R.Q. (respiratory quotient) and the O_2 consumption or CO_2 output. In three metabolic states there is no simple relationship: during rapid glycogen deposition, e.g. during rapid infusion of glucose (*Depocas*, 1964) and during and shortly after feeding; after prolonged starvation (*Cahill et al.*, 1966); and during a short burst of exercise (*Young et al.*, 1967; *Wahren et al.*, 1971). In the post-absorptive state, however, there is good evidence of near-equality in the only three cases we have found. The ratio: (rate by R.Q.)/(rate by label) is used as an index.

In all the following examples the R.Q. values were in the range 0.80–0.83, i.e. the metabolic states were comparable although the authors' descriptions of them differ.

1. In man the basal R.Q. is 0.83 (*Richardson*, 1929). *Long et al.* (1971) determined disposal rates and CO_2 expiration in 11 basal subjects. Excluding one very aberrant result ($P \leq 0.001$) the ratio: (glucose C disposal)/(CO_2 C expired) was 0.421 ± 0.029 ($\pm$ S.E.M.), giving (rate by R.Q.)/(rate by label) = 1.06.

2. In the dog starved for 18h *Steele et al.* (1968) determined the disposal rate, CO_2 excretion and non-esterified fatty acid concentration and quote results for N excretion. *Paul et al.* (1966) gave the regression of R.Q. on non-esterified fatty acid concentration, from which the R.Q. was calculated to be 0.80, and the ratio 1.00 in the dogs used by *Steele et al.* (1968).

3. In anaesthetized rats at 30°C with R.Q. = 0.82 the ratio was 0.84 (*Depocas*, 1959, 1962).

We therefore take glucose disposal rates to be equal to rates of oxidation of total carbohydrate in post-absorptive rats and more doubtfully so in 24h-starved rats. In the latter *Depocas* (1964) found that starvation did not change the fraction of label injected as [^{14}C]glucose that was expired as $^{14}CO_2$, suggesting that the balance of the reactions involved in disposal was similar. [Because of isotopic exchange (*Depocas*, 1964; *Steele et al.*, 1968) $^{14}CO_2$ production cannot be equated with glucose oxidation.]

There are four distinguishable rates of body O_2 consumption by the resting rat, depending on how much it moves (*Bramante*, 1961, 1968). The highest, the average over 1h or more in a quiet but not motionless rat, is the relevant one here. This rate increases by 43% in post-absorptive rats when their ambient temperature is decreased from 29° to 20°C (*Stoner & Marshall*, 1971). The increase in glucose disposal rate (Table 2) was similar (33%) in post-absorptive rats, but was less, although not significantly so, in starved ones. The disposal rates at 20°C corresponded to the utilization of 39% and 14% of the body O_2 consumption by carbohydrate oxidation in post-absorptive and starved rats respectively. *Depocas* (1959) found a value of 35% in post-absorptive rats at 30°C.

Control of disposal rate. The effects of glucose concentration were consistent with the usual view that an increase in glucose concentration causes a release of insulin and a consequent increase in the rate coefficient, k_v . The effects of temperature are not so directly explicable. As already noted the increase in rate on lowering the temperature was caused by an increase in the rate coefficient, not by an increase in blood glucose concentration (Table 1). The rate coefficient, k_v , is a complex constant, which includes the rate coefficients for the disposal of glucose from all the glucose pools in the body and the ratios of these pool sizes to the plasma pool size, Q_1 (see eqns.

[4.3]–[4.5]). Hypothetically the coefficient could be increased by increasing the glucose space by improving the blood supply. As, however, even a lethal ischaemic injury only decreases the glucose space by about 16% (Ashby *et al.*, 1965) the changes in glucose space after a 9°C change in ambient temperature are

unlikely to have much effect, and it is necessary to suppose that the rate coefficients in the individual pools are diminished. These rate coefficients are the equivalent of permeability coefficients, and what is postulated is an increase in cell permeability on lowering the environmental temperature of the rat. Such a

Table 5. *Injured rats: linear regression of glucose label in blood pool, q_1 , against glucose in blood pool, Q_1 , at set times after injection*

Units: q_1 in $10^{-3} \times \text{c.p.m./100g body wt.}$; Q_1 in mg of glucose/100g body wt. The equation is in the form given above the data, a 'bar' representing a mean value. The slope is c , n is the number of rats. Times (t) were measured from the time of injection.

Groups	t (min)	$q_1 - \bar{q}_1 \pm \text{S.E.M.} = (c \pm \text{S.E.M.})(Q_1 - \bar{Q}_1)$	(n)
Scalded at 29°C	5	$q_p - 221.2 \pm 4.7 = (10.10 \pm 3.12)(Q_p - 7.10)$	(16)
	25	$q_p - 115.5 \pm 4.1 = (6.21 \pm 4.19)(Q_p - 7.12)$	(10)
	55	$q_p - 56.3 \pm 1.9 = (6.56 \pm 2.45)(Q_p - 7.19)$	(11)
	125	$q_p - 19.4 \pm 1.4 = (1.20 \pm 2.72)(Q_p - 6.63)$	(14)
Scalded at 20°C	6	$q_b - 191.1 \pm 4.8 = -(1.33 \pm 4.78)(Q_b - 8.39)$	(7)
	30	$q_b - 97.4 \pm 7.1 = (0.99 \pm 4.55)(Q_b - 8.51)$	(9)
	65	$q_b - 59.8 \pm 2.6 = (8.76 \pm 1.63)(Q_b - 8.25)$	(10)
	140	$q_b - 25.2 \pm 2.0 = (4.99 \pm 1.11)(Q_b - 8.14)$	(10)
Ischaemic at 20°C	5	$q_b - 219.7 \pm 6.9 = (2.40 \pm 3.08)(Q_b - 10.91)$	(7)
	25	$q_b - 118.1 \pm 3.7 = (10.97 \pm 1.37)(Q_b - 10.46)$	(8)
	60	$q_b - 66.9 \pm 2.7 = (6.00 \pm 1.43)(Q_b - 10.40)$	(7)
	140	$q_b - 34.2 \pm 3.4 = (4.27 \pm 1.36)(Q_b - 9.85)$	(7)

Table 6. *Injured rats: linear regression of blood glucose pool, Q_1 , and plasma volume, V_p , per 100g body wt. on time after injury, t*

The equations are given in the same form as in Table 5. For the analysis of variance only the slopes and errors on the slopes of the Q_1 - t curves were required. The equation for Q_1 is the upper one in each case.

Group	First and last times (min)	$Q_1 - \bar{Q}_1 \pm \text{S.E.M.} = (c \pm \text{S.E.M.})(t - \bar{t})$	(n)
Scalded at 29°C	86, 306	$V_p - \bar{V}_p \pm \text{S.E.M.} = (c' \pm \text{S.E.M.})(t - \bar{t})$	(n)
		$Q_p - 6.84 \pm 0.11 = -(0.0019 \pm 0.0020)(t - 174)$	(62)
Scalded at 20°C	148, 236	$V_p - 2.581 \pm 0.018 = (0.00144 \pm 0.00034)(t - 174)$	(62)
		$Q_b - 8.32 \pm 0.26 = -(0.0031 \pm 0.0077)(t - 177)$	(36)
		$V_p - 2.500 \pm 0.028 = (0.00019 \pm 0.00079)(t - 178)$	(37)
Ischaemic at 20°C	67, 205	$Q_b - 10.39 \pm 0.42 = -(0.0070 \pm 0.0078)(t - 124)$	(29)
		$V_p - 2.480 \pm 0.039 = -(0.00403 \pm 0.00073)(t - 126)$	(28)
Controls	—	$V_p = 4.068 \pm 0.037^*$ (26)	

* Normal post-absorptive rats at 20°C.

Table 7. *Injured rats: linear regression of plasma glucose concentration, C_p , on blood glucose pool, Q_1*

The equations are in the form given in Table 5. Values of C_p are in mg/ml of plasma, of Q_1 , in mg/100g body wt.

Group	Range of C_p	$C_p - \bar{C}_p \pm \text{S.E.M.} = (c \pm \text{S.E.M.})(Q_1 - \bar{Q}_1)$	(n)
Scalded at 29°C	1.88–3.69	$C_p - 2.66 \pm 0.02 = (0.403 \pm 0.025)(Q_p - 6.84)$	(62)
Scalded at 20°C	1.89–4.04	$C_p - 2.88 \pm 0.03 = (0.349 \pm 0.021)(Q_b - 8.32)$	(36)
Ischaemic at 20°C	2.11–5.03	$C_p - 3.51 \pm 0.07 = (0.308 \pm 0.032)(Q_b - 10.39)$	(29)

co-ordinated effect seems to imply central regulation. It could be mediated by an increased release of insulin or a lessening of resistance to the action of insulin at the cell surface.

Rates of glucose disposal in post-absorptive injured rats

Basic data and calculations. The calculation of the variation of k_v and $R(t_c)$ with plasma glucose concentration, C_p , required for each group of rats the regression of q_1 on Q_1 at each of the four sampling times (Table 5), of Q_1 on time after injury (Table 6), and of C_p on Q_1 (Table 7). It is not relevant to further analysis that several of the regression coefficients in Tables 5 and 6 do not differ significantly from zero. In rats at 20°C values of C_b , not C_p , were determined, and values of C_p were calculated by the equation given by Heath (1973b). Handling and injection did not bring about any increase in C_p in these rats; therefore values of k_v and $R(t_c)$ were those appropriate to the resting state, and were comparable with values of $k_v(e)$ and $R(e)$ in normal rats.

Statistical analysis gave for each group of rats the slightly curved relationships between k_v and C_p and between $R(t_c)$ and C_p shown with standard error ranges in Figs. 2 and 3.

As the linear equations relating q_1 and Q_1 (Table 5) are likely to be unreliable at extreme values of Q_1 and C_p , the ranges of C_p shown are shorter than the experimental ones by the omission of the top 10% and bottom 10% of the values of C_p .

Fig. 2. Mean rate coefficient, k_v , for disposal of glucose from injured rats as functions of plasma glucose concentration

The envelopes represent standard error ranges.

The values of k_v shown are those for plasma glucose turnover calculated for rats at 20°C by eqn. (7).

Mean rates of disposal and O_2 consumption. The mean rates at the mean plasma glucose concentrations in the three groups were: 1.28 ± 0.04 in scalded at 29°C, 1.31 ± 0.08 in scalded at 20°C and 1.27 ± 0.06 mg/min per 100 g in ischaemic at 20°C. The similarity accords with that in O_2 consumption, with no effect of environmental temperature (Stoner, 1969).

Compared with normals, one rate, that in scalded rats at 29°C, was significantly higher ($P < 0.05$) than in post-absorptive normals at 29°C, and two rates, in scalded rats at 29°C and ischaemic ones at 20°C, were significantly lower ($P < 0.05$) than in post-absorptive normals at 20°C (Table 2). Probably the rates were intermediate, and were therefore slightly out of line with the O_2 consumption, which was identical with that in normal rats at 29°C. The small discrepancy could be due either to increased carbohydrate oxidation relative to that of other substrates or to an altered balance between isotopic exchange and other reactions.

Effects of severity of injury. Each method of inducing trauma seems likely to have inflicted the same amount of tissue damage, within narrow limits, on each rat subjected to it. For hind limb ischaemia there is direct evidence that this is so (Stoner, 1965). The decrease in plasma volume characteristic of non-haemorrhagic injuries (Wiggers, 1950) was also very constant from rat to rat. Thus, from the variances of the mean values of plasma volume (V_p) in normal and injured rats in Table 6 the likely standard deviations of the decreases of plasma volume were calculated. The F test showed ($P > 0.9$) that the standard deviations

Fig. 3. Mean disposal rates, $R(t_c)$, of glucose in injured rats as functions of plasma glucose concentration

The envelopes represent standard error ranges.

were less than the following values (ml/100g) in each case: scalded at 29°C, ± 0.014 ; scalded at 20°C, ± 0.137 ; ischaemic at 20°C, ± 0.208 .

The injuries can be ranked in severity. The ischaemic injury causes 85% mortality within 24h (Stoner, 1961), whereas scald at 20°C causes no deaths in 48h (Stoner, 1968). Mortality from scald at 29°C has not been determined; but as the rats in this group were the only ones to show signs of recovery, i.e. an increasing plasma volume (Table 6) and decreasing plasma glucose concentration (Heath, 1973b), this injury seems to have been the least severe.

Although each injury was reproducible and two, scald at 20°C and ischaemia, were certainly very different in severity, i.e. potential lethality, plasma glucose concentrations, rate coefficients and rates overlapped (Figs. 2 and 3). The overlap in the plasma glucose concentrations in scalded and ischaemic rats at 20°C includes over half the concentrations in each group, although no rats in the former group were expected to die but 85% in the latter group were. Thus, although all rats were hyperglycaemic, the amount of increase of plasma glucose concentration was not a useful index of severity of injury, although it is in man (Green *et al.*, 1949; Murdoch, 1953).

The rate coefficients were better correlated with plasma glucose concentration than with the severity of the injury. At no glucose concentration were their values after different injuries significantly different from each other (Figs. 2 and 3). Those in the two groups injured at 20°C formed a striking continuum, with almost identical, and highly significant, mean regression coefficients (-0.041 ± 0.006 in the scalded animals, -0.039 ± 0.005 in the ischaemic ones). In rats at 29°C the results were somewhat different: the fall in rate coefficient was not quite significant ($P \sim 0.07$) and the disposal rate rose with plasma glucose concentration ($0.05 > P > 0.01$), unlike in rats at 20°C. It is not clear what caused these differences.

Considering only rats at 20°C, the disposal rate was nearly independent of glucose concentration over a twofold range, whereas the rate coefficients varied inversely with it. The rate coefficients were always much lower than in normal post-absorptive rats at 20°C. [Before comparison the value of $k_v(e)$ in normal rats given in Table 1 should be adjusted to about 0.33/min to allow for differences in V_p and glucose space.] Thus in injured rats the relationship was the opposite to that in normal ones. An increase in plasma glucose was accompanied by a decrease in rate coefficient. As the most severe injury, ischaemia, only decreases the space accessible to glucose by about 16% (Ashby *et al.*, 1965) the decrease in rate coefficient cannot be explained by impairment of circulation and must be caused by a decrease in cell permeability.

Possible control mechanisms. Co-ordination of an increase of glucose concentration with a decrease in

cell permeability implies involvement of the central nervous system. Afferent impulses of various types, including those from pain, volume and pressure receptors, are received centrally and are likely to induce an integrated response to injury, of which the increase in concentration and decrease in permeability are two facets. On this view it is not surprising that two dissimilar injuries should cause similar metabolic changes, as the response is more likely to be to the intensity and type of the stimuli than to where they originate in the body; or that similar amounts of tissue damage should cause different intensities of responses, as the complexity of the integration must leave ample room for variation between individuals. The integrating centre is most likely to be the hypothalamus, which is known to function abnormally after injury. Its capacity for thermoregulation is greatly impaired (Stoner, 1972) and its noradrenaline content is decreased (Stoner & Elson, 1971).

Of the processes mediated by the hypothalamus the following seem likely to contribute.

(1) Adrenaline release. This causes breakdown of muscle glycogen but not, *in vivo* in the rat, of liver glycogen (Sherlock, 1949), and, in man, inhibition of the release of insulin in response to hyperglycaemia (Amatuzio *et al.*, 1954; Porte *et al.*, 1966). The importance of adrenaline release is best seen by considering ischaemic injury. The form of the injury used in the present study takes place in two stages: while the tourniquets are on the hind limbs, and after removal of the tourniquets. As hypovolaemia and major failure of thermoregulation only occur after removal of the tourniquets, the time of removal is taken as the time of injury. Adrenaline is, however, released shortly after the tourniquets are applied (Stoner & Westerholm, 1969), and accounts for the rapid breakdown of muscle glycogen, mostly to lactate, with consequent moderate hyperglycaemia (Stoner, 1958) from the conversion of lactate into glucose by the liver (Cori & Cori, 1928). Although the presence of hyperglycaemia, sometimes with plasma glucose concentrations as high as 3mg/ml, shows that the insulin response is not normal, the rate of utilization of carbohydrate is somewhat increased (Threlfall & Stoner, 1954). The actual decrease in rate found after removal of the tourniquets in spite of the very much greater hyperglycaemia would therefore require a much greater release of adrenaline. There is no evidence for this; and in other species, although the release of insulin by hyperglycaemia is greatly inhibited by injury, the plasma insulin concentrations are usually a little above normal and are never more than slightly decreased (Ross *et al.*, 1966; Allison *et al.*, 1968; Carey *et al.*, 1970; Cryer *et al.*, 1972; Cerchio *et al.*, 1971). It therefore seems likely that the inverse concentration-permeability relationship found cannot be wholly explained by adrenaline release.

2. Autonomic stimulation and glucagon release. In

the dog stimulation of the hepatic nerve causes glycogenolysis in the liver (Edwards & Silver, 1972), and it has been stated that stimulation of the pancreatic nerve causes release of glucagon (Marliss *et al.*, 1972). These processes could account for the conversion into glucose of nearly all the liver glycogen within 2–3 h of ischaemic (Stoner, 1958) or scald (Heath, 1973a) injury. There is other, indirect evidence for glucagon action after injury in the rat (Heath, 1973a).

3. Growth hormone release. Growth hormone antagonizes the effect of insulin on glucose uptake. The effect requires 1–2 h to develop (see Daughaday & Kipnis, 1966, for references), and should, therefore, appear by the start of the present experiments if the injury causes immediate release of the hormone. In man very big increases in growth hormone concentrations in blood have been observed during surgery (Glick *et al.*, 1965; Ross *et al.*, 1966; Salter *et al.*, 1972), up to ten times more than those which cause insulin antagonism in healthy males by 8 h after injection (Abrams *et al.*, 1971). (No attempt was made to study earlier effects.) The existence of resistance to insulin action at a later stage after injury is well-established (Howard, 1955; Allison *et al.*, 1968; Hinton *et al.*, 1971).

In principle the processes described seem able to account for the observed concentration–permeability effects. Whether in fact they do so can only be decided from experiments on the concentrations and effects in the rat of the relevant hormones.

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