act on hyaluronic acid. The concentration of material *in vivo* is unknown, indeed the meaning of the term concentration becomes ill-defined when applied to a system known to be inhomogeneous in phase and distribution of components. Over the experimental range studied, the conclusions are valid independently of the proportions of water to solids. The most concentrated material, approaching the gel state, flowed only with difficulty. The measurements do not show any trend that might indicate that the conclusions are invalid outside the range studied. Whereas prolonged action of hyaluronidase may be expected to lead to quite different conditions (e.g. sample η_R 1.1), the minimal effective action may be presumed to be the most important one clinically.

Nature of fast component and action of trypsin. Fast component is concluded to be a protein of relatively large particle size, which is somehow trapped together with hyaluronic acid complex during ultrafiltration. Thus even after severe action of hyaluronidase (sample η_R 1.1), the sedimentation behaviour of fast component was unaffected, but its boundary disappeared after the action of trypsin.

The sedimentation coefficients of fast component and their variation with concentration, found here, are in agreement with the measurements of Johnston (1955) on human synovial fluids. Johnston suggested from indirect evidence that fast components arose from hyaluronic acid, or could arise due to various interactions of hyaluronic acid. The present findings with hyaluronidase and trypsin do not support this view.

Whatever the constitution of hyaluronic acid complex may be, the trypsin experiments show that the translational hydrodynamic interaction

between hyaluronic acid and buffer does not depend on any material open to attack by trypsin.

SUMMARY

1. The action of hyaluronidase and trypsin on the sedimentation and viscosity behaviour of hyaluronic acid complex was studied.

2. It is concluded that hyaluronidase does not affect the frictional interaction involved in moving buffer relative to hyaluronic acid complex, except after prolonged action.

3. It is suggested that during the use of hyaluronidase as a spreading agent, it facilitates the movement of fluid together with polysaccharide through the tissue spaces, rather than enabling a movement of fluid through polysaccharide.

4. Trypsin did not affect the frictional interaction involved in moving buffer relative to hyaluronic acid complex.

5. Fast component of bovine-synovial-fluid hyaluronic acid complex was found to be degraded by trypsin but not by hyaluronidase.

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The Metabolism of [^{14}C]Glucose, [^{14}C]Fructose and [2- ^{14}C]Pyruvate after Limb Ischaemia in the Rat

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The general response to an injury includes numerous changes in carbohydrate metabolism (see Stoner, Threlfall & Green, 1952; Stoner & Threlfall, 1960). These changes may be important, since in small mammals after severe injury there are rapid falls in the temperature of the liver and brain which cannot, in the early part of the response, be

explained by alterations in tissue blood flow or the availability of oxygen (Stoner, 1954, 1958a). The liver mitochondria from injured rats behave normally *in vitro* (Aldridge & Stoner, 1960). This has led us to seek the cause of the temperature changes in the earlier stages of carbohydrate metabolism with [^{14}C]glucose, [^{14}C]fructose and [2- ^{14}C]pyruvate.

To avoid some difficulties in interpretation a period after the injury (4 hr. bilateral hind-limb ischaemia) has been chosen when the hepatic blood flow is still normal, i.e. 1.5–3.5 hr. after removal of the tourniquets. During this period hyperglycaemia is maximal, metabolism appears to be proceeding at a steady rate and the tissue temperatures, although below normal, are falling only slowly (Stoner, 1958*a*). This is therefore a particularly favourable period in which to study the response to injury and all the experiments were carried out during it, comparison being made with suitable controls.

In the injured rats the concentration of [^{14}C]-glucose in the plasma was found to fall more slowly than in the controls after its intravenous injection. The excretion of $^{14}\text{CO}_2$ was slower in the injured rats after the injection of any of the three labelled compounds. In order to interpret the results quantitatively, we have used the concept of 'pools'. A pool is a mathematical concept: each pool contains one compound only; inside it, the compound can move freely, but it can only move from pool to pool at a finite rate. Glucose, for example, appears to be distributed between three pools in the body of the rat (Baker, Shipley, Clark & Incefy, 1959). The concept is useful only when the pools can be given anatomical boundaries, as in the present paper.

METHODS

Radioactive chemicals. Several samples of uniformly labelled [^{14}C]-glucose (specific activity 60–145 $\mu\text{C}/\text{mg.}$) and [^{14}C]-fructose (specific activity 63–64 $\mu\text{C}/\text{mg.}$), sodium [$2\text{-}^{14}\text{C}$]-pyruvate (specific activity 28 $\mu\text{C}/\text{mg.}$) and $\text{NaH}^{14}\text{CO}_3$ (specific activity 12 $\mu\text{C}/\text{mg.}$) were obtained from The Radiochemical Centre, Amersham. No further assay of the radioactivity of these samples was attempted. They were diluted with 0.9% NaCl to give stock solutions (5 $\mu\text{C}/0.1\text{ ml.}$) which were stored at -20° . The accuracy of the dilution and dispensing of these small amounts was not greater than $\pm 5\%$.

Animal techniques. Male albino rats of the Porton strain (body wt. $208 \pm 24\text{ g. s.d.}$), fed without restriction until the beginning of the experiment on diet 41b (Bruce & Parkes, 1956), were used. The production of hind-limb ischaemia with rubber tourniquets applied during a short period of ether anaesthesia and the administration of a similar period of anaesthesia to the controls were carried out as before (Stoner, 1958*a, b*). The environmental temperature was $18\text{--}22^\circ$.

The period of limb ischaemia was from 9.0 a.m. to 1.0 p.m. The labelled compounds were injected at 2.30 p.m. in both injured and control rats to take account of the diurnal fluctuations in metabolism. The ^{14}C -labelled compound [5 $\mu\text{C}/100\text{ g. body wt.}$, in 0.1 ml. of 0.9% (w/v) NaCl] was injected into a tail vein except with $\text{NaH}^{14}\text{CO}_3$, which was given intraperitoneally.

Three types of experiment were carried out:

(1) Determination of $^{14}\text{CO}_2$ -excretion rate. Immediately after the injection the rat was placed in a desiccator (vol.

4.8 l.) through which a stream of dry, CO_2 -free air was drawn at the rate of about 500 ml./min. and the expired CO_2 collected as described by Gould, Sinex, Rosenberg, Solomon & Hastings (1949). The expired air was usually collected for two 30 min. periods and then for a further hour.

(2) Determination of [^{14}C]-glucose concentration in the plasma after the first 30 min. from injection. After the injection the rats were kept in desiccators as described above, anaesthetized with sodium pentobarbitone (Nembutal, Abbott; 5 mg./100 g. body wt. intraperitoneally), 30, 60 or 120 min. after injection and blood samples were obtained from the thorax. Heparin was used as anti-coagulant. Pyruvate (unlabelled) was also determined in some experiments. Then sodium monoiodoacetate was added to the blood.

(3) Determination of [^{14}C]-glucose concentration in the plasma during the first 30 min. after injection. The rats were anaesthetized with sodium pentobarbitone throughout the experiment. Two blood samples (less than 0.2 ml.) were obtained at intervals from each rat from an exposed femoral vein in a syringe moistened with heparin solution.

Aortic blood samples for CO_2 content were obtained as described previously (Stoner, 1958*a*). The colon temperature was determined with a mercury thermometer inserted through the anus to a depth of 8 cm.

Analytical methods. The total CO_2 output was determined gravimetrically. The Na_2CO_3 collected from the expired air was precipitated, from duplicate portions, as BaCO_3 with BaCl_2 and the BaCO_3 filtered on to sintered-glass disks.

When possible, haematocrit values were obtained as described by Stoner (1958*b*). Blood CO_2 was determined by a modification (Stoner, 1958*a*) of the method of Born, Dawes & Mott (1955). Blood pyruvate was determined by the method of Friedemann & Haugen (1943). Blood glucose was determined in triplicate on Somogyi (1930) extracts by the glucose-oxidase method of Huggett & Nixon (1957), slightly modified in the final stage by the addition of H_2SO_4 to give a final concentration of 0.06 N (Saifer & Gerstenfeld, 1958), the colour being read at 410 m μ . The colour comparisons in both these methods were carried out with a Unicam SP. 500 spectrophotometer.

The specific activity of the plasma glucose was determined on phenylglucosazone prepared from whole blood as described by Feller, Strisower & Chaikoff (1950), since in freshly drawn rat blood the glucose is in the plasma compartment. The small samples from the femoral vein were directly deproteinized with the Somogyi reagents without accurate measurement of the volume of the blood and a single determination of the glucose content of the extract was made as described above. The purified osazone was oxidized by the method of Lindenbaum, Schubert & Armstrong (1948), but the CO_2 was collected in NaOH instead of Ba(OH)_2 . The Na_2CO_3 formed was precipitated by the addition of BaCl_2 and the BaCO_3 filtered on to sintered-glass disks.

The radioactivity of the BaCO_3 samples was determined at infinite thickness with an end-window counter (EHM 2S; G.E.C.) attached to conventional scaling apparatus. The specific activity of the samples was obtained by reference to a methyl methacrylate source of ^{14}C standardized against a sample of phenylglucosazone of known activity.

To test the techniques employed, the elimination of $^{14}\text{CO}_2$ after the intraperitoneal injection of $\text{NaH}^{14}\text{CO}_3$ was determined in control and injured rats. In both, the rate of excretion of $^{14}\text{CO}_2$ declined exponentially, with flow constants of 0.047 and 0.035/min. in the control and injured rats respectively. The control value was the same as that found by Gould *et al.* (1949) under similar conditions.

Mathematical methods and terminology. A terminology similar to that of Bergner (1959) has been adopted to describe the rates of movement of labelled and unlabelled compounds from pool to pool. The mathematical methods are given in the Appendix.

RESULTS

The general effects of hind-limb ischaemia on the rat are described in previous papers (Stoner *et al.* 1952; Threlfall & Stoner, 1954; Stoner & Threlfall, 1954; Threlfall & Stoner, 1957; Stoner, 1958*a, b*; Stoner & Threlfall, 1960). The mortality rate after this injury is about 85% under the present experimental conditions with an average survival time of about 13 hr.

Total output of carbon dioxide. The total CO_2 output between 1.5 and 3.5 hr. after 4 hr. bilateral hind-limb ischaemia was much less than from the controls (Fig. 1), in keeping with the fall in total O_2 consumption under these conditions (Stoner, 1958*a*).

Metabolism of sodium [2- ^{14}C]pyruvate. The cumu-

lative excretion of $^{14}\text{CO}_2$ after the intravenous injection of sodium [2- ^{14}C]pyruvate in the control and injured rats is shown in Table 1. Whereas exponential curves could be fitted to these points within the limits of experimental error, for the reasons given in the Discussion, a more complicated expression was preferred which, incidentally, fitted the experimental points better.

Blood pyruvate. The concentration of blood pyruvate did not alter significantly in the injured rats during the period of observation nor was it significantly different from that in the controls (Table 2). Thus in the injured rats the period of raised blood-pyruvate levels which immediately follows the removal of the tourniquets (Stoner *et al.* 1952) was finished by the beginning of the experiment. The control values were higher than usual (Engel, Winton & Long, 1943), probably because the rats were disturbed by handling.

Metabolism of [^{14}C]glucose and [^{14}C]fructose. The cumulative excretion of $^{14}\text{CO}_2$ after the intravenous injection of [^{14}C]glucose and [^{14}C]fructose in control and injured rats is shown in Fig. 2. The results for both sugars in either control or injured rats were so similar that they could be fitted by the same curve. The excretion of $^{14}\text{CO}_2$ from both [^{14}C]glucose and [^{14}C]fructose was severely depressed after injury. The curves for the controls were markedly sigmoid.

Blood glucose. The concentration of blood glucose in the controls (Table 3) remained fairly constant during the experiment. The values obtained in the rats after limb ischaemia showed that the period of observation was within the phase of maximum hyperglycaemia (Stoner, 1958*b*).

Specific activity of plasma glucose. The observed specific activity of the plasma glucose at different times after the intravenous injection of [^{14}C]glucose in the control and injured rats is shown in Fig. 3. In both there had been a big fall in the specific activity by the time the observations began and thereafter it fell at a progressively slower rate. The gradients for the later parts of the curves (30–120 min.) could be obtained by the method of least squares from the collected logarithmic results on single samples from rats anaesthetized immediately before killing them to obtain the specimen of blood. This method could not be applied to the earlier parts of the curves because of their steepness and the variations between individual rats. This difficulty was overcome by using anaesthetized rats, two blood samples being taken from each rat and the gradient for the interval between them being determined from the ratio of their specific activities. From the results (Table 4) equations were derived, as shown in the Appendix, to express the changes in the specific activity of the plasma glucose in the control and injured rats from 5 min. to 2 hr. after the intravenous injection of $5\ \mu\text{C}$

Fig. 1. Mean cumulative excretion of CO_2 from control rats (—) and rats 1.5 hr. after a 4 hr. period of bilateral hind-limb ischaemia (---). Control rats were given a short period of ether anaesthesia at the time of the application of the tourniquets to the experimental rats. Vertical lines through the points represent S.E.M. Figures in parentheses give the number of rats on which each point is based.

Table 1. Cumulative excretion of ¹⁴CO₂ from sodium [2-¹⁴C]pyruvate

The cumulative excretion of ¹⁴CO₂ after the intravenous injection of sodium [2-¹⁴C]pyruvate (5.0 μC/100 g. body wt.) in control rats and rats 1.5 hr. after a 4 hr. period of bilateral hind-limb ischaemia. The calculated amount of ¹⁴CO₂ excreted in the control and injured rats according to the formula

$$A = \alpha_p A_0 \left[1 - e^{-k_p t} - \frac{k_p}{k_4 - k_p} (e^{-k_p t} - e^{-k_4 t}) \right]$$

is also shown. In this, A₀ = the dose and α_p = the fraction oxidized, in the controls 0.84 and in the experimental rats 0.57. In the controls k_p = 0.16/min. and k₄ = 0.029/min. In the experimental rats k_p = 0.07/min. and k₄ = 0.03/min. Numbers of rats are shown in parentheses.

Time after injection (min.)	Excretion of ¹⁴ CO ₂ (μC/100 g. body wt.)			
	Control rats		Experimental rats	
	Observed (mean ± s.e.)	Calculated (A)	Observed (mean ± s.e.)	Calculated (A)
30	2.058 ± 0.140 (6)	2.058	1.126 ± 0.121 (5)	1.076
60	3.291 ± 0.130 (6)	3.305	1.942 ± 0.208 (5)	2.044
120	4.077 ± 0.148 (6)	4.049	2.807 ± 0.267 (5)	2.699

Table 2. Concentrations of blood pyruvate

Blood-pyruvate concentrations in rats 1.5 and 3.5 hr. after a 4 hr. period of bilateral hind-limb ischaemia (9.0 a.m.-1.0 p.m.) and in control rats bled at the same time. Numbers of rats are shown in parentheses.

Time	Blood pyruvate (mg./100 ml.) (Mean ± s.d.)	
	Control rats	Experimental rats
2.30 p.m.	2.10 ± 0.83 (7)	2.64 ± 0.66 (7)
4.30 p.m.	2.54 ± 0.72 (7)	2.52 ± 0.32 (7)

Fig. 3. log Specific activity of the blood glucose in control rats (●) and in rats 1.5 hr. after a 4 hr. period of bilateral hind-limb ischaemia (○) at intervals after the intravenous injection of 5 μC of [¹⁴C]glucose/100 g. body wt. Points joined by a line represent values obtained in the same rat, and the single points represent values obtained in individual rats. For further details see text and Table 4.

Fig. 2. Mean cumulative excretion of ¹⁴CO₂ from [¹⁴C]-glucose (left) and [¹⁴C]fructose (right) from control rats and rats 1.5 hr. after a 4 hr. period of bilateral hind-limb ischaemia. Control rats were given a short period of ether anaesthesia at the time of the applications of the tourniquets to the experimental rats. In each case the upper curves are from the control rats and the lower are from the injured rats. Vertical lines through the points represent s.e.m. Figures in parentheses give the number of rats on which each point is based. To show the similarity between the excretion of ¹⁴CO₂ from [¹⁴C]glucose and [¹⁴C]fructose the curves fitted to the glucose results have been drawn for the fructose results.

Table 3. Concentrations of blood glucose

Blood-glucose concentrations in the control rats and in rats after a 4 hr. period of bilateral hind-limb ischaemia. The [¹⁴C]glucose was injected in the experimental rats 1.5 hr. after removal of the tourniquets. Numbers of rats are shown in parentheses.

Time (min.) after injection of [¹⁴ C]glucose	Blood glucose (mg./100 ml.) (Mean ± s.d.)	
	Control rats	Experimental rats
30	128 ± 14 (6)	319 ± 58 (6)
60	134 ± 9 (5)	233 ± 25 (4)
120	112 ± 11 (3)	258 ± 105 (4)

Table 4. Rate of fall in the specific activity of plasma glucose in control and injured rats

The average rate constants over different time intervals are given. For the first 30 min. after injection average rate constants were calculated from the specific activities of two samples from the same rat by the formula $2.303 (\log S_2 - \log S_1) / (t_2 - t_1)$, where S_n is the specific activity at t_n . After the first 30 min. the gradient was determined by the method of least squares, logarithmic values obtained from single samples on individual rats being used. The data for these calculations are given in Fig. 3. These results are compared with those calculated from equation (3) and the constants given in the Appendix.

Control rats				Injured rats			
Time after injection (min.)	No. of rats	Average rate constant (min. ⁻¹)		Time after injection (min.)	No. of rats	Average rate constant (min. ⁻¹)	
		Expt.	Calc.			Expt.	Calc.
4-12	2	-0.1002 (mean)	-0.0964	9.4-22.1	1	-0.0350	-0.0336
12-30	1	-0.0525	-0.0553	9.4-22.8	1	-0.0235	-0.0333
30-120	12	-0.0246 (0.0035*)	-0.0252	12-30	1	-0.0340	-0.0302
				30-60	8	-0.0191 (0.0090*)	-0.0181
				60-120	6	-0.0108 (0.0004*)	-0.0108

* 95% fiducial error, calculated according to Snedecor (1946).

of [¹⁴C]glucose/100 g. body wt. These equations are:

Control: Fraction of radioactivity remaining in plasma glucose

$$= 0.80e^{-0.144t} + 0.20e^{-0.024t}.$$

Ischaemic shock: Fraction of radioactivity remaining in plasma glucose

$$= 0.659e^{-0.082t} + 0.341e^{-0.00955t}.$$

These equations have only two components because, unlike those given by Baker *et al.* (1959), they do not include the initial phase of mixing in the plasma which formed the first of their three components. The time constants for our controls are very similar to the corresponding ones obtained by them in post-absorptive rats, i.e. 0.15 and 0.018, showing that the use of sodium pentobarbitone in some of our experiments did not affect the results.

Blood content of carbon dioxide. Two hours after removal of the tourniquets the CO₂ content of the arterial blood was significantly ($P < 0.001$) reduced, being 84.8 ± 9 (s.d.) mg./100 ml. of blood compared with 119.0 ± 6 (s.d.) mg./100 ml. of blood in the controls.

DISCUSSION

To assess the results obtained with [¹⁴C]glucose in the normal rat and to define the changes after physical injury it is necessary to have a model susceptible to mathematical treatment. Such a model can be set up in the usual way by supposing

Fig. 4. Scheme for the proposed system of pools. The radioactivity in a pool is represented by q and the amount of glucose by Q . Movement between the pools is represented by the flow constants, k . Alternative routes for k_2 are indicated by (A) and (B). Addition of unlabelled material to the system is indicated by I .

that the glucose in the body is in two discrete pools, arranged as pools 1 and 2 in Fig. 4. The three-pool system used by Baker *et al.* (1959) is not applicable to our results as our first determinations were made several minutes after the injection. Our pool 1 is

therefore analogous to pool 1 + pool 2 of Baker *et al.* (1959).

The usual assumption of a steady state has been made (i.e. that the pool sizes are constant) and is justified in the controls by the relative constancy of their blood-glucose and blood-pyruvate concentrations and by their steady excretion of carbon dioxide. It is supported by results in parallel experiments (Stoner, 1958*a, b*) showing that in such rats consumption of oxygen and carbohydrate is proceeding at a constant rate.

In Fig. 4 the quantity of unlabelled glucose in pools 1 and 2 is given by Q_1 and Q_2 . The movement of glucose from pool to pool and out of the system can be represented by rate constants. Thus the rate of movement of glucose from pool 1 is $k_3 Q_1$ for its metabolism and $k_1 Q_1$ for its movement to pool 2. These two pools are considered to contain all the free glucose of the body and irreversible loss of glucose from them implies the conversion of the glucose into a metabolite. It has been assumed that pools 1 and 2 are separated by a diffusion barrier for which $k_1 = k_{-1}$. It must be remembered that the glucose pools do not include glycogen or other metabolites but only glucose itself. The total irreversible loss of glucose from the two pools is $k_3 Q_1 + k_2 Q_2$. As the pool sizes remain constant this loss is replaced by a steady flow of unlabelled glucose into pool 1, represented by I_1 , or a similar flow into pool 2, represented by I_2 , or by both.

In expiration experiments of this type whatever ^{14}C -labelled compound is injected, quantitative recovery of ^{14}C as $^{14}\text{CO}_2$ in the expired air is never obtained. When the compound is rapidly oxidized most of the ^{14}C is recovered rapidly but the remainder is excreted only very slowly. The fractions

of the irreversible losses of glucose from pools 1 and 2 which are rapidly oxidized are represented by α_1 and α_2 , so that the amount of glucose lost from pool 1 and rapidly oxidized to carbon dioxide would be $\alpha_1 k_3 Q_1$. The flow constants (k) are expressed per minute, and the pool contents, except for pool 3, are expressed as mg. of glucose/100 g. body wt., so that the rate constants of the glucose pools are in terms of mg. of glucose/min./100 g. body wt. Pool 3 will be shown to be the bicarbonate-carbon dioxide pool and its contents are expressed as mg. of carbon dioxide/100 g. body wt. I_3 represents the flow of carbon dioxide into this pool from sources other than glucose.

Pools 1 and 2 must occupy different anatomical locations, which will be delineated as clearly as possible. Pool 3, that of a different compound, can obviously share the same body space with pools 1 and 2.

At the beginning of the experiment [^{14}C]glucose is injected into pool 1. The flow of labelled glucose can be represented in the same way as the flows of unlabelled glucose with q instead of Q to represent quantities of radioactive glucose expressed as fractions of the injected ^{14}C . Since there is only one injection of [^{14}C]glucose there are no radioactive equivalents of I_1 and I_2 and the level of radioactive glucose falls steadily. The methods of calculating the flow constants and pool sizes are given in the Appendix, to which reference should be made for all mathematical details. The calculated flow constants are given in Table 5.

In the controls, pool 1, into which the [^{14}C]glucose is injected, amounts to 21.7 mg. of glucose/100 g. body wt. This pool must therefore contain the plasma glucose. It will also contain the intra-

Table 5. *Characteristics of the pool systems shown in Fig. 4*

Calculations are described in the Appendix. (A) and (B) refer to the two possible pathways for the glucose leaving pool 2.

	Control rats	Rats with ischaemic shock
Q_1 (mg. of glucose/100 g. body wt.)	21.7	46.0
Q_2 (mg. of glucose/100 g. body wt.)	21.7	41.7
Q_3 (mg. of CO_2 /100 g. body wt.)	198	120
k_1 (min. ⁻¹)	0.0480	0.02487
k_2 (min. ⁻¹)	0	0.00257
k_3 (min. ⁻¹)	0.0600	0.01925
k_4 (min. ⁻¹)	0.0290	0.0325 (A); 0.030 (B)
$k_3 Q_1$ (mg. of glucose/min./100 g. body wt.)	1.30	0.885
Percentage of $k_3 Q_1$ oxidized ($100 \alpha_1$)	94	67.5 (A); 72.0 (B)
$k_2 Q_2$ (mg. of glucose/min./100 g. body wt.)	0	0.107
I_1 (mg. of glucose/min./100 g. body wt.)	1.30	0.99
$k_4 Q_3$ (mg. of CO_2 /min./100 g. body wt.) (from Fig. 1)	55.0	32.5
Percentage of CO_2 derived from glucose	36	40 (B)
$\left(\frac{1.468 \alpha_1 k_3 Q_1}{k_4 Q_3} \times 100 \right)$		
I_3 (mg. of CO_2 /min./100 g. body wt.)	3.4	1.75 (A); 1.91 (B)

cellular liver glucose. This is a new interpretation of this pool for which there is much evidence. Thus the liver cell is freely permeable to glucose (Gey, 1956; Cahill, Ashmore, Earle & Zottu, 1958); $^{14}\text{CO}_2$ is excreted at the same rate from rats given ^{14}C glucose and ^{14}C fructose (Fig. 2), which can only be explained if most of the fructose is rapidly converted into glucose, a process taking place in the liver of the rat (Hers, 1957); and the high rate of excretion of $^{14}\text{CO}_2$ after giving ^{14}C glucose and ^{14}C fructose is explicable only if both rapidly penetrate cells and oxidation of glucose takes place in the same cells as those which convert fructose into glucose. The observations of Shreeve (quoted by Baker, Shreeve, Shipley, Incefy & Miller, 1954) that ^{14}C fructose in the plasma was rapidly converted into ^{14}C glucose has been confirmed by the chromatography of blood extracts after the intravenous injection of ^{14}C fructose (unpublished results).

The plasma and liver glucose together account for about 70% of the glucose in pool 1. The total blood volume of the controls was taken to be about 7 ml./100 g. body wt., their mean haematocrit value was 40% and the mean blood-sugar level was 125 mg./100 ml. Since the glucose of rat blood is extracellular this means that its concentration in the plasma was 208 mg./100 ml. and the total plasma glucose was 8.75 mg./100 g. body wt. Similarly their liver water was about 2.4 ml./100 g. body wt., giving a free-glucose level in the liver of 5.0 mg./100 g. body wt. The extra glucose in pool 1 is that distributed in those parts of the interstitial space which rapidly exchanged with the plasma before the first samples were taken. In a rat with the above characteristics this would amount to 3.8 ml. of interstitial fluid/100 g. body wt.

The inclusion of the liver in pool 1 means that I_1 must be very much greater than I_2 , since in a rat not actively absorbing glucose from the gut the liver is the only significant source of plasma glucose. Accordingly it has been assumed that, during the period of these experiments, $I_2 = 0$. It has thus been possible to calculate the values of Q_2 and I_1 (Table 5).

Since, with the exception of the liver, glucose is an extracellular constituent of the body, pool 2 must represent that part of the interstitial space which the ^{14}C glucose could penetrate only relatively slowly. Assuming that the concentration of glucose in this pool is the same as that in the plasma it would occupy about 10.4 ml. of interstitial fluid/100 g. body wt. Added to the plasma and interstitial fluid in pool 1 this would give a total extracellular space of 18.4 ml./100 g. body wt., which is similar to the values obtained in other ways (Manery, 1954).

The interstitial fluid of the body is distributed between the two pools. Some of the fluid (that in pool 1) must come into equilibrium much more

rapidly than the rest and is probably in the more vascular tissues. In other tissues such as muscle, Cori, Closs & Cori (1933) and Stoner *et al.* (1952) have shown that the glucose concentration in the interstitial fluid did not quickly reflect the changes in the plasma concentration (cf. also the discussion of this point by Coxon & Robinson, 1959). Although it is an oversimplification to consider the extracellular space as a two-pool system (Cotlove, 1954), the fact that an extracellular space has been calculated which is similar to that found by more direct methods is valuable confirmation that the model is basically sound.

The total glucose pool ($Q_1 + Q_2$) is in good agreement with those of Baker *et al.* (1959). Much greater glucose spaces have been obtained in the rat from single-injection experiments (Feller *et al.* 1950), but an inadequate model was used for their calculation.

The value of I_1 (Table 5) is about the same as that found by Baker *et al.* (1959) in rats in the post-absorptive state. As the system is in a steady state, I_1 also represents the total utilization of glucose ($I_1 = k_3 Q_1 + k_2 Q_2$).

From the anatomy of the model, $k_2 Q_2$ will mainly represent the irreversible loss of glucose to muscle. The results of Robinson & Coxon (1957) and Coxon & Robinson (1959) suggest that, in the dog, after a single intravenous injection of ^{14}C glucose its rate of oxidation to $^{14}\text{CO}_2$ by muscle is very slow indeed in comparison with its oxidation by liver, brain and kidney. Thus $k_2 Q_2$ is mainly the rate at which glucose is converted into glycogen in muscle. For reasons given in the Appendix the best value of k_2 appears to be zero, which agrees with the finding that there is no net synthesis of glycogen in the controls at this time (Stoner, 1958b).

The excretion of $^{14}\text{CO}_2$ in the expired air from ^{14}C glucose is very rapid. Since the only metabolic loss of glucose in the controls ($k_3 Q_1$) is from pool 1, the glucose in that pool must be able to reach a site of oxidation very quickly. From the arguments given above most of this glucose must be oxidized by the liver, but some may also be oxidized by the brain and by the 21 g. (approx.)/100 g. body wt. of other tissues (kidney, spleen, gut, etc.) whose cells are bathed in the interstitial fluid in pool 1.

The sigmoid $^{14}\text{CO}_2$ -excretion curves for ^{14}C glucose and ^{14}C fructose can only be explained by delay in a further pool. The rate of $^{14}\text{CO}_2$ excretion is not accounted for by k_3 since, if it were,

$$d[\text{CO}_2]/dt = k_3 q_1$$

and the rate would fall rapidly with time, whereas it increases. A succession of pools in the path of glucose oxidation can be visualized:

If this is a closed system with no introduction of unlabelled materials except into Q_1 then

$$k_3 Q_1 = k_n Q_n = \dots = k_{CO_2} Q_{CO_2}.$$

The rate of excretion of $^{14}CO_2$ will be controlled by the smallest flow constant, which from the above equation must be associated with the largest pool. From the available data only one such pool (pool 3, Fig. 4) can be calculated. Assuming the above system to be a closed one the size of this pool would be equivalent to 42 mg. of glucose/100 g. body wt., which precludes its being composed of pyruvate or other glycolytic or tricarboxylic acid-cycle intermediates, only small amounts of which are present (LePage, 1948; Frohman, Orten & Smith, 1951; Neish, 1953; Stoner & Threlfall, 1960). With Baker *et al.* (1954) it is concluded that this pool consists of bicarbonate-carbon dioxide and is the 'bicarbonate' pool. Consequently the system is not a closed one and the carbon dioxide from glucose will form only part of the total carbon dioxide of this pool.

As pointed out above, only a fraction (α) of the ^{14}C injected in a labelled compound is recovered as $^{14}CO_2$ in the expired air, so that some of the $[^{14}C]$ glucose leaving pool 1 in the direction of pool 3 will never reach it, but will be stored as metabolites from which $^{14}CO_2$ is formed extremely slowly. These metabolites may be any substance from glycogen to compounds formed by carbon dioxide-fixation. The best fit to the experimental data for the excretion was obtained by assuming that α_1 is 0.94 (Table 5), rather more than with $[2-^{14}C]$ pyruvate (0.84). The constant for the flow out of the bicarbonate pool, k_4 , was found to be 0.029/min.

Pool 3, as the bicarbonate pool, must receive bicarbonate from sources other than glucose at a rate represented by I_3 (Fig. 4). The total rate of output from the pool is given by the rate of excretion of carbon dioxide (Fig. 1), which must also, by definition, equal $k_4 Q_3$. The input is given by $I_3 + \alpha_1 k_3 Q_1$. As input and output must be equal to maintain Q_3 constant, Q_3 and I_3 can be found (Table 5). Trial calculations show that rates of flow $k_n Q_n$ and I_x are not likely to be in error by more than 0.1 mg./min./100 g. body wt., and total fractions (α values) oxidized by more than $\pm 5\%$. However, the flow constants, k_n , and the pool sizes, Q_n , could be in error by $\pm 20\%$ and as the errors tend to accumulate on k_4 it may have any value between 0.02 and 0.04/min. Consequently Q_3 may have any value in the range 130–265 mg. of carbon dioxide/100 g. body wt. The lower of these values is about 30% higher than the estimates, including our own, based on the injection of $H^{14}CO_3^-$ ions (Gould *et al.* 1949; Shipley, Baker, Incefy & Clark, 1959). There is, however, an obvious difference between the intracellular production of $^{14}CO_2$, as here, and its injection into the

extracellular space. Complete mixing in the bicarbonate pool is known to be slow (Kornberg, Davies & Wood, 1952), and thus estimates of the size of the bicarbonate pool made during the mixing period after a single dose of $H^{14}CO_3^-$ ions will be too low (Drury, Wick & Almen, 1956). I_3 and $\alpha_1 k_3 Q_1$ are not affected by these errors. The values given in Table 5 show that 36% of the expired carbon dioxide is produced by the oxidation of glucose initially in pool 1.

$^{14}CO_2$ from $[2-^{14}C]$ pyruvate will also pass through pool 3 and its excretion should also be controlled by k_4 . As the intermediate pools are small, if the excretion of $^{14}CO_2$ was strictly exponential its flow constant should equal k_4 . The flow constant found in this way from the data in Table 1 is smaller (0.0216/min.) than k_4 but the simple picture from which it was calculated is essentially unreasonable, as pyruvate must take some time to leave the plasma and reach the site of its metabolism, mainly by the liver. Although the penetration of pyruvate will be rapid it will not be instantaneous. With pyruvate, let q'_1 be the specific activity of the plasma $[2-^{14}C]$ pyruvate and q'_2 the specific activity of the liver pyruvate, then $dq'_1/dt = k_p q'_1$, $dq'_2/dt = k_p q'_1 - k_4 q'_2$ and $d[^{14}CO_2]/dt = k_4 q'_2$, where k_p is the flow constant for the penetration of pyruvate into its site of oxidation. Solution of these equations gives the equation shown in the legend to Table 1. In fitting this equation to the results k_4 was assumed to be the same as in the glucose experiments, as it must be for all excretion of carbon dioxide from carbohydrate sources, and k_p was chosen to give the best fit (Table 1). In the controls $k_p = 0.16/\text{min.}$, so that penetration is very rapid.

The results obtained on injured rats were fitted to the same mathematical model, with the constants shown in Table 5. Although the results in the injured rats were naturally more variable than in the controls, trial calculations showed that the likely errors on the constants are very little more.

In the shocked rats Q_1 and Q_2 were greatly increased (Table 5). In these rats the average haematocrit value was 56%, giving a reduced plasma volume of 2.2 ml./100 g. body wt. However, the total extracellular space is considered to be unchanged, undergoing redistribution as a result of the swelling of the hind limbs (Green & Stoner, 1950; Rosenthal & Millican, 1954). The average blood sugar in these rats was 285 mg./100 ml. (647 mg./100 ml. of plasma), so that the plasma would contain 14.25 mg. of glucose/100 g. body wt. After limb ischaemia the liver water in the shocked rats was about 2.3 ml./100 g. body wt., which would contain 14.9 mg. of glucose/100 g. body wt. These would account for 63% of the glucose in pool 1. The remaining 37% would be in the inter-

stitial fluid, with which rapid exchange was possible, and in the shocked rats this would amount to 2.6 ml. of interstitial fluid/100 g. body wt.

From the previous assumptions Q_2 would correspond to a glucose space of 6.4 ml. of interstitial fluid/100 g. body wt. With the plasma and interstitial fluid of pool 1 this would give an extracellular space of 11.2 ml./100 g. body wt., as against 18.4 ml./100 g. body wt. in the controls. The apparent reduction is thought to be due to the fall in blood flow through the peripheral tissues decreasing the volume of interstitial fluid available for equilibration with the plasma [^{14}C]glucose.

The total glucose utilization (I_1) was reduced after limb ischaemia by 24%. Unlike in the controls, k_2 was probably significant and positive, i.e. glucose was lost irreversibly from pool 2.

This glucose may be either oxidized immediately to carbon dioxide (scheme A) or deposited in the tissues, perhaps as glycogen, and not oxidized to any appreciable extent during the experiment (scheme B). Both cases can be solved (Table 5) and give very similar results. Scheme B is adopted for discussion because oxidation of [^{14}C]glucose to $^{14}\text{CO}_2$ is very slow in muscle. Then $k_2 Q_2$ could represent deposition of glycogen, for, although no glycogen is deposited in the controls during the experiment, in the shocked rats it is deposited in the muscle of the heart and hind limbs. As much as 20 mg. of glycogen/100 g. body wt. can be laid down in the hind-limb muscles of shocked rats during these experiments and it has a higher specific activity than that of the glycogen elsewhere (Stoner, 1958b). The calculated deposition ($120 k_2 Q_2$) would be 12.8 mg./100 g. body wt. Further work is needed to establish the validity of scheme B under these conditions.

In injured rats α_1 is certainly smaller (0.72) and Q_3 is probably smaller (80–160 mg. of carbon dioxide/100 g. body wt.) than in the controls. The first cannot be explained yet. It implies that much glucose is converted into compounds other than carbon dioxide. The low value of Q_3 agrees with the low value found for the arterial-blood carbon dioxide; both may be explained by a fixed-acid acidosis. The fall in the plasma volume and the decreased blood flow through the peripheral tissues reducing the fraction of the total body 'bicarbonate' with which the metabolically formed $^{14}\text{CO}_2$ could come into equilibrium may also have contributed to the reduction in Q_3 . Exit from this pool is controlled by a flow constant, k_4 , of 0.030/min. This constant is not significantly different from that in the controls.

The excretion of $^{14}\text{CO}_2$ after the injection of [$2\text{-}^{14}\text{C}$]pyruvate in the shocked rats was also roughly exponential with time but the constant was even lower than in the controls (0.0149/min.).

The results could, however, be fitted rather more satisfactorily to the equation given in Table 1, which allows for the initial distribution of the pyruvate, with $k_p = 0.07/\text{min.}$ and $k_4 = 0.030/\text{min.}$

Apart from the loss of glucose from pool 2 the main changes after limb ischaemia were: (1) The rate of loss of glucose from pool 1 by metabolism ($k_3 Q_1$) fell from 1.30 to 0.885 mg./min./100 g. body wt., a 32% reduction. (2) The rate of glucose oxidation to carbon dioxide ($\alpha_1 k_3 Q_1$) fell from 1.22 to 0.638 mg./min./100 g. body wt., a 42% reduction. Both differences are highly significant. Errors on each value, as already stated, should not exceed 0.1 mg./min./100 g. body wt. (3) The proportion of pyruvate oxidized fell 33% (differences in α_p).

The temperature coefficients *in vivo* for the reactions involved in the metabolism of glucose are not greater than 2 (Ingle & Nezamis, 1949; Bartlett, Bohr & Inman, 1955). Assuming that $Q_{10} = 2$, these changes can only be explained by lower temperatures in injured rats if, for glucose metabolism, the temperature was 5.5° lower, and, for glucose oxidation, 10° lower. Even at the end of the experiment the temperatures in the liver and colon were only 4–5° lower than in the controls. As the values of $k_3 Q_1$ mainly depend on [^{14}C]glucose plasma concentrations determined early in the experiment when the temperatures were only 2–3° lower, differences in temperature cannot explain these changes. The discrepancy is so great that it strongly suggests that the converse is true and that the temperature drops because the rate of oxidation of glucose falls.

How glucose metabolism is inhibited after injury is not yet known. Inhibition appears to take place at two points: glucose leaves pool 1 more slowly ($k_3 Q_1$ smaller) and what leaves is oxidized less efficiently to carbon dioxide (α_1 smaller). The corresponding α_p for pyruvate is also smaller (Table 1), which suggests that there is a difference in the fate of about one-third of the pyruvate after injury.

The similarity in the $^{14}\text{CO}_2$ -excretion curves for [^{14}C]glucose and [^{14}C]fructose in the shocked rats suggests that most of the latter was rapidly converted into [^{14}C]glucose and treated as such. This idea is supported by the finding that the plasma glucose of the shocked rat becomes labelled after the intravenous injection of [^{14}C]fructose (unpublished results). Wynn (1956) has described a similar depression of fructose metabolism in surgical patients subjected to severe hypothermia (28.5–30.5°). The inability of the shocked rat to form more $^{14}\text{CO}_2$ from [^{14}C]fructose than [^{14}C]glucose differentiates this condition from other disturbances of carbohydrate metabolism such as diabetes mellitus and acidosis (see Hers, 1957).

APPENDIX

Suppose that the glucose in the rat is distributed between two pools, 1 and 2, related as shown in Fig. 4, where:

k_1 is flow constant controlling the rate of transfer of glucose from pool 1 to pool 2. $k_{-1} = k_1$ (obverse of above). If it assumed that transfer is equally ready either way, the correct equality is:

$$k_1 Q_1 = k_{-1} Q_2,$$

but as Q_1 and Q_2 are found to be very similar, little error arises from the use of the simpler identity, which greatly simplifies calculation). k_2 and k_3 are flow constants controlling loss of glucose from the system via pools 2 and 1. All flow constants are in units/min.

q_1 and q_2 are fractions in pools 1 and 2 of the total [^{14}C]glucose injected. At the start of the experiment all the [^{14}C]glucose is in pool 1, so, when $t = 0$, $q_1 = 1$ and $q_2 = 0$.

Q_1 and Q_2 are quantities of endogenous glucose in pools 1 and 2 (in mg./100 g. of rat). These are constant throughout the experiment by definition.

I_1 and I_2 are rates of introduction of unlabelled glucose into pools 1 and 2 (in mg./min./100 g. of rat). This introduction must take place to keep Q_1 and Q_2 constant in spite of losses of $k_3 Q_1$ from pool 1 and of $k_2 Q_2$ from pool 2.

Now

$$dq_1/dt = -(k_1 + k_3) q_1 + k_2 q_2, \quad (1)$$

$$dq_2/dt = -(k_1 + k_2) q_2 + k_1 q_1. \quad (2)$$

By solving, and inserting the condition that $q_1 = 0$ at $t = 0$, the equation is obtained:

$$q_1 = H_1 e^{-g_1 t} + (1 - H_1) e^{-g_2 t}, \quad (3)$$

where

$$g_1 + g_2 = 2k_1 + k_2 + k_3, \quad (4)$$

$$g_1 g_2 = k_1 k_2 + k_2 k_3 + k_3 k_1, \quad (5)$$

$$(g_1 - g_2) H_1 = k_1 + k_3 - g_2. \quad (6)$$

The results for q_1 consist of a series of average rate constants over different time ranges, as explained in the Results section. Those for uninjured rats can be fitted precisely by $H_1 = 0.7967$, $g_1 = 0.1571$ and $g_2 = 0.02384$, but, put into equations 4-6, these values give $k_2 = -0.0027$.

At the start of the experiment [^{14}C]glucose can only reach pool 2 from pool 1, i.e. k_2 cannot be negative at this stage, but as the experiment proceeds it could theoretically reach pool 2 by other routes (recycling), so that $k_2 = -0.0027$ would represent an average flow constant for the whole experiment. This average flow, however, implies that much labelled glucose is outside the system of pools 1 and 2, which is inconsistent with the finding that 94% of the [^{14}C]glucose injected is rapidly converted into CO_2 . We have therefore assumed that $k_2 = 0$, and found the values of H_1 , g_1 and g_2

which gave the best fit (that shown in Table 4). The values of H_1 , etc., are given below. The figures in parentheses represent the likely ranges, as values at these limits about double the sum of the squares of the deviations from the experimental results: $H_1 = 0.80$ (0.77-0.81), $g_1 = 0.144$ (0.142-0.146), $g_2 = 0.024$ (0.0235-0.0250) and $k_2 = 0$ (0-0.0020).

For shocked rats the best fit is given by $H_1 = 0.659$, $g_1 = 0.062$ and $g_2 = 0.00955$. The values of k_1 , k_2 and k_3 calculated from these constants are given in Table 5.

Pool size. Let the calculated q_1 at t be q_t , and the experimental specific activity in pool 1 be a_t . Then at $t = 0$, $a_0 = a_t/q_t$. The values of a_0 obtained are:

t (min.)	Controls		t (min.)	Injured
65	238 (4)	} 230	60	109.6 (3)
125	222 (2)		120	108.6 (3)
				} 109

The numbers of rats are given in parentheses. The amount injected into pool 1 was $5 \mu\text{C}$, which contained Q_1 mg. of glucose/100 g. rat, so $Q_1 = 5000/a_0$. Values of Q_1 are given in Table 5.

As shown in the text, pool 1 includes the liver, so $I_1 \gg I_2$. We then put $I_2 = 0$, whence, since Q_1 and Q_2 do not change with time:

$$dQ_1/dt = 0 = I_1 + k_1 Q_2 - (k_1 + k_3) Q_1, \quad (7)$$

$$dQ_2/dt = 0 = k_1 Q_1 + (k_1 + k_2) Q_2, \quad (8)$$

whence Q_2 and I_1 can be found.

Utilization of glucose.

$$\text{Total utilization} = I_1 = k_3 Q_1 + k_2 Q_2. \quad (9)$$

Expiration of $^{14}\text{CO}_2$ after injection of [^{14}C]glucose. The expiration of $^{14}\text{CO}_2$ is too rapid to be explained by the small constant k_2 , and must therefore be explained in terms of k_3 . But, if $d[\text{CO}_2]/dt = k_3 q_1$, where $[\text{CO}_2]$ is the fraction of the ^{14}C injected expired as $^{14}\text{CO}_2$, the rate must fall rapidly with time, whereas in fact it increases. This can be explained if there is another pool interposed between pool 1 and expiration, and may be linked with the system in two ways, as shown in Fig. 4.

In case A it is assumed that all the glucose which leaves the system is oxidized at the same rate to CO_2 ; and in case B that glucose leaving via pool 2, represented by $k_2 Q_2$, is not oxidized, but is laid down as glycogen.

Case A:

$$[^{14}\text{CO}_2] = 1 - q_1 - q_2 - q_3. \quad (10)$$

From equations already given it can be shown that

$$q_2 = C(e^{-g_1 t} - e^{-g_2 t}), \quad (11)$$

where

$$C = (k_1 + k_3 - g_1) H_1 / k_1 = (k_1 + k_3 - g_2) H_2 / k_1,$$

q_1 is given by (1),

$$dq_3/dt = k_2 q_2 + k_3 q_1 - k_4 q_3. \quad (12)$$

Integrating, as $q_3 = 0$ when $t = 0$,

$$q_3 = A(e^{-k_4 t} - e^{-q_1 t}) + B(e^{-k_4 t} - e^{-q_2 t}), \quad (13)$$

where

$$\left. \begin{aligned} A &= H_1 q_1 (g_2 - k_2) / k_1 (g_1 - k_4), \\ B &= H_2 g_2 (g_1 - k_2) / k_1 (g_2 - k_4). \end{aligned} \right\} \quad (14)$$

The value of k_4 is chosen to give the best fit to the experimental results.

Case B:

$$[^{14}\text{CO}_2] = 1 - q_1 - q_2 - q_3 - Y,$$

where Y represents the fraction lost from the system via pool 2, and so not oxidized. q_1 and q_2 are as before (equations 1 and 11).

$$dq_3/dt = k_3 q_1 - k_4 q_3, \quad (15)$$

whence

$$q_3 = A(e^{-k_4 t} - e^{-q_1 t}) + B(e^{-k_4 t} - e^{-q_2 t}), \quad (16)$$

where

$$A = k_3 H_1 / (g_1 - k_4), \quad \text{and} \quad B = k_3 H_2 / (g_2 - k_4) \quad (17)$$

$$dY/dt = k_2 q_2, \quad (18)$$

whence

$$Y = k_2 C[(1 - e^{-q_2 t})/g_2 - (1 - e^{-q_1 t})/g_1]. \quad (19)$$

For control animals, $k_2 = 0$, so the cases become indistinguishable. The best fit is obtained by taking $k_4 = 0.0265$, giving the fractions of total ^{14}C injected which are expired by different times shown in Table 6. Pool 3, however, appears to be the bicarbonate pool, and it is certain that some metabolites will be reabsorbed as compounds other than bicarbonate, and will not therefore reach pool 3. It is therefore reasonable to suppose that only a fraction, α_1 , reaches this pool. The best fit is obtained with $\alpha_1 = 0.94$, as shown in Table 6.

Table 6. $^{14}\text{CO}_2$ from control animals

The $^{14}\text{CO}_2$ expired is expressed as a fraction of the total $^{14}\text{CO}_2$ injected.

	Time (min.)		
	30	60	120
Expt.	0.256	0.572	0.824
Calc.:			
$k_4 = 0.0265$	0.259	0.569	0.869
$k_4 = 0.0290; \alpha = 0.94$	0.261	0.556	0.832

Table 7. $^{14}\text{CO}_2$ from shocked animals

	Time (min.)		
	30	60	120
Expt.	0.096	0.241	0.412
Calc. A:			
$k_4 = 0.0325, \alpha = 0.675$	0.096	0.232	0.425
Calc. B:			
$k_4 = 0.018, \alpha = 1$	0.091	0.236	0.483
$k_4 = 0.030, \alpha = 0.72$	0.098	0.229	0.411

For shocked animals either case A or case B may be correct. Whichever is correct, α must be low to give a good fit (Table 7).

Size of pool 3.

$dQ_3/dt = 0 = I_3 + \alpha_1 k_2 Q_2 + \alpha_2 k_2 Q_2 - k_4 Q_3$ (case A), or: $I_3 + \alpha_1 k_3 Q_1 - k_4 Q_3$ (case B). In case A it is assumed that $\alpha_1 = \alpha_2$. As low values of α are found for pyruvate oxidation in shocked rats, and pyruvate must be on the metabolic pathway of glucose from both pools, this is plausible.

SUMMARY

1. The excretion of $^{14}\text{CO}_2$ in the expired air after the intravenous injection of [^{14}C]glucose, [^{14}C]fructose and [$2\text{-}^{14}\text{C}$]pyruvate has been studied in normal rats and rats 1.5 hr. after they had been subjected to a 4 hr. period of bilateral hind-limb ischaemia.

2. The disappearance of [^{14}C]glucose from the blood stream after its intravenous injection was also studied in these two groups.

3. A mathematical model was developed to account for the distribution and metabolism of [^{14}C]glucose in the controls. This model comprised two pools for the body glucose and a third pool of bicarbonate-carbon dioxide in the path of its excretion as carbon dioxide.

4. The anatomical location of these pools has been described as far as possible.

5. The application of the model to the results in the shocked rats showed that the total amount of glucose being metabolized was reduced by 32% and that the amount of glucose being oxidized was decreased by 48%.

6. The rate of excretion of $^{14}\text{CO}_2$ from [^{14}C]fructose was the same as from [^{14}C]glucose in both the controls and the shocked rats, so that there was no evidence for the preferential utilization of fructose by the shocked rat.

7. The oxidation of [$2\text{-}^{14}\text{C}$]pyruvate was depressed by 33% in the shocked rats.

8. It was concluded that these changes were not the result of the fall in body temperature after limb ischaemia. It is possible that these changes lead to the fall in heat production.

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The Reaction of the Disulphide Groups of Insulin with Sodium Sulphite

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The reaction of simple disulphides with sulphite has been studied in some detail in recent years. Stricks & Kolthoff (1951) showed that, with cystine and oxidized glutathione, the equilibrium

is dependent on pH, as this affects the relative proportions of RS^- + RSH present. Cecil & McPhee (1955) and McPhee (1956) studied the kinetics of the reaction of sulphite with a number of simple disulphides. They observed that under the conditions used reaction took place with SO_3^{2-} but not with HSO_3^- ions. The net charge on the disulphide molecule was found to have a considerable

effect on the rate of reaction with SO_3^{2-} ion, the positively charged compounds reacting rapidly and the negatively charged ones slowly. All disulphides investigated reacted to completion under suitable conditions.

In the present work, the behaviour of disulphide bonds in insulin has been studied in the light of the earlier investigations. Insulin was chosen as a suitable protein for various reasons. At the time the work was started it was the only protein in which both the arrangement of amino acids and the position of the disulphide bonds were known (Ryle, Sanger, Smith & Kitai, 1955). Fig. 1, showing the structure of the molecule, is reproduced from this paper. It will be seen that there are three disulphide bonds, two joining the A and B chains and

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