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MOVEMENTS OF RADIOACTIVE CARBON DIOXIDE WITHIN THE ANIMAL BODY DURING OXIDATION OF ^{14}C-LABELLED SUBSTANCES

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In the preceding paper (Coxon & Robinson, 1959) we have described a study of the transport in dogs of radioactive carbon dioxide into and out of different regions of the body following a single intravenous injection of radioactive bicarbonate. The present report is concerned with a similar investigation, on a regional basis, of the movements of isotopic carbon dioxide following the administration of radioactive glucose to dogs and monkeys. In the latter case the situation is complicated by comparison with that which arises after the injection of isotopic bicarbonate in that radioactive carbon dioxide is endogenously generated from the glucose over a considerable period of time. As in the simpler case of exogenous labelling of the animal's body-content of carbon dioxide we have measured the arteriovenous differences in specific activity of the blood carbon dioxide in a number of vascular territories at varying intervals of time after a single intravenous injection of ^{14}C-glucose (or other oxidizable substance). The results of these measurements will be discussed in terms of the differing characteristics of local pools of carbon dioxide which they reveal; and the effect of these heterogeneous pools upon the time course of changes in the specific activity of carbon dioxide in the arterial blood and expired air will be considered with particular reference to the validity of model systems employed by other authors in the interpretation of such changes. A brief note on some of the results has already appeared (Robinson & Coxon, 1957).

METHODS

The general arrangements for collecting and analysing samples of blood and expired air were identical with those previously described (Coxon & Robinson, 1959). In the majority of experiments the animals were under pentobarbitone anaesthesia but in one instance thiopentone (which is said to produce less change in blood sugar levels) was substituted without, however, affecting the results.

Uniformly labelled glucose, obtained from the Radiochemical Centre, Amersham, was injected intravenously in tracer quantities. The dose given was approximately 1 μc radioactivity/kg body

weight, contained in 1 mg of sugar. The animals used comprised fifteen mongrel dogs weighing between 10 and 30 kg and one rhesus and one pigtail monkey, weighing 2·5 and 4·5 kg, respectively. (The doses given to the monkeys are indicated with the results of the individual experiments.) Carboxyl-labelled acetate was given to three dogs in a dose of either 1 or 2 μc/kg, approximately, and carboxyl-labelled palmitate was administered to one dog in a dose of 5 μc/kg body weight.

Arterial blood was sampled in all experiments; details of the method of obtaining venous blood from different regions are mentioned with the results. Catheterization of deeply situated veins was carried out under fluoroscopic control. Coincident timing of the arterial and venous sampling was ensured by using coupled syringes.

RESULTS

Arterial blood

The time course of changes in the specific activity of the carbon dioxide (SA_{CO_2}) in arterial blood is illustrated in Fig. 1, where the results from four

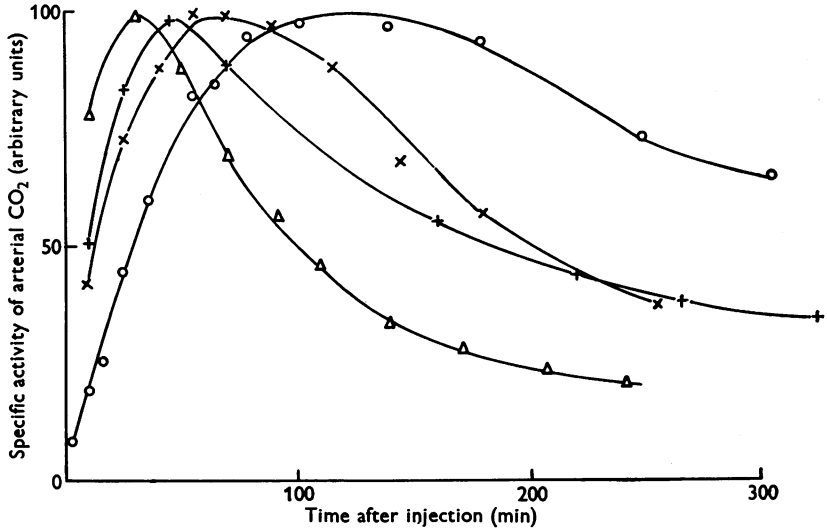

Fig. 1. The changes with time in specific activity of arterial carbon dioxide following the injection of ^{14}C-glucose. Each curve is from a different experiment and the peak specific activity is in each case put equal to 100, the other values being then adjusted by a corresponding factor.

animals are plotted. The general form of this curve was similar in all experiments but there were variations in the detailed contour which made it impossible to combine the results of all experiments in a single curve by simply adjusting the scale, as was done for some earlier experiments when $H^{14}CO_3^-$ had been injected (Coxon & Robinson, 1959). This indicates that, as might be expected, there are more contributory variables when ^{14}C is introduced in the form of glucose. The time of peak specific activity had a mean value of 62 min in the present experiments but there was considerable scatter, as may be seen from the individual graphs.

Expired air. In the seven samples in which it was determined the specific

activity of the carbon dioxide (SA_{CO_2}) in expired air gave a value very close to that of blood. The ratio of the specific activities of arterial and expired CO_2 varied between 0·96 and 1·05 with a mean of 1·00 in the 24 determinations made.

Accurate measurement of rate of loss of the injected ^{14}C as $^{14}CO_2$ was not a primary aim of these experiments, and the periods of collection of expired CO_2 were short. However, it was possible to calculate the $^{14}CO_2$ output in any given period from the mean rate of total CO_2 production and a mean value for expired CO_2 specific activity during the period, obtained by integration of the specific activity curve. In this way it was calculated that the fraction of the injected ^{14}C which was expired in the first hour after injection varied from 5 to 17% of the injected dose, being higher in experiments where the peak specific activity of arterial CO_2 was reached early. Between 24 and 38% of the injected ^{14}C was expired in the first 3 hr after the injection.

Venous blood

Mixed venous blood was sampled from the pulmonary artery on seven occasions during one experiment and the mean ratio of the specific activity of its CO_2 to that in arterial blood was found to be $0·98 \pm 0·03$ in the seven pairs of samples.

Femoral and lumbar venous blood. Femoral venous blood was sampled simultaneously with arterial blood in five experiments. Figure 2 shows the changes in specific activity of the CO_2 with time, in arterial and femoral venous blood, in a typical experiment. It will be seen that the venous CO_2 gave a specific activity–time curve of similar general shape to that in arterial blood, but the venous SA remained below the arterial for the first 2–3 hr after the injection of labelled glucose. If the ratio of venous to arterial specific activity (which we shall call the $V:A$ ratio) be plotted against time, then curves of the type shown in Fig. 3 are obtained. The results of the three experiments on which the femoral curves in Fig. 3 are based show that the ratio begins with a value of about 0·5 and rises slowly to unity over a period of 3 hr or more. Ratios appreciably in excess of unity were not encountered, as had happened in earlier experiments with bicarbonate.

Since the ratio of venous total CO_2 to arterial total CO_2 had a mean value of only 1·15, it is obvious that the low $V:A$ ratio for specific activity found in the early stages of our experiments cannot be attributed simply to the addition of inactive CO_2 to the blood during its passage through the limb.

In one experiment a large vein draining the lumbar muscles (as was found at autopsy) was mistaken on the X-ray screen for the renal vein and was catheterized. Blood from this gave similar $V:A$ ratios to those found in femoral venous blood (see Fig. 3).

Hepatic venous blood. In two experiments arterial and hepatic venous blood was sampled simultaneously after ^{14}C-glucose injection. The hepatic

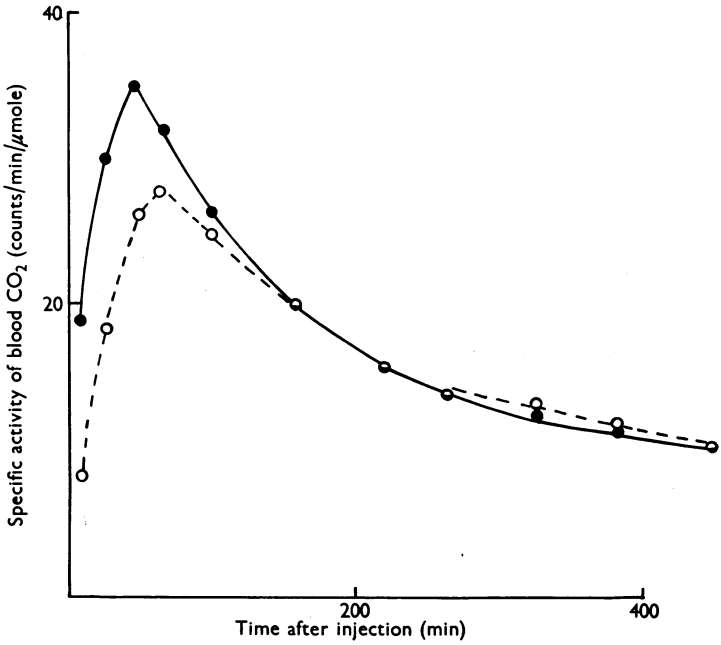

Fig. 2. The changes in specific activity of the CO_2 with time in femoral venous (○) compared with arterial (●) blood in a typical experiment.

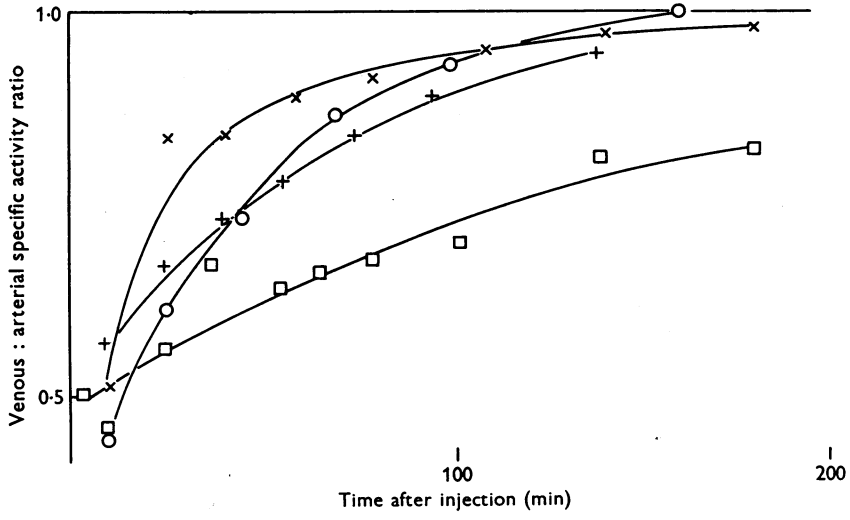

Fig. 3. Changes with time in venous:arterial specific activity ratios for carbon dioxide in the hind limb (+, ○, □) and lumbar muscles (×); each symbol refers to a different experiment.

venous blood CO_2 had a higher specific activity than the arterial for about 150 min after the injection. The full results for one such experiment are given in Table 1 and it will be noted that the $V:A$ specific activity ratio was initially between 1·1 and 1·2. The second experiment gave a more erratic series of values but the general course of events was the same, as is shown in Fig. 4.

TABLE 1. Hepatic arteriovenous differences in specific activity of blood CO_2 after ^{14}C-glucose I.V. injection

Time after injection (min)	Blood CO_2 specific activity: Arterial (counts/min/ μmole)	Blood CO_2 specific activity: Venous (counts/min/ μmole)	$V:A$ specific activity ratio
9·2	6·3	7·4	1·17
24·6	13·0	15·0	1·15
39·5	14·7	17·0	1·16
61·2	14·7	15·8	1·07
80·4	12·5	13·1	1·05
100·4	11·4	12·0	1·05
122·3	9·3	10·0	1·07
147·5	7·6	7·8	1·02
171·1	6·7	6·6	0·98
197·6	5·7	5·6	0·97

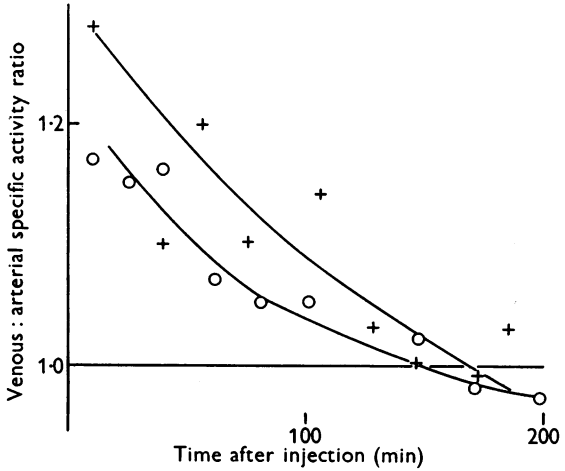

Fig. 4. Changes with time in hepatic venous:arterial specific activity ratio, following I.V. injection of ^{14}C-glucose, in two experiments.

Renal venous blood. Renal venous blood was sampled in two experiments. Rather irregular specific activity curves were obtained. The renal venous CO_2 clearly had a specific activity higher than the arterial CO_2 in the first 30 min after injection, the initial $V:A$ specific activity ratio being about 1·2; but the two specific activities become equal more quickly than in any other region, as may be seen from Table 2, which gives the results of one such experiment. The high blood-perfusion rate of the kidney makes studies of arteriovenous differences more difficult here than in other regions. In both our experiments

the catheter was in the left renal vein, so that some spermatic and ovarian venous blood may have been included in the samples. The para-amino hippurate extraction ratio, however, was of the order of 75%, suggesting that most of the blood had flowed through actively functioning renal tissue.

TABLE 2. Renal arteriovenous differences in specific activity of blood CO_2 after ^{14}C-glucose I.V. injection; results from one of two similar expts.

Time after injection (min)	Blood CO_2 specific activity: Arterial (counts/min/ μmole)	Blood CO_2 specific activity: Venous (counts/min/ μmole)	$V:A$ specific activity ratio
13·0	12·8	14·8	1·16
30·4	18·4	19·0	1·03
45·9	18·2	19·3	1·06
66·5	19·0	19·0	1·00
90·5	18·8	18·8	1·00
121·2	16·5	15·8	0·96
152·5	14·3	14·3	1·00
186·5	13·2	12·7	0·96

TABLE 3. Cerebral arteriovenous differences in specific activity of blood CO_2 after ^{14}C-glucose I.V. injection; results from one of two similar expts.

Time after injection (min)	Blood CO_2 specific activity: Arterial (counts/min/ μmole)	Blood CO_2 specific activity: Venous (counts/min/ μmole)	$V:A$ specific activity ratio
11·3	3·5	8·2	2·34
24·6	6·1	11·1	1·82
39·3	7·0	11·4	1·63
55·9	7·0	11·5	1·64
94·5	4·8	9·1	1·90
112·5	3·9	6·9	1·77
137·3	3·7	6·0	1·62

Cerebral venous blood sampled from superior longitudinal sinus of dog.

Cerebral venous blood. Some indication that blood leaving the brain might contain CO_2 of relatively high specific activity was first provided by the results of experiments which will be mentioned again later, in which external jugular venous blood had been sampled. However, the characteristics of the cerebral venous blood were most clearly shown in a dog experiment in which samples were taken from the superior sagittal sinus by means of a needle inserted through a burr hole previously made in the skull. The results of this experiment are given in full in Table 3.

Two experiments on monkeys, in which cerebral venous blood was sampled from the internal jugular vein, confirmed in a qualitative fashion the results obtained in the dog. In the monkey, as in man, according to Kety & Schmidt (1945), the internal jugular vein contains a higher proportion of blood of cerebral origin than in the dog. Plots of the $V:A$ ratios in the dog and in one of the monkey experiments are shown in Fig. 5.

Simultaneous sampling from more than one region

In one experiment on a monkey, blood samples were taken simultaneously from internal jugular and femoral veins and from an artery. The relationship between the specific activities of CO_2 in these three situations is shown in Fig. 6. The nature of this relationship is entirely consistent with what would be expected from a survey of the combined results of dog experiments in which different regions were studied in different animals.

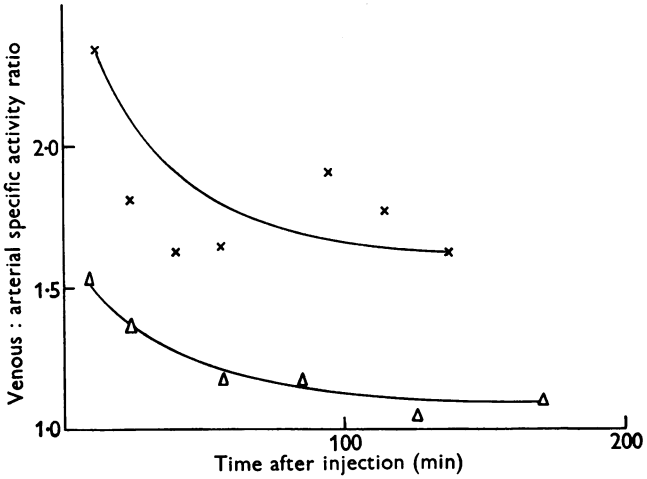

Fig. 5. Changes with time in the venous:arterial ratios of specific activity of carbon dioxide in blood traversing the brain following the intravenous injection of ^{14}C-glucose in a dog (×) and a monkey (△).

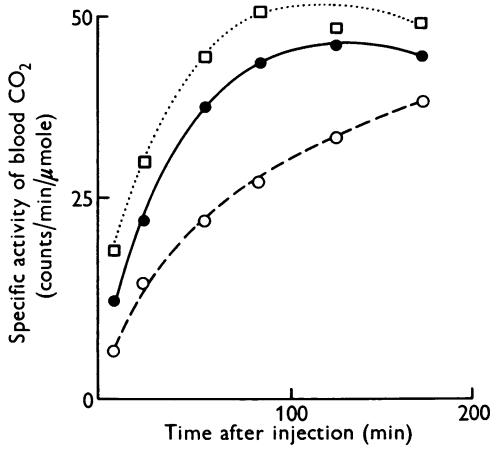

Fig. 6. Changes with time in specific activity of carbon dioxide in arterial (●), internal jugular (□) and femoral venous (○) blood, following the intravenous injection of 20 μc of ^{14}C-glucose into a pigtail monkey weighing 4·5 kg.

The results of studies on blood from the external jugular vein of dogs, such as those set out in Table 4, conform with what might be expected of an effluent which represented a mixture of blood from muscular and supporting tissues (having the same general characteristics as those of the hind limb) with blood from cerebral tissue. This is in agreement with the anatomical distribution of the tributaries of the external jugular vein in the dog.

TABLE 4. Arteriovenous differences in specific activity of blood CO_2 in the external jugular blood of a dog

Time after injection (min)	Blood CO_2 specific activity: Arterial (counts/min/ μmole)	Blood CO_2 specific activity: Venous (counts/min/ μmole)	$V:A$ specific activity ratio
10	82·4	73·2	0·89
30	105	103	0·98
50	92·8	95·4	1·03
70	72·9	81·0	1·11
91	59·4	65·2	1·10
110	48·4	58·0	1·20
136	35·5	42·1	1·19
171	29·5	40·6	1·38
207	24·8	34·2	1·38
242	22·2	28·1	1·27

Experiments using labelled substrates other than glucose

Carboxyl-labelled ^{14}C-acetate gave results which were similar to those with ^{14}C-glucose, but the changes in specific activity after intravenous administration of acetate in three dogs pursued a shorter time course than those resulting from the administration of ^{14}C-glucose. As is illustrated in Fig. 7, the peak specific activity in arterial blood occurred very much earlier than with glucose —at 6 min as against a mean value for glucose of 62 min. Moreover, the cross-over of arterial and femoral venous SA–time curves occurred at about 80 min instead of at 150 min as with glucose. On one occasion the hepatic venous blood was examined after the administration of ^{14}C-acetate. In this the specific activity of the carbon dioxide exceeded that of the arterial blood in the sample taken at 5 min. The $V:A$ ratios of specific activity for the liver effluent are compared with the femoral values (from different dogs) in Fig. 8. This shows the early attainment of a ratio of unity in the case of both femoral blood and hepatic blood.

^{14}C-palmitate. One experiment was undertaken in which sodium palmitate labelled in the carboxyl group was given to a dog, the dose being 4·5 μc/kg. Femoral venous blood was sampled together with arterial in this experiment. The values for the femoral $V:A$ ratio of specific activity are plotted alongside those for acetate in Fig. 8, where it will be seen that the ratio attains unity rather sooner in the case of palmitate. It must be remembered, of course, in this connexion that the ^{14}C in the carboxyl-labelled palmitate molecule would be found in the first 2-carbon fragment to be split off and oxidized.

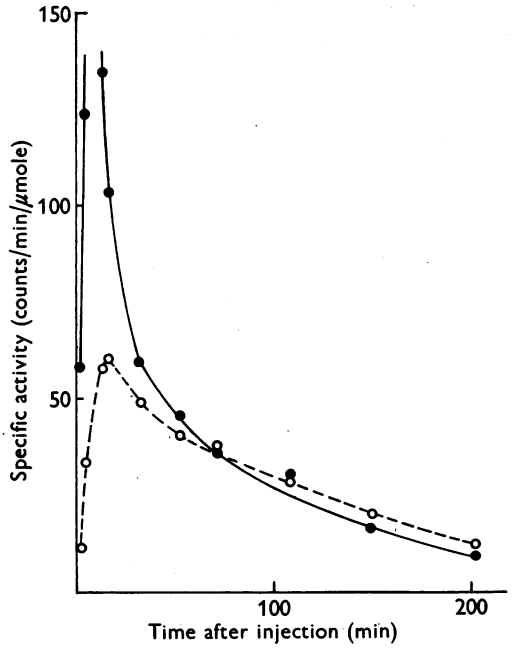

Fig. 7. Changes with time in specific activity of the carbon dioxide in arterial (●) and femoral venous (○) blood following intravenous ^{14}C-acetate in 3 dogs.

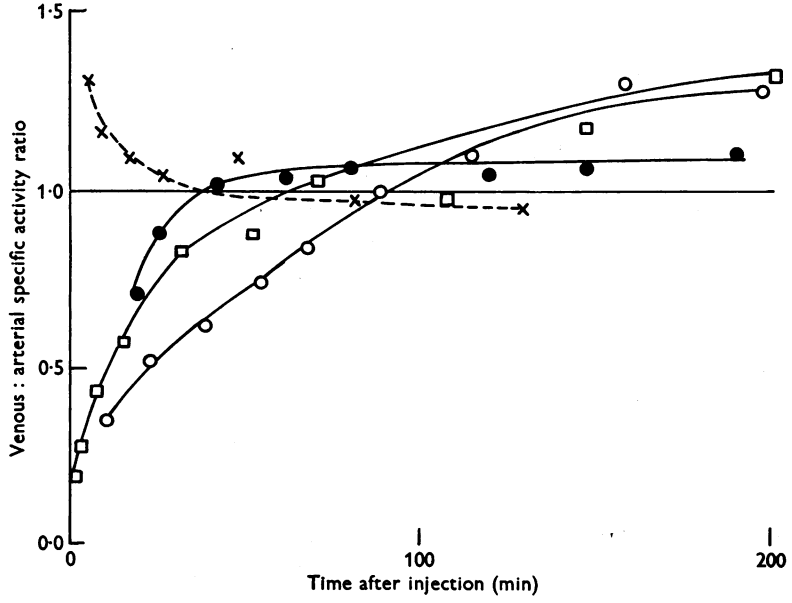

Fig. 8. Changes with time in venous: arterial ratio of specific activity of carbon dioxide in femoral venous (□, ○) and hepatic venous (×) blood following intravenous injection of ^{14}C-acetate, and in femoral venous blood following intravenous injection of ^{14}C-palmitate (●). Each curve is from a different dog.

^{14}C-lactate. Some scattered early observations by one of us (Coxon, unpublished) revealed that in cats the femoral $V:A$ ratio changed with time after the injection of ^{14}C-lactate, in a similar manner to that observed in the present experiments with other substrates in dogs. For example, it was generally below unity until 30 min after the injection and above unity when determined 2 hr after the injection.

DISCUSSION

Changes in total concentration and in isotopic composition undergone by the CO_2 of the blood traversing an organ should give an indication of the direction of net movement of $^{14}CO_2$ into and out of the organ, and the actual quantities of isotope exchanged in this way could be roughly estimated by using such values in combination with estimates of blood flow and applying the Fick principle. However, if the significance of such movements of $^{14}CO_2$ is to be evaluated in relation to the production of $^{14}CO_2$ in the course of oxidative metabolism in the cells of an organ, it is necessary to take into account several factors other than the metabolic rate which enter into the over-all chain of events which affect the clearance from the organ of the CO_2 produced in it.

The factors involved in the transport of $^{14}CO_2$ between tissues and blood after the isotope has been injected in the form of a single rather massive dose of radioactive bicarbonate have been considered in the previous paper (Coxon & Robinson, 1959). The more complicated case where $^{14}CO_2$ is produced within the body from glucose or other oxidizable precursor may be visualized by reference to the scheme shown in Fig. 9.

Clearly, in a system of this kind, the specific activity of the CO_2 (SA_{CO_2}) leaving an organ will be dependent both upon the specific activity of the carbon from which it is derived by oxidation and also upon the total CO_2 pool in the organ with which it must mix before being finally conveyed away in the venous blood. Now it may be inferred both from what is known of the intracorporeal distribution of glucose and from the results of Steele, Wall, de Bodo & Altszuler (1956), that a two-way movement of isotopic glucose into and out of tissues must occur as well as a two-way movement of CO_2, such as was found, for example, in our own earlier experiments with ^{14}C-bicarbonate (Coxon & Robinson, 1959). Thus the rate at which the glucose pool in the tissues mixes with blood glucose will be an important determinant of the specific activity of glucose at its site of oxidation, and the rapidity with which this mixing is effected will depend on the size of the pool. If the rate of mixing were blood-flow-limited, it might depend also upon the blood-perfusion rate of the tissue. Hence a region which holds a large pool of glucose mixing slowly with the blood glucose may in the early stages of an experiment be oxidizing glucose of a lower specific activity than that of the blood glucose, yet later on the specific

activity of the glucose at the oxidation site may exceed that of the blood glucose, since the specific activity of the latter will be falling with time. Such a region would therefore be likely to discharge CO_2 of relatively low specific activity early in an experiment but of relatively high specific activity later in the experiment. On the other hand a region with a small and/or rapidly mixing glucose pool would tend to discharge CO_2 of relatively high specific activity at an early stage.

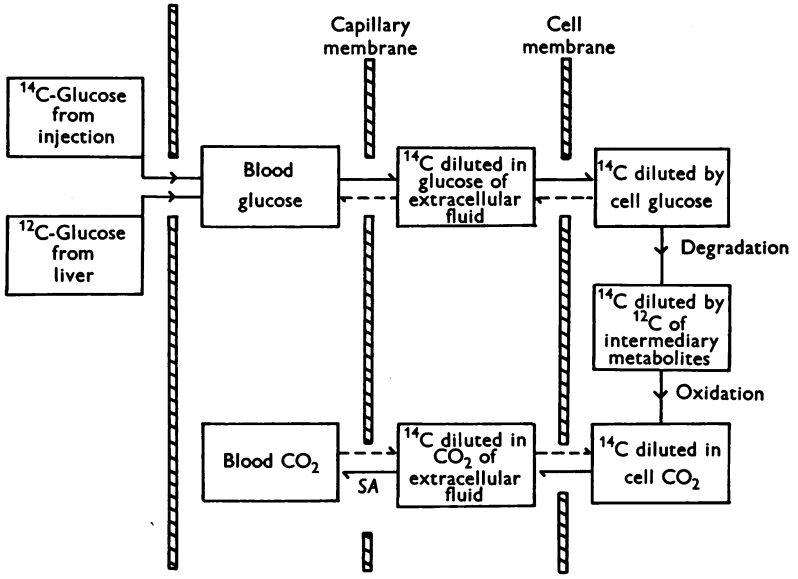

Fig. 9. Diagram illustrating stages in conversion of injected ^{14}C-glucose into exhaled $^{14}CO_2$. *SA* = *SA* of CO_2 of extracellular fluid.

It appears from the findings of Steele *et al.* (1956) that the concentration of free glucose in a tissue may give a fair indication of the size of its glucose pool, since the glucose pool of the body as a whole (defined as the glucose which readily dilutes injected ^{14}C-glucose) seems to consist almost entirely of free glucose dissolved in the blood and extracellular fluid. However, dilution of ^{14}C during its conversion from glucose to CO_2 might also result from mixing with pools of intermediary metabolites if these were present in any quantity, and evidence of such intermediate pools was found, in fact, by Sacks (1957). Omission of the effects of such pools from calculations of the proportion of CO_2 derived from glucose under various conditions may introduce appreciable inaccuracy. Examples of calculations which may be subject to such limitations are to be found in the report by Sacks (1956) concerning the origin of $^{14}CO_2$ in the effluent blood from brain and in the estimate by Baker, Shreeve, Shipley, Incefy & Miller (1954) of the proportion of expired $^{14}CO_2$ derived from

glucose. The results of the latter authors are discussed in greater detail below (p. 506).

We had hoped when we began our experiments to be able to use arteriovenous differences in the specific activity of blood CO_2 in order to estimate the rate of oxidation of particular substrates in the individual regions studied. In fact, however, it has become clear that the influence of pool sizes and rates of mixing in various tissues so dominate the picture that estimates of rates of oxidation from arteriovenous differences in the radioactivity of CO_2 are fraught with great uncertainty. The remainder of this discussion will therefore be devoted to a consideration of the local factors which influence the rate of appearance of radioactive carbon dioxide in the venous blood from different vascular areas during the oxidation of labelled precursors, and an attempt will be made to assess the influence of such local variables upon the rate of appearance of $^{14}CO_2$ in the mixed venous blood, and hence in the expired air.

Significance of arteriovenous differences of CO_2 specific activity in different regions

The lungs. The lungs do not alter the specific activity of blood CO_2 after ^{14}C-glucose injection. This was shown to be so in the bicarbonate experiments, and the situation could be different with other labelled substrates only if they were broken down to CO_2 by the lungs themselves. Since the specific activities of arterial CO_2, mixed venous blood CO_2 and expired CO_2 were found to be closely similar after ^{14}C-glucose injection, it may be concluded that the changes in specific activity of blood CO_2 must occur in the greater circulation.

The brain. The brain raises the specific activity of blood CO_2 for at least 3 hr after ^{14}C-glucose injection in both dog and monkey. Qualitatively this agrees with what is known of cerebral metabolism. Brain appears to be dependent largely, if not entirely, on the blood sugar for its energy source (McIlwain, 1955), although Sacks (1956, 1958) found some evidence of utilization of fumarate and butyrate by human brain and Geiger (1958) also quotes some results suggesting non-glucose sources of substrates for brain metabolism. However, glucose remains by far the most effective antidote to hypoglycaemic coma and in man the brain consumes some 70% of the glucose entering the circulation from the hepatic veins and also some 24% of the oxygen intake of the body as a whole. It is therefore in accordance with expectation that our findings indicate a rapid conversion of blood glucose to CO_2 in the cerebral tissues. They also are in good agreement with the results of Sacks (1957), who made similar determinations of arteriovenous differences in specific activity of CO_2 across the brain following the administration of ^{14}C-glucose to man. The specific-activity-raising effect in the dog's brain was maintained for longer than in any other organ studied. This sustained output of $^{14}CO_2$ would be compatible with a small cerebral free-glucose pool in the presence of a high perfusion rate

and a small bicarbonate pool; it could also be due in part to the oxidation in the later stages of an experiment of intermediates which had become labelled in earlier stages and were turning over relatively slowly. In connexion with the latter possibility it may be noted that J. R. Henderson & R. V. Coxon (unpublished) have found that the glycogen of brain attains a specific activity which greatly exceeds that of glycogen from liver or muscle some hours after an injection of ^{14}C-glucose.

Liver and splanchnic region. The situation here is complicated by the fact that some four-fifths of the blood entering the liver has previously passed through the vascular bed of the spleen or gut. However, if one regards the whole territory of the portal circulation as a single region so far as arteriovenous differences are concerned, its effect on the specific activity of CO_2 is a 'raising' one; and since the outflow from the hepatic veins constitutes about one quarter of the total venous return to the heart, the hepatic effluent will make a contribution to the specific activity of the CO_2 in mixed venous blood almost as great as that of brain. For there, although the arteriovenous difference may be as great as 100% of the arterial value, the total blood flow is about one fifth of that through the liver. The fact that the liver appears to be active in the oxidation of blood glucose in these experiments is of some interest in view of the difficulty of measuring glucose utilization in the liver in intact animals by conventional methods employing the arteriovenous difference of unlabelled sugar (cf. Myers, 1950) owing to the practically unique capacity of the organ to extrude sugar into the blood. The ability of liver cells to oxidize ^{14}C-glucose *in vitro* is of course well known from work with tissue slices (Bloom, Stetten & Stetten, 1953). The contribution of the gut to the $^{14}CO_2$ content of hepatic-vein blood is probably very small in fasting subjects, for Myers (1950) found only a very small glucose uptake by the gut in fasting men and the relatively high rate of utilization of glucose by isolated rat intestine reported by Fisher & Parsons (1953) was found in association with absorptive activity.

The limbs. For the limbs our findings were entirely at variance with those characteristic of the other regions so far discussed; for instead of a positive arteriovenous difference in the *SA* of the blood CO_2, there was for at least 2 hr after injection of labelled glucose a negative difference. This negative difference was considerably greater than could be accounted for by simple dilution of arterial $^{14}CO_2$ by $^{12}CO_2$ derived from oxidation of non-radioactive material in the limb, since the change in specific activity was several times greater than the arteriovenous differences in total CO_2. It could therefore only be due, like the similar negative difference found in experiments where ^{14}C-bicarbonate was injected (Coxon & Robinson, 1959), to dilution in the pool of CO_2 in the limb. Since about 40% of the cardiac output passes through tissues of the same general type as those making up the hind limb and since

in the early stages of an experiment the lowering of specific activity of CO_2 as between samples from the femoral artery and femoral vein is about 50%, it is apparent that under these conditions about 20% (i.e. 50% of 40%) of the $^{14}CO_2$ of the blood would be drained off into such tissues each minute, the blood volume being approximately equal to the minute output of the heart. It also follows from the magnitude of the arteriovenous difference in specific activity across the hind limb—and across similar tissues such as those drained by the external jugular and lumbar veins—and from their total blood flow that some 40% of the blood going to make up the venous return to the right side of the heart will contain CO_2 with a specific activity less than that of arterial blood. In view of the magnitude of this *SA*-lowering effect the question arises as to whether any $^{14}CO_2$ is being produced from ^{14}C-glucose in the tissues of the limb during the time when the effect is evident, since any CO_2 so produced would be radioactive. The fact that the magnitude of the fall in *SA* of CO_2 across the limb in the presence of ^{14}C-glucose is of the same order as when ^{14}C-bicarbonate is injected (Coxon & Robinson, 1959) and no appreciable quantity of ^{14}C-glucose is at hand, may appear to support the suggestion that glucose is not metabolized in the resting limb. However, the conditions in the two cases are not truly comparable, for in the experiments with ^{14}C-glucose, the *SA* of the arterial blood CO_2 was rising owing to the metabolic activity of such organs as the brain, whereas in the experiments where ^{14}C-bicarbonate was injected, it was steadily falling. In experiments where bicarbonate was injected, isotopic equilibrium between the tissue and venous CO_2 was established in about 30 min, as is indicated by the cross-over of the arterial and venous specific activity–time curves; in the experiments where glucose was injected, on the other hand, the curves coincided only after 2–3 hr. As has been implied earlier in this discussion, the arteriovenous difference across a region, in the presence of changing specific activities of arterial CO_2 and of glucose, serves only to compare that region with others contributing to the $^{14}CO_2$ of the blood; and hence, in the case of the limb while the arteriovenous difference is negative, this may mean only that $^{14}CO_2$ production there is being overshadowed by its more rapid production elsewhere. There is other information from studies of arteriovenous differences for non-isotopic glucose (Andres, Cader & Zierler, 1956) which strongly suggests that glucose is not a major fuel of resting muscle in man, but our present results, though consistent with this suggestion, do not provide positive support for it in the dog. In addition to this factor, the sluggishness of mixing in the limb pool of CO_2 may be paralleled by a similar retarded mixing in the glucose pool, which again would lower the *SA* of $^{14}CO_2$ produced from it in the early stages of an experiment as compared with that evolved by oxidation in other organs when blood glucose was more immediately broken down.

Collateral evidence of the size and mixing rates of regional pools of glucose and carbon dioxide

In view of the inferences drawn in preceding paragraphs regarding the extent and the rates of mixing of the glucose and carbon dioxide pools of different organs, it is important to consider what evidence, if any, of an independent kind is available against which to check these inferences. Such evidence is fragmentary, but in Table 5 we have assembled values for the

TABLE 5. Estimation from published values of magnitude of organ pools of glucose and carbon dioxide and of their perfusion rates

Organ ...	Brain	Liver	Kidney	Resting muscle	Bone	Authors
Proportion of cardiac output received (%)	4·9 (dog) 10·3 (monkey)	25·1	25·4	26	1·4	Levy & Blalock (1937)
Weight of organ (g/kg body-weight)	10 (dog) 23 (monkey)	35	6·6	450	170	Skelton (1927); Dhéré & Lapicque (1898); Schmidt, Kety & Pennes (1945); Green (1950)
Blood flow (ml./100 g organ)	54 (monkey)	80	400	6·4	0·67	Schmidt *et al.* (1945); Blalock & Mason (1936); Levy & Blalock (1937); Cargill & Hickam (1949); Himwich & Castle (1927) Green, Lewis, Nickerson & Heller (1944); Andres *et al.* (1956); Edholm, Howarth & McMichael (1945)
Glucose concentration in organ (mg/100 g);	8 (rat)	82	51	22	—	Gey (1956)
Relative blood-perfusion rate (ml./mg glucose in organ)	6·8	0·98	8·4	0·32	—	Robinson (1958)
Contribution to glucose pool of whole body (mg/kg body wt.)	0·8	29	3·3	99	—	Robinson (1958)
Total CO_2 concentration (m-mole/100 g tissue)	1·3 (cat, rabbit)	1·4	1·1	1·1	60	Irving, Ferguson & Plewes (1930); Drury, Wick & Almen (1956)
Relative blood-perfusion rate (ml./m-mole of organ CO_2)	42	57	390	6·4	0·01	Robinson (1958)
Contribution to body pool of CO_2 (m-mole/kg body wt.)	0·1	0·51	0·07	5·0	100	Robinson (1958)

glucose and carbon dioxide content of several of the bodily regions which we have studied. Also, since we have given some prominence to blood flow as a possible limiting factor in the rate of mixing of tissue pools, the table also includes estimates from the literature of the blood flow through various regions. Some of the values in the table are from species other than the dog and are therefore only applicable to the present purpose in a very approximate way,

but they probably give a fair indication of the broad differences between the regions in question.

Bearing these limitations in mind, it becomes apparent from the table that the brain and kidney have distinctly high blood-perfusion rates relative to their contents of glucose and might therefore be expected to be the sites of rapidly mixing glucose pools, as was deduced from the behaviour of the arteriovenous specific activity curves for CO_2. Liver and resting muscle have smaller blood flows in relation to their glucose contents, the ratio being lowest in the case of muscle; the latter value, therefore, is consistent with our findings, which indicated a comparatively slow release of $^{14}CO_2$ from the resting dog's leg in which muscle is a major constituent. In the case of liver there appears to be an inconsistency between the two types of evidence, but this may be connected with the unique capacity of the liver to release glucose into the blood and with its double circulation.

As regards the CO_2 pools of different regions, which have been discussed more exhaustively elsewhere (Coxon & Robinson, 1959), the general picture, based on estimates of CO_2 content and blood flow, conforms with what is inferred from the arteriovenous measurements. The position of the kidney in any classification based on pool size and blood flow is rendered uncertain by its particularly high blood flow (which makes arteriovenous differences harder to measure) and by the non-uniformity which must arise from the existence of different tubular segments containing urine of varying composition. Thus in a general way the information which is to hand on the pool sizes and blood-perfusion rates in the regions in which we have studied $^{14}CO_2$ release is compatible with the suggested interpretation of our findings.

Experiments with substrates other than glucose

When either acetate or palmitate instead of glucose was the source of $^{14}CO_2$, the arteriovenous differences in SA_{CO_2} were qualitatively similar to those found with glucose as the oxidizable precursor. However, the time course of the changes observed was different; in particular the peak in the arterial SA–time curve after the injection of ^{14}C-acetate appeared much sooner than in experiments with ^{14}C-glucose (cf. Fig. 8); and also the femoral $V:A$ ratio of specific activities reached unity considerably sooner than when glucose had been injected—though somewhat later than when, in other experiments, radioactive bicarbonate had been exogenously administered. These relationships would be compatible with the not unreasonable idea that the pool of acetate in the metabolizing tissues was much smaller than the glucose pool. The high initial $V:A$ ratio for the liver when labelled acetate was supplied, which exceeded that found with glucose as the source of labelling, suggests that the liver's pool of acetate is turning over more rapidly than its glucose pool. The fact that the $V:A$ ratio for specific activity reaches and

exceeds unity in the hind limb more rapidly with ^{14}C-palmitate than with ^{14}C-acetate (Fig. 8) may possibly suggest some relation to Gordon's (1957) report on the extraction from peripheral blood of unesterified fatty acid, which may therefore be a favoured fuel for resting muscle. However, it should again be emphasized that the effects of local pools of precursor and product are such that the arteriovenous difference in specific activity will only provide an estimate of the rate of production of $^{14}CO_2$ in the region studied as compared with the rates of production in other regions, since these must determine the arterial concentration of $^{14}CO_2$ at any given time. The manner in which the various regions exert their influence on the time course of the specific activity of arterial CO_2 will be examined in more detail in the next section.

Effect of different regions on the time course of changes in specific activity (SA) of arterial carbon dioxide

The information discussed in the foregoing paragraphs provides a possible foundation for working out an empirical reconstruction of the arterial SA_{CO_2}–time curve by summing algebraically the *SA*-raising and *SA*-lowering effects of different regions. We did not actually measure blood flow, but the agreement between our measurements of cardiac output and those in the literature suggests that, as a first approximation, published figures may justifiably be used to compute the fractions of the cardiac output traversing the various regions, and thus their relative contributions to the total venous return. By combining such published figures for blood flow with our own figures for arteriovenous differences in SA_{CO_2}, it is possible to calculate, for the times at which our measurements were made, the total raising or lowering effect of each region studied upon the *SA* of the CO_2 in the mixed venous blood reaching the heart. This blood has only to pass through the lungs (where no detectable change in *SA* occurs) to become arterial blood, and hence changes with time in mixed venous *SA* will be reflected in rapidly succeeding changes in arterial SA_{CO_2}. Estimates of the raising and lowering effects of the organs studied by us are shown in the last line of Table 6. Now, since measurements of arteriovenous differences in specific activity represent for practical purposes instantaneous changes in specific activity of CO_2 in the blood circulating through the organs sampled, the algebraic sum of such changes should provide a measure of instantaneous change in mixed venous and hence in arterial SA_{CO_2}. These instantaneous changes are an index of the rate of change at that instant. Formally expressed this relationship may be written:

$$\alpha\left(\frac{\mathrm{d}Sav_1}{\mathrm{d}t}\right)+\beta\left(\frac{\mathrm{d}Sav_2}{\mathrm{d}t}\right)+\gamma\left(\frac{\mathrm{d}Sav_n}{\mathrm{d}t}\right)=\frac{\mathrm{d}Sa_{\mathrm{Art}}}{\mathrm{d}t}, \qquad (1)$$

where $\mathrm{d}Sav_1$, $\mathrm{d}Sav_2$, etc. are arteriovenous differences in SA_{CO_2} in regions

1, 2..., n, and α, β, ...γ are coefficients allowing for the fraction of the total venous return contributed by these regions.

In Fig. 10 arteriovenous differences in SA_{CO_2} in different organs are plotted against time. In Fig. 11 the algebraic sum of these arteriovenous differences is plotted against time, and, from the arguments advanced above, this curve

TABLE 6. Effects of positive and negative arteriovenous differences in specific activity of blood carbon dioxide (SA_{CO_2}) in various organs on SA_{CO_2} of mixed venous blood returning to heart

Arteriovenous increment or decrement in SA_{CO_2}	Time (min) 10	25	40	60	80	100
In liver	+1·2	+2·0	+2·3	+1·0	+0·6	+0·5
In kidney	+1·7	+1·4	+0·5	0	0	0
In brain	+2·0	+2·0	+2·0	+1·9	+1·7	+1·5
In limbs	−2·5	−3·5	−4·4	−3·8	−1·7	—
Over-all change in SA_{CO_2} during one circulation	+2·4	+1·9	+0·4	−0·9	+0·6	—

The arteriovenous increments and decrements tabulated are derived from the measured values in a series of experiments chosen so that the peak in the arterial specific activity–time curve lay near 50 min. The observed arteriovenous differences were multiplied by a factor such that the peak arterial SA_{CO_2} would correspond to 15 counts/min/μmole, and in the case of the brain were divided by 5 since the blood flow through the brain is one fifth of that through the other organs listed.

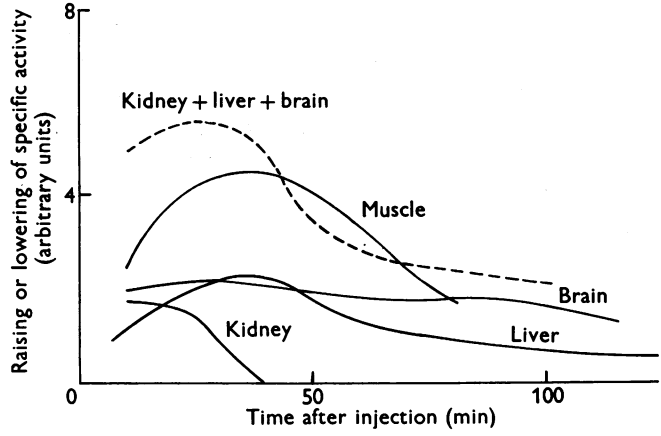

Fig. 10. Calculated effects of venous outflow from brain, liver and kidney in raising, and of resting muscles in lowering, the specific activity of carbon dioxide in mixed venous blood.

should give a measure of the slope of the arterial SA_{CO_2} plotted against time. It will be seen that this latter curve must pass through a maximum and a minimum at the points in time where the horizontal axis is cut in Fig. 11. Once the changes in slope with time are established, a theoretical arterial SA_{CO_2}–time curve can be constructed, and, by choosing a suitable scale, can be superimposed upon an observed curve. This has been done in Fig. 12, and on comparing the experimental with the calculated graph it will be apparent

that there is very good correspondence up to 40 min, which is shortly before the SA reaches its peak at $t_{max.}$; beyond this point agreement is less good. Since the predicted curve was based, owing to scarcity of values, on somewhat imprecise calculations, some doubt must remain as to whether the relatively slight deviations between it and the experimental curve are real. If, without prejudging this question, a cause of the deviation is sought within our admittedly incomplete data, it will be found that the principal reason for the

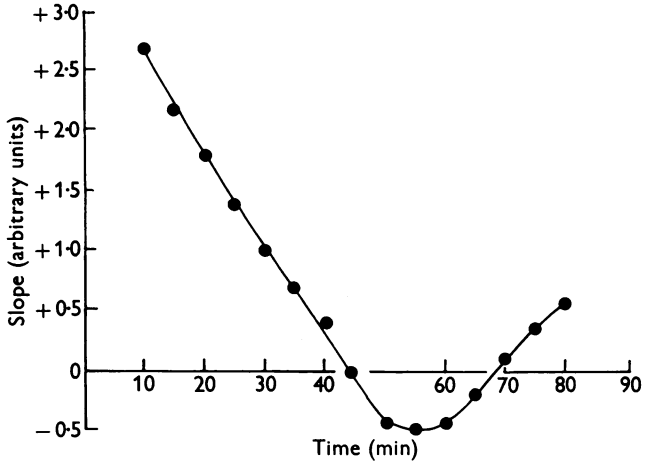

Fig. 11. Change of slope with time of arterial SA_{CO_2}–time curve predicted from results in Table 6.

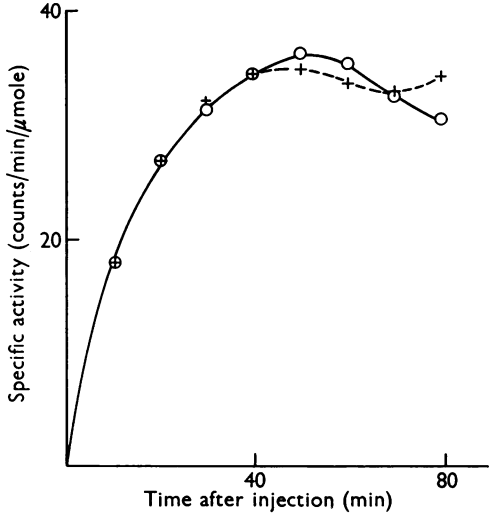

Fig. 12. Comparison of shape of an experimental curve (○) of arterial SA_{CO_2} against time with that of a curve predicted from Fig. 11 (+). The two curves have been drawn to coincide both as regards slope and height at the 10-min point; the theoretical curve at the other points shown has been assigned a slope according to the relationship with time depicted in Fig. 11.

secondary rise in the predicted curve between 70 and 80 min lies in the continued discharge of CO_2 of relatively high SA from the brain. This is evident from inspection of Fig. 10, but it must be emphasized that the brain curve in that figure was based on only a single experiment. This was the only experiment of the kind performed on a dog, but our other experiments in which venous blood from the brains of monkeys was sampled, and also the results of Sacks (1957) on the human brain, tend to confirm in a qualitative way the accuracy of the brain curve shown. This being so, the diluting effect of blood of lower SA_{CO_2} from other sources must be greater in the real situation than in the theoretical one which we have envisaged in our calculations. Hind-limb results may well prove insufficiently representative of the 'diluting' organs and it should also be noted that the outflow from the heart, which has a high metabolic rate, was not studied in our experiments; and it may be that the effluent from the coronary circulation exerts a marked diluting effect during the second hour.

Interpretation of arterial specific activity–time curves

Our reconstruction of the arterial SA_{CO_2}–time curve is entirely empirical and in this respect our approach differs from that of some other authors who have endeavoured to relate the observed curves to the predicted behaviour of

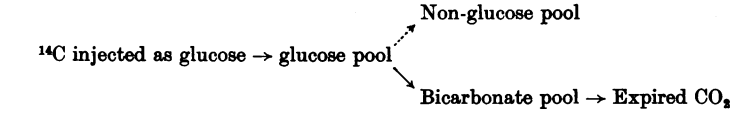

Fig. 13. Simplified scheme after Baker *et al.* (1954) showing the metabolic path of ^{14}C injected as glucose and appearing in expired air as $^{14}CO_2$. ——— and ····· represent events in the real animal. Only events represented by ——— are assumed to occur in deriving a theoretical '100% CO_2 from glucose' curve for CO_2 production.

model systems by making various assumptions with regard to the behaviour of these systems. An example of such an approach to the interpretation of the curve is that adopted by Baker *et al.* (1954). This employs a hypothetical two-pool model, the characteristics of which are summarized in Fig. 13. It is assumed that the sizes of the glucose and bicarbonate pools are constant and that the rate of entry of glucose from the liver and the rate of discharge of CO_2 from the lungs also remain steady, and that all the expired CO_2 is derived from glucose. Then, if λ_1 is the rate of decay of specific activity in the glucose pool and λ_2 is the rate of decay of specific activity in the bicarbonate pool, it can be shown that the specific activity of the expired CO_2 (b) at time t is given by:

$$b = a_0 \frac{\lambda_2}{\lambda_2 - \lambda_1} \quad (e^{-\lambda_1 t} - e^{-\lambda_2 t}), \tag{2}$$

where a_0 is the initial specific activity of the body's glucose.

This expression is valid so long as the injected ^{14}C-glucose mixes rapidly with the body's pool of ^{12}C-glucose; it further requires that there be a relatively rapid and irreversible transformation of glucose to carbon dioxide without serious hold-up in intermediate pools, and that the CO_2 pool itself be effectively homogeneous. It is also clear that algebraically the equation is only meaningful so long as λ_1 and λ_2 are not equal to each other, since, if they were, the denominator of the right-hand side would become zero.

Using such a model, Baker *et al.* calculate the rate of change of the *SA* of expired CO_2 and call this their '100% CO_2 curve', recognizing that it will differ from the real curve obtainable from a resting animal in which the R.Q. will be less than unity and much of the expired carbon dioxide will in reality be derived from sources other than glucose. The '100% CO_2 curve' so calculated turns out in fact to be of the same shape as the observed curves from four human subjects who were studied, but is displaced upwards on the *SA* axis. This displacement is ascribed mainly to the production of CO_2 from precursors other than glucose and the correspondence in shape is taken as evidence of the general applicability of the theoretical two-pool model to the living subject; since, as Baker *et al.* point out, the interposition of other intermediate pools would displace the curve on the time axis and change its contour. In further support of the validity of their model, Baker *et al.* calculate a value for λ_2 from equation (2) by solving the equation for λ_2 at the time of maximal *SA* ($t_{max.}$) taken from their experimental curve. The calculated value for λ_2 is then compared with a value for λ_2 directly determined on other experimental subjects, obtained by labelling the bicarbonate pool by means of an injection of $NaH^{14}CO_3$. The agreement is claimed to be reasonably good, but, in fact, in one of their four experiments the calculated value for λ_1 was nearer to the observed value of λ_2 than was the calculated value of λ_2. Secondly, since in three of their four experiments the calculated value of λ_2 was greater than λ_1, while in the other experiment λ_2 turned out to be less than λ_1, it may be questioned whether the non-equality of these constants, as required in the application of equation (2), could be safely presumed. Furthermore, the number of points available to Baker *et al.* for plotting that part of their 100% curve which preceded $t_{max.}$ was very small, and, since $t_{max.}$ was not represented by a really sharp inflexion in their published curves, the exactness of correspondence in contour with the observed '100% CO_2' curves is difficult to establish unequivocally. It must be granted, however, that the general impression of good correspondence is borne out by their graphs. Apart from these possible inherent weaknesses in the validation of the two-pool model by Baker *et al.*, our present results do show clearly that at the time of the peak in arterial SA_{CO_2} the bicarbonate pool in our dogs was not homogeneous.

We have made no measurements of the radioactivity of blood glucose but it is evident from the results of Steele *et al.* (1956) that mixing in the glucose

pool of a dog was incomplete until about 60 min from the time of a single intravenous injection of ^{14}C-glucose. This is a little after $t_{max.}$ on the arterial *SA*–time curve in many of our experiments. Now Baker *et al.* have made calculations of the proportion of expired CO_2 coming from the oxidation of glucose by assuming that the fraction of expired CO_2 derived from glucose will have at $t_{max.}$ the same *SA* as that of the body's glucose pool at that time, the glucose pool being sampled from plasma glucose. This, in view of the probably incomplete mixing of the glucose pool and the certainly incomplete mixing of the CO_2 pool at this time, is clearly not strictly accurate. For example, CO_2 derived from oxidation of ^{14}C-glucose in the brain at $t_{max.}$ will still be undergoing dilution in the CO_2 pools of the limbs, as is illustrated in Fig. 10.

Our observations point to the desirability of applying theoretical arguments in terms of pools and turnover rates to regions of the body rather than to the whole body. Ideally the regions studied would be narrowed down to the point where they comprised morphologically and metabolically homogeneous cell populations with a uniform and constant vascular supply, but in practice the study of quite crudely demarcated subdivisions of the body may be expected to give a much truer picture of the course of events than is attainable by treating the whole body as a single catenary system. This has been appreciated by a number of authors, as is evident from Robertson's (1957) review of models employed in the interpretation of isotopic data from intact animals. However, comparatively few measurements have been made on subcompartments of the animal body, and the present results appear to suggest that such studies may go a considerable way towards bridging gaps between physiological events in the real animal and their counterparts in mathematically idealized model systems.

It should be noted in conclusion that, when experiments are designed with the primary aim of determining the rate of oxidation of a labelled substrate in an intact animal, the complexities of the problem can be considerably reduced by the employment of a continuous infusion instead of an instantaneous injection of the isotopic metabolite, so that its specific activity in the arterial blood may be kept reasonably constant. This technique has been successfully used by Steele *et al.* (1956) and by Searle, Strisower & Chaikoff (1956).

SUMMARY

1. Measurements of the specific activity of carbon dioxide in arterial blood and in the venous blood from a number of organs have been made after the injection of ^{14}C-glucose, ^{14}C-acetate and ^{14}C-palmitate into dogs and monkeys.

2. Organs can be classified into those which, during the hour following the injection, either raise or lower the specific activity of the carbon dioxide in blood passing through them.

3. Arteriovenous differences across brain, liver and kidney showed a raising of specific activity, resting muscles a lowering.

4. On the basis of the different behaviour of different organs in respect of $^{14}CO_2$ production from ^{14}C-glucose an empirical explanation is offered of the time course of the changes in specific activity of expired CO_2 during the oxidation of ^{14}C-glucose.

5. The application of these findings to model systems used in the interpretation of data from animal experiments employing isotopic tracers is discussed.

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