2. The esters were detected on the paper by an adaptation of the hydroxylamine-ferric chloride test for carboxylic esters, by the iodine method of Brante (1949), by the phosphotungstic acid-stannous chloride method of Chargaff *et al.* (1948) and, in the case of acetylcholine, by pharmacological assay.

We are grateful to the Medical Research Council for a grant (V.P.W.) and the University of Ceylon for a travelling fellowship (S.W.).

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The Metabolism of ^{14}C-Labelled Bicarbonate in the Cat

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(*Received* 12 *September* 1951)

Studies on the metabolism of ^{14}C-labelled urea in mice (Leifer, Roth & Hempelmann, 1948; Skipper, Bennett *et al.* 1951) and cats (Kornberg, Davies & Wood, 1951*b*) have shown conclusively that urea is broken down in the mammalian body. Its rate of breakdown in these experiments has been measured from the rate of expiration of the ^{14}C-labelled carbon dioxide produced. In order to interpret the results of such studies on cats, it was necessary to have information on the rate of excretion from the blood of ^{14}C-labelled carbon dioxide, and on the rate of incorporation of ^{14}C into urea. The results of earlier studies on mice and rats injected with ^{14}C-labelled bicarbonate (Armstrong & Schubert, 1949; Armstrong & Zbarsky, 1949; Gould, Sinex, Rosenberg, Solomon & Hastings, 1949; Greenberg & Winnick, 1949; Schubert & Armstrong, 1949; Skipper, White & Bryan, 1949; Skipper, Nolan & Simpson, 1951; Skipper, Bennett *et al.* 1951) did not supply the required data, because of the great differences in body size between these animals and cats, and because the isotope in these experiments had been administered intraperitoneally.

Experiments are described in which ^{14}C-labelled sodium bicarbonate was injected intravenously into anaesthetized cats and the expired carbon dioxide continuously collected in a special respiration circuit. By this technique, information was obtained on the rate of expiration of labelled carbon dioxide from ^{14}C-labelled bicarbonate, and on the existence of exchange mechanisms between blood and tissue carbon dioxide in the animal. The results of these studies are very similar to those obtained independently in human subjects (Hellman, 1951). From determinations of blood urea and bicarbonate it was also possible to measure the rate of incorporation of ^{14}C into urea synthesized during the experiment, and to confirm that the urea carbon is derived from carbon dioxide.

Part of this work has been communicated to the Biochemical Society (Kornberg, Davies & Wood, 1951*a*).

EXPERIMENTAL

Treatment of cats. A weighed cat was anaesthetized with ether followed by chloralose (75 mg./kg. body weight) and was placed on an operating table covered by a large stainless steel tray to avoid contamination of the laboratory. Cannulae were inserted into the trachea for collection of expired CO_2, into the right external jugular vein for administration of intravenous injections, and into the right femoral artery for collection of blood samples during the course of the experiment. Both ureters were tied off. The animal was then connected through its tracheal cannula to the respiration circuit schematically represented in Fig. 1.

Respiration circuit. This system is so constructed that atmospheric pressure is maintained at the tracheal cannula, so that there is no resistance to free respiration. This is achieved by the two aquarium pumps P_1 blowing, and P_2 drawing air through the system at approximately 400 ml./min. Constancy of the pressure inside the circuit is controlled by three leaks L and a rubber balloon B which acts as a gas reservoir, these being placed at points which do not at any time come into contact with $^{14}CO_2$. Two soda-lime towers S remove atmospheric CO_2 from the incoming air, which can

be freely inspired by the cat through a valve inserted in the tracheal cannula. The expired CO_2 is absorbed in one or other of the two absorption trains A_1 or A_2, only one of which is in circuit at any one time. They are changed by means of the two three-way taps T_1 and T_2, and replaced by fresh absorption trains at frequent known intervals. The air, now again CO_2-free, is passed down a water pump to avoid the possibility of accidentally contaminating the laboratory.

evolved was quantitatively absorbed in a slight excess of 0·3 N-NaOH, in the apparatus described below. The liquid was adjusted to pH 9·3 by careful addition of 0·05 N-HCl, using thymolphthalein as internal indicator. This gave a solution which was sufficiently alkaline to retain its $^{14}CO_2$ content, but which could be safely injected into the cat. Samples (1 ml.) of solutions prepared as above, containing 0·3–0·6 mg. carbon, and corresponding to 510000, 700000

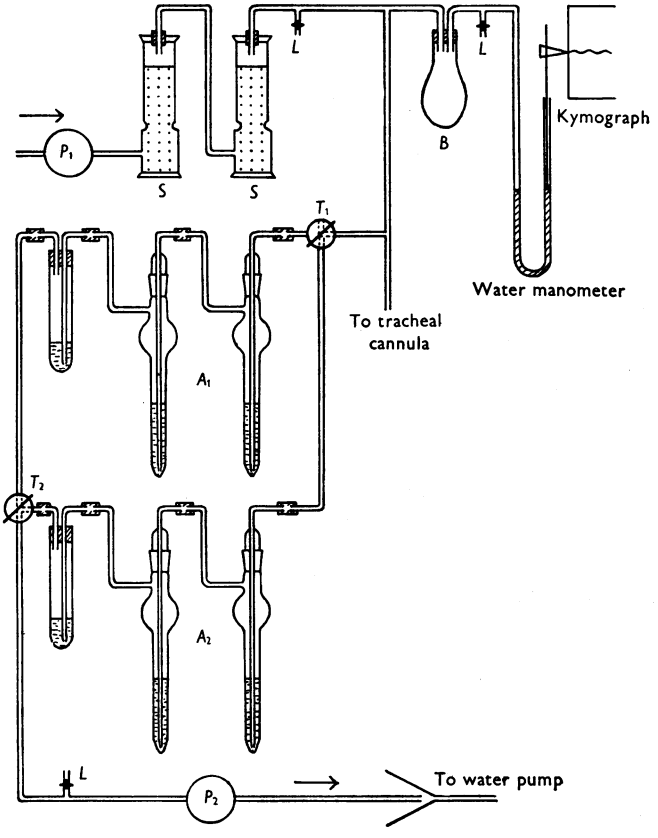

Fig. 1.

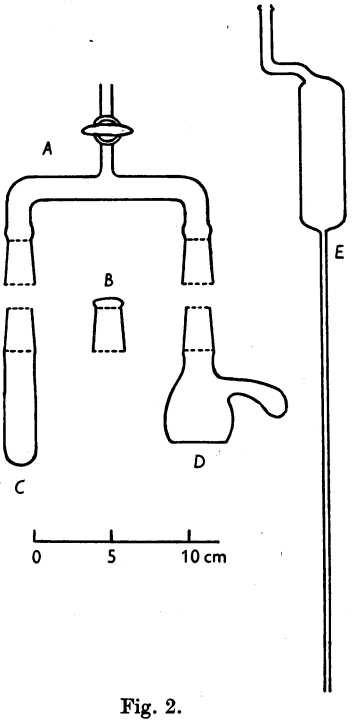

Fig. 2.

Fig. 1. Respiration circuit for absorption of expired $^{14}CO_2$. P_1, P_2, aquarium pumps; L, leaks with screw-clips; B, rubber balloon acting as gas reservoir; S, soda-lime towers; T_1, T_2, three-way taps; A_1, A_2, absorption trains (only A_2 is in circuit).

Fig. 2. A, vacuum transfer unit; B, cap for small tubes; C, small tube for $Ba(OH)_2$; D, side-bulb cup; E, long pipette fitted with rubber bulb, for removal of liquid from absorption units.

The absorption trains, A_1 and A_2, consist of two all-glass units which are connected in series, and each of which contains 10 ml. approx. 6 N-NaOH and 3–4 drops 0·75% (w/v) egg albumin. This latter substance acts as a frothing agent and, by forming a layer of alkaline bubbles in the units, ensures complete absorption of the CO_2. The efficiency of absorption is checked by a third tube containing 5 ml. saturated $Ba(OH)_2$, which solution should, and in fact does, remain unclouded throughout the period of absorption.

Preparation and injection of labelled materials. $NaH^{14}CO_3$ solutions were prepared from $Ba^{14}CO_3$. Samples (7–15 mg.) of $Ba^{14}CO_3$ (equivalent to 50–100 μc.) were acidified with lactic acid (British Pharmacopoeia) *in vacuo*, and the $^{14}CO_2$ and 1 020000 counts/min. respectively under our conditions of radio-assay (see later), were injected quantitatively in the three experiments on HCO_3^- metabolism to be described.

Collection and analysis of samples

Carbon dioxide. When the ^{14}C-labelled material had been injected, the expired CO_2 was continuously collected over the next 4–5 hr., any one absorption train being used for periods varying from 5 to 20 min. The $NaOH$-Na_2CO_3 solutions in the units were then quantitatively transferred by means of long pipettes with a rubber bulb at one end (Fig. 2*E*) to ampoules which were immediately sealed. The

units were then washed, fresh NaOH and frothing agent introduced, and the trains reassembled for use later in the experiment.

The liquid obtained from any one absorption train was made up to 50 ml. with water, and 1–2 ml. samples taken for analysis. All analyses were carried out in an apparatus modified by Davison from that of Barker (described by Kamen, 1948). These samples were pipetted into the main compartment of a side-bulb cup (Fig. 2*D*) and approximately 1 ml. lactic acid introduced into the side bulb by means of a bent Pasteur pipette. The side-bulb cup was attached to the transfer unit (Fig. 2*A*), a small tube (Fig. 2*C*) containing 3 ml. 0·22 N-$Ba(OH)_2$ and 2 drops thymol blue, being attached to the other end. The system was evacuated, the tap closed and the contents of the side bulb tipped into the main compartment. CO_2 was rapidly evolved and rapidly absorbed in the $Ba(OH)_2$. Quantitative absorption was ensured by warming the side-bulb cup and transfer unit in the hand, the system being shaken continuously for 5 min. whilst the small tube was cooled under running cold water. CO_2-free air was admitted into the system, and the small tube, which contained $BaCO_3$, was detached and stoppered with a flat cap (Fig. 2*B*). The amount of CO_2 absorbed was determined by titration of the unneutralized $Ba(OH)_2$ with 0·20 N-HCl. The precipitate was washed twice with water and once with acetone in a centrifuge, suspended in acetone, and plated on weighed aluminium disks, as described later.

At various times during the experiments, blood samples (1 ml.) were taken from the arterial cannula and received in stoppered tubes containing 0·2 ml. N-NaOH to prevent loss of CO_2. The tubes were stored at −12°. When required for analysis, the frozen clotted samples were thawed, homogenized with 1 ml. 1·7 N-NaOH and 4 ml. water, washed into measuring tubes with 1 ml. water and the volumes read. About half the liquid was measured into the main compartment of a double-armed Warburg vessel, with 1 ml. 3 M-acetate buffer, pH 5·0, in one side arm and 0·8 ml. jack bean urease solution buffered to pH 5·0 (Davies & Kornberg, 1951) in the other. The vessels were equilibrated at 25° and first the buffer was added from one side arm. The amount of gas evolved gave the 'total CO_2' content of the sample. The 'total CO_2' content of any tissue was defined as the acid-volatile CO_2, which included dissolved CO_2, H_2CO_3, HCO_3^-, CO_3^{--} and carbamino compounds. As alkali had been added initially to all samples, the 'total CO_2' at the time of analysis was present in the form of CO_3^{--}.

When equilibrium had again been reached, the jack bean urease was added from the other side arm. The urea in the sample was hydrolysed, and an equivalent amount of CO_2 evolved. These estimations were accurate to ±5%.

Another sample of the dilute blood homogenate, together with 1 ml. 0·125 M-Na_2CO_3 (containing 1·5 mg. C, added as carrier) was added to a side-bulb cup, and 1 ml. 1·5 N-H_2SO_4 was pipetted into the side bulb. The side-bulb cup was attached to one end of a transfer unit, a tube containing 3 ml. 0·22 N-$Ba(OH)_2$ and 2 drops thymol blue attached to the other, the system evacuated and the cup contents mixed. The CO_2 evolved was assayed by the techniques described. The cup was detached, 2 ml. 0·125 M-Na_2CO_3 and 0·5 ml. 3 M-acetate buffer, pH 5·0, were added to the contents and the system was evacuated. Any trace of radioactive C remaining in the system as bicarbonate had thus been diluted with 3 mg. non-radioactive C, which was then driven off on evacuation. CO_2-free air was admitted, 0·5 ml. 1·0% (w/v) urea solution was added to the contents of the cup, and 1 ml. jack bean urease solution pipetted into the side bulb. The cup was attached to one end of another transfer unit, a tube containing $Ba(OH)_2$ and thymol blue as above being attached to the other end. The system was evacuated and the cup contents mixed.

After 2 hr. all the urea in the sample had been decomposed, and the quantity and radioactivity of the $^{14}CO_2$ evolved was determined.

Tests showed that this procedure made it possible to determine independently highly active HCO_3^- and urea of low activity, present in the same sample of blood, without the results being vitiated by contamination.

Samples (1 g.) of cat tissues were taken during the experiments, blotted to remove adherent blood, and stored in weighed tubes containing 5 ml. 2 N-NaOH to retain all acid-volatile CO_2. The tissues disintegrated on standing and were washed quantitatively into the main compartments of side-bulb cups with addition of 1 ml. 0·125 M-Na_2CO_3 to act as a carrier. The tissue 'total CO_2' was decomposed by addition *in vacuo* of lactic acid from the side bulb, and the $^{14}CO_2$ evolved was measured.

Measurement of radioactivity

The washed $BaCO_3$ samples were suspended in acetone, shaken vigorously in the small stoppered tubes, and poured into stainless-steel cylinders which fitted firmly into the circular grooves of weighed aluminium plates. Acetone leaked slowly past the junction of cylinder and plate, leaving the fine precipitates of $BaCO_3$ (20–30 mg.) deposited on the plates as uniform layers of area 3·8 sq.cm. The cylinders were covered and allowed to dry at room temperature overnight. When dry, the cylinders were removed, the plates weighed, and the radioactivity assayed with a thin mica-window Geiger-Müller counter, corrections having been made for background counts and for the dead-time of the system. At least 5000 counts were taken for each sample, giving a standard error of less than 1·5%. The efficiency of counting was frequently checked by counting a uranium standard. There was no detectable loss for at least 3 days in the activities of the samples, kept covered in Petri dishes, through exchange of their ^{14}C content with atmospheric CO_2.

All counts were corrected to infinite thickness for self-absorption in the $BaCO_3$ by means of an experimentally determined activity-saturation curve (cf. Calvin, Heidelberger, Reid, Jolbert & Yankwich, 1949), and the specific activities expressed as the corrected counts/min./mg. C. All analyses and counts were performed in duplicate and agreed to ±3%.

RESULTS

Expiration of $^{14}CO_2$

After intravenous injection of ^{14}C-labelled sodium bicarbonate, the rate of expiration of $^{14}CO_2$ is initially very rapid, and corresponds to an exponential equation with three components. The curves obtained in our experiments were found empirically to correspond within 2% to the function

$$(100 - P) = 28{\cdot}4e^{-0{\cdot}243t} + 41{\cdot}5e^{-0{\cdot}0234t} + 30{\cdot}2e^{-0{\cdot}00119t},$$

where P is the percentage of injected ^{14}C left in the cat at t min. after the injection (Fig. 3). The three components therefore have half-lives of 2·85, 29·6 and 582 min. respectively. In the first 30 min., 50 % of the injected ^{14}C is expired, the total output over the 5 hr. experiments being 79–81 %.

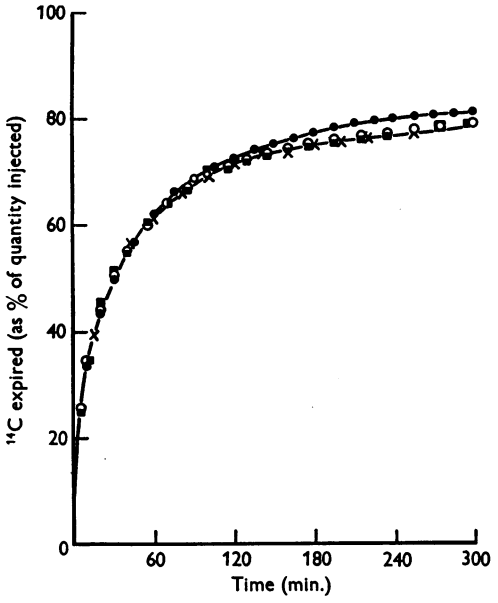

Fig. 3. Expiration of $^{14}CO_2$ after intravenous injection of $NaH^{14}CO_3$. ○—○, cat *A*, 1·83 kg.; ●—●, cat *B*, 1·55 kg.; ×—×, cat *C*, 1·69 kg.; ■—■, curve of $(100\text{-}P) = 28{\cdot}4e^{-0{\cdot}243t} + 41{\cdot}5e^{-0{\cdot}0234t} + 30{\cdot}2e^{-0{\cdot}00119t}$, where P is the percentage of injected ^{14}C remaining in cat after t min.

Semi-logarithmic plots of the specific activities of the expired $^{14}CO_2$ against time (Fig. 4) give smooth curves. Analysis of the radioactivity of the blood 'total CO_2' showed that the specific activities of expired CO_2 and blood 'total CO_2' are identical at any time, and these curves thus also represent the change in the specific activities of blood 'total CO_2' with time. The three curves obtained in our experiments cut the ordinate at specific activities of approx. 16 000, 16 000 and 28 000 counts/min./mg. carbon respectively. As the blood volume of the cat is approx. 70 ml./kg. body weight, and as the amounts of bicarbonate injected were negligible, this corresponds in each case to a blood 'total CO_2' concentration of 24–27 mM. This is the 'total CO_2' content of blood (Krebs, 1950) and shows that only at zero time is the radioactive material present entirely in the blood. After 3 hr. the rate of expiration of $^{14}CO_2$ is nearly constant and is less than 2 % of the injected isotope/hr. The blood and various soft tissues, which were weighed, have low, slowly decreasing, activities in their 'total CO_2' at this time (Table 1), and after 4 hr. together contain approx. 1 % of the injected ^{14}C, i.e. approx. 5 % of the ^{14}C still remaining in the cat. The activity of bone is 15–40 times that of any other tissue, and, since bone

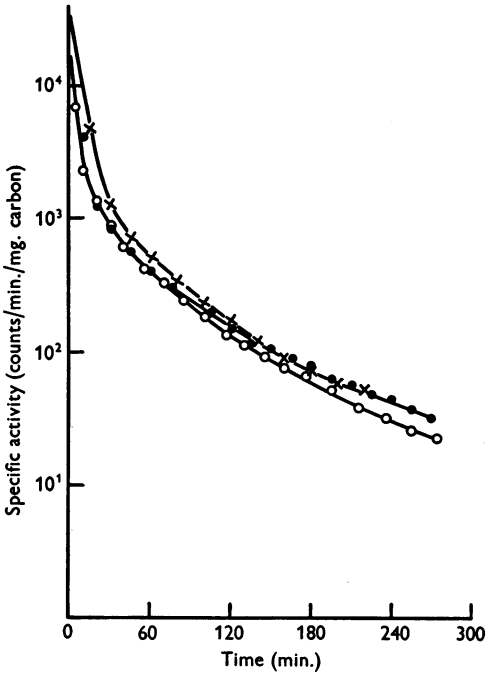

Fig. 4. Changes in specific activity of expired $^{14}CO_2$ after intravenous injection of $NaH^{14}CO_3$. ○—○, cat *A*, 1·96 kg.; ●—●, cat *B*; 1·55 kg.; ×—×, cat *C*, 1·69 kg.

forms approx. 18 % of the body weight of the animal (Custor, 1873) over 6 % of the injected ^{14}C, i.e. over 30 % of the isotope still remaining in the cat, is present in this tissue at this time.

Table 1. *Radioactivity of 'total* CO_2*' of tissues after intravenous injection of* $NaH^{14}CO_3$ *into a cat*

(Cat, 1·5 kg., received 1×10^6 counts/min. intravenously at time 0.)

Time (min.)	Tissue radioactivity (counts/g. wet wt.)				
	Blood	Jejunum	Muscle	Marrow	Femur
32	360	143	—	—	—
58	155	84	85	—	—
91	82	43	—	—	—
120	51	28	23	—	—
150	30	15	—	—	—
182	21	11	10	—	—
240	13	6	5	11	220

Urea synthesis

As the ureters of the experimental animals were tied at the start of each experiment, no ^{14}C was lost in the urine, and urea synthesis could be followed

both by the rise in the concentration and radioactivity of blood urea (Table 2). The urea synthesized in the early stages of the experiments is formed from CO_2 which is in equilibrium with blood bicarbonate of high specific activity, and the major part of the incorporation of ^{14}C into urea occurs in the first 40 min., the blood urea by this time containing 2·0 % of the ^{14}C injected. After 5 hr. 3·1 % of the administered isotope was incorporated into urea.

Table 2. *Concentration and specific activity of blood urea after intravenous injection of* $Na^{14}HCO_3$

(Cat I (1·55 kg.) received $5·1 \times 10^5$ counts/min., cat II (1·83 kg.) $7·0 \times 10^5$ counts/min., at time 0. Ureters tied.)

Cat no.	Time (min.)	Blood-urea concentration (mg./100 ml.)	Specific activity (counts/min./mg. carbon)	Percentage of injected ^{14}C incorporated in urea
I	0	31·0	0·00	0·00
	5	31·0	56·8	0·80
	16	32·3	102	1·50
	25	32·6	117	1·74
	40	35·5	124	2·00
	70	36·0	145	2·37
	100	36·5	154	2·56
	130	42·0	150	2·67
	160	40·0	154	2·80
	192	44·0	143	2·87
	220	47·5	135	2·91
	250	47·7	136	2·96
	280	50·1	134	3·06
II	0	62·5	0·00	0·00
	20	65·0	64·5	1·64
	35	70·0	70·1	1·92
	48	68·2	78·7	2·10
	78	69·7	88·7	2·42
	115	70·6	94·7	2·62
	153	77·5	91·4	2·77
	198	77·7	94·7	2·88
	253	80·0	94·5	2·96
	285	84·0	94·4	3·10

The total quantity of urea carbon synthesized during the experiments was obtained by multiplying the rise in plasma urea-carbon concentration by the 'urea space' of the cat. The 'urea space', i.e. the total volume of water in which the urea of the body is dissolved, has been found to be 65 % of the body weight of the cat (Kornberg *et al.* 1951*b*). Table 3 shows that the ratio

$$\frac{\text{carbon incorporated into urea}}{\text{carbon incorporated into urea + carbon expired as } CO_2}$$

was 0·037 or 0·038. Comparison of the distribution of radioactivity in urea and expired CO_2 show that the ratio

$$\frac{^{14}\text{C-urea synth. from labelled bicarbonate}}{^{14}\text{C-urea synth. from labelled bicarbonate} + ^{14}CO_2 \text{ expired}}$$

was also 0·037 or 0·038. This means that within the experimental error of the average values (± 2 %) the urea carbon synthesized during the experiments must have been derived solely from carbon in equilibrium with blood bicarbonate, and confirms the results obtained *in vitro* by Grisolia & Cohen (1948), and *in vivo* by Mackenzie & du Vigneaud (1948), Sprinson (1949), and Armstrong & Zbarsky (1949).

DISCUSSION

Rate of expiration of $^{14}CO_2$

When ^{14}C-labelled sodium bicarbonate or carbonate is injected intraperitoneally into mice or rats, ^{14}C is rapidly expired as $^{14}CO_2$. Skipper *et al.* (1949), Skipper, Nolan *et al.* (1951), Skipper, Bennett *et al.* (1951) using mice, recovered 96 % of the injected dose in this form within 24 hr., 92·8 % having been expired within the first hour and over 50 % in the first 10 min. The total elimination in rats is very similar to that in mice, recoveries of 93–98 % being recorded (Armstrong & Zbarsky, 1949; Gould *et al.* 1949; Greenberg & Winnick, 1949) but the rate of expiration is slightly lower, 50 % of the injected ^{14}C appearing as $^{14}CO_2$ in the first 18 min. (Gould *et al.* 1949). The experiments described in the present paper show that in cats only 50 % of the injected ^{14}C is expired in the first 30 min., and that the total isotope expired in 5 hr. is 79–81 % of that injected. It is of interest that both the rate and total output of $^{14}CO_2$ was less in cats than in mice and rats, even though the material was injected intravenously into the cats, in contrast to the intraperitoneal injections

Table 3. *The source of the carbon of urea*

Cat no.	I	II
Body wt. (kg.)	1·55	1·83
Urea 'space' (ml.)	1010	1190
Blood-urea concentration (mg./100 ml.):		
At start	31·0	62·5
At end	50·1	84·0
(*A*) Total urea synthesized (mg. carbon)	44·0	59·0
(*B*) Total respiratory CO_2 output (mg. carbon)	1120	1500
$\frac{(A)}{(A)+(B)}$	0·037	0·038
(*A'*) ^{14}C fixed as urea (counts/min.)	15 600	21 700
(*B'*) $^{14}CO_2$ expired (counts/min.)	411 000	547 000
$\frac{(A')}{(A')+(B')}$	0·037	0·038

with the smaller animals. The rate of excretion of $^{14}CO_2$ after intravenous injection of labelled sodium bicarbonate can be formulated as an exponential equation with three components; at no time does it correspond to the first-order reaction described by Gould *et al.* (1949) after intraperitoneal injection of labelled bicarbonate into rats.

The 'bicarbonate pool'

The data of Gould *et al.* (1949) show that the specific activity of the expired $^{14}CO_2$ in their experiments falls at a uniform rate for the first 60 min., and that the initial specific activity obtained by extrapolation of the curve to zero time indicates a 'bicarbonate pool' of 2 m-moles/100 g. rat. As the total acid-volatile CO_2 in the extracellular and intracellular fluid of an animal of this size amounts to only 1 m-mole, the authors stated that 'the $C^{14}O_2$ must have rapidly entered into mobile equilibrium with 1 m-mole of CO_2 elsewhere in the body. One possibility is rapid exchange with the CO_2 of bone, and another is incorporation into dicarboxylic and tricarboxylic acids.' This conclusion and the earlier one of Brues & Buchanan (1948) that the total mass of carbon in mobile equilibrium with CO_2 in the body is greater than the total amount of CO_2 and bicarbonate in solution, is confirmed by our present findings, which provide evidence for the existence of exchange mechanisms between blood and tissue 'total CO_2'. After injection of labelled bicarbonate, ^{14}C is taken up into the tissues at varying rates from blood bicarbonate of initially very high radioactivity. The ^{14}C content of any tissue reaches a maximum when the specific activities of blood and tissue 'total CO_2' become nearly equal.

In these early stages of the experiments the distribution of ^{14}C amongst the blood and tissues will thus be in the order of their rates of CO_2 exchange. The 'fixed' isotope is released again into the blood at rates governed by many factors, such as the 'total CO_2' content, diffusion constants, blood supply, and metabolic activity of the tissue. The concentration of 'total CO_2' in cat blood is approx. 25 mM and a 2 kg. cat thus contains about 45 mg. carbon as acid-volatile CO_2 in its blood. Since, however, the rate of expiration of CO_2 is of the order of 90 mg. carbon/15 min. an amount of carbon equivalent to the total amount in the blood is expired about every 7 min. This rapid removal through the lungs of ^{14}C from the blood will preferentially deplete of their isotope content those tissues with the highest rates of exchange of CO_2, so that after 4 hr. the distribution in the tissues of the ^{14}C still remaining in the cat is, in general, in inverse order of these exchange rates (Table 1, cf. Skipper, Nolan *et al.* 1951). Of the ^{14}C injected as labelled bicarbonate in the present experiments, after 4 hr. only about 1 % could be accounted for as the residual labelled bicarbonate of the blood, muscles and viscera, but approx. 6 % was 'fixed' in bone. This large amount of incorporation is mainly due to the high 'total CO_2' content of bone. These facts present striking evidence for the occurrence of CO_2 exchange mechanisms in the body.

Further evidence is provided by the data on the expiration of $^{14}CO_2$ from the cat. As already stated, the graph of the percentage of injected ^{14}C expired against time is an exponential curve with three components (Fig. 3). These have half-lives of 2·85, 29·6 and 582 min. respectively, and, though the detailed nature of the three components is uncertain, they show that the 'bicarbonate pool' is certainly not homogeneous. This conclusion is also indicated when attempts are made to calculate the 'bicarbonate pool' of the cat by the procedure of Gould *et al.* (1949). The semi-logarithmic plot of specific activity against time (Fig. 4) is a smooth curve in each experiment, which at no time becomes linear. Attempts to calculate the 'bicarbonate pool' by dividing the ^{14}C remaining in the cat at any point by the ^{14}C-bicarbonate concentration of the blood at that point give a steadily rising 'pool' which after some time exceeds the body weight of the animal. The underlying assumption of this procedure, that all the ^{14}C remaining in the cat at any time is rapidly miscible with, and has the same specific activity as, blood bicarbonate, must therefore be erroneous, and 'fixation' of ^{14}C in some form not rapidly miscible with blood bicarbonate must therefore have occurred. This is supported by the fact that in the long-term experiments with mice and rats the 'total CO_2' of bone and other tissues of low metabolic activity (cf. Skipper *et al.* 1949; Skipper, Nolan *et al.* 1951; Neuberger, Perrone & Slack, 1951) have specific activities much higher than that of blood.

The synthesis of urea

Recent studies on the breakdown of labelled urea in mice (Leifer *et al.* 1948; Skipper, Bennett *et al.* 1951) and cats (Davies & Kornberg, 1950; Kornberg *et al.* 1951*b*) have shown that urea is not a stable end product of nitrogen metabolism, but is broken down in the body: after intravenous injection of ^{14}C-labelled urea into cats, approx. 2 % of the isotope was expired in 5 hr. as $^{14}CO_2$. This slow evolution of CO_2 from urea may be likened to the processes occurring in a 'pool' with a low rate of CO_2 exchange, and the mechanisms of incorporation of ^{14}C into urea from ^{14}C-labelled bicarbonate should thus be similar to those postulated above. After 5 hr., 3·1 % of the ^{14}C-labelled bicarbonate is present as urea carbon, but though the rate of urea synthesis was uniform throughout the experiments (Table 2), 50 % of this incorporation occurred in the first 30 min. Most of this 'fixation' of ^{14}C thus took place when the radioactivity of blood bicarbonate was very high, the rate of release of 'fixed' ^{14}C being

governed by the rate of breakdown of urea. These facts thus fully support the views expressed above on the 'fixation' of ^{14}C in other tissues of the body.

It was first shown by Grisolia & Cohen (1948) *in vitro* and by Mackenzie & du Vigneaud (1948) *in vivo* that the specific activities of bicarbonate carbon and urea carbon are the same, and that the urea carbon is, within the experimental error, derived wholly from CO_2. The present findings (Table 3) confirm this view; this means that in our experiments with fasted cats less than 2 % of the urea synthesized was formed from precursors other than labelled bicarbonate, such as arginine derived from the diet or from protein breakdown.

SUMMARY

1. Apparatus and techniques are described for *in vivo* studies of the metabolism of ^{14}C-labelled bicarbonate in the cat.

2. The rate of expiration of $^{14}CO_2$ after intravenous injection of $NaH^{14}CO_3$ is initially very rapid, 50 % being expired in the first 30 min., the total expired in 5 hr. being 79–81 % of the injected ^{14}C. The rate of expiration can be represented by an exponential equation with three components of half-lives 2·85, 29·6 and 582 min. respectively.

3. Of the ^{14}C injected, 3·1 % was incorporated into urea in 5 hr., 50 % of this incorporation occurring in the first 30 min. Within the experimental error (± 2 %) all the urea carbon synthesized was derived from carbon in rapid equilibrium with blood bicarbonate.

4. After 4 hr. approximately 6 % of the injected ^{14}C was present in bone, the carbon of which exchanges relatively slowly with blood bicarbonate. The blood, muscles and viscera contained approximately 1 % of the isotope at that time.

5. These results show that the bicarbonate pool is not homogeneous, but consists of several component CO_2-exchange mechanisms operating at widely differing rates.

We wish to thank Prof. H. A. Krebs, F.R.S., for his interest and advice, Mr T. Smith for technical assistance, Dr Q. H. Gibson and Mrs M. Davison for details of the design and use of the vacuum transfer apparatus, Prof. S. Gurin for advice on the preparation of solid samples for radio-assay, and Mr J. F. Scott for mathematical assistance. One of us (H.L.K.) wishes to acknowledge the receipt of a grant from the Medical Research Council.

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