UPTAKE AND RETENTION OF FIXED CARBON* IN ADULT MICE

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Permissible continuous levels of $C^{14}O_2$ in air and of C^{14} in tissue which have been suggested (9, 10, 18) have been largely derived on hypothetical grounds by applying limiting assumptions to established physiological data. Although the present studies began as a general experimental approach to the problem of potential $C^{14}O_2$ radiotoxicity the results seem to be of more general interest because of the central role of carbon in metabolism. A discussion of some of the unsolved problems of CO_2 metabolism which motivated this work follows:—

(*a*) Of the total carbon existing in an organism what fraction has been fixed from the metabolic CO_2 pool and of this fraction what portion has been derived from *air* CO_2? The former may be designated as the *fixed carbon fraction* (FCF),* and the latter the *air carbon fraction* (ACF).* These fractions may be expected to vary between different organisms, between different parts of organisms, and with age, environment, diet, etc. Knowledge of these fractions and the factors which influence their magnitude will facilitate calculation of tolerable $C^{14}O_2$ levels in air.

The only obvious method of measuring these fractions is to place organisms in a constantly labeled atmosphere until the isotope concentration in the entire organism becomes constant. When this happens the ratio of isotope concentration in the organism to that in the atmosphere represents the ACF,[1]

* Fixed carbon includes all organic carbon atoms which have at some time existed *in the organism* as CO_2. Air-derived carbon includes all such atoms which have been taken into the organism *from the air as* CO_2. The fixed carbon therefore includes the air-derived carbon. (It includes in addition all carbon which has been oxidized to CO_2 and again reduced to organic form *in the organism.*) The following relationships hold:

$$\text{FCF} = \frac{\text{Fixed carbon}}{\text{Total carbon}}$$

$$\text{ACF} = \frac{\text{Air-derived carbon}}{\text{Total carbon}}$$

[1] In an isotopic steady state thus attained with $C^{14}O_2$-labeled air the specific radioactivity (s.a.) of air-derived carbon will equal the s.a. of air CO_2 and the s.a. of the

and a similar ratio between isotope concentration in the organism to that in the CO_2 pool of the organism represents the FCF.[1]

There is a considerable body of evidence that certain carbon positions and certain compounds tend to accumulate fixed carbon to an appreciable extent. Positions 3 and 4 of glucose (28) and the corresponding carbons of carbohydrate intermediates (27) and their amino acid homologues (13), urea carbon (13, 14, 19), and certain carbon positions of uric acid (25) have all been shown to contain appreciable quantities of fixed carbon. But carbons 1, 2, 5, and 6 of glucose (23), and the fatty acids (20) may also become isotopically labeled with CO_2 carbon. It is not unlikely that most of the carbon positions in an organism may have some small fraction represented by fixed carbon.

(*b*) What influence does the atmospheric partial pressure of CO_2 have on the magnitude of these fractions? Benson and Calvin (3) have shown that the amount of CO_2 fixed in the dark by *Scenedesmus* algae is proportional to the CO_2 concentration at very low partial pressures, but that the amount fixed becomes independent of the concentration when the latter is above 0.1 per cent. There appear to be no data bearing on this question in animals.

(*c*) What are the "turnover" characteristics of fixed carbon? Previously reported experiments have shown considerable differences in the amount of fixed carbon taken up by various organs and tissues following relatively short or inconstant exposures to isotopic CO_2 or one of its salts (1, 6, 11, 21, 24). These differences are undoubtedly due in part to the differences existing in the true fractions as discussed above and in part to the differences in the rates at which true values are experimentally approached. A separation of these two factors has been partially achieved in this work. The results obtained are useful for making qualitative comparisons between turnover rates of fixed carbon in various organs and tissues, but a rigorous analysis of this type of data in terms of true turnover rates seems impossible and has not been attempted.

EXPERIMENTAL

The only practical method of furnishing a constantly labeled atmosphere to animals as large as mice is to ventilate them at a constant rate with a reservoir of constantly

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fixed carbon will equal the s.a. of CO_2 in the organism. Hence for a particular chemical or morphological component in an organism:

$$\text{ACF} = \frac{\text{s.a. of carbon in component}}{\text{s.a. air } CO_2}$$

$$\text{FCF} = \frac{\text{s.a. of carbon in component}}{\text{s.a. of } CO_2 \text{ in organism}}$$

In this experiment the s.a. calculated for alveolar air is used to approximate the s.a. of CO_2 in the organism.

labeled air. Enrichment of the air with oxygen seems to overcome the depressing action seen with moderately increased CO_2 levels.

The equipment used in the experiment of this report is diagrammed in Fig. 1. Sealed metabolism chambers (approximately 10 liters) connected in series were ventilated with the contents of 8 gas cylinders containing oxygen-enriched air (30 per cent) and 35 mc. (8.51 mM) of $C^{14}O_2$. The total pressure in the cylinders was 1950 pounds/sq. in. and the total volume 344 liters. A large inner tube provided a low pressure gas reservoir and a mercury piston pump kept the ventilation rate constant at 1 liter per minute. Waste CO_2 was completely trapped in a series of carboys containing concentrated KOH.

A flask containing calcium chloride was placed after each chamber in the flow train to reduce humidity. A 3-way stopcock on each side of these traps allowed collection of CO_2 samples for counting without disturbing the gas flow.

The exposure lasted 34 days. Animals were placed in the chambers according to the rotation scheme of Fig. 2 which enabled exposures to be made for varying time periods, and yet provided that each chamber always contain the same number of mice. This was accomplished by removing one animal at the same time another was inserted. In this manner CO_2 concentrations were kept relatively uniform.

The mice in chambers I and II were CF No. 1 females, 8 weeks old at the beginning of the experiment. The animals in chamber III were older females. Two of these were bred 4 days before the beginning of the experiment but failed to deliver young despite the fact that semen plugs were observed after coitus, and one was later exchanged with an animal of similar age. The third animal in chamber III had a small mammary carcinoma at the beginning of the experiment which increased its mass several fold during the next 34 days.

The chambers were opened every 2 days to feed, water, and weigh the animals and to clean the cages. This procedure required about 30 minutes or 1 per cent of the exposure time.

Urine and feces were collected at 4, 8, and finally 24 hour intervals. Collection was continued for 16 days in chambers I and III (at this time the first animal was sacrificed and replaced) and for the entire 34 days in chamber II, since in this chamber no animals were exchanged. The urine was filtered into lusteroid tubes and frozen. Feces samples were frozen and dried. Urine, feces, and expired air were collected from chamber II for 2 weeks following the exposure, the chamber being ventilated with CO_2-free air during that time.

The CO_2 concentration was measured in each chamber with a Haldane apparatus every 8 hours during the exposure. Samples for counting were simultaneously prepared using traps containing solid $Ba(OH)_2$ and NaOH (carbonate-free).

Urine urea carbon was isolated for counting by first acidifying aliquots of urine with HCl and drying *in vacuo* to remove CO_2 and carbonates. After treatment with buffered urease for 1 hour the samples were reacidified and CO_2 was collected in carbonate-free NaOH. $BaCO_3$ was precipitated and counted.

After the determination of urea carbon s.a. had been completed on aliquots of all samples, a pooled sample was prepared from the urine collected in chamber II during

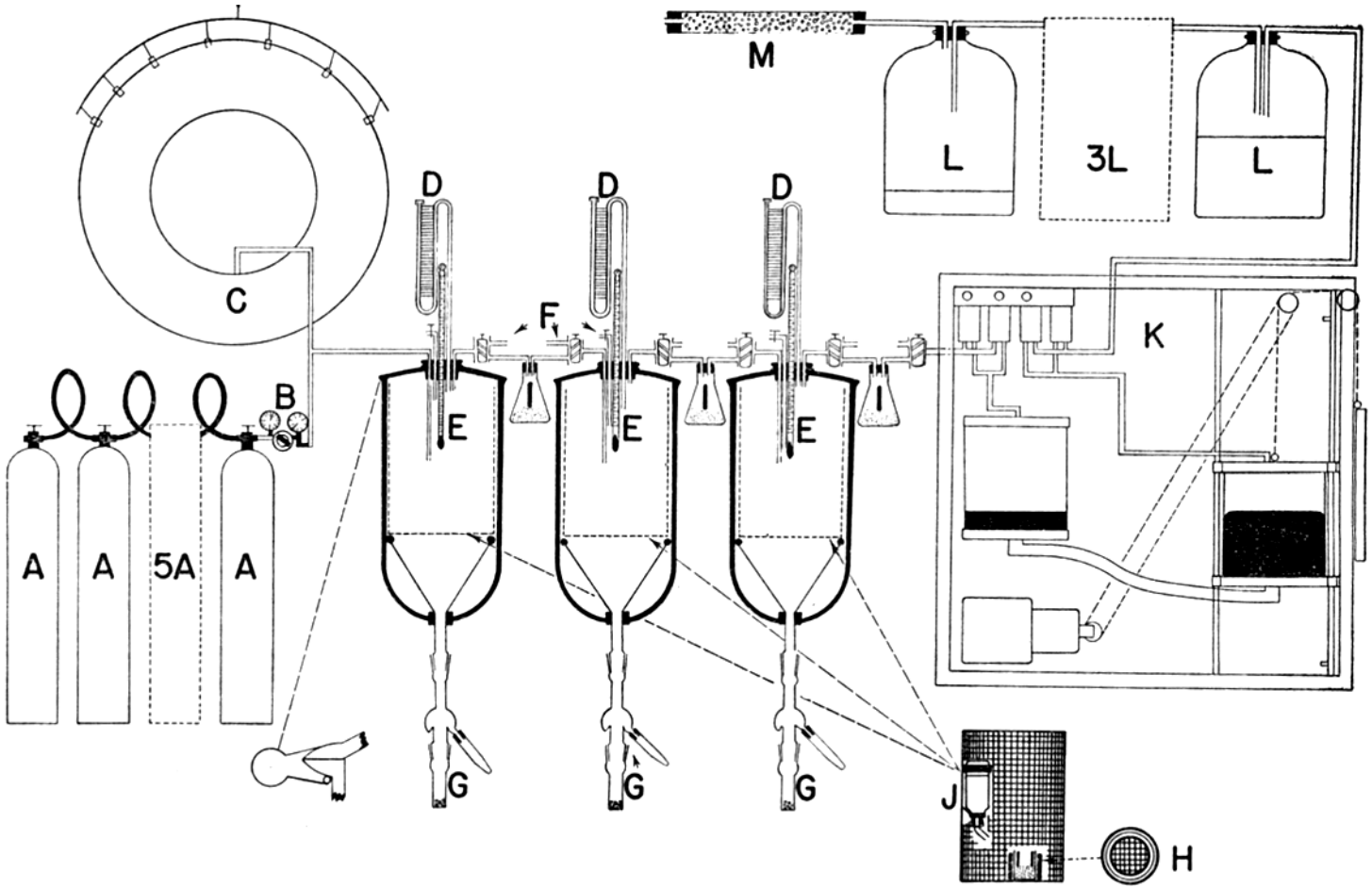


FIG. 1. Apparatus for exposure of mice to $C^{14}O_2$-labeled air. *A*, gas cylinders; *B*, two-stage pressure regulator; *C*, large inner tube; *D*, water manometers; *E*, thermometers; *F*, sampling connections; *G*, urine and feces separators; *H*, spillproof-feeder; *I*, $CaCl_2$ traps; *J*, removable wire cages; *K*, mercury piston pump; *L*, carboy CO_2 traps containing KOH; *M*, soda lime trap.

the latter half of the exposure. Portions of the pooled urine were then analyzed for both total and urea carbon content and radioactivity.[2]

The radioactivity of the total fecal carbon was measured by homogenizing dry feces with a glass homogenizer using ether as a suspending fluid. The suspension was

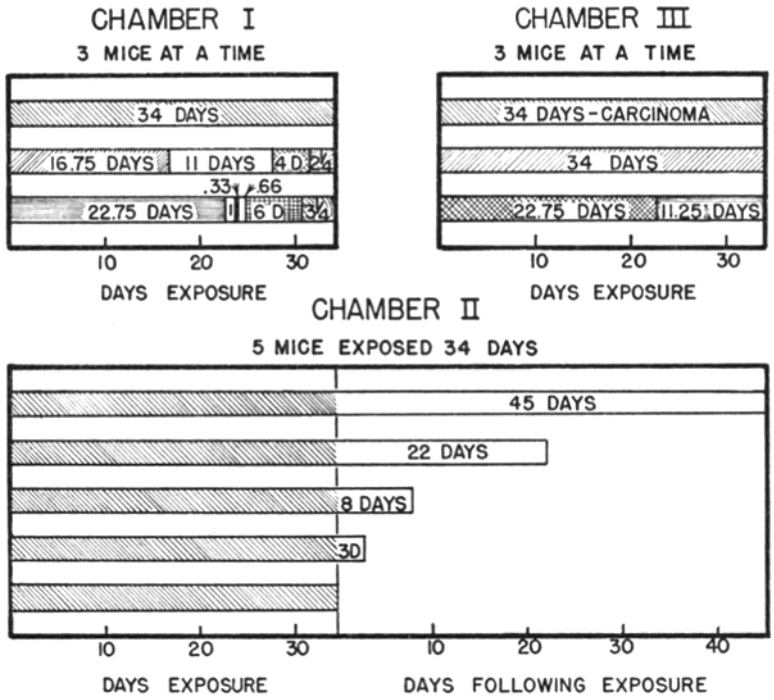


FIG. 2. Scheme of exposure of mice to $C^{14}O_2$-labeled air. Each horizontal bar represents a mouse in a chamber. Bar divisions indicate the time when a given animal was removed and replaced by another animal. The total exposure time (in days, D) of each mouse is indicated on the bar segment.

plated out at greater than "infinite thickness" on aluminum planchets and dried. The planchets were then covered with a dilute solution of urease, allowed to stand, dried in air, then *in vacuo*, and counted. This procedure lowered the activity of samples which were counted prior to such treatment.

A sample of feces pooled from chamber II during the final half of the exposure was crudely fractionated by exhaustive extraction in a Soxhlet apparatus; first with

[2] The method used for all determinations of carbon and for counting CO_2 gas will be submitted for separate publication. It consists of oxidation of organic samples with the Van Slyke and Folch combustion mixture (26) and measurement of the resulting CO_2 manometrically after its purification by a series of traps. The radioactivity measurement is made in a proportional gas counter (4).

ether, then with ethyl alcohol, and finally with water. The three extracts and the residue were dried over steam, weighed, and assayed for carbon content and radioactivity.[2]

When the exposure of each animal was completed it was killed with ether and dissected. Each organ was freed of as much fat and connective tissue as possible. After being opened longitudinally the gastrointestinal tract was washed with distilled water to remove the contents. The small intestine was divided into segments of equal length. The segment adjacent to the stomach is herein designated duodenum and jejunum and the caudal segment is designated ileum despite the arbitrary separation. The "neck glands" include only the salivary glands and the cervical lymph nodes. Since the pancreas in mice is not a well demarcated organ, considerable serosal tissue is included in this sample. The mesentery includes all adipose tissue, connective tissue, lymph nodes, and vessels remaining in the abdominal cavity after removal of the other organs mentioned in the results. The eye samples include only the optic bulbs and not orbital contents.

All organs and tissues were placed in separate weighed vials, frozen, and dried *in vacuo* at 0–5°C. The vials were then sealed and stored in a freezer at −30°C until analyzed.

After all animals had been sacrificed the dried samples were collected into groups of like tissues for analysis. Since the radioactivity in these samples was too low to measure accurately as $BaCO_3$ the materials were counted directly. This was done by first homogenizing the samples in ethyl ether with a glass homogenizer, then centrifuging, and decanting. Resuspension of the sediment in a small amount of ether allowed the material to be plated on aluminum planchets. When more than 50 mg. of dry material were available the residual ether was allowed to evaporate from the centrifuge tube and the dry residue was pulverized with a glass rod. The resulting fine, flocculent powder could then be tamped into an aluminum planchet with a press.

The supernatant ether of several sets of samples was pooled into groups and allowed to evaporate. The residual lipids were then plated on aluminum planchets for counting.

Blood that had been withdrawn from the inferior vena cava into a syringe moistened with a solution of heparin was centrifuged and the plasma was separated, frozen, and dried. It was later redissolved in a small amount of distilled water and plated on aluminum planchets for counting. The erythrocytes were washed twice with saline by successive centrifugation, and the final saline decanted. After being frozen and dried the residue was pulverized and tamped into planchets. The skins were washed in water, alcohol, and ether and dried flat. Discs were cut from each skin with a cork borer and counted on both sides, the average counting rate being used in determining the s.a.

Counts on solid samples were made with either thin mica window or "Q" gas Geiger counters depending upon the amount of radioactivity present and were of sufficient duration to give a theoretical standard deviation of less than 2 per cent. Representative samples of all sets were analyzed as CO_2 in a proportional gas counter in order to standardize s.a. on the basis of carbon content.[2]

RESULTS

Table I shows the mean CO_2 concentration in the chambers during the exposure. Since the chambers were connected in series the concentration was least in I and greatest in III. The CO_2 outputs of the mice were calculated from the CO_2 concentrations and the flow rate through the chambers.[3]

Fig. 3 gives the relative s.a. of the gas in the three chambers during the exposure. The gradual increase in s.a. was later shown to be the result of a reversible combination of the $C^{14}O_2$ with metal oxides in the gas cylinders. Thus as the tank pressure diminished the $C^{14}O_2$ partial pressure in the gas phase also tended to diminish reversing the binding reaction. This phenomenon became especially marked during the last few days of the experiment when the "half-time" of emptying of the tanks became short. The activity per unit

TABLE I

Mean CO_2 Concentrations, Mean Relative $C^{14}O_2$ Levels, and Mean CO_2 Outputs of Mice in Metabolism Chambers during a 34 Day Exposure to $C^{14}O_2$-Labeled Air

Chamber	Mean CO_2 concentration	Mean s.a. × mean CO_2 concentration	Mean CO_2 output
	per cent	*relative per cent*	*ml./gm./hr.**
I	0.43	96.5	3.3
II	1.15	100.0	3.0
III	1.68	98.6	3.0

* Corrected to S.T.P.

volume doubled during the entire period with half of the increase taking place during the final 4 days.

The differences in s.a. between successive chambers are almost exactly accounted for by the differences in total CO_2 concentration (Table I). Thus the isotope removed by the animals was a negligible fraction of that passing through the chambers and the $C^{14}O_2$ concentration was equal in the three chambers.

Fig. 3 includes the s.a. data on the urea carbon of the urine excreted in the three chambers. Since no animals were replaced during the first 16 days a comparison of these values is a valid test of the effect of CO_2 concentration on its fixation in urea. The results show the amount of C^{14} fixed to be nearly independent of the total CO_2 concentration under these conditions. The arithmetic means of all comparable values in the three sets of data show that the s.a. of urea of chamber II was 99.0 per cent of that in I and the s.a. in III

[3] Assuming an R.Q. of 0.85 the mean CO_2 output of these mice which were quite active during the 34 day period was more than twice the "basal" output as calculated from the formula of Brody (8) and higher than any of the experimental values quoted by him.

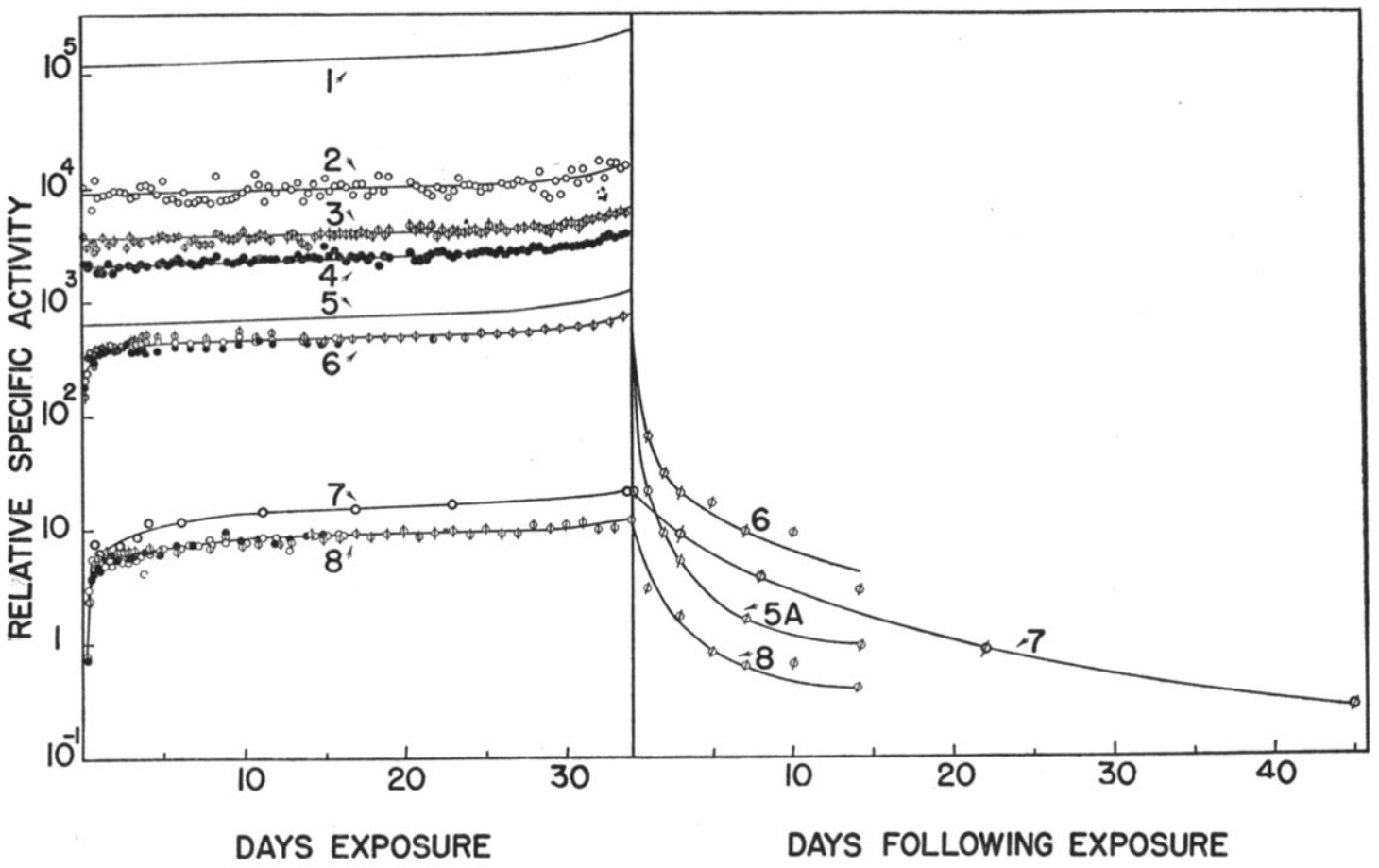


FIG. 3. Uptake and retention of $C^{14}O_2$ in the excreta of adult mice. Relative specific activities: 1, CO_2 normal air (0.03 per cent CO_2) calculated; 2, CO_2 chamber I; 3, CO_2 chamber II; 4, CO_2 chamber III; 5, CO_2, alveolar air (5.5 per cent CO_2) calculated; 5A, expired CO_2 chamber II; 6, urine urea carbon chambers I, II, and III; 7, liver carbon; 8, total fecal carbon chambers I, II, and III.

was 90.5 per cent of that in I. Fig. 3 shows the same general phenomenon to be true in the s.a. of feces.

There were two instances in this experiment in which animals were exposed for identical periods at different concentrations of CO_2 but at the same concentration of $C^{14}O_2$. One mouse in chamber I and one mouse in chamber III were exposed for the initial 22.75 days in the first instance. In the second instance one animal of chamber I, one animal of chamber II, and 2 animals of chamber III were exposed for 34 days. Comparisons of the s.a. values for the various tissues of these animals are shown in Table II.

In the 34 day animals there appeared to be somewhat less isotope retained at the higher CO_2 concentrations, but in the 22.75 day animals the small difference seen was in the opposite direction. Since there was a fourfold difference in the total CO_2 concentration between chambers I and III it seems that the total CO_2 concentration in this range has no detectable influence on the amount of isotope retained.

To explain these facts it is necessary to assume that the uptake and retention of labeled molecules are accompanied by a similar uptake and retention of unlabeled molecules according to the concentration of each. *Hence the uptake, fixation, and retention of air CO_2 are proportional to its concentration.*

This generality allows a comparison to be made between s.a. levels in various components of the mouse with those calculated for normal atmospheric air, *i.e.* air containing 0.03 per cent CO_2, and for those calculated for alveolar air; *i.e.*, air containing 5.5 per cent CO_2. Both extrapolated curves appear in Fig. 3.

The rationale of this extrapolation follows: It appears from this experiment as well as from previous ones (10) that after a relatively short time the net uptake of $C^{14}O_2$ is negligible when its concentration remains unchanged in the atmosphere. The rate at which the isotope is entering the animal must then closely approximate the rate at which it is leaving. All C^{14} which is leaving (except a calculated 2 per cent excreted in urine and feces) does so *via* the lungs. Hence, the partial pressure of $C^{14}O_2$ in the alveolar air must nearly equal that in the ambient air and the CO_2 s.a. levels in these loci are related as the inverse ratio of the respective CO_2 concentrations.[4] From this it follows that a given level of $C^{14}O_2$ in air will determine the s.a. of alveolar air despite changes in the ambient CO_2 concentration, *unless the latter becomes high enough to increase the alveolar CO_2 concentration.*

The s.a. of the urine urea carbon rose within hours to a certain fraction of the air s.a. and then remained at this fraction for 34 days. From this s.a. level one may predict that about 0.34 per cent of the carbon in urea originates from

[4] A mammal inhaling normal atmospheric air containing 0.03 per cent CO_2 increases the concentration of CO_2 to about 5.5 per cent in the alveoli (16). To each molecule of CO_2 thus inhaled 182 molecules are added. If the inhaled CO_2 is isotopically labeled the s.a. is reduced by a factor of $\frac{1}{183}$ upon entering the alveoli.

TABLE II

Effect of Total CO_2 Concentration on $C^{14}O_2$ Uptake in Mouse Tissues

Organ or tissue	Relative specific activity*					
	22.75 day exposure animals		34 day exposure animals			
	Chamber		Chamber			
	I‡	III§	I‡	II‖	III§	III§
Pancreas	0.95	1.02	1.05	1.10	1.03	0.98
Thymus	0.86	0.85	0.97	1.04	0.91	0.99
Uterus, tubes, ovaries	0.83	0.84	0.95	0.95	0.78	0.88
Liver	1.01	1.08	0.98	0.95	0.87	0.91
Adrenals	0.79	0.86	1.03	0.89	0.87	0.76
Plasma	0.85	1.09	1.14	0.91	0.97	1.03
Subcutaneous fascia	0.54	0.55	0.67	0.66	0.53	0.60
Spleen	0.68	0.62	0.80	0.83	0.74	0.75
Kidney	0.74	0.82	0.83	0.84	0.81	0.79
Stomach	0.70	0.78	0.75	0.80	0.71	0.69
"Neck glands"	0.67	0.63	0.72	0.71	0.64	0.65
Cecum	0.82	—	0.69	0.81	0.74	0.65
Colon	0.83	0.82	0.73	0.74	0.70	0.77
Cerebrum	0.43	0.46	0.59	0.59	0.52	0.50
Esophagus	0.52	0.61	0.59	0.71	—	0.50
Tongue	0.48	0.54	0.65	0.61	0.56	0.58
Cerebellum	0.43	0.45	0.58	0.55	0.55	0.47
Lungs	0.61	0.63	0.66	0.68	0.61	0.68
Duodenum, jejunum	0.73	0.81	0.71	0.74	0.62	0.70
Ileum	0.73	0.74	0.69	0.72	0.65	0.67
Skin	0.27	0.29	0.55	0.50	0.52	0.42
Muscle	0.43	0.40	0.47	0.44	0.44	0.36
Mesentery	0.45	0.46	0.62	0.57	0.44	0.49
Heart	0.48	0.49	0.54	0.57	0.54	0.55
Erythrocytes	0.27	0.32	0.44	0.47	0.38	0.48
Spinal cord	0.32	0.26	0.30	0.21	0.35	0.31
Eyes	0.22	0.12	0.25	0.31	0.29	0.17
Costal cartilage	0.22	0.20	0.21	0.24	0.24	0.24
Fur	—	—	0.18	0.18	0.12	0.19
Mean.....	0.59	0.62	0.69	0.66	0.61	0.62

* Values equal to ACF $\times 10^4$.

Mean CO_2 concentrations in chambers. $C^{14}O_2$ concentrations identical.

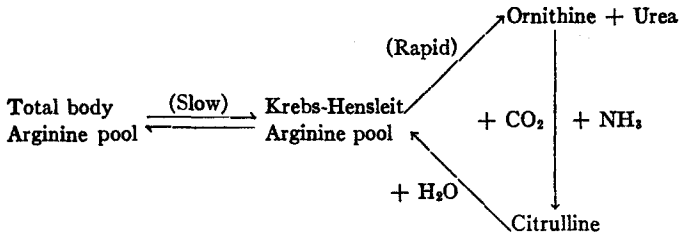
‡ 0.43 per cent.

§ 1.68 per cent.

‖ 1.15 per cent.

the air when the latter has the normal CO_2 concentration and that about 62 per cent originates from alveolar CO_2. Analysis of the pooled urine sample showed non-urea carbon s.a. to be only 21 per cent of urea s.a.

During the exposure the urea carbon activity was only two-thirds of that calculated for alveolar air, but following the exposure the s.a. of the expired CO_2 fell far below that of urea carbon. Grisolia and Cohen (15) have shown that the s.a. of citrulline formed *in vitro* in the presence of KCl-washed liver residue, ornithine, and glutamate is nearly equivalent to the s.a. of the $NaHC^{14}O_3$ added to the system. Urea produced on enzymatic degradation of citrulline also has an equivalent s.a. Furthermore, Mackenzie and du Vigneaud (17) had previously shown that when methyl-labeled methionine is fed to a rat, the s.a. of urine urea carbon is identical to that in the expired air for the following 2 days, and these investigators indicate that all urea carbon originates from the CO_2-bicarbonate system. This conclusion has recently been verified by Armstrong and Zbarsky (2) who found that urea carbon attained essentially the same s.a. as that of the expired air 24 hours after the beginning of a constant intraperitoneal injection of $Na_2C^{14}O_3$ solution, this relationship being maintained for 3.9 days. It is possible that this discrepancy may be reconciled by two seemingly reasonable postulates: The first is that there exists an appreciable concentration gradient of CO_2 between the alveoli and the tissues in which the urea is formed causing the CO_2 in the cells of these tissues to have a correspondingly lower s.a. Urea being formed would then have the lower s.a. of the intracellular CO_2. In the experiment of Mackenzie and du Vigneaud the $C^{14}O_2$ going into urea presumably was formed from methionine oxidation *in these cells*. Hence no gradient existed. In the investigation of Armstrong and Zbarsky absorption of the isotope from the peritoneal cavity would take place mainly *via* the portal circulation and this would bring it into immediate and intimate contact with the liver cells in which urea is formed. The second postulate involves the dynamics of arginine participation in the Krebs-Hensleit urea cycle as diagrammed here:



Presumably only a small amount of the total body arginine actively participates in the cycle at a given time. It is more than likely that this small "Krebs-Hensleit pool" is turning over with a larger pool of protein-bound arginine which is not participating in the cycle. If this exchange were relatively slow a short term isotope experiment would not "feed" enough labeled carbon into the large arginine pool to appreciably elevate the urea s.a. after the administration of the isotope ceased. But, during an exposure to the isotope of

long duration, such as this one, the guanidino group of the total arginine pool would probably become well saturated with isotopic carbon and would return the isotope to the urea cycle as the slow exchange continued thus elevating urea s.a. above CO_2 s.a. It is clear that additional evidence must accumulate before these uncertainties can be eliminated.

Fig. 3 shows that the uptake of fixed carbon in feces was only slightly less rapid than the uptake in urea but the s.a. attained after equilibration was much less. The data indicate that only 0.0054 per cent of total fecal carbon originates from air CO_2 or that 1.0 per cent comes from alveolar CO_2. Counts on the crude fecal fractions showed that the alcohol and water extracts contained 53 per cent of the radioactivity but only 25 per cent of the total carbon.

All the s.a. data of the tissue samples were calculated and plotted as the ACF using the normal atmospheric concentration of CO_2 (0.03 per cent) as the standard reference value.[5] The FCF may be approximated by multiplying the ACF by 183.[4] The true FCF could be determined only if the actual s.a. of intracellular CO_2 were known.

The unexpected gradual increase in the s.a. of air CO_2 during the exposure introduced certain difficulties into the interpretation of the data. Since animals were exposed at different times over the 34 day period (see Fig. 1) it became necessary to correct the s.a. values obtained on tissue analyses so that they corresponded in some manner with the s.a. of air CO_2 during the time that the particular animal was in the chamber. A somewhat arbitrary but seemingly rational method of doing this was used. The data obtained from the chamber II animals (all exposed 34 days and then sacrificed at intervals during the next 45 days) were plotted and the time required for *half* of the acquired activity to disappear from each tissue was noted. The measured s.a. of each tissue sample in the experiment was then divided by the calculated s.a. of normal air CO_2 *at the time of removal less the observed "half-time."* This tended to correct for discrepancies between tissues having large differences in the rate of isotope uptake and hence gave more consistent fitting of points than the other equally arbitrary methods tried. In cases in which this value was less than the mean air s.a. during the exposure the latter value was used. It must be emphasized, however, that application of alternative computational methods does not change the final curves greatly.

The results obtained from analyses of the defatted organs and tissues of the animals in chambers I and II are displayed in Figs. 4 to 6. The ordinate scale (logarithmic) in each graph represents the ACF computed as described above. In most of the organs and tissues analyzed the net rate of uptake of isotopic fixed carbon decreased as the time of exposure increased. In the liver

[5] The ACF for any low concentration (x per cent) of air CO_2 may be obtained by using the factor $\frac{x}{0.03}$ on the data presented.

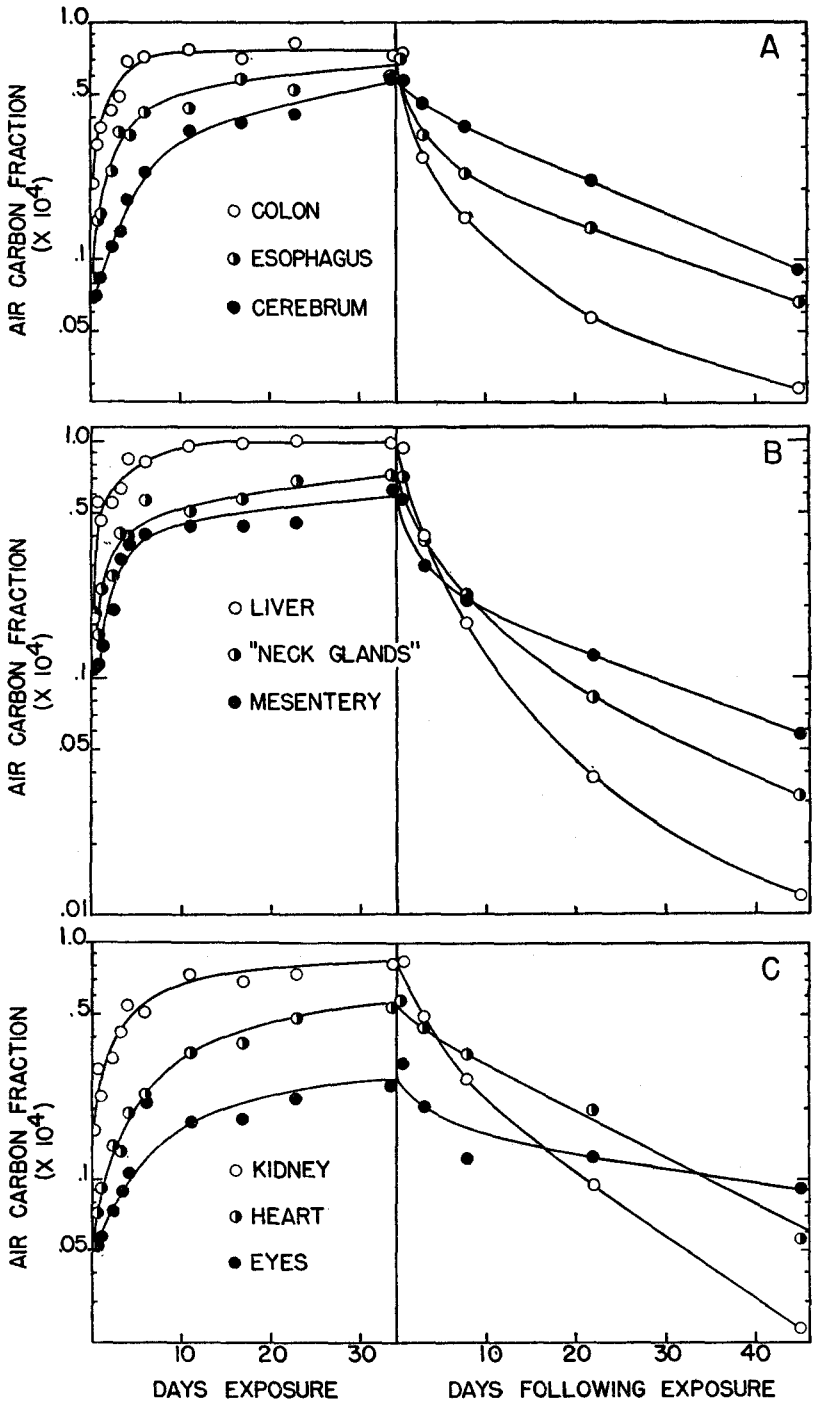


FIG. 4. Uptake and retention of fixed carbon in the tissues of adult mice.

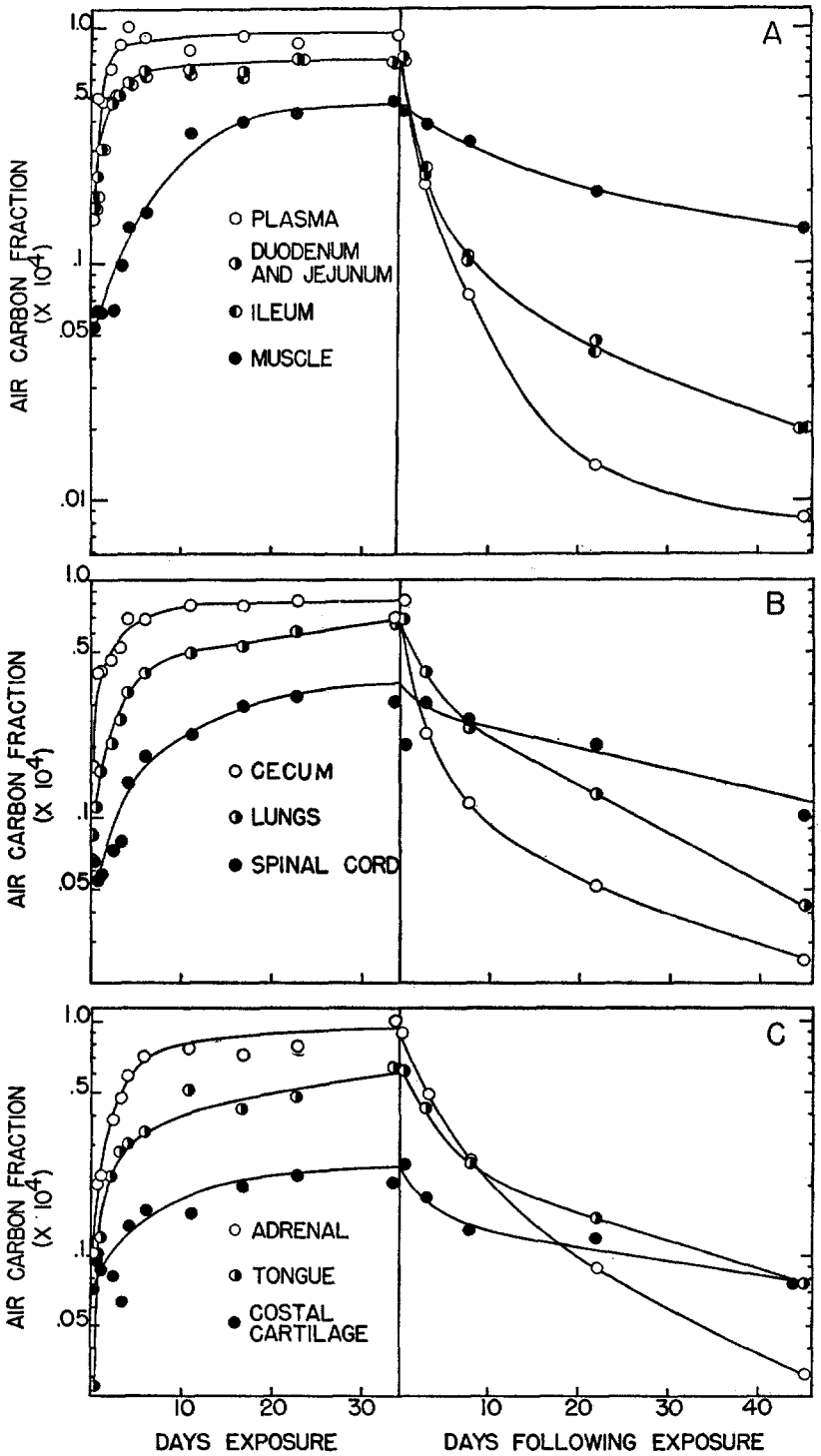


FIG. 5. Uptake and retention of fixed carbon in the tissues of adult mice.

(Fig. 4, B) the ACF had approximately reached its limiting maximum value after 16 days and in the plasma (Fig. 5, A) no significant uptake of fixed carbon was seen after 3 days. The curves for the different segments of the small and

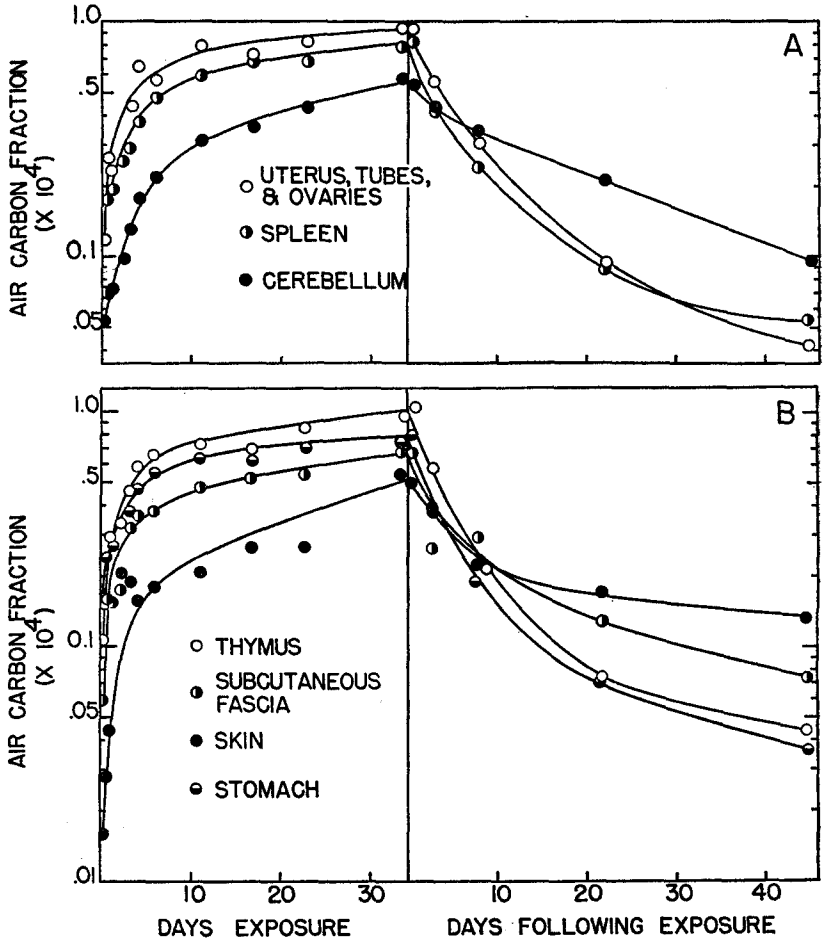


FIG. 6. Uptake and retention of fixed carbon in the tissues of adult mice.

large intestine (Fig. 4, A; 5, A–B) did not differ greatly and the ACF of each had become essentially constant after 10 days.

The fixed carbon content of most of the other organs and tissues was still increasing a measurable amount after 34 days. The different structures of the central nervous system (Fig. 4,A; 5,B; 6,A) and the skeletal (Fig. 5,A) and cardiac (Fig. 4,C) muscle gave evidence of a slower rate of turnover of fixed carbon. A comparison of the curves obtained for the cerebrum and cerebellum

with the one obtained for the spinal cord indicates that grey matter may metabolize fixed carbon more rapidly than does white matter.

With the exception of those cells and structures such as erythrocytes and hair, which are known to be wholly or partially inert from the metabolic point of view, the pattern of uptake of isotope during the exposure and retention after the exposure was qualitatively similar for all tissues. Each curve seems to be approaching or has reached a limiting value after 34 days. Following the exposure the ACF values decline at rates comparable to the rates of increase during the exposure.

If one makes the approximating assumption that all of the isotopic fixed carbon is evenly distributed throughout the total fixed carbon after 34 days, the limiting value may be estimated even in those tissues where the ACF is still increasing at this time. Using the notation

ACF_∞ = limiting value of the ACF,
a = ACF after 34 days of exposure and
b = ACF 34 days after the termination of the exposure,

it is evident that a/ACF_∞ represents the fraction of the total fixed carbon that *has been replaced* by tracer carbon during the exposure. If the fixed tracer carbon is now assumed to have the same distribution as the total fixed carbon then b/a represents the fraction of the total fixed carbon *which has not been replaced* after 34 days and $1\text{-}b/a$ represents the fraction that *has* been replaced. Thus,

$$\frac{a}{ACF_\infty} = 1 - \frac{b}{a}$$

and

$$ACF_\infty = \frac{a^2}{a - b} \qquad (1)$$

The values of ACF_∞ calculated for the various tissues by means of this equation are shown in Table III. It is certain that some bias has been introduced into these values by the approximating assumption that the total fixed carbon pool is uniformly tagged at the end of the exposure. In the tissues having components which turn over so slowly that "saturation" of them had not taken place during the exposure, loss of isotopic carbon from the rapidly turning over components of these same tissues would more than likely cause the total isotope loss to be disproportionately high during the postexposure period thus raising the value of $a - b$ and lowering ACF_∞ below its true value. It can be seen that the organs and tissues having slow rates of isotope uptake, as judged by the curves of Figs. 4 to 6, also have lower calculated values for ACF_∞. It is therefore likely that the ultimate ACF of the various tissues differs less than is indicated in Table III. The true ACF thus seems to be approximately 10^{-4} for most tissues and in no case was it found to be less than a third of

this amount. This seems to mean that only about 0.01 per cent or less of the total non-lipid organic carbon in adult mice can be derived from the CO_2 of the air when the concentration in the latter is 0.03 per cent. As will be shown below this is also true of the carbon extracted with ether.

Examination of the various curves of isotope retention after the exposure indicates considerable complexity in the turnover characteristics of fixed carbon in all tissues. None seems to have the exponential type of "biological decay" that characterizes systems in which the turnover of isotope is greatly dominated by a single homogenous component as has been demonstrated in

TABLE III

Calculated Limiting Values of ACF in Various Organs and Tissues

Organ or tissue	ACF_∞*	Organ or tissue	ACF_∞*
	$\times 10^4$		$\times 10^4$
Pancreas	1.18	Esophagus	0.76
Thymus	1.05	Tongue	0.75
Uterus, tubes, and ovaries	1.01	Cerebellum	0.75
Liver	0.98	Lungs	0.75
Adrenals	0.97	Duodenum, jejunum	0.75
Plasma	0.96	Ileum	0.73
Spleen	0.88	Skin	0.73
Subcutaneous fascia	0.86	Muscle	0.71
Kidney	0.84	Mesentery	0.69
Stomach	0.82	Heart	0.68
"Neck glands"	0.81	Erythrocytes	0.64
Cecum	0.78	Spinal cord	0.62
Colon	0.77	Eyes	0.45
Cerebrum	0.77	Costal cartilage	0.38

* Calculated from equation (1).

the turnover of water (7), sodium (12), and other substances. However, it is also evident on inspection of these curves that some organs such as heart, kidney, and brain approximate the exponential isotope excretion pattern more closely than do others such as the gastrointestinal tract and the liver. It is clear that one cannot compare turnover rates of fixed carbon in various organs except in a very arbitrary manner until much more information is at hand concerning the details of the dynamic processes involved. But since even an arbitrary comparison is of some interest one has been made in Table IV. Here the tissues are listed in order of their apparent "biologic half-times" for fixed carbon between 0 to 3 days and again between 23 to 45 days as computed from the postexposure data.

The data obtained on erythrocytes are shown in Fig. 7, the ordinate scale being linear in this case. The ACF of these cells increased at a constant rate

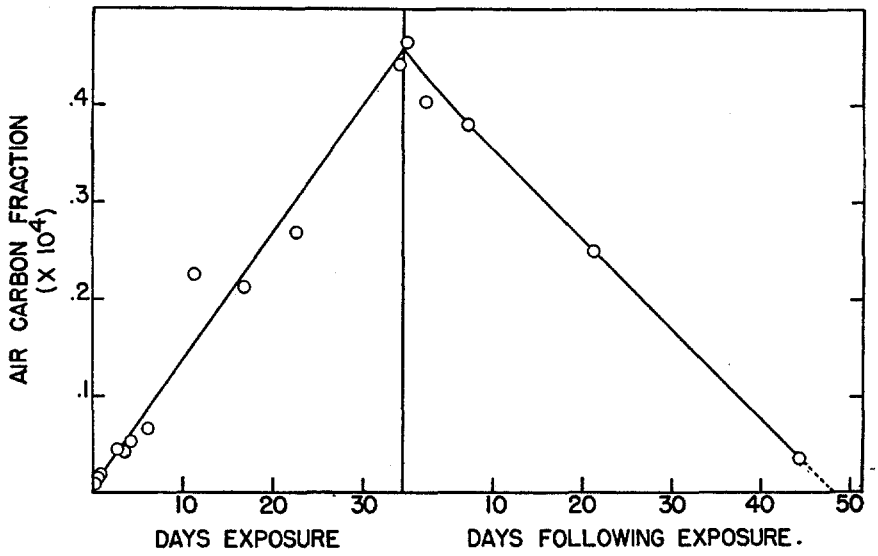


FIG. 7. Uptake and retention of fixed carbon in mouse erythrocytes.

TABLE IV

Relative Turnover Times for Fixed Carbon in Representative Tissues

Organ or tissue	0 to 3 days* "Half-time"	Organ or tissue	20 to 45 days* "Half-time"
	days		*days*
Plasma	1.5	Kidney	11.5
Ileum	1.5	Heart	12.7
Duodenum, jejunum	1.8	Liver	13.8
Colon	2.1	Lungs	15.0
Cecum	2.1	Adrenals	15.2
Liver	2.4	"Neck glands"	16.7
Stomach	2.9	Pancreas	17.0
Adrenals	3.1	Cerebrum	18.2
Spleen	3.1	Ileum	18.6
"Neck glands"	3.1	Cerebellum	19.6
Esophagus	3.1	Uterus, tubes, ovaries	20.5
Thymus	3.7	Duodenum, jejunum	21.0
Uterus, tubes, ovaries	3.8	Esophagus	22.0
Kidney	3.9	Stomach	22.5
Pancreas	4.3	Cecum	22.5
Lungs	4.4	Colon	23.3
Tongue	5.3	Tongue	25.4
Skin	7.1	Thymus	29.5
Cerebellum	7.5	Spleen	31.5
Heart	8.8	Plasma	31.6
Cerebellum	9.0	Muscle	46.0
Costal cartilage	9.0	Costal cartilage	50.0
Muscle	13.5	Skin	63.0

* Calculated from postexposure data.

during the exposure and then decreased constantly after the exposure, indicating that no appreciable turnover of fixed carbon takes place in these cells once they are formed. It also means that old red cell components which contain

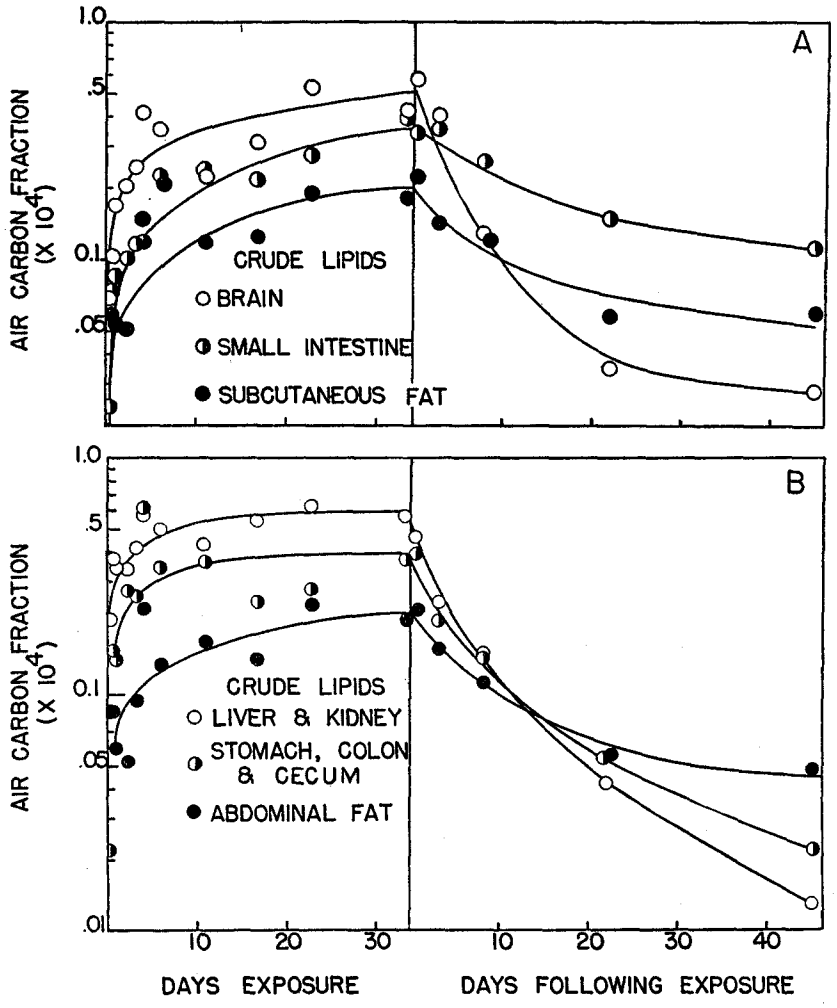


FIG. 8. Uptake and retention of fixed carbon in crude lipid fractions of various organs and tissues of adult mice.

fixed carbon are not reincorporated into new cells without first undergoing great dilution. Since the postexposure curve intersects the abscissa at 49 days this length of time may be considered to represent the life span of the mouse erythrocyte, which is of considerably shorter duration than has been found for the human erythrocyte using an analogous technique (22).

The s.a. of the growing mammary carcinoma of the chamber III mouse was found to be 96 per cent of the value found in the liver of the same animal.

The results obtained on the ether-extractable material of some of these tissues are shown in Fig. 8. The rather large variation of some individual values from the curves can be quite closely correlated with the mass of adipose tissue found on dissection. In animals which had small depots of fatty tissue both subcutaneously and intraabdominally the values were generally much higher than those found in animals with larger depots. It is interesting that this correlation was reflected in organs such as brain, liver, and kidney in which the lipid content is relatively small and not closely related to the size of the storage depots. In organs in which the fixed carbon turnover of the defatted tissue was rapid the lipid turnover also tended to be rapid but in no case except brain was the lipid turnover as rapid as it was in the lipid-free counterpart. Also in no case except brain did the ACF of the lipids become as high as that of the lipid-free fraction. In the brain the initial rate of incorporation of fixed carbon is considerably higher in the lipid fraction than in the remainder of this tissue. Because of the large spread in individual values an estimate of the ultimate ACF for lipids would be quite unreliable. The curves suggest that these values would probably be lower than 10^{-4} but would certainly not differ by more than a factor of 3 or 4 from this amount.

DISCUSSION

The data of this experiment strongly indicate that the distribution of total fixed carbon is quite uniform between the various organs and tissues and their crude fractions. Certain compounds and certain carbon positions in these compounds have been repeatedly shown to become heavily labeled when isotopic CO_2 is administered, but the distribution of these substances seems to be such that no appreciable concentration of radioactivity appears in any single location.

Analyses not included in this report show this to be also true of the organic substances of bone.[6] However, the inorganic carbon of bone has been shown to attain a s.a. which is considerably greater than that attained by the organic fraction 6 days after the implantation of a pellet of $CaC^{14}O_3$ into the peritoneal cavity of a rat (1) and again after 5 days of continuous intraperitoneal injection of $Na_2C^{14}O_3$ (2). It may be considered certain that the s.a. of bone carbonate cannot exceed that of the alveolar CO_2 when the only isotope source is the inhalation of this gas. Since osseous tissue contains about 20 times as much

[6] The data obtained on the bones of these animals were incomplete since much of the bone carbonate activity had exchanged with air CO_2 or was otherwise lost during the preparation of samples. Before the results at hand concerning the organic fractions of bone are reported it is intended to supplement these data with a similar experiment designed to measure the turnover characteristics of bone carbonate.

organic carbon as it does inorganic carbon as calculated from the composition of bone (5) it may be further computed that if the s.a. of bone carbonate reaches the level of alveolar CO_2 the s.a. of total bone carbon will be increased about 3.5-fold.

On the basis of this accumulated information it is now possible to arrive at a reasonable permissible level of $C^{14}O_2$ in air *for mice.* A radiation dose of 0.3 rep per week[7] (the present accepted maximum tolerable dose) will be received by any tissue containing 0.015 μc. of C^{14} per gm. of tissue. In a survey of permissible levels of radioiostopes Perry (18) has selected for each isotope a "standard critical organ" this being the site where the element in question is present in the greatest concentration. Adipose tissue which in the living state may contain as much as 50 to 65 per cent of its mass as carbon was selected as the "standard critical organ" for C^{14}. In view of the possibility that bone, despite its lower carbon content (*ca.* 16 per cent), may attain an ACF which is 3.5 times as great as other tissues it must also be considered as the possible critical organ. Assuming that the ultimate ACF of fat may be as high as 10^{-4}, mice would need to be continuously exposed to air having 215 μc. per gm. of carbon or 31 μc. per c.m. in order to reach the maximum permissible C^{14} level. Assuming that the ACF of bone may reach 3.5×10^{-4} the air level would need to be 57 μc. per c.m., so adipose tissue may indeed be the critical organ. The value obtained is thus approximately 70 times more lenient than the value, 0.45 μc. per c.m., derived by Perry (18) as the tolerable level in air for the human on the basis of continuous exposure.

SUMMARY

1. Mice were continuously exposed to air containing $C^{14}O_2$. The specific radioactivities of urea carbon, total fecal carbon, and numerous components of tissue carbon were compared as a function of the duration of exposure with the radioactivity of the air CO_2.

2. The data indicate that the total CO_2 fixed from the air is proportional to its concentration in the air.

3. When the CO_2 concentration in the air is normal (0.03 per cent) about 0.34 per cent of the carbon of urea originates from air CO_2. A lesser proportion of the non-urea carbon of urine has its origin from air CO_2.

4. Only about 0.0054 per cent of the total fecal carbon is derived from air when the CO_2 concentration is 0.03 per cent. The constituents, which are extractable with alcohol and water, contain considerably higher proportions of fixed carbon than either the insoluble residue or the ether-extractable material.

5. The rates of uptake at the beginning of the exposure and the rates of

[7] 1 rep = 83 ergs of radiant energy absorbed per gram of tissue.

loss at the termination of the exposure differed strikingly among the tissues studied.

6. However, the ultimate ratio of fixed air CO_2 carbon to total carbon in these tissues seemed to be approaching limiting values which would not vary by more than a factor of 3 from one another. It appears that of the total organic carbon in an adult mouse approximately 0.01 per cent may originate directly from the CO_2 of the air when the animal respires in air having a CO_2 concentration of 0.03 per cent, and that 1.8 per cent or more of the total carbon may originate from CO_2 within the animal.

7. Data are presented which indicate the life span of the mouse erythrocyte to be 49 days.

8. Calculations made on the basis of these experimental data and the accepted standard for permissible radiation in the human would allow mice to be continuously exposed to air containing 31 μc. per c.m. without ever exceeding the accepted permissible level for humans.

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