the first hour after administration. Later, the excretion is slowed down progressively, and after 12 hr about 85% of the dose is excreted as $C^{14}O_2$. In these animals no significant activity is found in the tissues collected and the same observations have been made for the excreta collected during the experiment. About 88%–99% of the total activity was recovered. Since the amount of residual C^{14} in the body at the time of sacrifice was very small, failure to find measurable amounts of C^{14} in the tissues is not to be interpreted as evidence that none was there.

The injections of barium carbonate produced an increase of the total quantity of CO_2 expired by the mice as shown in Fig. 2. The total CO_2 expired becomes three to five times higher than the normal value, which is reached only several hours after injection.

The half-life of retention by the body can be estimated from a plot of the data on semilogarithmic paper in which the percentages of the injected dose remaining in the animal are plotted on the logarithmic axis and time on the regular axis. As shown in Fig. 3, it is possible

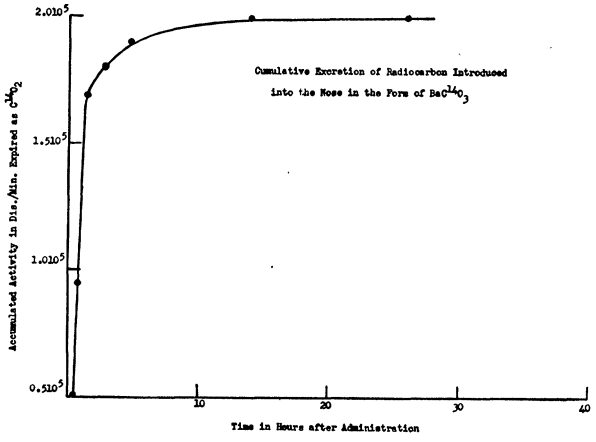

FIG. 4.

to obtain curves analogous to radioactive decay curves. The curves obtained present two processes corresponding to two biological half-lives of 1 hr and 9 hr, respectively. We observe first a rapid excretion of the injected C^{14}, followed by progressively slower excretion. The biological half-lives reported here apply only to the particular experimental conditions described in this paper.

In order to obtain more information about the mechanism of excretion of radiocarbon by the lungs, some experiments were performed by injecting mice intravenously with sodium carbonate containing C^{14}. The curves representing the cumulative excretion of C^{14} in the form of $C^{14}O_2$ are very similar to those obtained with barium carbonate injections. About 70% of the injected dose is excreted during the first 3 hr following administration. After that time, negligible amounts of radiocarbon are expired by the lungs. If the data are plotted on semilogarithmic paper, only one biological half-life of about 1 hr is obtained; most of the C^{14} is expired following a half-life of 1 hr. Comparing these results with those obtained with barium carbonate injections, we find again the rapid excretion by the lungs of C^{14} as $C^{14}O_2$. As with sodium carbonate injections, no detectable activity could be found in the liver, spleen, lungs, kidney, and excreta. The fact that in these experiments all the radiocarbon was not recovered can be explained by a loss of material during the injections of the small amounts of solutions.

Fig. 4 represents the typical data obtained when C^{14} is introduced into the nose in the form of $BaC^{14}O_3$. The results are expressed as accumulated activity in disintegrations per min expired as CO_2. Because experimental difficulties were encountered, it is rather difficult to know the exact total dose given to the animal. However, an estimation can be made indicating that up to 70% of the administered dose is excreted within the first 4 hr following administration.

From these data we may conclude that most of the C^{14} absorbed by the organism as barium carbonate is expired by the lungs as $C^{14}O_2$, and that the dangers to those who work with C^{14} are not as great as expected.

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Studies on the Metabolism of Administered Cytochrome C by the Aid of Iron-labeled Cytochrome[1]

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This journal repeatedly has been the site of discussion of the therapeutic and prophylactic value of cytochrome C injections (*3, 6, 8–10, 13*). It may, therefore, be of interest to report here some recent findings on the metabolism of administered cytochrome C as traced by the fate of its constituent iron. The production of Fe^{55}-labeled cytochrome C has been reported previously (*1*). The purity of this preparation based on spectrophotometric analysis and protein dry weight determination was 73%; noncytochrome iron present was less than 2.5% of cytochrome iron. Two male albino rats were injected intravenously with this preparation and exposed immediately thereafter to a simulated altitude of 20,000 ft. Twenty-four hours after injection about 10 ml of blood was drawn by heart puncture, and the animal was decapitated. Cytochrome was isolated from kidney, liver, spleen, and heart, according to Rosenthal and Drabkin (*12*), and from muscle by a procedure similar to that of Keilin and Hartree (*5*). A fractionation for ferritin, according to Granick (*4*), was carried out on portions of the livers and on the kidneys of one of the animals. Fractions obtained and organs were treated according to Peacock

[1] The radioiron used in this investigation was supplied by Carbide and Carbon Chemicals Corporation, Oak Ridge, Tennessee, on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

et al. (7), Vosburgh, or Flexner, and Cowie (*14*) in the case of larger samples. The results are summarized in Table 1. It seems from the recovery obtained that no major site of radioactivity was missed. The lower recovery in the first rat is due to less complete analysis of fractions and organs.

The distribution of radioactivity suggests that injected cytochrome C is very rapidly disposed of by the body. None of the administered material is found intact with certainty after 24 hr. A large portion is spilled in the urine. It was only in the first 2-hr urine fraction of rat 2 that cytochrome could be found spectrophotometrically. All radioactivity in this fraction, 38.4% of the injected dose, could be accounted for by the cytochrome found. The radioactivity dialyzing from the urine was very little and may be due to cytochrome which passed the dialyzing membrane. The kidney is the principal site of the radioactive material remaining in the body. Although considerable radioactivity appears in the ferritin fraction (5.8%), the bulk is associated with the residue. The disposal of this material may have taken place along pathways indicated by the work of Rather (*11*) on hemoglobin injections. The small amounts of radioactivity following the isolated cytochrome are very likely due to contamination by breakdown products rather than true admixture of intact radioactive cytochrome.[2]

Alternate pathways of disposal of the administered material might be pointed out by the fact that rat 1, which did not immediately excrete as much of this material in the urine as rat 2, had considerably more radioiron in liver, liver ferritin, muscle, and bone marrow. The high activity found in muscle and skin is surprising. Although precautions were taken, the possibility of contamination in skin and feces samples could not be excluded. Blood trapped in the organs, however, could not cause a serious error after heart puncture and decapitation.

In addition to the rapid appearance of radioiron in the ferritin fractions, the fact that plasma contained only one-tenth the radioactivity of red cells may be considered the most convincing evidence for the rapid breakdown of injected cytochrome. Assuming 10 mg of hemoglobin iron in rat 2 and an average life of 50 days for rat hemoglobin, 200 μg of iron is needed for hemoglobin resynthesis in 24 hr. According to the radioactivity found in the red cells, 0.24% thereof has already been made available from the breakdown of injected cytochrome.

Considering the almost complete absence of radioactivity in the cytochrome fractions isolated from the organs, which carry the main part of respiratory metabolism, the complete absence in brain, and the minimal amount found in heart, the beneficial action of cytochrome administration, as repeatedly reported by clinical workers, cannot be due to the known catalytic function of cytochrome in cellular metabolism and hardly to any other property of the intact cytochrome molecule itself, unless a profound species difference would exist.

[2] Interference from radioactive breakdown products of the injected material is excluded if unlabeled cytochrome is given to rats which have labeled endogenous cytochrome. Experiments of this type gave no evidence of incorporation of the injected cytochrome (*2*).

TABLE 1

RADIOACTIVITY RECOVERED 24 HOURS AFTER INJECTION OF Fe-LABELED CYTOCHROME IN MALE RATS

Recovered from	Rat 1 (body wt 322 g, injected with 3.9 mg cytochrome, 6610 cpm ± 3%*) % of injected dose	Rat 2 (body wt 288 g, injected with 4.6 mg cytochrome, 11476 cpm ± 3%*) % of injected dose
Kidney, total	36.6	35.2
cytochrome	0.10	0.56
Liver, total	12.1	5.9
cytochrome	0.12	< 0.03
Spleen, total	1.0	0.50
cytochrome	< 0.03	0.03
Heart, total	0.12	0.15
cytochrome	< 0.03	< 0.03
Muscle, total	9.3	4.7
cytochrome	< 0.03	< 0.03
Brain	< 0.03	< 0.02
Lung	0.18	0.14
Gonads	0.19	0.38
Thyroid, adrenals, thymus		0.29
Bone	2.6	1.7
Skin		4.7
Blood, whole	2.6	
red cells		2.4
plasma		0.2
Stomach	0.20	0.17
Stomach content		0.10
Intestines, total	1.51	
small		0.36
large		1.26
Intestine content	0.56	0.40
Abdominal cysts		0.49
Feces	1.8	1.5
Urine	18.4	39.4
Total recovery,† %	87.1	99.9

* Geiger tubes of different efficiency were used in the two analytical series.

† The values are corrected for losses which could be defined. The actual uncorrected recoveries are 80% and 93% for rats 1 and 2, respectively.

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