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Distribution of Radioactive Carbon Administered as Carbonate in the Body and Excreta of Mature Rat.*

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Several studies have demonstrated the assimilation of carbon-dioxide by heterotropic organisms and mammalian tissue slices.[1] The intact animal also incorporates the carbon of carbon-dioxide as shown in experiments which utilized either the heavy (C^{13}) isotope,[2,3,4] or the short-lived radioactive (C^{11}) isotope[5] as tracers. Investigations with the intact animal have been limited by the difficulty of detecting C^{13} in high dilution or because of the rapid decay of C^{11}. It was found that the carbon of carbon-dioxide is incorporated in the liver glycogen,[3,5] bone,[4,5] urea,[2,4] and in certain amino acids of liver protein[4] of the rat. Further evidence for the uptake of the carbon of carbon-dioxide in bone was afforded[6] by radioautographs in experiments with rats using the long-lived C^{14} as a tracer. The radioautographs also indicated that C^{14} was present in the liver and kidney for periods up to 2 weeks.[6] We have investigated in further detail the quantitative distribution of C^{14} from carbon-dioxide in the intact animal and in the excreta. Because of the higher sensitivity of the methods for the determination of C^{14} over those of C^{13}, when the isotopes are present in high dilution, it has been possible to compare the assimilation of the labeled carbon in a greater number of tissues than has previously been possible. In this study the tracer isotope has been given parenterally as $Na_2C^{14}O_3$ and, as a means of prolonging the period of high C^{14} activity of the body fluids, as $CaC^{14}O_3$.

Experimental. Rat I—A male rat, weighing 624 g was given an intraperitoneal injection of 3.74 ml of a 0.043 molar solution of $Na_2C^{14}O_3$ (prepared by a method similar in principle to that of Allen, Gest, and Kamen)[7] and immediately placed in an apparatus (Fig. 1) which allowed the continuous collection of the exhaled carbon-dioxide and the separate collection of the urine and the feces for prolonged periods. At the end of 96, 120 and 144 hours additional 1.0 ml amounts of the $Na_2C^{14}O_3$ solution were administered by intraperitoneal injection. Each ml of the $Na_2C^{14}O_3$ solution contained about 0.1 millicurie C^{14} (2.05×10^6 counts per minute under our conditions of counting C^{14}). The collection of expired CO_2 was complete as indicated from experiments in which a known amount of $Na_2C^{14}O_3$ was placed in the apparatus and decomposed with acid with a resulting average recovery from 5 trials of 100 ± 2% of the theoretical $C^{14}O_2$. The diet, supplied without opening the apparatus, consisted of whole milk saturated with dextrose. The rat was accidentally killed by suffocation at about the 160th hour when its weight was 617 g.

Rat II—A male rat weighing 473 g was given about 0.40 millicurie of C^{14} (8.3×10^6 counts per minute) by implanting, through an abdominal incision, a pellet of $CaC^{14}O_3$

* This study was supported by a grant from the Research Grants Division of the U. S. Public Health Service. Some of the work described in this paper is to be submitted by Arthur Lindenbaum to the Graduate School of the University of Minnesota as a part of a thesis in partial fulfillment of the requirements for the Ph.D. Degree.

1 Wood, H. G., *Physiol. Rev.*, 1946, **26**, 198.

2 Swendseid, M. E., Ph.D. Thesis, Graduate School, University of Minnesota, 1941.

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5 Solomon, A. K., Vennesland, B., Klemperer, F. W., Buchanan, J. M., and Hastings, A. B., *J. Biol. Chem.*, 1941, **140**, 171.

6 Bloom, W., Curtis, H. H., and McLean, F. C., *Science*, 1947, **105**, 45.

7 Allen, M. B., Gest, H., and Kamen, M. D., *Arch. Biochem.*, 1947, **14**, 335.

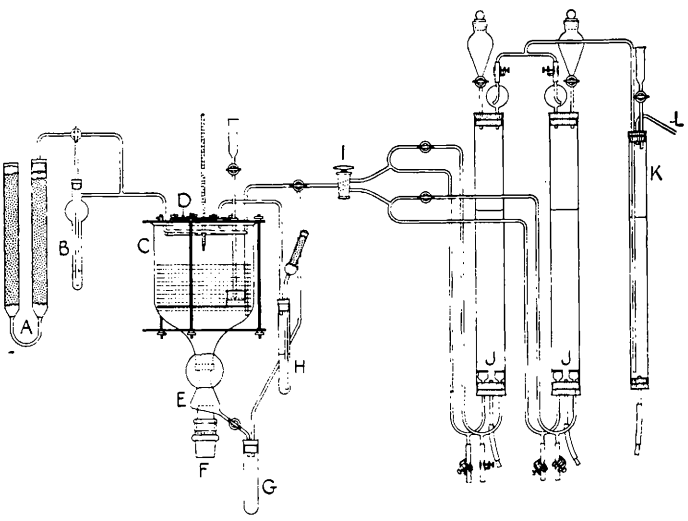

FIG. 1.

Apparatus for collection of expired carbon-dioxide and excreta of a rat. A, soda-lime tubes in air intake; B, bubble rate indicator which can be by-passed through 3-way stopcock shown; C, cage made from 10-liter bottle with bottom replaced with brass plate held as shown and sealed to glass with dentist's carding wax. Turbulent flow of gas in cage produced by air entering cage through multiple pin holes, directed upwards, in spiral tube near top of cage. Wire basket for support of animal covered with baked-on Glyptal varnish; D, port; E, urine and feces separator anchored to cage with plaster-of-paris; F, feces receptable; G, urine receptacle; H, auxiliary air intake; I, stopcock to direct exhaust air to one or the other CO_2 absorber; J, sintered glass disks in columns containing one liter 10% sodium hydroxide; K, trap; L, to suction.

TABLE I.

Comparison of C^{14} Assay by Direct Count of Sample with that Obtained by Wet Combustion and Subsequent Counting of $BaC^{14}O_3$.

Rat No.	Sample	Specific activity† × 10^6	
		Direct count	$BaCO_3$ from wet comb.
I	Glycerol	2.76 ± .088	2.81 ± .25
"	Feces (0-24 hr)	136 ± 1.1*	131 ± 2.6*
"	" (24-48 hr)	14.9 ± .16*	13.2 ± .81*
"	" (48-72 ")	4.5 ± .23*	5.3 ± .39*
"	Defatted bone	19.5 ± .36	20.2 ± .89
"	Incisor dentin	34.0 ± .091	34.8 ± 1.1
II	Liver fatty acids	9.54 ± .15	10.8 ± 1.1
"	Protein—spleen	34.1 ± .34	34.0 ± .74
"	" —kidney	30.8 ± .33	28.0 ± .80
"	" —lung	21.7 ± .21	20.0 ± .80
"	" —heart	12.1 ± .39	11.2 ± 1.1
"	Liver glycogen	24.9 ± .28	26.4 ± .93
"	Plasma globulin	60.6 ± .65	61.8 ± 1.2

† % of injected dose per mg carbon.

* These results are given as the percentage of the total injected dose × 10^3.

in each of the lower quadrants of the peritoneal cavity. The pellets weighed 86.6 mg and 118.3 mg respectively, and were prepared from a mixture of 246 mg $CaC^{14}O_3$ and 10 mg gelatin suspended in warm distilled water. After the excess water was removed by slow evaporation, the $CaC^{14}O_3$-gelatin mixture was molded into pellets by manual manipulation

TABLE II.
Excretion of C^{14} by Rat I After Intraperitoneal Injections of C^{14} as Sodium Carbonate.

Injection No.	Time elapsed after first injection	% injected dose excreted			Specific activity × 10⁶†	
		Expired air*	Urine	Feces	Expired Air	Urine
1	0-1 hr	70.8	—	—	6.12 × 10⁵	—
"	1-3 "	21.4	—	—	22,100	—
"	3-6 "	1.5	—	—	2,080	—
"	6-12 "	0.68	—	—	405	—
"	12-24 "	0.54	—	—	175	—
"	0-24 "	94.65	0.45	0.31	15,400	2,710
"	24-48 "	0.77	0.018	0.014	82.5	140
"	48-72 "	0.21	0.011	0.0070	33.6	103
"	72-96 "	0.13	0.0072	0.024	21.0	67.6
"	96 hr total	96.0	0.49	0.36	3,450	980
2	96-120 " "	103‡	0.090‡	0.15‡	17,800‡	536‡
3	120-144 " "	98.1‡	0.21‡	0.14‡	15,500‡	197‡
4	144-168 " "	91.2‡	0.071‡	0.26‡	25,800‡	518‡
—	0-168 " "	96.8	0.33	0.28	2,230	362

* The rat exhaled 560 ± 80 mM of CO_2 each 24 hr.
† Per cent of injected dose per milligram of carbon.
‡ These results represent the excretions of the C^{14} given in injections 2, 3, and 4 occurring during 24-hour periods following each administration. No correction was applied for the residual excretions of C^{14} from the previous injections.

TABLE III.
Excretion of C^{14} by Mature Rat II After Implantation of $CaC^{14}O_3$ in the Peritoneal Cavity.

Time elapsed after administration	% injected dose excreted			Specific activity* × 10⁶		Relative specific activity (Long bones = 100)	
	Expired air	Urine	Feces	Expired air	Urine	Expired air	Urine
2-21 hr	1.09	.0052	.00	255	39.0	480	73
21-45 "	3.77	.0156	.0047	580	88.5	1,100	170
45-69 "	9.42	.0414	.0052	1,380	212	2,600	400
69-93 "	15.4	.0595	.0150	2,190	283	4,100	530
93-117 "	20.8	.0802	.0253	2,830	374	5,300	700
117-141 "	20.9	.0666	.0165	2,820	388	5,300	730
141-142 "	0.80	—	—	2,650	—	5,000	—

* Per cent of injected dose per mg carbon.

while wearing rubber gloves and a surgeon's mask. The pellets were then dried in an oven. Collections of excreta and expired air were made, beginning 2 hours after the operation, over the time intervals indicated in Table III. Six days after the implantation of the pellets the animal was sacrificed by slitting its throat. A roentgenogram made at this time showed only a faint image of the disintegrated pellets. The weight of the animal just before being sacrificed was 474 g.

The blood was collected in a centrifuge tube containing heparin. Hemin was prepared by the method of Shemin and Rittenberg.[8] The solution remaining from the hemin isolation was treated with 10% sodium tungstate. The precipitate, which is presumed to consist mainly of globin, was washed with water, dried and pulverized. Albumin and globulin were isolated from the plasma by the method of Koch[9] and each protein was washed with trichloracetic acid solution.

The isolation and purification of liver glycogen, after removal of a sample of the organ for total C^{14} determination, and of muscle glycogen was carried out by the method of Stetten and Boxer.[10] Myosin was prepared from skeletal muscle using the pro-

8 Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 1946, **166**, 621.

9 Koch, F. C., *Practical Methods in Biochemistry*, William Wood, Baltimore, Second Edition, 1937, p. 53.

10 Stetten, G. E., and Boxer, D., Jr., *J. Biol. Chem.*, 1944, **155**, 231.

TABLE IV.
Distribution of C^{14} in the Tissues and Their Components in Rat II After Implantation of $CaC^{14}O_3$ in the Peritoneal Cavity.

Sample description	% of total administered dose, × 10^3	Specific activity† (SA), × 10^6	Relative (SA) (long bones = 100)
Long bones (less marrow)	22.0	53.1 ± .72	100
Short bones	68.0	49.9 ± .54	94
Inorganic CO_2 (long bones)	—	428 ± 2.5	810
Inorganic CO_2 (short bones)	—	408 ± 2.4	770
Long bone protein	—	10.6 ± .64	20
Short bone protein	—	12.1 ± .21	23
Long bone marrow	—	52.0 ± 1.3	98
Molar teeth	0.37 ± .010	—	—
Incisor teeth	1.30 ± .032	—	—
Molar dentine	—	28.7 ± .81	54
Incisor dentine	—	28.1 ± .53	53
Inorganic CO_2 of incisor dentine	—	474 ± 12	890
Incisor enamel	—	54.2 ± 4.0	102
Whole blood*	11.2	16.8 ± 1.1	32
Hemin	—	3.1 ± .15	5.8
Red blood cell protein	—	4.06 ± .27	7.6
Plasma albumin	—	74.1 ± .48	140
Plasma globulin	—	60.6 ± .65	110
Liver (intact)	365	48.7 ± .54	92
Liver glycogen	—	24.9 ± .28	47
Liver fatty acids	—	9.54 ± .15	18
Muscle protein	62.9	6.93 ± .16	13
Muscle glycogen	—	6.8 ± .9	13
Myosin from muscle	—	5.47 ± .17	10
Body fat	30.5	1.73 ± .11	3.3
Fatty acids from body fat	—	1.27 ± .090	2.4
Glycerol from body fat	—	10.1 ± .20	19
Fat from viscera	—	2.13 ± .068	4.0
Fat from bones and teeth	—	1.70 ± .055	3.2
Protein—G.I. tract	25.3	37.1 ± .41	70
" —spleen	2.64	34.1 ± .34	64
" —testicles	3.97	27.5 ± .26	52
" —kidney	4.79	30.8 ± .33	58
" —lungs	3.28	21.7 ± .17	41
" —heart	1.72	12.1 ± .39	23
" —brain + spinal cord	—	6.39 ± .17	12
Brain + spinal cord (intact)	1.50	5.24 ± .15	9.8
Pelt	130	2.20 ± .22	4.1
Debris + pellet remains	34,600	—	—

* The hemoglobin concentration was 14.9%.
† Per cent of injected dose per mg carbon.

cedure of Greenstein and Edsall.[11] The precipitate of myosin was extracted with alcohol and ether before being dried at 110°C.

Large samples of body and mesenteric fat were obtained by dissection and were separately processed. After the fats had been separated from non-lipid material by extraction in a Soxhlet apparatus with an alcohol-ether mixture they were dissolved in ether and washed several times with 5% $NaHCO_3$ solution and with water. The ether solutions were dried by the addition of anhydrous Na_2SO_4. After several hours the solutions were filtered and the ether removed by evaporation.

Aliquots of the fat samples were saponified with 40% NaOH and the resulting fatty acids and glycerol separated.[12] The fatty acids were freed of cholesterol and other non-saponifiable ether soluble substances by extracting a solution of the fatty acids in dilute

[11] Greenstein. J. P., and Edsall, J. T., *J. Biol Chem.*, 1940, **133**, 397.

[12] Koch, F. C., *Practical Methods in Biochemistry*, William Wood, Baltimore, Second Edition, 1937, pp. 25-26.

NaOH with ether. The free fatty acids resulting from acidification of the NaOH solution were washed 4 times with boiling distilled water, dissolved in ether and treated with active charcoal. The solution was filtered, dried with anhydrous Na_2SO_4 and the ether removed as previously described. A sample of liver fatty acids was prepared in the same manner from an ether extract of the acidified residue of this organ remaining from the isolation of the liver glycogen. The glycerol fractions were further purified as follows: An aqueous solution was extracted with ether, treated with active charcoal, filtered and concentrated on a hot plate. The resulting glycerol was water clear.

The proteins from each of several of the visceral organs, the testes and the central nervous system were prepared by suspending the pulverized, fat-free organ in 40% trichloracetic acid, centrifuging and subsequently extracting the precipitates with 10% trichloracetic acid. The precipitates were washed with water, alcohol and ether.

After skinning, the carcass was boiled in slightly alkaline water, permitting the bones and teeth and the skeletal muscles to be isolated. The bones and the teeth were dried at 110°C and extracted in a Soxhlet apparatus for 72 hours with an ether-alcohol mixture and dried at 110°C. The skeleton was divided into the *long* bones which consisted of the humeri, femora, tibiae, fibulae, radii and ulnae, and the *short* bones which comprised the rest of the skeleton. The marrow was removed from the long bones after they had been rendered fat-free and both fractions of the skeleton were finely pulverized. The preparations of bone protein consisted of the insoluble material obtained by digesting a part of each of the skeleton fractions in 40% trichloracetic acid, centrifuging, and subsequent washing of the precipitates with 10% trichloracetic acid, hydrochloric acid, water, alcohol, acetone and ether. The enamel and dentin of the molar and incisor teeth were separated[13] after the activity count of the whole teeth in the pulverized state had been made.

The pelt was digested with hot 20% sodium hydroxide, homogenized in a Waring blendor, evaporated to dryness and pulverized. All solutions resulting from processing the tissues, including the liver, and the organs and tissues not mentioned above were pooled to give a sample labeled "Debris." This mixture was evaporated to dryness and pulverized.

While dissecting the rat several small particles which apparently consisted of unabsorbed $CaC^{14}O_3$ were found. Their C^{14} content, present as inorganic carbon, was found to be 12.9% of the total administered dose. The fat-free residue from the mesenteric fat depot contained an amount of C^{14}, as inorganic carbon, which accounted for about 20% of the total dose of C^{14}. The fact that approximately 30% of the administered $CaC^{14}O_3$ remained unabsorbed does not alter the values for the relative specific activity of the samples given in Tables III and IV.

Rat III—About 145 mg of powdered $CaC^{14}O_3$ (0.065 millicurie or 1.3×10^6 counts per minute) were distributed over the peritoneal viscera of a 422 g male rat. Collections of expired air were made at intervals over a period of 40 days following the implantation of the $CaC^{14}O_3$. After sacrifice of the animal the carcass, less pelt, was ground in a meat chopper and homogenized with a small quantity of water in a Waring blendor, dried at 110°C and pulverized. The pelt was prepared for activity counts as previously described.

Method of Assay for C^{14} and Total Carbon Content. All samples were assayed for C^{14} using the technic described by Armstrong and Schubert.[14] When required the samples were subjected to wet combustion by adapting the method of Van Slyke and Folch[15] to a simple and rapid gravimetric procedure.[16] The evolved CO_2 was collected in a saturated

[13] Manly, R. S., and Hodge, H. C., *J. Dental Research*, 1939, **18**, 133.

[14] Armstrong, W. D., and Schubert, J., *Anal. Chem.*, 1948, **20**, 270.

[15] Van Slyke, D. D., and Folch, J., *J. Biol. Chem.*, 1940, **136**, 509.

[16] Lindenbaum, A., Schubert, J., and Armstrong, W. D., *Anal. Chem.*, in press.

solution of barium hydroxide and the resulting precipitate of $BaC^{14}O_3$ was washed and transferred to the brass filter dishes for weighing and counting. All samples of the $BaC^{14}O_3$ were stored in a desiccator until counted to prevent exchange with atmospheric carbon-dioxide.[17] The inorganic carbon of bone, dentin and enamel was determined and prepared for counting by collecting, in barium hydroxide, the CO_2 evolved from treatment of an aliquot of the pulverized material with 3 M perchloric acid.

Several materials were counted directly as thick samples without combustion. Some of the samples which were counted directly were also oxidized, as described above, and the $C^{14}O_2$ collected and counted as $BaC^{14}O_3$. The agreement between the direct counts and the counts of $BaC^{14}O_3$ was good (Table I). When samples contained a higher percentage of carbon than is present in $BaCO_3$ it was advantageous to make a direct count because the activity was proportionately greater.†

Preparation of Radioautographs. A fat-free humerus and lower incisor from Rat I were dried, and attached to large corks, with a viscous mixture of acetone and "Duco" household cement. When dry the specimens were ground on a fine-grained carborundum stone until a plane section, about midway through the marrow or pulp cavity was obtained. The marrow or pulp cavity was then filled with the cement and the specimen was reground. The ground surface of the bone or tooth was placed in direct contact with Eastman No-Screen X-ray film in a light-tight container. At the end of two weeks the films were developed. Samples of bone from an untreated rat, when prepared and exposed in an identical manner, gave no image on the film. A kidney of Rat II was cut through the plane of the long axis of the hilum and the flat surface was kept in contact with the film for 2 weeks at 10°C.

Results and Comment. The deviations of the results given in the tables are derived from the statistical error of the radioactivity measurements.‡ The data given in Tables II, III and IV summarize the findings regarding the excretion of C^{14} and the incorporation of this isotope in the tissues and derived components of Rats I and II.

Within 6 hours following the injection of $Na_2C^{14}O_3$ about 95% of the total injected dose of C^{14} appeared in the expired CO_2 (Table II). This finding agrees with that of Swendseid,[2] who injected mice with $NaHC^{13}O_3$ and followed their respiratory output of $C^{13}O_2$ for 3 to 4 hours. A sharp decrease followed the initial rapid rate of excretion of $C^{14}O_2$ and the rate became very much reduced about the 3rd or 4th day (Rat I). The results with regard to expired air can be taken to indicate that the $C^{14}O_2$ excreted after the first 24-48 hours, was, in large part, derived from C^{14} which had been fixed for a time in the animal's body. These results are of interest in regard to the health hazards associated with work with C^{14} since they indicate the probability of rapid excretion of a major part of the $C^{14}O_2$ inhaled during short exposures to this gas. Of the C^{14} given Rat I, 98% was recovered and 0.45% of the administered isotope was found in the animal at the time of death.§

The administration of C^{14} as pellets of

[17] Armstrong, W. D., and Schubert, J., *Science*, 1947, **106**, 403.

† Dr. Peter Yankwich (University of California) has indicated in a personal communication that the scattering of the beta-rays of C^{14} by $BaCO_3$ is somewhat higher than by organic biological materials. The agreement which we have observed between results calculated from counts made of unoxidized organic materials and of $BaC^{14}O_3$ derived from the same samples would, therefore, not be expected under all conditions of counting C^{14} in solid samples. It is suggested that a comparison of direct counts with those of $BaC^{14}O_3$ be made with the specific arrangement of counter and sample to be employed before direct C^{14} assays of biological materials are adopted as a routine procedure.

‡ Square root of the sum of the squares of the standard deviations of the sample and background.

§ The specific activities of the carbon and the fraction of the total dose of C^{14} present in the tissues and certain derived components of the tissues of Rat I were determined by methods essentially like those employed with Rat II but are not reported in detail. These data will be supplied upon request.

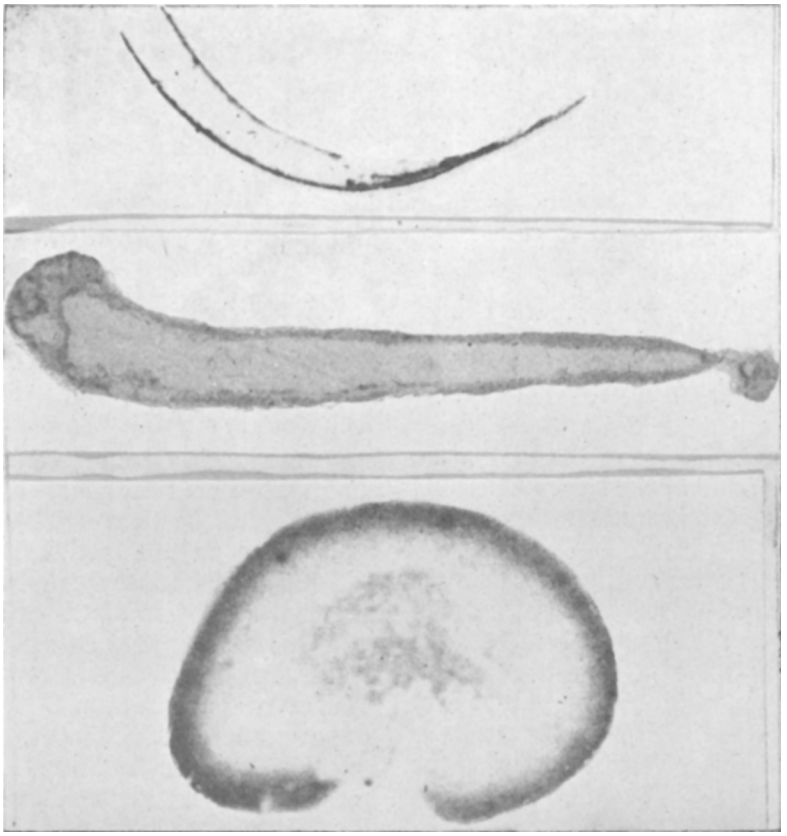

FIG. 2.
Radioautographs of a lower incisor (top), humerus (middle), and kidney (bottom) × 3.

$CaC^{14}O_3$ prolonged the period of elimination of the labeled carbon and resulted in the body fluids having a high and constant C^{14} specific activity for the 48-hour period preceding the sacrifice of Rat II (Table III). The largest amount of the administered C^{14} was present in the expired air of Rat III on the 7th-8th day and no detectable C^{14} was present in the expired air of this animal on the 22nd day following the implantation of $CaC^{14}O_3$. The C^{14} retained by Rat III, 40 days after administration, was $0.38 \pm .05\%$ of the total administered dose, $0.21 \pm .03\%$ being present in the pelt. These results indicate that a large fraction of C^{14} is excreted even when the isotope is present in the body as an insoluble inorganic compound.

In Rat II about 0.75% of the administered dose or about 1.0% of the adsorbed dose, was retained by the tissues. The C^{14} recovered in the tissues, excreta and expired air of Rat II was 108% of the administered amount. This result as to material balance is not in as good agreement with theory as was obtained with Rat I because of the uncertainties in the determination of the unabsorbed C^{14} from the pellets of $CaC^{14}O_3$.

It is apparent that the greatest incorporation of C^{14} in bone and teeth took place in the inorganic carbonate fraction. This turnover of carbon probably occurred mainly by exchange of carbonate groups of the mineral phase for labeled carbonate groups. The finding of C^{14} in the bone protein is of special significance since it is evidence, independent of the process of exchange of ions by the mineral phase, that bone tissue of a mature animal is continuously reformed. The C^{14} content of the inorganic carbon of bone and of the bone protein accounted for nearly 90%

of the total activity present in the skeleton of Rat I. There is a possibility that the bulk of the residual C^{14} activity may reside in the citric acid component of the skeleton since it is known that about 1% by weight of the dry fat-free bone is citric acid.[18]

The radioautograph of the incisor tooth of Rat I (Fig. 2) shows that the C^{14} was concentrated in a layer near to, or immediately adjacent to, the pulp cavity. Since this region is the location of the most recently formed dentin, the heavy concentration of C^{14} in this position is accounted for by deposition in the mineral phase of the dentin formed during the course of the experiment. The radioautograph of the kidney of Rat II shows that the C^{14} was concentrated in the cortical region.

The greater specific activity of C^{14} in the enamel of the incisor teeth than in the dentin (Rat II, Table IV) is probably due to the presence in enamel of a greater amount of inorganic carbon, which has a high uptake of C^{14}, than is present in dentin. No results as to the C^{14} content of the enamel of molar teeth were obtained because of poor yields of enamel from these teeth.

In the fat the greater part of the activity resides in the glycerol fraction but a definite incorporation of C^{14} in the fatty acids took place. The marked incorporation of C^{14} in the protein of the visceral organs is of interest. The relative amount of C^{14} incorporated by these proteins is in general agreement with their relative metabolic activity as determined by experiments with isotopic nitrogen.[19]

Other workers[2,5] were unable, in short term experiments, to demonstrate the incorporation of isotopic carbon (C^{11} or C^{13}), administered as inorganic carbon, in the muscle and liver glycogen of unfasted animals. Our results (Table IV) show, however, that such incorporation can be detected when the study is conducted under conditions which maintain the isotopic inorganic carbon content of the body fluids at a high level over a longer time.

Summary. Radioactive carbon was administered to unfasted mature rats either by intraperitoneal injection of $Na_2C^{14}O_3$ or by implantation in the peritoneal cavity of $CaC^{14}O_3$. Quantitative studies were made of the relative amounts of C^{14} excreted in the urine, the feces, expired air and incorporated by the tissues and derived components. A significant incorporation of C^{14} was found in the inorganic carbonate fraction of bone and in bone protein, the dentin, and enamel of the teeth, fatty acids, glycerol, hemin, red cell protein, plasma proteins, central nervous system protein, liver and muscle glycogen, muscle protein, and in the proteins of the testes and of the thoracic and abdominal viscera.

[18] Dickens, F., *Biochem. J.*, 1941, **35**, 1011.

[19] Schoenheimer, R., *The Dynamic State of Body Constituents*, Harvard University Press, Cambridge, Mass., 1942.

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Fractionation of Serum Cholesterol.*

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During the last few years considerable evidence has accumulated indicating that the cholesterol of normal plasma is, to a very large extent, bound with the plasma proteins. Since cholesterol bound with plasma proteins would be completely soluble in the plasma, it is possible that its physiological behavior might be quite different from that of cholesterol which exists in the plasma unbound with

* We are indebted to Charles C. Haskell & Co., Richmond, Va., for financial support of this investigation.