The results indicate that in the smallest spherical nuclei of liver tumors the amount of desoxyribonucleic acid is the same as in normal hepatic nuclei of similar size. Comparable results were independently obtained by Leuchtenberger,[13] who measured nuclei in transplanted mouse sarcoma. Considering the abnormal chromosome arrangements which are known to exist in neoplasms, (Boveri[14]) the presence in these tumors of nuclei with more or less nucleic acid cannot be excluded. The conflicting results of previous investigations may be attributed in part to the analyses of tissue samples containing different numbers and different sizes of nuclei.

Summary. The amount of desoxyribonucleic acid in single spherical nuclei of normal rat liver was compared with that of similar nuclei in tumor tissue by microphotometric determination of the intensity of the Feulgen reaction. It was found that the amount of desoxyribonucleic acid contained in nuclei of similar size is the same in hepatoma and cholangioma as in the normal liver.

[13] Leuchtenberger, C., personal communication.

[14] Boveri, T., The Origin of Malignant Tumors, Williams and Wilkins, Baltimore, 1929.

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17312. Excretion of Radiocalcium by Normal Rats.*

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The objectives of the present experiments were to determine the effect of various amounts of labeled calcium on the relative amounts of radiocalcium‡ excreted in the urine and feces, and to determine the distribution or radiocalcium in the tissues and contents of the gastrointestinal tract during the first few hours after the administration of a dose of labeled calcium.

Materials and methods. Young adult rats of the Sprague-Dawley strain were used. Immediately after the subcutaneous injection of a dose of labeled§ calcium each animal was placed in a separate wire metabolism cage over a urine-feces separator.[1] The animals shown in Table II were supplied with water only, but each of the other animals had access to both water and the stock diet, Purina Laboratory Chow, during the experimental period.

At the end of the experimental period the animals were dispatched by a blow on the head. In those instances in which the segments of the gastrointestinal tract and their contents were to be separately assayed for radioactivity, especial care was taken to remove the entire contents. The collected urine and cage washings, the feces, and the other samples were dried, and then dry ashed in an electric muffle at 550°C. In each instance the ash was dissolved in dilute hydrochloric acid and suitable aliquots transferred to small aluminum pans and dried under an infra red lamp. Dry sample weights were kept small (0.5 to 1.5 mg/cm^2 on sample pan surface) and comparable to the standards in order to minimize the error due to self-absorption of radioactivity. Suitably prepared standards and the samples were then assayed for radio-

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‡ The radiocalcium (Ca^{45}) used in these experiments was allotted by the United States Atomic Energy Commission, and supplied by the Monsanto Chemical Company.

§ 3 to 10 microcuries of Ca^{45}.

[1] Gross, I., and Connell, S. V. B., *J. Physiol.*, 1923, **57**, lx.

TABLE I.
Excretion of Radiocalcium after Injection of Various Amounts of Labeled Calcium.

No. of rats	Body wt., g	Dose of labeled Ca, mg	% of dose excreted	
			Urine	Feces
8	208 ± 12*	0.07	0.59 ± 0.25	12.60 ± 5.75
4	293 ± 30	0.21	0.61 ± 0.42	9.56 ± 1.13
3	224 ± 40	0.41	0.93 ± 0.65	11.36 ± 1.25
3	213 ± 12	2.50	5.90 ± 1.75	15.17 ± 1.14
3	190 ± 8	4.90	2.95 ± 0.94	12.46 ± 1.10
6	211 ± 30	7.00	2.49 ± 1.55	9.41 ± 5.40
10	233 ± 35	14.00	6.05 ± 2.15	7.74 ± 4.50

* Standard deviation of the mean.

activity by using a scale-of-sixteen Geiger-Muller counter, equipped with a thin mica window (1.8 mg/cm^2) tube.

Results and comments. The data summarized in Table I show that under the conditions of our experiments subcutaneously administered radiocalcium was excreted mainly in the feces. Variations in the relative amounts of radiocalcium excreted in the urine and feces were large for each dose of labeled calcium injected, but the results indicate clearly that the amount of calcium in the dose was an important factor in determining the relative amounts of radiocalcium excreted in the urine and feces. As would be expected, retention of the radiocalcium varied widely and probably reflects differences in the rate of absorption of the labeled calcium, or differences in the calcium requirement of the animals.

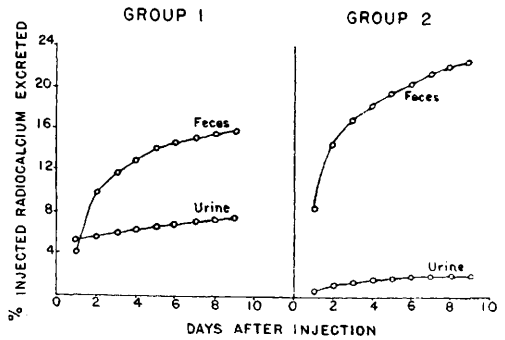

FIG. 1.

Group 1 consisted of 4 rats (avg wt. 265 g), each of which received two subcutaneous doses of 7 mg of labeled calcium within the first hour of the experimental period. Group 2 consisted of 4 rats (avg wt. 220 g), each of which received a single subcutaneous dose of 0.07 mg of labeled calcium at the beginning of the experimental period.

Fig. 1 shows graphically the excretion of radiocalcium by 2 groups of rats during the 9 days following administration. The animals of Group 1, each of which received an injection of 7 mg of labeled calcium at the beginning of the experimental period and a second injection of 7 mg at the end of the first hour, excreted 5.2% of the administered radiocalcium in the urine and 4% in the feces during the first 24 hours. Thereafter the amounts of the radiocalcium which appeared daily in the feces exceeded the amounts excreted in the urine. Summation of the fractions of the administered radiocalcium found in the urine and feces during the 9-day experimental period reveals that approximately 7.27% of the dose was excreted in the urine and 15.9% in the feces.

The results obtained for the animals of Group 2, Fig. 1, show that when the quantity of labeled calcium injected was only 0.07 mg the average amount of the administered radiocalcium excreted in the urine during the first 24 hours was only 0.66%, while the average amount excreted in the feces was 8.49%. In the case of this group of animals approximately 2.10% of the dose of labeled calcium was excreted in the urine and 22.75% in the feces during the 9-day experimental period. From these experiments (Fig. 1) it is apparent that the gastrointestinal tract was the principal route by which the labeled calcium was excreted. Furthermore, it appears that only when a plethora of the labeled calcium was being eliminated during the first 24 hours did the kidneys respond by excreting a large fraction of the administered radiocalcium.

It is generally agreed that a considerable amount of excreted calcium enters the lumen

TABLE II.
Distribution of Radiocalcium in Tissues and Contents of Gastrointestinal Tract.

Rat No.	Body wt., g	Hr after dose of 7 mg labeled Ca	% of dose recovered from tissues				% of dose recovered from contents			
			Stomach	Small intestine	Cecum	Colon	Stomach	Small intestine	Cecum	Colon
1	205	1	0.14	0.40	0.01	0.08	0.23	1.79	0.29	0.12
2	190	1	0.11	0.60	0.02	0.11	0.12	0.91	0.10	0.06
3	204	1	0.10	0.69	0.03	0.07	0.15	0.92	0.14	0.16
4	185	1	0.20	0.40	0.02	0.07	0.11	1.10	0.12	0.07
Avg	196		0.14	0.52	0.02	0.08	0.15	1.18	0.16	0.10
5	178	3	0.17	0.49	0.03	0.14	0.09	1.24	0.57	0.35
6	196	3	0.12	0.63	0.04	0.12	0.05	1.23	0.17	0.13
Avg	187		0.14	0.55	0.03	0.13	0.07	1.23	0.37	0.24
7	190	6	0.11	0.44	0.03	0.13	0.05	0.41	1.71	0.17
8	187	6	0.12	0.57	0.01	0.10	0.08	0.48	1.17	1.57
9	186	6	0.14	0.71	0.04	0.28	0.03	0.41	1.41	0.14
Avg	188		0.12	0.57	0.03	0.17	0.05	0.43	1.43	0.63

of the intestine as a constituent of the intestinal secretions[2] and the bile,[3] and that this calcium, supplemented by a fraction of the dietary calcium is reabsorbed. Evidence of an active excretion of calcium through the wall of the small intestine,[4] or the colon,[5-7] however, has not been equally acceptable.

In our experiments (Table II) the employment of radiocalcium has made possible the orientation of various portions of the gastrointestinal tract with respect to the excretion of calcium. The distribution patterns of radiocalcium in the tissues and contents of the gastrointestinal tracts of animals, which were sacrificed at 1, 3, and 6-hour intervals after the subcutaneous injection of 7 mg of labeled calcium, are shown in Table II. Of particular interest was the relatively large fraction of the excreted radiocalcium found in the contents of the small intestine and the smaller fractions recovered from the contents of the cecum and colon at the 1-hour interval. In Rats 1 and 2 the small intestine was severed at the midpoint and the tissues and contents of the two halves separately assayed for radioactivity. The tissues of the upper and the lower half of the small intestine of Rat 1 contained 0.24 and 0.16% of the administered radiocalcium, respectively, whereas the contents of the upper and lower half contained 0.43 and 1.36%, respectively. In the case of Rat 2 the tissues of upper and lower half of the small intestine contained 0.28 and 0.32% of the administered radiocalcium, respectively, and the contents 0.13 and 0.78% respectively.

Unfortunately separate assays were not made on the tissues and contents of equal parts of the small intestine of Rat 5 or 6. However, such assays were made on Rats 7, 8, and 9. In these animals approximately the same amounts of radio-activity were found in the tissues of the upper and lower half of the small intestine, but in 2 instances the amounts of radiocalcium in the contents of the 2 segments differed appreciably. In Rat 7 the contents of the upper and lower half of the small intestine contained 0.12 and 0.29% of the injected radiocalcium, respectively, and in the case of Rat 9 the fractions of the administered radiocalcium recovered in the 2 segments were 0.09 and 0.32%, respectively. The contents of the upper and lower half of the small intestine of Rat 8 contained 0.20

[2] Logan, M. A., *Physiol. Rev.*, 1940, **20**, 522.

[3] Greenberg, D. M., and Troescher, F. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 488.

[4] Walsh, E. L., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 839.

[5] Bergeim, O., *J. Biol. Chem.*, 1926, **70**, 51.

[6] Cowell, S. J., *Biochem. J.*, 1937, **31**, 848.

[7] Kosman, A. J., and Freeman, S., *Fed. Proc.*, 1943, **2**, 16.

and 0.28% of the injected radiocalcium, respectively.

It is very doubtful that the major portion of the excreted radiocalcium found in the lower half of the small intestine could have resulted from the failure of the upper half to reabsorb secreted calcium. Duckworth and Godden[8] have observed in the rat that the net absorption of ingested calcium is not affected by a diet containing 30% of fiber. Since such a diet should cause a great increase in the intestinal secretions, it would appear that the efficiency of reabsorption of secreted calcium is very high in the rat. Therefore, we are inclined to regard that fraction of the excreted radiocalcium found in the lower half of the small intestine as indicative of an active excretion of calcium through the wall of the intestine. In this connection, the extreme rapidity with which the fecal excretion of calcium has been shown to occur is of great interest. Norris and Kisieleski[9] recovered 5 to 8% of a dose of labeled calcium from the contents of the intestines of rats one minute after intravenous administration.

The presence of relatively small fractions of excreted radiocalcium in the contents of the cecum and colon at the 1 hour interval (Table II), and the larger amounts in the contents of these segments at later intervals, suggest that the bulk of the excreted radiocalcium entered the small intestine at points above the ileocecal valve. This observation is in accord with evidence that has been advanced[10] that there is little or no active excretion of calcium into the large intestine.

Summary. 1. The excretion of subcutaneously administered radiocalcium has been studied in normal rats.

2. The amount of labeled calcium injected appeared to be an important factor in determining the relative amounts of radiocalcium excreted in the urine and feces. When the quantity of labeled calcium injected was increased from 0.07 mg to 14 mg the fraction of the excreted radiocalcium which appeared in the urine in the first 24 hours increased from a relatively small value to a value approaching or slightly exceeding the amount of radiocalcium excreted in the feces. During a 9-day experimental period the amount of radiocalcium excreted in the feces, when either 0.07 or 0.14 mg of labeled calcium was administered, greatly exceeded the amount excreted in the urine.

3. The distribution of radiocalcium in the tissues and contents of the stomach, small intestine, cecum, and colon was determined at 1, 3, and 6-hour intervals after the administration of a dose of labeled calcium. The results of these experiments indicate that there is little or no active excretion of calcium through the wall of the large intestine.

[8] Duckworth, J., and Godden, W. J., *Biochem. J.*, 1941, **35**, 16.

[9] Norris, W. P., and Kisieleski, W., Cold Spring Harbor Symposia on Quantitative Biology, 1948, **13**, 164.

[10] Henry, K. M., and Kon, S. K., *Biochem. J.*, 1939, **33**, 173.

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