We have investigated the incorporation of isotopically labeled guanine (containing N^{15} in the 1 and 3 nitrogens and in the 2-amino group[4]) into the nucleic acids of this strain of mouse bearing this tumor. The guanine was administered in five intraperitoneal injections, of 2.4 mg each, over a 6 day period, beginning on the 7th day after implantation of the tumor. Inasmuch as isotopic nitrogen, particularly from the 2-amino group, can be contributed to body pool ammonia[4] it is not unlikely that the isotope found in the protein residue, pyrimidines and even the adenine (Table I) arose from this source. There was, however, a small but definite incorporation into the guanine of the nucleic acids, but there is no evidence of a specific uptake by the tumor since the incorporation into the guanine of the tumor tissue and into that of the nontumorous viscera of the same mice was significant in each case.

When guanine was adminisered to an unspecified strain of the white rat[5] or to Sherman strain rats (Rockland Farms), either orally[4] or intraperitoneally,[6] there was no significant incorporation of the guanine into the tissue nucleic acids. The species difference in the purine metabolism of the Sherman rat and the C57 black mouse (bearing adenocarcinoma Eo771) makes it necessary to be conscious of other such possibilities in mammalian purine metabolism.

Incorporation of Guanine into Nucleic Acid of C57 Mice Implanted with Adenocarcinoma Eo771.

	Atom % excess N^{15}
Injected guanine	6.40
Viscera:	
Nucleic acids	.030
Total purines	.049
Guanine	.072
Pyrimidines	.024
Tissue residue (after extraction of nucleic acids)	.015
Tumor:	
DNA	
Total purines*	.046
Adenine†	.038
Guanine	.050
PNA	
Total purines*	.058
Adenine†	.037
Guanine	.105

* Isolated as the copper salts after separation of the pentose (PNA) and desoxypentose (DNA) nucleic acids by the Schmidt-Thannhauser procedure.

† Calculated from N^{15} values of adenine picrate.

[3] Sugiura, K., Hitchings, G. H., Cavalieri, L. F., and Stock, C. C., in press.

[4] Brown, G. B., Roll, P. M., Plentl, A. A., and Cavalieri, L. F., *J. Biol. Chem.*, 1948, **172**, 469.

[5] Plentl, A. A., and Schoenheimer, R., *J. Biol. Chem.*, 1944, **153**, 203.

[6] Brandt, A., Roll, P. M., and Brown, G. B., unpublished data.

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Behavior of Thyroid Toward Elements of the Seventh Periodic Group. II. Rhenium.*† (17482)

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On the basis of speculations made in a previous report[1] in which we suggested that not only iodine but all the elements of the seventh periodic group are selectively filtered by the thyroid, we have extended our work to include rhenium. As with Cl, Br, I, Mn and At, we find that Re also accumulates in the thyroid in greater concentration than

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[1] Baumann, Emil J., and Metzger, N., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 536.

in any other tissue examined, thus lending further support to our hypothesis.

Metallic Re powder was irradiated in the Oak Ridge nuclear reactor. It was dissolved in 2 ml of concentrated nitric acid and appropriately diluted for parenteral administration. This perrhenic acid solution contained 2 radioactive Re isotopes, Re^{186} and Re^{188} having half lives of 90 and 18 hours, respectively, as well as a large amount of stable Re. In nearly all the experiments to be described, the 18 hour half life isotope was allowed to decay to such an extent that by the time of our experiments it was an unimportant factor in our measurements. Of necessity, however, a relatively large amount of carrier Re was given with each dose—from 3 to more than 100 μg depending on the age of the irradiated Re. The amount of stable Re given in each case was therefore greater than a tracer quantity.

Adult albino rats weighing 250 to 400 g were fed on a diet consisting of mixed cereals, dried milk, meat scrap, yeast, and salts moistened with fresh milk. Fresh vegetables were given separately. Since we were studying only the filtration process of the thyroid, it was desirable to inhibit completely the reactions whereby thyroxine and diiodotyrosine are synthesized. To this end, thiouracil‡ was added to the mixture to make a 1% concentration. For comparison with normals, however, thiouracil was omitted from the food of 8 rats.

One ml of a HRe^*O_4 solution having an activity of 200,000 to 300,000 counts per minute§ was injected intraperitoneally into each rat. The animals were sacrificed with $CHCl_3$ usually 4 hours after the injection. In a number of instances in which the effect of time on the Re* concentration of tissues was studied, rats were killed 1 to 24 hours after injection. Autopsies were performed at once and tissues prepared for analysis. As much blood as possible was drained from the tissue before hashing. One to 2 g samples of blood, lung, liver and muscle were weighed into 15 mm diameter metal planchets, moistened with 10-20 drops of 1% NaOH, dried for an hour at 105°C and then at 120-130°C. The thyroid of each rat was weighed in a planchet, moistened with 1-2 drops of NaOH and dried in the same way. The samples were then ashed at 450-500°C for 18 hours. The ash was coated with 0.5-1.0 ml of 1% celloidin and the activity measured with a thin mica window Geiger counter. Appropriately diluted samples of the injected solution measured with the same apparatus were used as standards to establish the decay curve for the Re* preparation in each series of experiments. Known amounts of Re* were added to blood and to muscle samples and ashed as described. The maximum loss in these controls due to self absorption and possibly to some loss during ashing was found to be not more than 15% and usually less than 10%. No corrections are made for these errors in the data given.

Fig. 1 shows the distribution of Re* in thyroid, lung, liver, muscle, and blood of 8 normal rats measured as described. Fig. 2 shows similar observations on 8 representative rats which had been under the influence of thiouracil for from 5 to 20 days before Re* was injected. The thyroids weighed 45 to over 100 mg depending on the duration of thiouracil feeding. They were very hyperemic and hyperplastic. The thyroids of the control rats weighed 14 to 20 mg, a few being slightly enlarged and slightly hyperemic.

Four hours after Re* injection, an average of 4.5% of the administered dose per g of tissue was found in the thyroids of normal rats, the range was 1.4 to 7.2% per g. For thiouracil treated rats which had been given 1 ml of a Re* solution containing 3 to 12 μg of carrier, the average concentration was 12.8% per g of thyroid with a range of 5.1 to 25.5% per g. When larger amounts of carrier were used, smaller proportions of the injected dose were found in the thyroids. For example, with 4 thiouracil treated rats a Re* solution containing 250 μg Re carrier

‡ We thank the American Cyanamid Company for supplying the thiouracil.

§ The activity of the Re shipments varied from 25,000 to 100,000 cpm per μg at the time of shipment as determined from data submitted with the irradiated preparations and from the catalogue of isotopes of the Atomic Energy Commission.

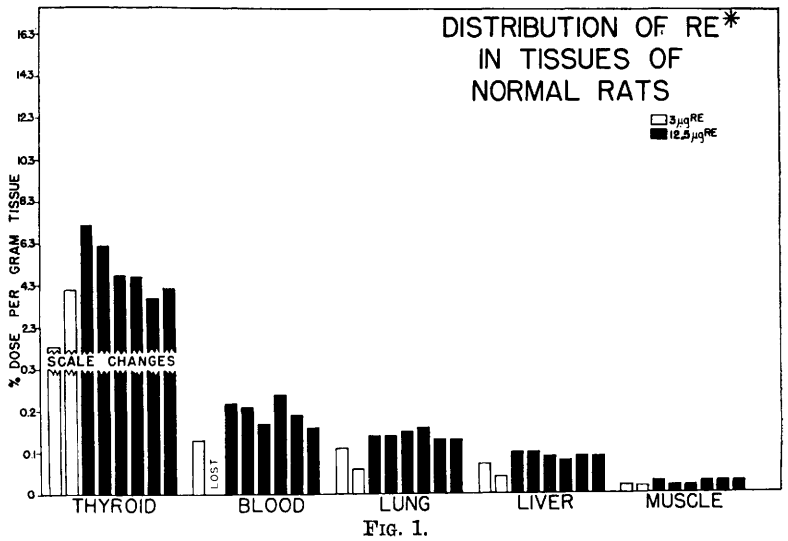

FIG. 1.

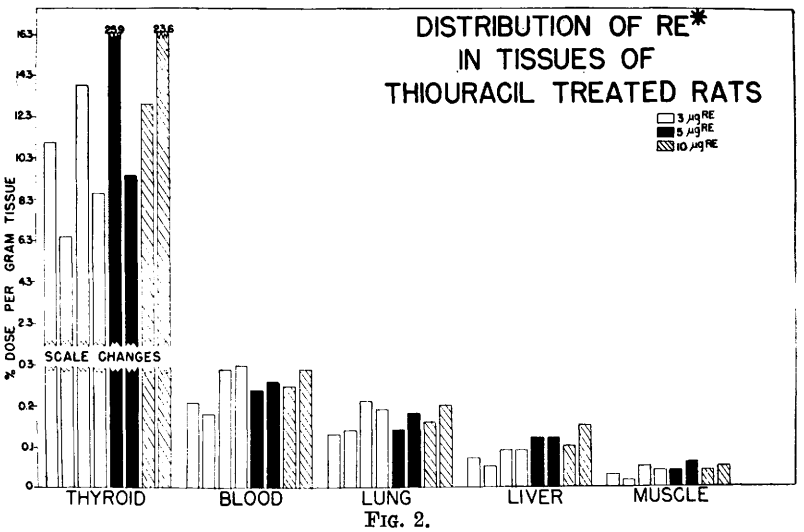

FIG. 2.

per ml was injected; 4 hours later, the thyroids were found to contain only 2.2 to 4.2% of the dose per g of tissue. Since the capacity of any tissue to take up any substance is limited, it is to be expected that with increasingly large amounts, a smaller fraction of the administered dose will be retained. As with iodine, the percentage of administered Re* that can be taken up varies in a general way, directly with the mass of thyroid and inversely with amount of carrier.

The amount of Re* found in other tissues was very much smaller—only 0.5% to 5% of the injected Re* per g of tissue. The Re* concentrations of blood, liver, lung and muscle of the thiouracil treated rats differed little from the corresponding concentrations found in the control group. On the average, lung of normal rats contained 0.13% of the injected Re* per g and in thiouracil fed rats 0.16%. Corresponding average figures for liver were 0.08% and 0.10%, and for muscle 0.03% and 0.04%, respectively. In all, experiments were made on 41 thiouracil fed rats with similar results in every case. Fig. 3 shows a comparison of the average Re* uptake of the 8 normal and the 8 representative thiouracil fed rats shown in Fig. 1 and 2.

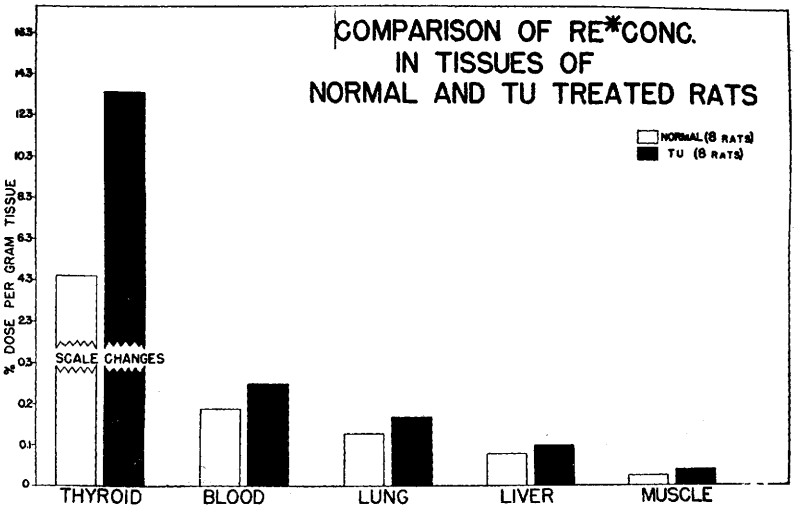

FIG. 3.

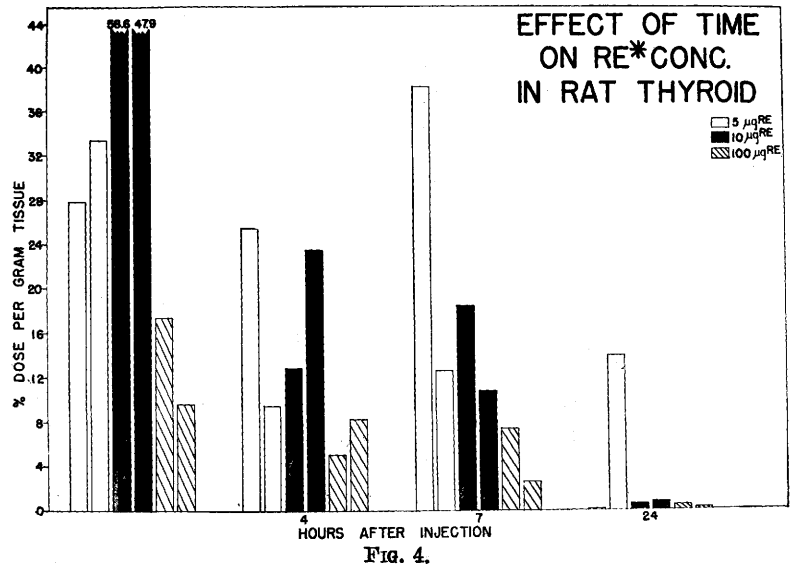

FIG. 4.

The ratio of the Re* concentration in the thyroid to that in the blood is invariably large, 25 to 100 or more; while the ratio of Re* concentration of other tissues to blood is always less than one. It follows, therefore, that the process whereby such a high thyroid Re* content comes about is an active selective filtration, and not simply a passive reflection of the amount of Re* in the blood passing through this organ.

In view of the common origin of both thyroid and lung from the primordial gill clefts, it is worthy of note that the Re* concentration of lung is exceeded only by that of the thyroid.

To determine the effect of time on the distribution of the Re* in the body after injection of a single dose, 3 experiments were performed. In each case 1 ml of a Re* solution was injected intraperitoneally into 8 thiouracil fed rats. At 1, 4, 7 and 24 hours after injection 2 animals were sacrificed. The amount of carrier given per rat in these 3 experiments was 5, 10 and 100 μg and the activity of the preparations per ml was 270,000, 282,000, and 431,000 c/m, respec-

TABLE I.
Excretion of Injected Re* (%).

	Re injected		Urine			Feces		
	Carrier, mg	Re* activity, cpm, ×1000	1st day	2nd day	Total	1st day	2nd day	Total
Rat 12a	0.010	309	84.4	0.7	85.1			
" 12b	0.010	309	79.4	2.2	81.6			
Rabbit 14b	0.50	1310	94.8	1.1	95.9	.1	.8	.9
" 16a	1.00	492	79.8	12.4	92.2	.0	.0	.0
" 16b	1.00	492	92.1	2.8	94.9	.0	.0	.0

tively. The uptake of Re* by the thyroids is shown in Fig. 4.

In all tissues examined, the greatest concentration of Re* usually appeared at the one hour interval, decreasing rather slowly to 7 hours after injection, and then more rapidly; so that 24 hours after, not more than 1/3 and usually less than 1/10 of the amount found one hour after the injection remained. After another day, the tissue activity was little more than at background levels. Although there was considerable variation among individuals (*e.g.* see Fig. 4 at the 24 hour interval) this general statement of our findings held whether 5, 10, or 100 μg of carrier were given. Again, with the larger doses as, *eg.*, when 100 μg of carrier Re were given, the percentage of the dose found in the thyroid and other tissues was much less than with the smaller amounts of carrier.

From these data one may assume that Re is rapidly excreted. From the urines of 2 rats, 85 and 82% of an intraperitoneal Re injection was recovered in 2 days of which all except 1-2% was excreted in the first day. Because of the difficulty of collecting quantitatively the little urine excreted by a rat, similar experiments were made with 3 rabbits. Bladders were compressed at the end of each day. Here 96, 92, and 95% of the injected Re was recovered in 2 days. The feces of 2 rabbits showed negligible radioactivity, while 0.9% of the injected dose was recovered in the feces of the 3rd rabbit. Contamination of feces by urine could not be excluded. The details of these tests are given in Table I.

Total recovery of injected Re* was attempted on 2 rats 3.5 hours after intraperitoneal injection of 1 ml of a Re* solution containing 13 μg of carrier Re, having an activity of 125,000 c/m. The 2 rats were sacrificed with $CHCl_3$ and tissues prepared for analysis. Only 72% and 85%, respectively, were recovered. The Re* was distributed as follows:

	Rat 1, %	Rat 2, %
Thyroid	1.09	1.23
Liver	1.32	1.24
Skeleton and muscle	12.6	14.8
Blood	2.33	1.63
Other viscera	25.4	16.7
Skin and hair	15.1	25.2

A large part of the unrecovered rhenium can be accounted for by the loss of urine during anesthetization. In general, the distribution is similar to that known for chlorine.

Summary. Rhenium is preferentially and actively filtered from the blood by the thyroid. After injecting a single dose, the Re* concentration in that organ is 25 to 100 or more times greater than that found in any other tissue. These findings therefore add support to our hypothesis that the thyroid will selectively filter from the blood all the elements of the seventh periodic group. The greatest concentration of Re* in tissues occurs within one hour. For several hours it decreases slowly and then more rapidly so that more than 90% is recovered from the urine in 24 hours.

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