TABLE I.
Evaluation of Effect of Darvisul on SK Virus in Mice.

Dilution of virus	Treated No. survivors		Controls No. survivors	
	5th day	14th day	5th day	14th day
2 × 10-4	0/25	0/25	0/25	0/25
2 × 10-5	1/25	0/25	1/25	0/25
2 × 10-6	7/25	2/25	7/25	2/25
2 × 10-7	21/25	18/25	23/25	18/25

Date of Experiment = October 3, 1948.
Numerator = survivors. Denominator = total number inoculated.

intraperitoneally to 25 mice in amounts of 0.5 ml at 9 a.m., 0.25 ml at 1 p.m., 0.25 ml at 4 p.m., and 1.0 ml at 7 p.m. so that the total daily dosage was 16 mg. Control mice, 25 in number, received equivalent volumes of salt solution intraperitoneally.

The results are presented in Table I. It is apparent that no difference between treated and control mice was observed. It is concluded, therefore, that under the conditions of the experiment Darvisul had no therapeutic effect upon the infection of mice with the SK strain of murine encephalomyelitis virus.

16984

Behavior of the Thyroid toward Elements of the Seventh Periodic Group. I. Halogens and Thiocyanates.*

EMIL J. BAUMANN AND NANNETTE METZGER.
From the Laboratory Division, Montefiore Hospital, New York City.

It will perhaps surprise many readers of this report to learn that the thyroid will filter from the blood not only iodine but the other halogens as well. Further, while it is generally known that feeding thiocyanates to man and other animals will cause the development of goiter, thyroid hyperplasia and goiter have also been produced by feeding relatively large amounts of any of the halogens other than iodine. The evidence for these statements will be assembled, much of it from the literature, and an hypothesis offered that attempts to show that bromides, chlorides, fluorides, as well as thiocyanates all bring about thyroid enlargement by the same means, and to explain why the responses of the thyroid to all of these substances are similar.

Filtration of Halogens and Thiocyanates by the Thyroid. The first step in formation of thyroxine consists in filtering iodide from the blood. This filtration by the thyroid is remarkably effective, its concentrating power being of the order of 10,000. In an early experiment of Marine and Feiss[1] nearly 90% of a dose of 5 mg of KI was found in the goitrous thyroid of a dog in 10 minutes. In many more recent experiments with radioactive iodine similar results have been noted. Even when the synthesis of thyroxine is prevented by administration of thiourea and related substances, the thyroid can still filter iodine from the blood, first shown by Baumann, Metzger, and Marine[2] and subsequently by others. Selective filtration by the thyroid is not limited to iodine; in fact the

* A report of this work was made before the American Society of Biological Chemists at Chicago, May 14, 1947, *Fed. Proc.*, 1947, **6**, 237.

1 Marine, D., and Feiss, H. O., *J. Pharm. Exp. Therap.*, 1915, **7**, 557.

2 Baumann, E. J., Metzger, N., and Marine, D., *Endocrinol.*, 1944, **34**, 44.

gland behaves in a similar fashion with all the elements of the 7th group of the periodic table that have been studied, *viz.*, Cl, Br. Mn, and At, as well as with thiocyanates. It may be predicted that fluorine, rhenium, and technetium (element 43) will be found to behave in the same way.

Baumann, Sprinson, and Marine[3] found more Br in rabbit thyroids than in other tissues, especially when the gland was hyperplastic, an observation that had been made by many other investigators. Ray and Desach,[4] after injecting subcutaneously small amounts of $MnCl_2$ daily for 6 days, noted an accumulation of Mn in all tissues, but they found that the thyroid had a concentration 2 to 10 times as great as any other tissue studied. And somewhat similar experiments were made with Astatine by Hamilton and Soley[5] using the tracer technic. This element, prepared by α particle bombardment of Bi by Corson, Mackenzie, and Segrè[6] is radioactive. It was found to behave as a metal rather than like the halogens, *e.g.*, it is not precipitated by $AgNO_3$ from nitric acid solution while it is precipitated by H_2S from an acid solution. Hamilton and Soley fed tracer doses of this isotope to guinea pigs with normal or with hyperplastic thyroids. They found many times more Astatine in the thyroid than in any other tissue they examined.

Experimental. We compared the concentration of Cl^- and SCN^- in thyroid and other tissues of albino rats. Both normal and goitrous animals were used. The goitrous rats were fed our stock food mixture to which 1% thiouracil† was added for 2 to 5 weeks. The tissues of two or more rats were pooled and treated as follows:

Finely minced tissues were analyzed for Cl^- by digestion over a free flame with 5 ml standard $AgNO_3$ and a few drops $KMnO_4$ if necessary (Eisenman[7]). For thiocyanate determination the finely minced tissues were weighed into 25 or 50 ml Erlenmeyer flasks and digested with 10-20 ml 4% alcoholic KOH in a boiling water bath. To limit evaporation a small funnel covered with a watch glass was placed in each flask while heating. After half an hour, the funnel and cover glass were removed, the contents of the flask evaporated to dryness, neutralized with 5 N H_2SO_4, adding 0.2 ml in excess and finally 1.5 ml 10% Na_2WO_4. After the precipitated protein had flocked out, the mixture was made up to 10 ml, centrifuged and filtered. A 75-80% aliquot was analyzed by the method of Baumann, Sprinson, and Metzger.[8]

Adult rats were used to measure the Cl^- content of tissues. Our stock diet consisting of mixed cereals, dried milk, yeast, meat scrap, and salts contains about 1.3% NaCl. Due to the special role of chlorides in the physiology of tissue fluids, one would hardly expect to find as much Cl^- in thyroid as in blood. However, thyroid did contain 2 or 3 times as much Cl^- as muscle while the Cl^- concentration of tissues having much blood, such as liver, was only slightly less than in thyroid. Some of the data of these tests are given in Table I.

In the thyiocyanate experiments adult rats were injected intraperitoneally with 2 ml of a 5% NaSCN solution and sacrificed by chloroform anesthesia 4 to 5 hours later. Blood was taken from the heart, the tissues dissected and prepared for analysis. Some of these data are given in Table II. A similar experiment was made on a 2.5 kg rabbit in which 7 ml of 5% NaSCN were injected intraperitoneally. This proved to be irritating, the rabbit showing signs of discomfort for about 15 minutes, but no hyperemia of the peritoneum was found at autopsy 5 hours later.

It will be seen from Table II that thyroid tissue always contained a greater concentration of SCN^- than any other tissue, though

[3] Baumann, E. J., Sprinson, D. B., and Marine, D., *Endocrinol.*, 1941, **28**, 793.

[4] Ray, T. W., and Desach, J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 228.

[5] Hamilton, J. G., and Soley, M., *Proc. Nat. Acad. Sci.*, 1940, **26**, 483.

[6] Corson, D. R., MacKenzie, K. R., and Segrè, E., *Phys. Rev.*, 1940, **57**, 459.

† We thank the American Cyanamid Company, who supplied the thiouracil.

[7] Eisenman, A. J., *J. Biol. Chem.*, 1929, **82**, 411.

[8] Baumann, E. J., Sprinson, D. B., and Metzger, N., *J. Biol. Chem.*, 1934, **105**, 269.

TABLE I.
Comparison of Cl- Concentration in Thyroid and in Other Fresh Tissues.

No. rats	Treatment	Thyroid Cl, mg per g	Muscle Cl, mg per g	Pancreas Cl, mg per g	Liver Cl, mg per g
3	Normal	1.48	.50		1.26
5	"	1.19	.47		1.11
5	"	1.30	.50	1.20	1.07
5	"	1.27	.49	1.05	1.12
7	"	1.29	.51	1.00	1.13
4	"	0.75	.44		
2	Thiouracil	0.81	.43		
2	"	1.30	.47		1.03
2	"	1.32	.46		0.98
2	"	1.99*	.44	1.30	1.12
2	"	1.56*	.95		1.27

* These rats had snuffles-pneumonia.

TABLE II.
Comparison of Concentration of SCN- in Thyroid and in Other Fresh Tissues.

No. animals	Thyroid, wt. g	Thyroid SCN, mg per g	Muscle SCN, mg per g	Liver SCN, mg per g	Blood SCN, mg per g	Kidney SCN, mg per g
2 rats	.209	.49	.21	—	.30	.43
2 "	.147	.64	.18	—	.31	—
2 "	.119	.71	.48	.42	.53	.66
3 "	.259	.73	.51	.38	.40	.62
3 "	.213	.70	.37	.40	.58	.66
2 "	.125	.80	.60	.50	.54	.74
Rabbit	.179	.59	.30	.29	.26	.37

the kidney which is the excretory organ for thiocyanate had only a slightly lower concentration than the thyroid. The level of tissue SCN^- was largely determined by the elapsed time between injection and death.

The daily intake of iodine necessary to maintain a normal thyroid in an albino rat is only 2 or 3 μg. If 50 to 250 mg of halides are added daily to the food of rats, a not very excessive amount, they would receive 16,000 to 80,000 times as much halide as iodide. And since all halogens are selectively filtered by the thyroid one could reasonably expect the added halide to have a mass action-like effect on the iodine normally ingested, thus preventing it from reaching the thyroid. Such is the case. Experiments have been made with fluoride, chloride, bromide, and thiocyanate (which behaves like the halogens) and with each of these substances, thyroid hyperplasia was produced.

Thus Goldemberg[9] found that rats given NaF for several months develop very hyperplastic thyroids, as much as 8 times the normal size. Hibbert[10] produced thyroid hyperplasia in rats on low iodine intake by giving 2% $CaCl_2$ or 3% NaCl. These observations were confirmed and extended by Remington[11] who fed diets containing 1 to 4% of NaCl. The thyroid iodine was reduced to one-third of the amount in the control animals. Morruzzi[12] and Simon[13] found on continued feeding of NaBr to young rats that thyroid I was decreased and goiter developed. The many more recent experiments in which goiter has been produced by giving thiocyanates to man and other animals are well known and need not be specifically cited.

Our observations and those from the literature are summarized in Table III. Table III indicates (1) that the thyroid selectively

[9] Goldemberg, L., *C. R. soc. biol.*, 1926, **95**, 1169.

[10] Hibbert, J. S., *Arch. Surg.*, 1933, **26**, 648.

[11] Remington, R. E., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 652.

[12] Morruzzi, G., *Boll. soc. ital. biol. sper.*, 1940, **15**, 733.

[13] Simon, I., *Boll. soc. ital. sper.*, 1941, **16**, 562.

TABLE III.
Summary of Thyroid Response to Administration of Group VII Salts.

	Selectively filtered by thyroid	Reduces thyroid iodine	Produces thyroid hyperplasia and goiter
F	?	?	+
Cl	+	+	+
Br	+	+	+
I	+	—	—
SCN	+	+	+
Mn	+	?	?
At	+	?	?

filters from the blood SCN^-, the halides, and other members of group VII of the periodic table. This inference is based on the greater concentration of these ions found in the thyroid compared with other tissues; (2) that the reduction of thyroid iodine is brought about by giving 0.1 to 0.4 g SCN^-, F^-, Cl^-, Br^- to rats daily for several weeks (or greater doses to larger animals); and (3) that the development of thyroid hyperplasia follows as a consequence of the lowering of thyroid iodine.

Discussion. The investigations mentioned above as well as others have all been concerned with but one of these ions. There has been no very satisfactory explanation offered of how any of them act. In fact most workers have been content to go no farther than to present their findings. For example Wolff, Chaikoff, Taurog, and Rubin[14] in studies on thiocyanate goiter concluded that SCN^- inhibited the uptake of I by the thyroid, but no explanation of the inhibition was offered. In a study of the uptake and discharge of I by propylthiouracil and thiocyanate-induced goiters, VanderLaan and Bissell[15] found that SCN^- did interfere with the uptake of I but this could be prevented by giving large amounts of I simultaneously. Propylthiouracil, on the other hand, inhibited thyroid hormone synthesis but did not interfere with the "trapping" mechanism, an effect similar to that described by Baumann, Metzger, and Marine[2] for thiourea a number of years before. This study was extended by J. E. and W. P. VanderLaan[16] who found that SCN^- inhibited the preferential accumulation of small amounts (<1.0 mg) of iodide and that this inhibition persisted for 5 hours. Furthermore they found that bromides in a dose 500 times greater than that of the injected iodide will not cause significant I displacement from the thyroid. In this experiment apparently a single dose of bromide was used though no details are given. It does not necessarily conflict with those of Morruzzi[12] and of Simon,[13] for the latter fed bromides continuously for months and uniformly found thyroid iodine greatly diminished under these conditions.

In all the thyroid-halogen-SCN^- work so far considered, massive doses of halide or thiocyanate have almost always been used, many hundreds or thousands of times as much as the I ingested. We believe that these anions exert a mass action-like effect; as a consequence inorganic iodide is washed out of the thyroid and new supplies of I are prevented from being taken up. The iodine present as thyroxine and diiodotyrosine is used up in the course of a few weeks, and since new supplies of I cannot be received, thyroid hyperplasia and goiter develop as soon as the thyroid I falls below a critical level. Not only do the halides and SCN^- behave this way toward I, but we[3] have found that large doses of chlorides remove bromides from the thyroid. We believe that continued administration of relatively large amounts of any halide will wash out any other halide present in small amount.

Thiocyanates behave in the same way as do the halogens. For they too accumulated preferentially in the thyroid and at the same

14 Wolff, J., Chaikoff, I. L., Taurog, A., and Rubin, L., *Endocrinol.*, 1946, **39**, 140.

15 VanderLaan, W. P., and Bissell, A., *Endocrinol.*, 1946, **39**, 157.

16 VanderLaan, J. E., and VanderLaan, W. P., *Endocrinol.*, 1947, **40**, 403.

time washed iodide out, thus producing thyroid hyperplasia. This similar behavior of SCN^- is not surprising; rather it is to be expected from the position of SCN^- at the end of the Hofmeister anion series (acetate> Cl^->Br^->I^->SCN^-, etc.) Since the effects of these anions on protein swelling, surface tension, and on many other phenomena increase as the SCN^- end of the series is approached, it is reasonable to suppose that the ability of the thyroid to filter these ions from the blood would be affected similarly.

A question that arises is how the thyroid differentiates between the halogens and other ions. A point of similarity is found in the arrangement of their electrons. All have 7 in their outer shells consisting of 2 in the s and 5 in the p groups. We suggest that this characteristic electron arrangement of the halogens is one that may enable thyroid tissue to effect this differentiation. At the same time it would explain why *all* halides are filtered from the circulating medium in preference to other ions. While thiocyanates closely resemble the halides in their chemical properties, and while the behavior of tissues towards halides and thiocyanates is similar we do not know whether their nuclear structure is correspondingly alike.

Further to support this view one should note the preferential accumulation of Mn and At by the thyroid. Like the halogens these elements of the seventh periodic group also have 7 electrons in their outer shells but instead of 2 s and 5 p electrons, the arrangement is believed to be reversed. And as stated in the first part of this paper one would also expect the remaining elements of group VII, *viz.*, technetium (element 43), and rhenium, to be preferentially filtered from the blood.

It becomes a matter of great interest to determine the relative avidity of the thyroid for these elements, to determine whether the preference follows a definite order, and to note the effect of the reversal of the electrons in the outer shell.

Summary. 1. Thyroid filters preferentially and accumulates thiocyanates as well as all the elements of the seventh periodic group that have been studied, *viz.*, Cl, Br, I, Mn, At.

2. All halides and thiocyanates when administered in relatively large amounts compared with iodine wash inorganic iodide from the body and prevent new supplies of iodine from being received by the thyroid, thus producing thyroid hyperplasia and goiter. It is suggested that these ions exert, at least in part, a mass action-like effect.

3. It is further suggested that similarity in arrangement of electrons in the outer shell of the elements of the seventh periodic group enables the thyroid to differentiate between them and other elements.

16985

Water Content of Rat Uterus and Oviducts During Proestrus and at End of Heat.*

D. LOUISE ODOR AND RICHARD J. BLANDAU. (Introduced by C. E. Tobin.)

From the Department of Anatomy, University of Rochester School of Medicine and Dentistry.

During the period of sexual receptivity in the rat the uterine cornua are distended with fluid which is retained by the tonic contractions of the cervix.[1,2] The appearance of the fluid within the lumina coincides with a significant decrease in uterine tissue water.[3] Also during heat several of the ampullar loops of the oviducts become greatly distended with

* This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

1 Long, J. A., and Evans, H. M., *Mem. Univ. Calif.*, 1922, **6**, 1.

2 Blandau, R. J., *Am. J. Anat.*, 1945, **77**, 253.

3 Astwood, E. B., *Am. J. Physiol.*, 1939, **126**, 162.