and serum from a person known to have brucellosis in evolution, the immune serum antigen mixture leaves a black spot practically unaltered at the place where it was set. A control mixture containing normal serum is washed upwards. The different behavior of both mixtures is very striking and takes place in less than 1 minute (Fig. 5).

The intensity of the test varies in degree as shown in Figs. 7, 8, and 9. Fig. 6 is a normal serum control.

The main difference in the action of normal and immune serum seems to depend on the fact that normal serum acts as a protective colloid upon the antigen (as it acts upon the ink), preventing its fixation in the paper from which it may be readily washed away by the ascending fluid. When immune serum is used in the test the protective effect fails to act upon a "colloid" previously modified in its electrical condition because of the attachment of antibody. Under such conditions the reaction becomes similar to that of a mixture of saline and antigen though considerably reinforced by the action of the specific globulin.

Because of this affinity to the filter paper by the immune serum-antigen mixtures, we believe that the designation of "surface fixation" may be explanatory of this phenomenon.

The union of the antigen and antibody seems to be very rapid which is to be expected from the findings on agglutination and precipitation reactions by the authors cited herewith and a very demonstrative addition to this view may be observed using method (b). When the antigen is placed in a previously arranged wheal of normal serum the addition of saline will push the antigen to the periphery producing a more or less uniform spread of stained organisms (Fig. 11). This occurs in a fraction of a second. If the antigen is set on a spot of immune serum, the addition of saline is without noticeable effect upon the antigen which remains at the place where it was placed (Fig. 10).

The intensity of the fixation phenomenon depends on the antibody content of the serum. It is demonstrable with serum from patients suffering from brucellosis in evolution and remains active even when agglutinins have become of very low titres. The application of this method as a test for doubtful cases of brucellosis has not been investigated *in extenso*. So far, only antigens prepared with Brucella have been studied. All organisms do not have the same ability to spread with the ascending fluid, which shows certain relationships to phenomena studied in paper chromatography.

Summary. A new method of demonstrating the union of antigen and antibody is described. The test is performed on the surface of filter paper and submitted to the washing effect of a stream of isotonic NaCl solution. While normal serum is washed together with the antigen, the immune serum produces the fixation of the antigen to the paper at the place where it was deposited.

Regarding its specificity, it has been observed that this test, studied only in brucellosis, produces responses which so far have agreed with the usual agglutination reactions.

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Disappearance of Hyaluronidase from the Blood of Albino Rats. (17571)

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Following the intravenous injection of hyaluronidase into the albino rat, edema of the extremities, elevation of the blood hematocrit and a decrease of the concentration of circulating plasma proteins result.(1,2) These effects are most marked in 15-45 minutes and

1. Elster, S. K., Freeman, M. E., and Dorfman, A., *Am. J. Physiology*, 1949, v156, 429.
2. Elster, S. K., Freeman, M. E., and Anderson, P., *J. Lab. and Clin. Med.*, 1949, v34, 834.

are no longer observed in 4-8 hours. The present investigation was undertaken to study the rate of disappearance of the intravenously administered hyaluronidase and the changes produced by it in the level of the nonspecific plasma hyaluronidase inhibitors.

Experimental: One hundred twenty-four male albino rats of the Sherman strain, weighing between 140 and 270 g were fed Purina Laboratory Chow and water *ad lib.* The hyaluronidase was prepared from fresh bull testes according to the method of Freeman *et al.*(3)* The material was assayed and measured 3,500 turbidity reducing units (T.R.U.) per mg of nitrogen. These units have been defined by the assay procedure of Dorfman and Ott.(4) Seventy-four animals were anesthetized with ether and the jugular vein was exposed. Seven thousand five hundred T.R.U. hyaluronidase in normal saline were injected intravenously. At 5, 15 and 30 minutes and 1, 2, 4, 8, 24 and 72 hours, groups of animals were bled from the aorta with a heparinized syringe. The blood was centrifuged and the plasma separated. The hyaluronidase content of these plasma samples was determined turbidimetrically. The plasma hyaluronidase inhibitor level(5) of these and of 13 normal male albino rats was measured. Since the methods of assay measure the excess of one over the other, the values for hyaluronidase and plasma inhibitor are relative.

Thirty-seven rats were placed in metabolism cages and their urines collected for a period of 18 hours. The animals were then injected intravenously with 7,500 T.R.U. hyaluronidase, and they were replaced in the metabolism cages. Urine specimens for the subsequent 18 hours were collected in containers kept at 0-5°C. The hyaluronidase content of 15 and the protein content of the remaining 22 samples were determined.(6)

TABLE I.
Plasma Hyaluronidase Level Following Intravenous Administration of 7500 T.R.U.

Time following inj.	No. of animals	Mean plasma level (T.R.U./cc)	Range (T.R.U./cc)
5 min.	11	604	369–1000
15 "	9	65.3	47–110
30 "	7	4.4	0–16
1-4 hr	10	0	0

TABLE II.
Plasma Inhibitor Level Following Intravenous Administration of 7500 T.R.U. Hyaluronidase.

Hr following inj.	No. of animals	Mean plasma level (μ/cc)
1	6	<40
2	6	<40
4	10	<40
8	5	46.6
24	5	69.0
72	5	72.2

Mean normal plasma inhibitor level (13 rats)—75.1 μ/cc.

Following the introduction of testicular hyaluronidase into the blood, its plasma concentration was maximal at 5 minutes (Table I). It rapidly diminished, and at 30 minutes 5 of 7 specimens had amounts insufficient for detection; of the remaining 2, one contained 15 and the other 16 units/cc.

In Table II are listed the changes of the plasma hyaluronidase inhibitor following the intravenous injection of the enzyme. The mean value of sera of 13 normal rats was 75.1 units/cc. Since hyaluronidase was present in the 5, 15 and 30 minute samples, the inhibitor level could not be measured. At 1, 2 and 4 hours the amount of hyaluronidase inhibitor was less than 40 units/cc; at 8 hours it was still diminished, and it returned to normal levels in 24 to 72 hours.

Very small amounts of hyaluronidase appeared in the urine of the rats following the administration of 7,500 T.R.U. The mean control urine volume of the 15 rats for the 18 hours preceding the injection was 8.5 cc. The mean urine volume for the same time period following injection was 7.1 cc. The average hyaluronidase content of the urine

3. Freeman, M. E., Anderson, P., Oberg, M., and Dorfman, A., *J. B. C.*, 1949, v180, 655.

* The authors are indebted to Major Monroe E. Freeman, MSC, for the hyaluronidase preparations.

4. Dorfman, A., and Ott, M. L., *J. B. C.*, 1948, v172, 367.

5. Dorfman, A., Ott, M. L., and Whitney, R., *J. B. C.*, 1948, v174, 621.

6. Simmons, J. S., and Gentzkow, C. F. Laboratory Methods of the United States Army, Lea and Febiger, 1944, Philadelphia.

was 22.3 T.R.U./cc, so that a total of 158 T.R.U. hyaluronidase was excreted. There was no increase of the urinary protein excretion of 22 rats. During the control 18 hour period, 5.7 cc of urine were excreted, containing 1.33 mg protein per cc, or a total of 7.6 mg protein. During the comparable post-injection interval, the animals passed 5.5 cc of urine, that contained 1.33 mg protein per cc for a total of 7.3 mg protein.

Discussion: Meyer(7) has written that "intravenously injected enzyme is rapidly eliminated from the circulation". Seifter(8) found in rabbits a short sojourn of the injected hyaluronidase in the blood as assayed with the mucin-clot method. It was considerably longer when measured by the spreading technique. In the rabbit and the dog, large amounts of the hyaluronidase were excreted in the urine. In the albino rat, intravenously administered hyaluronidase disappeared rapidly from the blood. Approximately 2% of the enzyme was found in the urine in an active turbidity reducing form. The amount of hyaluronidase injected into the animal represented 12.5 mg protein. The absence of significant proteinuria was added evidence of the lack of renal excretion of the enzyme. The fate of the injected enzyme has not been elucidated completely. Hyaluronidase most probably is a small protein molecule(9) and may be lost by simple diffusion from the vascular bed. This diffusion may be increased by the specific action of the enzyme *in vivo*, in which fluid and proteins are actively passed through the capillary wall.(1,2) A small amount of the enzyme was lost in the urine, and an unknown amount was inactivated by the blood inhibitors. As yet, the remainder has not been traced.

The plasma inhibitors were diminished by the administration of hyaluronidase, suggesting a direct action of one on the other. Recovery to normal levels did not occur for 24-72 hours. The source of the newly formed inhibitors has not been found. Wattenberg and Glick(10) have suggested that the inhibitors may be formed in the blood stream itself, since the tissues of the body had no anti-hyaluronidase activity.

Summary: Following the intravenous administration of testicular hyaluronidase to albino rats, the following changes were noted:

1. The maximum concentration of the enzyme was attained within 5 minutes and diminished rapidly, so that at one hour it was no longer detected by turbidimetric methods.

2. Approximately 2% of the hyaluronidase appeared in the urine in an active form.

3. The plasma hyaluronidase inhibitor level was depressed for 8 hours and returned to normal within 24-72 hours.

4. No significant proteinuria resulted.

7. Meyer, K., *Physiol. Rev.*, 1947, v27, 335.
8. Seifter, J., *Ann. N. Y. Acad. Sci.*, in press.
9. Freeman, M. E., personal communication.
10. Wattenberg, L. W., and Glick, D., *J.B.C.*, 1949, v179, 1213.

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Destructive Action of Astatine^{211} (Element 85) on the Thyroid Gland of the Rat.* (17572)

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The observation that astatine accumulates in the thyroid suggested that this new element might be of possible therapeutic application in hyperthyroidism as has already been demonstrated in the case of radioiodine.(1,2) As-

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tatine is not only radioactive with a half-life of 7.5 hours,(3) but unlike most of the artificial radio-elements, it emits alpha particles† rather than electrons and gamma rays. The radiobiological significance of the fact that astatine^{211} emits alpha particles is that each of these nuclear particles arising from the disintegration of an astatine nucleus will dissipate an initial kinetic energy of 6 Mev in about 50 μ of a soft tissue such as the thyroid. The beta rays from the 8-day radioiodine, I^{131}, possess a maximum energy of .6 Mev with a range of nearly 2,000 μ of tissue.

Methods. The At^{211} was produced by the bombardment of bismuth by 29 Mev alpha particles from the 60 inch cyclotron at the Crocker Laboratory. The astatine^{211} was distilled from the bismuth target and collected in 12 N HCl, which in turn was diluted and made isotonic by the addition of an appropriate quantity of sodium hydroxide. The procedures outlined above were derived from a report by Segre and his co-workers which describes the nuclear and chemical properties of astatine.(4) Preliminary thyroid uptake studies in the rat revealed that in a group of eight 200 g animals of both sexes that from 2% to 8% of the administered astatine^{211} was present in the thyroid at the end of 18 hours,(5) the necessary corrections for the decay of the astatine^{211} having been applied. These values are comparable to the results obtained in earlier studies in which guinea pigs were employed as the experimental animals.(1) More detailed studies on the distribution of astatine^{211} in other tissues are now in progress. Three 200 g white rats were selected to observe the radiobiological action of the alpha particle irradiation of the thyroid from the accumulated astatine^{211}. The astatine^{211} in an isotonic solution of NaCl was administered to two of the rats by intraperitoneal injection. They received 10 μc and 50 μc respectively. The third animal was used as a control. After 30 days the 3 animals

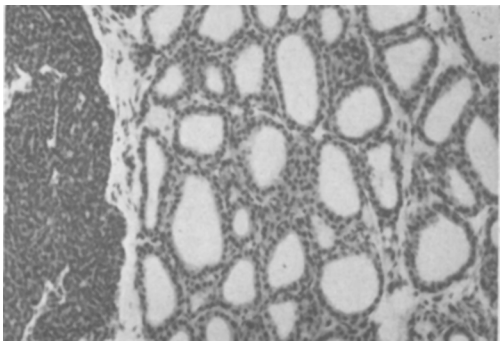

FIG. 1.
Photomicrographs of thyroid and parathyroid tissue of rats. H&E, × 140.
Normal control. (Bouin's fixation).

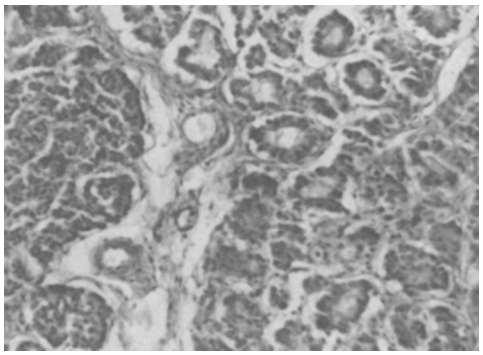

FIG. 2.
Thirty days after injection of 10 μc of At^{211}. (Alcohol fixation).

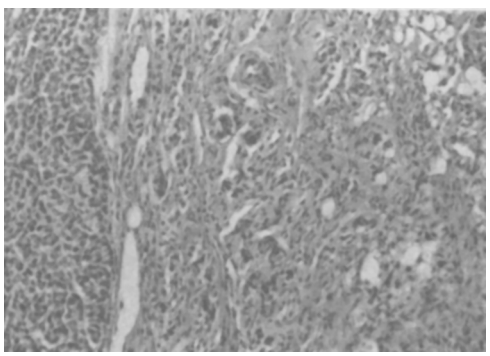

FIG. 3.
Thirty days after injection of 50 μc of At^{211}. (Alcohol fixation).

1. Hamilton, J. G., and Soley, M. H., *Proc. Nat. Acad. Sci.*, 1940, v26, 433.

2. Hamilton, J. G., *Radiology*, 1942, v39, 541.

3. Seaborg, G. T., and Perlman, I., *Rev. Mod. Phys.*, 1948, v20, 585.

† In addition there are emitted x-rays of 80 Kev which makes possible *in vivo* thyroid uptake studies in man.(2)

4. Johnson, G. L., Leininger, R. F., and Segre, E., *J. Chem. Phys.*, 1949, v17, 1.

5. Unpublished data.

were sacrificed and the thyroids removed. The tissue was fixed, embedded in paraffin, and the sections stained with haematoxylin and eosin for histological study. The sections were cut in such a manner that from each of the 3 thyroids a portion of the parathyroid gland was included.

Results. Photomicrographs of the sections from the control, 10 μc, and 50 μc studies are shown in Fig. 1, 2 and 3 respectively. At the 10 microcurie dosage level it is apparent that there was a marked degree of injury to the thyroid follicles as evidenced by their diminished size, loss of colloid, and increase in size of the follicular epithelium, Fig. 2. The general thyroid architecture is distorted to such a degree that it would seem probable that in this instance the degree of radiation injury approached a level of being totally irreversible even if a recovery period of from six months to a year had been allowed. The adjacent parathyroid tissue did not reveal any observable changes that would suggest radiation injury to this organ. The thyroid tissue in the rat which received 50 microcuries of astatine^{211} is not recognizable as such. Here there is complete obliteration of the follicles, colloid, and follicular epithelium. The thyroid tissue has been replaced by fibrous tissue with some infiltration of lymphocytes and fat cells. In this instance it appears that a complete and irreversible destruction of the thyroid gland took place, as a result of an extreme degree of radiation injury. It is significant to note that there was no histological evidence of manifest radiation injury in the adjoining parathyroid tissue.

Summary. The results from this preliminary study demonstrate the capacity of astatine to induce an extreme degree of radiation injury of the thyroid gland in the rat without apparent involvement of the adjoining parathyroid gland.

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Prevention of Procaine Convulsions by Presidon and Sodium Pentobarbital. (17573)

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While the use of procaine is extensive and relatively safe, an occasional subject is found sensitive to the drug. The toxic reactions to subcutaneous procaine are caused by stimulation of the central nervous system resulting in restlessness and tremors which may proceed to convulsions. There is also an initial period of respiratory stimulation followed by depression of the medullary centers resulting in dyspnea and respiratory collapse. The use of hypnotics in the prevention and treatment of toxic reactions to local anesthetics is based on animal studies which show that hypnotics, especially the barbiturates, depress the convulsive effects of local anesthetics.(1,2) Presidon (Pyrithyldione; 3,3-diethyl-2,4-dioxotetrahydropyridine) is a relatively short acting hypnotic with a wide margin of safety.(3,4) It was shown to inhibit the convulsions produced by caffeine,(3) picrotoxin and Metrazol.(4) It was found to be an effective hypnotic in human subjects.(5) We have extended the studies on the anticonvulsive properties of Presidon to the prevention of pro-

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4. Koppanyi, T., Herwick, R. P., Linegar, C. R., and Foster, R. H. K., *Arch. intern. pharmacodynamie*, 1940, v64, 123.
5. Freed, S. C., *J. Lab. and Clin. Med.*, 1946, v32, 895.