ized by the injections of fresh human and guinea pig sera. The protection is transient, lasting more than one hour but less than 4 hours. Inactivated human and guinea pig sera afford no protection. Whether the protective action is due to the complement in the fresh sera, or whether other thermolabile enzymes are responsible for the reaction, has as yet not been determined. We are not prepared at this time to identify the substance in the aqueous extract of lung tissue which initiates the clotting. Lung tissue extract which has been heated at 56°C for 30 minutes is inactive. This would suggest that either pro-thrombin or thrombin may be the substances responsible for the clotting since they are thermolabile, while kinases as a class are thermostabile. Further studies are now under way which, it is hoped, will throw light on this subject.

15031

Effects of Thiouracil on the Mammary Gland.*

J. F. SMITHCORS. (Introduced by S. L. Leonard.)
From the Department of Zoology, Cornell University, Ithaca, N.Y.

The thyroid-inhibiting substances, thiourea and thiouracil, induced hyperplasia of the thyroid, attended by typical symptoms of hypothyroidism as indicated by lowered metabolism, decreased food intake and retarded growth.[1-5] In treated animals no other tissues except the anterior lobe of the pituitary were reported altered histologically.[1,5] The weights of the pituitary, gonads, and adrenals of treated animals were in proportion to the decreased body weights. The physiological effects of the thyroid-inhibiting substances were nullified by giving thyroid hormone simultaneously.[2,3]

Since thyroidectomy in both male[6] and female[7] rats favored the stimulation of lobule-alveolar development and the inhibition of the extension of the ducts of the mammary glands, an investigation was made to compare the effects of thiouracil with those following thyroidectomy on the mammary structure of the rat.

Methods. Rats of the Long-Evans strain were arranged in groups of similar age and body weight, and as far as possible comparisons were made between animals of the same litter. Eighty-five rats of both sexes, with body weights ranging from 37-238 g, were used. In some experiments rats of both sexes were castrated before treatment and a few animals were thyroidectomized for additional controls. Since previous experience had shown that injections of estrogen magnified the effects of thyroidectomy on mammary growth,[6] several experiments were made in which total dosages of 50 γ of *a*-estradiol dipropionate† were injected during the last 10 days of the experimental period. In one group, thyroxin was administered at the rate of 5 γ daily to determine if the effects of thiouracil would be counteracted. This dose of thyroxin was found to nullify the effect of the doses of thiouracil given on the metabolic rate and thyroid

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† The estrogen alpha-estradiol dipropionate (Di-Ovocylin) was obtained through the courtesy of Dr. E. Oppenheimer of the Ciba Pharmaceutical Co., Summit, N.J.

Mammary gland of young castrated female rat which received 5 γ of alpha-estradiol dipropionate daily for 10 days.

hyperplasia.[8,9]

Thiouracil‡ was administered subcutaneously in an aqueous suspension in daily doses of 10 mg per 100 g body weight or in the drinking water as a 0.1% solution. Treatment was continued for periods of 18-35 days. At autopsy, the right abdominal mammary gland was removed and stained as a whole mount. Observations were made on the thyroids, adrenals, and body weights.

Results. The mammary glands of 30 normal male and female rats treated with thiouracil for 18-35 days were indistinguishable from glands of 13 control animals. In a few instances there was observed a slight thickening of the ducts and an inhibition of the duct extension in the treated rats. The thyroids were increased 3 to 4 times in weight and the adrenals weighed less those of the controls but were in proportion to the body weight. The treated animals failed to grow as well as the controls. The estrous cycle was lengthened to 7-9 days in the adult treated females.

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‡ The thiouracil was obtained through the courtesy of Dr. Stanton M. Hardy, Lederle Laboratories, Pearl River, N.Y.

Mammary gland of castrated female littermate which received thiouracil for 24 days, and the same amount of estrogen during the last 10 days.

Comparison of the glands of 4 castrated treated rats with those of the 4 controls likewise indicated little or no differences in mammary structure.

When estrogen was injected in the thiouracil-treated and control rats, the mammary glands were obviously different. The glands from 13 rats receiving thiouracil possessed well developed lobules and alveoli while those from the 8 controls showed the usual estrogen stimulation on growth of the end buds and extension of the duct system (compare Fig. 1 and 2). When thyroxin was administered at the level of 5 γ per day, in addition to the thiouracil and estrogen (7 rats), the mammary glands showed end bud growth and duct extension with only an occasional lobule. The extensive lobule-alveolar growth failed to appear in these glands. The thyroids of these rats failed to show the great degree of hyperplasia that occurred when thiouracil alone was given.

Discussion. Thiouracil was unable to produce the lobule-alveolar growth in the mammary glands of normal or castrated rats similar to that produced by thyroidectomy.[6,7] This difference cannot be explained by a failure of the drug to induce a hypothyroid state because when estrogen was administered the

lobule-alveolar development appeared as it did in thyroidectomized rats similarly treated. It is possible that the period of thiouracil treatment was not continued for sufficient time for the changes to occur although thyroid removal for a similar period (35 days) did bring about these changes.[7] A lengthened estrous cycle was also produced by thiouracil, similar to that found in thyroidectomized rats.[10]

Estrogen injections into castrated rats will in time produce lobule-alveolar development but not in the 10-day periods of these experiments.[11] The hypothyroid state apparently causes a precocious appearance of the lobules and alveoli in the mammary glands under these conditions. The injections of thyroxin to offset the effects of thiouracil in inducing hypothyroidism were manifested in the failure of lobule-alveolar growth to appear in the mammary glands. Whether hypothyroidism brings about these changes in the mammary glands by altered hormone metabolism or by altered tissue responses is still a matter of conjecture.

Conclusion. Thiouracil administered to normal or castrated male and female rats failed to induce an alteration of the mammary glands similar to that obtained following thyroidectomy. The mammary glands of rats treated with thiouracil and with estrogen for the last 10 days of the experimental period possessed well developed lobules and alveoli whereas the control glands showed end bud growth and duct extension. When thyroxin was given along with thiouracil and estrogen, the mammary glands resembled those from rats receiving estrogen alone and no precocious lobule-alveolar growth was noted.

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15032

The Use of Regenerating Liver as a Method of Assay.*

A. MARSHAK AND R. L. BYRON, JR.

From the Radiation Laboratory and Department of Surgery, University of California.

Although the rate of mitosis in regenerating liver has been studied,[1] the procedure used was so laborious and time-consuming as to preclude its use as a method for testing the effect of agents upon the growth of liver cells. By applying the technic for obtaining free nuclei previously described by one of us,[2] a method of assay has been developed by means of which it has been possible to test as many as 10 substances in a week. The results of these assays will be published elsewhere.[3] We wish here to present results of studies of the relation of the age of the animal to the rate of mitosis and the uptake of P^{32} by the regenerating liver.

Method. An incision was made on the midline extending about ¼ to ½ inch on either side of the xiphoid process in an anteroposterior direction. The abdominal wall was cut along the midline for ¼ inch posterior to the tip of the xiphoid process which was dissected free of its attached muscle and fascia. With gentle pressure on the sides of the animal the median and left lateral lobes were delivered to the surface and their fascial attachments cut. They were then tied off at the base with cotton or linen thread and cut off as close as possible to the tie. To minimize

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