would be heterogeneous, because the antigen was only partially purified PTE. Absorption of the antiserum with beef serum and thyroid extract apparently removed non-specific antibodies, as the absorbed antiserum revealed a single immunodiffusion precipitin line and a single immunoelectrophoretic arc when reacted with the antigen. PTE preparations, varying from very crude to highly purified, all a) enhanced the precipitin reaction of a known PP-PTE and b) inhibited the subsequent anti-PTE potency of the antiserum when mixed with it, indicating a common antigen in these preparations. It is concluded that this antigen is PTH and not an artifact introduced during processing of the PTG, nor one which failed to be removed during final purification. The antiserum exerted a specific anti-hormone effect on the biologic calcium mobilizing activity of bovine parathyroid hormone in rats. The increase in antigenic potency with progressive purification of the PTE preparations further indicates that the PTH-antiPTH serum reaction is immunologically specific for parathyroid hormone.

The inability to demonstrate immunologic activity in aqueous extracts of human parathyroid tissue may be due to insufficient human tissue available for extraction or to immunologic species specificity of the hormone. The fact that aqueous extracts of an equal weight of bovine parathyroid tissue did reveal immunologic activity by immunodiffusion inhibition suggests species specificity as the more likely explanation.

Summary. An antiserum to bovine parathyroid hormone has been produced in each of 4 rabbits studied, indicating that this hormone is antigenic. The absorbed antiserum induced a single precipitin line by immunodiffusion and immunoelectrophoresis with PTH of variable states of purity, and exerted a specific anti-PTH effect on biologic activity. There are apparent antigenic differences between beef and human parathyroid hormone.

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Effect of Thyroxine on Thyroid Uptake of Thiourea-S35.* (28832)

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Previous studies in guinea pigs revealed that thyrotropin (TSH) was capable of accelerating the intrathyroid metabolism of thiourea-S35 as measured by the formation of sulfate-S35, this being the major metabolite of oxidative desulfuration of the labeled antithyroid compound(1). However, in the same experiment, administration of potas-

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sium iodide largely abolished the thyrotropin-effect, possibly by interfering with the oxidation of the sulfur atom. In view of the similarity between thiourea and iodide with respect to their intrathyroid pattern of oxidation, it seemed reasonable to determine whether the metabolism of thiourea-S35 by the thyroid gland might not also be regulated by circulating thyrotropin. To assess this possibility, experiments using thiourea-S35 were carried out in guinea pigs in which the secretion of thyrotropin had been inhibited by a prolonged period of thyroxine administration.

Materials and methods. Two separate experiments were carried out at different times using 10 male guinea pigs (250–350 g), randomly divided into 2 groups in each experiment. Each group of animals was placed in a separate cage and maintained under identical conditions during the experimental period. One group of animals received single daily intraperitoneal injections of 40 μg of l-thyroxine dissolved in physiologic saline, while the control animals received only the saline. (The l-thyroxine was dissolved first in 0.1 NaOH and then diluted in saline to a final concentration of 100 μg per ml with a pH of approximately 9.0.) All injections were carried out for 25 consecutive days and on the morning of the 26th day, 300 μc of S35-labeled thiourea† (in 0.5 ml saline or distilled water with a specific activity between 10.0 and 50.0 millicuries per millimole) were administered intraperitoneally to both groups of animals. Five hours later the first guinea pig was anesthetized with ether and blood was taken from the heart with a heparinized syringe, after which the animal was killed by incising the heart and great vessels. The same pattern was followed in the remaining animals at 30 minute intervals, together with the removal of the thyroid glands which were weighed (wet) and homogenized in glass tubes containing 1 ml of distilled water per 50 mg of thyroid tissue.

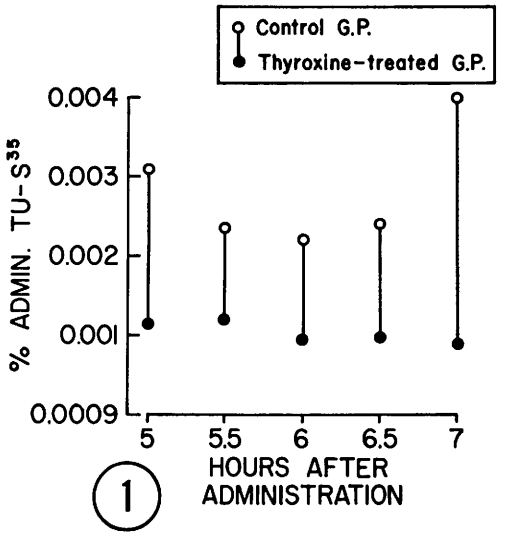
Since the major product of metabolism of thiourea-S35 in animals is sulfate-S35 (SO_4-S35) (2,3), a method was devised to separate the labeled sulfate from the labeled thiourea. After 1 ml of plasma or ½ ml of thyroid homogenate had been placed in centrifuge tubes, the proteins were precipitated by blowing in 2 ml of cold 10% trichloroacetic acid. The tubes were centrifuged and 1 ml aliquots of the supernates (containing thiourea-S35 plus sulfate-S35) were transferred to low-potassium crylite vials, to which 19 ml of scintillating solution‡ were added. A second 1 ml aliquot from each of the original supernates was then transferred to centrifuge tubes where the sulfate-S35 was removed in the following manner: addition of 0.8 ml of 10% trichloroacetic acid followed by 0.05 ml of a 1% solution of sodium sulfate and 0.15 ml of a 25% solution of barium chloride, resulted in precipitation of the stable and labeled sulfate. (Recovery experiments demonstrated that approximately 95% of sulfate-S35 which had been added to plasma or to thyroid homogenates was precipitated, while thiourea-S35 remained in solution.) The tubes were centrifuged and 1 ml aliquots of these supernates (containing only thiourea-S35) were transferred to the crylite vials to which scintillating solution was added, and the radioactivity in both sets of vials was measured using an automatic liquid scintillation spectrometer.§ The concentration of sulfate-S35 was obtained by subtracting the radioactivity values of thiourea-S35 from the total S-35 radioactivity; after the appropriate corrections were made for weight and volume, the percent of thiourea-S35 taken up by the thyroid gland was determined and the thyroid plasma ratio of sulfate-S35 was calculated.

Results and discussion. It is apparent (Fig. 1) that substantially more S35-labeled thiourea was taken up by the thyroid glands of the control animals than by those of the guinea pigs treated with the thyroxine. Furthermore, as reflected by the thyroid/plasma ratio of sulfate-S35 (Fig. 2), a significantly greater percentage of thiourea-S35 was degraded by the thyroid glands of the control guinea pigs than by those of the thyroxine-

† New England Nuclear, Inc., Boston, Mass.

‡ For preparation of scintillating solution see ref. 1.

§ Packard Instrument Sales Corp., La Grange, Ill.



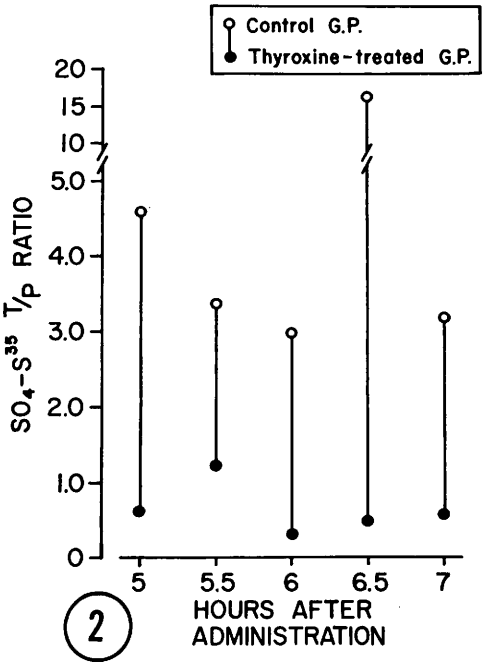


FIG. 1. Per cent of administered dose of thiourea-S35 found per total thyroid gland. Each circle represents a single guinea pig.

FIG. 2. Ratio of sulfate-S35 in thyroid and plasma (activity per gram of thyroid divided by activity per ml of plasma). The labeled sulfate originated from the intrathyroid metabolism of thiourea-S35.

treated animals ($P < 0.01$).

It is of interest that an earlier study using hypophysectomized rats showed that while the rate of intrathyroid metabolism of thiourea-S35 was considerably delayed, uptake of the labeled antithyroid compound by the thyroid gland was not impaired(3). Whether or not the difference between those results and these reported here is related to the species of animals, the specific activity of the thiourea-S35,‖ or the possibility of a direct inhibitory effect of thyroxine on the thyroid gland is not apparent. However, it seems clear that thyrotropin does indeed influence the uptake and metabolism of thiourea-S35 by the thyroid gland; consequently, these results offer further indirect evidence that antithyroid substances and iodide may have similar intrathyroid pathways of oxidative metabolism.

Summary. Guinea pigs pre-treated with thyroxine exhibited substantial reductions in thyroid uptake of thiourea-S35 and an impaired ability to metabolize the labeled compound at the same rate as that of the control animals.

‖ Administration of low specific activity thiorea-S35 to guinea pigs resulted in substantially less incorporation of radioactive material by the thyroid gland, probably as a consequence of dilution by the relatively large amount of stable thiourea.

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