C. *Hypnotic effect.* Hypnosis does not occur in animals receiving increasing dosage of Khellin. To study the influence of Khellin on hypnosis, the joint effect of Khellin and pentobarbital was determined in mice by the duration of sleep. The increase in sleeping time due to Khellin was taken as a measure of its central depressive effect. The results in Table III were obtained in groups of 20 and 40 mice which received a suspension of Khellin in 7% gum acacia orally half an hour before 50 mg/kg of pentobarbital was injected intraperitoneally. The duration of pentobarbital-hypnosis was definitely prolonged with 1 mg/kg of Khellin; with 5 mg per kg it was 4 times as long as that by pentobarbital alone.

Summary. Khellin has been shown to be effective in suppressing excitement in rats induced by caffeine. At slightly depressive levels, it is more effective against metrazol than against electrically induced convulsions. The central nervous depression produced by Khellin is mild but of long duration; with increasing dosage this is followed in mice by excitement.

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Factors Contributing to the Difference in Propylthiouracil Goitrogenesis in Rats and Guinea Pigs. (19056)

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In marked distinction to the rat, dietary thiouracil goitrogenesis in the guinea pig requires prolonged administration of the drug (1,2). On the other hand, administration of thyroid stimulating hormone readily produces a goiter(3). It seems probable that the explanation of this species difference would be concerned with one or all of the following classes of factors: (1) the concentration and effectiveness of thiouracil in the thyroid; (2) the rates of synthesis, excretion and utilization of the thyroid hormone; and (3) the threshold for increased thyrotrophin production and the goitrogenic capacity.

This report is concerned with two questions: (1) Is propylthiouracil equally effective in inhibiting thyroid hormone synthesis in both the rat and the guinea pig, and (2) Is thyroid hormone synthesized at comparable rates in these species. We have investigated these problems by measuring the effect of propylthiouracil on the rate of I^{131} incorporation into the protein of rat and guinea pig thyroid slices *in vitro*. That I^{131} incorporation under these conditions represents thyroid hormone synthesis has been shown by the work of Morton and Chaikoff(4).

Methods. Guinea pigs weighing 600-800 g, and Sprague-Dawley rats weighing 150-250 g were used regardless of sex. They had been fed stock diet for at least 2 weeks prior to use. The animals were sacrificed by a blow on the head, and the thyroids were rapidly removed, trimmed, weighed and transferred to iced containers. The glands were sliced as uniformly as possible. This usually meant division of each lobe into 3 to 4 slices for the guinea pig, and into 2 for the rat. When necessary, the lobes from several animals were combined to furnish sufficient tissue for the separation procedures outlined below. All glands were divided at the isthmus, and slices from one lobe were incubated with propylthiouracil, while those from the other lobe were used in the

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3. Williams, R. H., Weinglass, A. R., and Kay, G. A., *Am. J. Med. Sci.*, 1944, v207, 701.

4. Morton, M. E., and Chaikoff, I. L., *J. Biol. Chem.*, 1943, v147, 1.

control flasks. The thyroid slices were incubated for 3 hours at 38°C in Krebs-Ringer's solution which contained 10-30 μc of I^{131} per ml. In the flasks containing propylthiouracil, concentrations ranging from 10^{-2} M through 10^{-7} M were used. At the end of the experiment the reaction was stopped by boiling, and the tissue was homogenized in an all-glass instrument. The protein was precipitated with 20% trichloroacetic acid, kept at room temperature for one hour, and washed twice in 5% trichloroacetic acid. The precipitated protein was dissolved by heating in 4-10 ml of 4N NaOH for 6 to 12 hours, and appropriate aliquots evaporated on metal planchets without adjustment of pH. The radioactivity in these preparations was estimated with an end-window Geiger-Mueller tube and compared with standards appropriately prepared to permit corrections for absorption and decay. The results were calculated as:

$$\frac{\%\text{ of dose bound to protein}}{\text{mg thyroid tissue}}$$

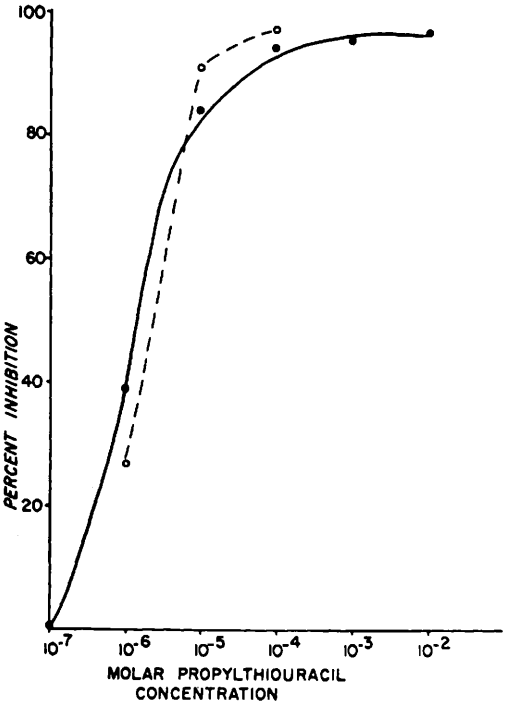

FIG. 1. The relation between propylthiouracil concentration and I^{131} binding to thyroid protein *in vitro*. Solid line, guinea pig thyroid; broken line, rat thyroid.

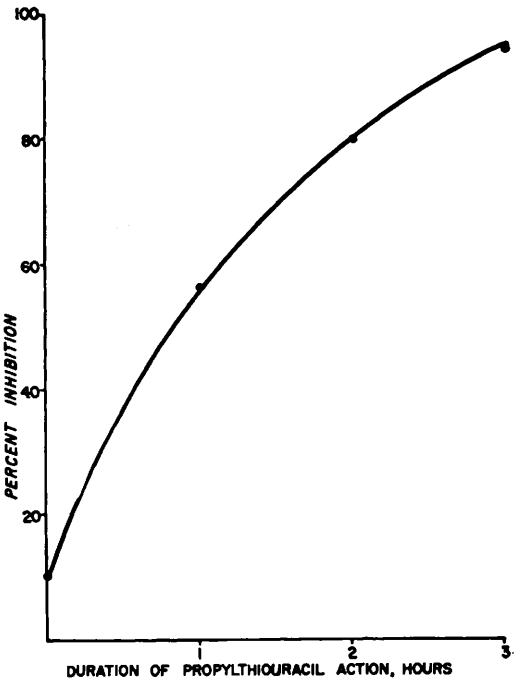

FIG. 2. The relation between duration of propylthiouracil action and I^{131} binding to guinea pig thyroid protein.

Results. In Fig. 1 the relationship between protein binding of I^{131} by surviving rat and guinea pig thyroid slices and the concentration of propylthiouracil in the incubation medium is shown. In both cases thyroid response was similar. At a concentration of 10^{-7} M, propylthiouracil was without effect; 10^{-6} M caused 25-40% inhibition; and 10^{-5} M resulted in almost complete inhibition of I^{131} uptake. The reality of these effects was tested by adjusting the propylthiouracil concentration in experimental flasks to 10^{-5} M at various intervals after incubation of guinea pig thyroid slices with I^{131} (Fig. II). When propylthiouracil was added at zero time, little or no I^{131} was incorporated into gland protein; and when added one or 2 hours after the addition of I^{131}, incorporation was reduced to approximately 80 and 60% of the 3-hour values, respectively. Consequently it appears that propylthiouracil *in vitro* inhibits thyroid hormone synthesis in guinea pig thyroid, and that the effectiveness of this drug in the guinea pig is not materially different from its effectiveness in the rat.

TABLE I. Relative Incorporation of I^{131} into Protein of Rat or Guinea Pig Thyroid Slices *in vitro*.

	No. of exp.	Avg. % of dose bound to protein/ mg of thyroid tissue	Stand. error
Rat	7	.494	.069
Guinea Pig	14	.0930	.0099

Table I presents data concerned with the relative activity of thyroid slices from both species with respect to I^{131} binding. It can be seen that rat thyroid slices incorporated I^{131} into the protein of the gland about five times as rapidly as did guinea pig thyroid slices. In the light of Morton and Chaikoff's work (4), it would appear that the rate of thyroid hormone synthesis is very much greater in the rat than in the guinea pig.

Discussion. The observations reported above show that, *in vitro,* propylthiouracil is equally effective in inhibiting thyroid activity in both the rat and the guinea pig. It is reasonable, therefore, to expect the same phenomenon to occur *in vivo* provided the drug reaches the acinar cells in adequate concentration. Recalculation of the data of Williams and coworkers(3,5) indicates that oral administration of the drug results in gland concentrations several hundred fold greater than that necessary to produce complete inhibition of iodine binding *in vitro.* These values were achieved within a very few days after the drug was given. Therefore, it hardly seems reasonable that the species difference in thiouracil goitrogenesis results from differences in the availability of the drug.

A more attractive hypothesis accounting for the refractoriness of the guinea pig to propylthiouracil is concerned with the balance between hormone production and its peripheral utilization. In the absence of thyroid activity, the duration of the euthyroid state would be inversely proportional to the rate of peripheral utilization of the hormone. Consequently the time lag before stimulation of pituitary thyrotrophin production and the subsequent appearance of the goiter should be proportional to the rate at which thyroid hormone was utilized.

Our observations, admittedly far from conclusive, provide evidence consistent with this hypothesis. It is obvious that in the intact euthyroid animal the rates of thyroid hormone production and utilization must be balanced. Measurement of the rate of hormone production should then shed light on the rate of utilization. Our data indicate that hormone synthesis by the rat thyroid occurs at approximately 5 times the rate for the guinea pig thyroid. Other factors remaining equal, it follows that peripheral hormone utilization and equivalent levels of hypothyroidism would occur in the guinea pig at one-fifth the rate for the rat. Thus the time lag for propylthiouracil goitrogenesis should be 5 times as long, about 50 to 70 days. This estimate agrees well with the observations reported by D'Angelo(1) and MacNair and Young(2).

Summary and conclusions. (1) The difference between the rat and guinea pig with respect to thiouracil goitrogenesis was investigated using the binding of I^{131} to surviving thyroid slices as a measure of thyroid hormone production. (2) Propylthiouracil in concentrations ranging from 10^{-7} M through 10^{-5} M inhibited hormone synthesis to an equal extent in both rat and guinea pig thyroid slices. *In vivo* studies have indicated the compound to concentrate in the gland to a comparable degree in both species. On the other hand, in the absence of propylthiouracil, hormone synthesis in rat thyroid slices occurred at 5 times the rate for guinea pig thyroid slices. (3) In view of these observations it is suggested that the species difference in thiouracil goitrogenesis can be accounted for by differences in the rates of peripheral utilization of circulating thyroid hormone.

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