Growth Repression in Rats by Thiouracil-Iodide Mixtures.* (30078)

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It is known that young rats suffer growth repression when maintained on diets containing 0.1% or more of thiouracil(1). Since the effects on growth and organ weights are qualitatively similar to those produced by thyroidectomy(2), the growth repression has been ascribed to thyroxine deficiency(3), and has been used to estimate the antithyroid potency of various drugs(1). Recently, Woodward and Berard(4) have reported submaxillary sialadenitis and growth repression in rats fed a diet containing 4% NaI for 28 days. No examination of the thyroid or its function was made, but this iodide dosage would be in the fibrolytic range as defined by Salter(1), hence appreciable thyroid damage and loss of function would be expected. In a previous experiment, using mixtures of thiouracil and KI at lower levels, we noted an apparent effect on growth of rats after several weeks treatment(5). However, as the animals were subjected to further experimental treatment, significance of the observation was uncertain. We therefore performed the present work to test specifically the effect of thiouracil-iodide mixtures on the growth of young rats, and to determine whether the effect of such mixtures was different from that caused by thiouracil alone.

Materials and methods. Male Sprague-Dawley rats, 25-65 days of age were used in all experiments, and divided so that average initial weights of all groups for any experiment agreed within 5 g. For experimental diets, ground Purina Fox Chow (1.3 ppm iodine), plus 5% lard as binder, was mixed with powdered thiouracil, NaI or KI singly or in combination, at a level of 3 μmoles/g of feed (0.0384% thiouracil, 0.0498% KI), and fed *ad libitum* in lipped containers to reduce wastage. From our experience with these drugs, it was expected that this level of thiouracil would approximately double thyroid weight without affecting the metabolic status of the animals, while the KI dosage would result only in increased thyroid iodine. Feed and drug consumption were recorded daily, animal weights weekly. When sacrificed after 14 weeks, length of animals was measured from breech to nose, and fresh organ weights obtained on a Roller Smith torsion balance. Several organs were prepared for histological examination (H. & E. Stain), others analyzed for total iodine. For the latter work, small tissues were ashed at 400° with KOH and the ash treated as described by Kirk(6). For larger tissues (50 mg dry wt), the oxygen combustion method of Bezem *et al* was used(7). 5-Iodothiouracil was also tested, but since it did not affect growth of rats, is not included in the results.

Results. (Fig. 1 and Table I). Significant

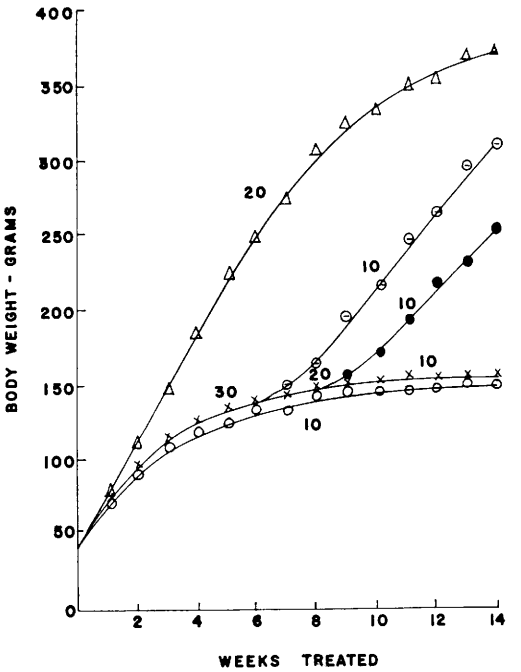

FIG. 1. Effect of antithyroid treatment on weight gain. △—△ Control. ×—× Thiouracil + KI. ⊖—⊖ Thiouracil + KI 6 wk, KI 8 wk. ●—● Thiouracil + KI 8 wk, plus thyroid powder 6 wk (1 mg/g diet). ○—○ Thiouracil + KI 6 wk, thiouracil 8 wk. Drug concentration 3 μmoles/g feed. Number of animals shown on curves.

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TABLE I. Effect of Antithyroid Treatment on Feed Consumption, Body Weights and Organs of Rats, Starting Age 24 Days; Treated 14 Weeks.*

	Dietary additive, 3 μmoles/g feed			
	Controls	KI	Thiouracil	Thiouracil + KI
Feed consumed, g/rat/day	16.5	17	16.6	*10.3*
Drug intake, mg/rat/day	—	8.5	6.4	4 (Thiouracil) 5.1 (KI)
Wt gain, g	336	340	330	*112*
Length in cm, breech to nose	23.3	23.4	23.3	*19.4*
Organ wt, mg/100 g body wt				
Thyroid	6.5	7.0	*16.0*	*15.6*
	(74)†	(*121*)	(*6.0*)	(*21.0*)
Pituitary	2.4	2.4	2.8	*4.5*
Spleen	185	190	170	*133*
	(.2)	(*2.0*)	(.3)	(*2.0*)
Heart	278	270	265	*246*
	(.4)	(1.1)	(.6)	(*3.3*)
Testes	946	940	981	*1622*
	(<.1)	(.2)	(<.1)	(*.5*)

* For italicized values compared with controls, $P \leq .05$.
† Total iodine, in parentheses, as μg/100 mg fresh tissue; 6-16 tissues of each kind analyzed. Values of less than 1 μg for a whole tissue are approximate only.

findings were the growth repression caused by the thiouracil-KI (TKI) diet, and ready reversibility of the repression by adding U.S.P. thyroid powder to the diet (1 mg/g), or by removing thiouracil; and conversely, maintenance of the growth repression, once established, by thiouracil alone. Diets containing either KI or thiouracil alone did not affect growth, hence these curves are not shown. Because of a possible Na/K imbalance in TKI-treated animals, NaI was tried in place of KI, but the same growth repression appeared. Also, since antithyroid treatment may cause involution of the adrenal cortex, a number of photomicrographs of histological sections, taken through the midzone, were measured with a polar planimeter to determine the relative areas of the cortex and medulla. The ratio of cortex to medulla was in the same range for both the control and the TKI-treated animals, and averaged 6.2 for both groups. To test the possibility of a functional impairment not discernible histologically, a separate group of TKI-treated animals was given daily injections of 0.1 mg desoxycorticosterone acetate after 5 weeks' treatment, but with no improvement of growth, and with no detectable changes in the adrenals. It was concluded, therefore, that the growth repression was not mediated through the adrenals.

Because of lower consumption of the TKI diet, (Table I), its palatability was tested by offering this diet, along with stock diet, to a group of normal animals, but they showed no preference for either. Utilization and nutritional value were also checked by placing control animals weighing 140-150 g on restricted feeding. These animals were able just to maintain this weight over a 2-week period on 10-11 g stock diet per day; *i.e.*, the same as the amount of TKI diet consumed per animal in the experimental group over a large part of the 14-week period. With regard to drug intake, animals changed from the TKI to the KI diet soon increased their feed consumption to match that of the controls, while animals changed from the TKI to the thiouracil diet showed no increase in feed consumption, hence continued to receive an average daily dosage of 4 mg thiouracil—a surprisingly small amount to maintain such a severe growth repression.

Absolute weights of pituitaries and testes of TKI-treated animals were very similar to those of control rats, hence were much heavier as a per cent of body weight. It has previously been shown that the rat testis requires little, if any, thyroxine for normal growth and maintenance(9,10), but is completely supported by a combination of gonadotrophin and growth hormone(11). Hence, secretion of the latter hormones appeared to be normal in TKI-treated animals. Expressed also as per cent of body weight, thymus, submaxillary gland, adrenals, kidneys, and liver were

about the same for all groups, but iodine content of thymus and liver was highest in the TKI group. Heart and spleen were smaller on either an absolute or relative basis. Thyroids of thiouracil- and of TKI-treated animals averaged 58 and 32 mg respectively. The difference between these figures is, in our opinion, larger than can be accounted for by the difference in thiouracil intake, hence the added iodide partially inhibited the thyroid weight increase. Histologically, however, thyroids of the two groups could not be reliably distinguished. Both showed about the same degree of epithelial hyperplasia and invagination, decrease in follicle size and loss of colloid.

It was interesting to note that, along with a smaller size, growth-repressed animals retained a juvenile appearance throughout the experiment with thin skin and soft, fine hair. When placed on diets that permitted resumption of growth, both skin and hair soon assumed more mature characteristics. Curves similar to those in Fig. 1 were obtained with 3 further groups of rats started at ages 35, 45 and 65 days. Since the older animals had heavier starting weights, differences from controls in final body weights were less extreme, but growth repression was still striking. In all instances, about 3 weeks treatment with TKI diet was reqired before growth repression was apparent.

Discussion. In an early experiment with thiouracil, Leathem(3) repressed growth of young rats on a diet containing 0.5% of the drug (about 13× the amount used here), and reported similar effects on growth and organ weights. Since similar results had been obtained with young rats by thyroidectomy (2), Leathem interpreted his growth findings as due to a voluntary reduction of food intake subsequent to a lowered metabolic efficiency induced by thyroxine deficiency. In view of the response of our TKI-treated animals to thyroid powder, and since no other primary deficiency was found, we interpret our present results in the same way. On the basis of this interpretation, the major question raised by the present work concerns the mechanism whereby a mixture of thiouracil and iodide suppress thyroxine synthesis much more strongly than thiouracil alone, but in such a fashion that after suppression is established, it is maintained by a small daily dosage of thiouracil alone. This statement assumes that the slight amount of iodine in the stock diet was inadequate to maintain whatever effect was initiated by the added iodide.

So far as gross thyroid changes are concerned, MacKenzie has reported a synergistic action between low levels of sulfaguanidine (2%) and high levels of NaI (5%) in increasing thyroid weight and hyperplasia, but antagonism between various levels of thiouracil and iodide(12). These experiments were terminated after 13-14 days, presumably before a growth effect could have appeared. Our results on thyroid weights agree with those of MacKenzie, but, assuming the growth repression to reflect thyroxine deficiency, imply a synergistic action between thiouracil and iodide at a more subtle level. Other explanations appear rather improbable. It is known, for example, that both thiouracil and excess iodide block organification of iodide by the thyroid, but excepting instances of iodide goiter, the action of iodide is very temporary(13). In view also of the relatively low dosages of the 2 drugs it does not appear possible that the 2 blocks acting independently could have suppressed thyroxine synthesis to the extent indicated by the growth repression. Iodide toxicity, assuming a greater sensitivity toward iodine in TKI-treated animals, seems to be ruled out by the low KI dosage and by continuation of the growth repression for 8 weeks by thiouracil alone. By comparison, the growth repression in NaI-treated animals as reported by Woodward and Berard(4) was produced on a diet containing 267 μmoles NaI/g of diet, or nearly 90× the iodide concentration used in our diets. The generally higher iodine content of tissues from the TKI group may reflect an abnormal iodine metabolism, but for the present is assumed to reflect only a higher than normal iodine intake plus a reduced renal clearance as reported for myxedematous patients(14). Hence, on the basis of immediate evidence, we conclude that thiouracil-iodide mixtures act synergistically on the thyroid so as to suppress thyroxine synthesis. As one possibility, the synergism may operate

by causing a prolonged and greatly increased sensitivity toward thiouracil.

A basic difficulty in attempting any interpretation of the mixed treatment is that while the effects of antithyroid treatment are well known, the actual mechanism by which the effects are produced is not known with certainty for any antithyroid substance. Further investigation of thiouracil-iodide mixtures may help illuminate such mechanisms, and also provide interesting results in growth and maturation studies.

Summary. Young rats grew normally on diets containing either thiouracil or iodide at a level of 3 μmoles/g of feed, but growth was severely repressed on diets containing this concentration of both substances. After several weeks' treatment, growth repression was quickly overcome by adding thyroid powder to the thiouracil-iodide diet, or by removing thiouracil. Conversely, after being established, the repression was completely maintained by thiouracil alone at the stated concentration. It is concluded that the primary cause of the growth repression was a thyroxine deficiency induced by a synergistic action of thiouracil and iodide on the thyroid gland.

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Effect of Feeding Different Amino Acid Diets on Growth Rate and Nitrogen Retention of Weanling Rats.* (30079)

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The essential amino acid requirements of weanling rats fed amino acid diets containing 10% protein (N × 6.25) have been reported from this laboratory† (1-3). The investigations had been undertaken as part of a study to develop a method of protein evaluation by relating the essential amino acid content of the protein to the essential amino acid requirements (4) of the animal or man rather than to whole egg protein (5,6).

The growth rate of weanling rats fed the best amino acid diets was 4-5 g/day. Recently, Rogers and Harper‡ have reported that weanling rats fed an amino acid diet containing 15.2% protein and high in arginine and containing asparagine, agar and 50% water, showed a rate of growth of 45-50 g/week, equivalent to that obtained with a diet containing 18% whole egg protein. Since it is necessary that amino acid requirements be known accurately before a correlation can be established between the amino acid content of the protein and its biological

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