Influence of Alpha-Naphthylthiourea (ANTU) on Iodine Metabolism.* (17779)

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In previous studies we demonstrated(1,2) that pre-treatment of rats with inorganic iodide administered either in the diet or parenterally afforded this species protection against several lethal doses of alpha-naphthylthiourea (ANTU). Other investigators subsequently obtained(3,4) similar results with rats. An extension of our initial studies revealed that large quantities of iodide provided guinea pigs(5) with no significant increase in resistance toward ANTU and the protective effect of iodide could not be demonstrated in thyroidectomized rats(2). The mechanism whereby iodide protects against the acute toxicity of ANTU has not been elucidated. DuBois and Erway(6) found that iodide diminished the strong inhibitory action of ANTU on tyrosinase and although this effect was not considered as an explanation for the prophylactic action of iodide against ANTU it suggests that iodide might protect some oxidative reaction from inhibition by ANTU. It had been shown that sublethal doses of thiourea derivatives other than ANTU(7,8) prevent the uptake of radioactive iodine by thyroid tissue. Thus, there exists a considerable amount of evidence suggesting that acute as well as chronic ANTU poisoning may involve interference with iodine metabolism. We, therefore, undertook an investigation of the effects of ANTU on the iodine metabolism of rats and guinea pigs and the present communication contains the results of iodine measurements on the tissues of normal and ANTU-poisoned animals receiving diets containing normal and high quantities of iodine. The influence of ANTU on the rate of uptake of iodine by the tissues of rats and guinea pigs was also ascertained using radioactive iodine (I^{131}) in tracer quantities in order to observe whether there was a correlation between the effects of ANTU on iodine uptake and the species susceptibility to the rodenticide.

Materials and methods. Sprague-Dawley rats weighing from 200 to 300 g and guinea pigs weighing 300 to 500 g were used in this study. In experiments concerned with the effect of ANTU on the iodine content of the tissues rats were fed Purina Laboratory Chow and guinea pigs were maintained on Purina Rabbit Chow. The high-iodine diet employed for studies with rats consisted of the synthetic 18%-casein diet used in our previous studies (2) with the addition of 5.3 mg of iodide as potassium iodide per g of diet. When low-iodine diets were fed to rats the same ration was employed with potassium iodide omitted from the salt supplement. Since guinea pigs did not readily accept the synthetic diet or Purina Rabbit Chow containing increased quantities of iodide they were fed a normal diet and iodine was administered in the drinking water at a concentration of 2.85 mg/ml. Rolled oats constituted the low-iodine diet for guinea pigs. ANTU was dissolved in propylene glycol and

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administered intraperitoneally with the strength of the solution being adjusted so that no animal received an amount of propylene glycol greater than 0.5% of its body weight. For studies with radioactive iodine a carrier-free solution of I^{131}, obtained from the Oak Ridge National Laboratory, was employed. The dose usually given was 10 microcuries administered intraperitoneally. Animals were fed a low-iodine diet for several days before administration of the isotope because this procedure resulted in a higher and more constant uptake of I^{131} by the tissues. At intervals after the administration of I^{131} the animals were sacrificed and the tissues were homogenized(9) in distilled water and diluted so that 2 ml of homogenate contained radioactivity within the range of accurate measurements. The I^{131} was measured using a Geiger-Muller apparatus with an end-window mica counter. For measurements of the iodine content of rat and guinea pig tissues animals were sacrificed by decapitation and 30% homogenates of the tissues were prepared. Total iodine was determined by the method of Perkin *et al.*(10). Separation of organically-bound from inorganic iodine was performed by the procedure of Palmer, Leland, and Gutman(11) as modified by Salter(12).

Experimental. The effect of ANTU on the iodine content of rat and guinea pig tissues. The iodine content of tissues from rats receiving a normal diet has been reported previously(12,13) and our values are essentially in agreement with those of other workers. The iodine content of tissues from 11 normal and 5 ANTU-poisoned rats is shown in Table I in which the distribution of iodine between the organic and inorganic fractions is given as well as the values for total iodine. These determinations demonstrated that the outstanding effect of acute ANTU poisoning on the iodine content of rat tissues was a decrease in thyroid iodine to about 50% of the normal value. Coincident with the decrease in thyroid iodine there occurred an increase in both the organic and inorganic iodine of the blood and an increase in the inorganic fraction in lung tissue accompanied by a decrease in organic iodine. The changes in the distribution of iodine in the lungs were interesting because pleural effusion and pulmonary edema are the most pronounced pathological manifestations of ANTU poisoning.

TABLE I.
Effect of ANTU on Total, Inorganic and Organic Iodine of Rat Tissues.

		Normal	6 hr after 10 mg/kg ANTU
Blood mg%	Total	.012 ± .0038*	.0216 ± .0022
	Inorg	.0046 ± .0003	.0137 ± .0057
	Org.	.0091 ± .0019	.0130 ± .0042
Lung mg%	Total	.0482 ± .0085	.0643 ± .0071
	Inorg.	.0102 ± .0057	.0208 ± .0080
	Org.	.0401 ± .0040	.0258 ± .0068
Liver mg%	Total	.0195 ± .0012	.0191 ± .0037
	Inorg	.0111 ± .0037	.0066 ± .0010
	Org.	.0133 ± .0022	.0089 ± .0015
Thyroid mg%	Total	129.2 ± 13.2	66.7 ± 6.3
	Inorg.	4.36 ± 1.1	1.6 ± 0.8
	Org.	104.1 ± 13.3	56.8 ± 13.6

* ± values equal 2 stand. dev. from the mean.

The iodine content of tissues from normal and ANTU-poisoned guinea pigs was also examined. The total iodine in the thyroid tissue of normal guinea pigs was 39.2 mg% which is approximately 30% of the normal value for rats. Seven hours after the administration of 200 mg/kg of ANTU the total iodine of guinea pig thyroids was 59.4 mg% which represented an increase on a weight basis. However, paralleling this increase in total iodine there was a decrease in thyroid weight to about one-half of the normal value. Thus, the thyroid iodine per animal showed an overall decrease after ANTU. A dose of 200 mg/kg of ANTU in guinea pigs is comparable to 10 mg/kg in rats in terms of the LD-50 of ANTU for the two species.

The effect of increased dietary iodine on the concentration of iodine in tissues. Previous studies in this laboratory(1,2) have shown

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TABLE II.
Influence of High-Iodine Diet on the Iodine Concentration of Rat Tissues.
(5.3 mg I/g diet fed for 5 days)

Iodine Fraction	Blood mg%	Lung mg%	Liver mg%	Thyroid mg%
Total	4.98 ± .61*	4.49 ± .63	1.33 ± .66	270 ± 25
Inorg.	3.43 ± .50	3.32 ± .58	.87 ± .35	36 ± 6
Organic	1.19 ± .30	.52 ± .29	.35 ± .10	216 ± 12

* ± values represent 2 stand. dev. from the mean of 8 measurements.

that increasing the dietary iodine causes a marked increase in the ability of normal rats to withstand ANTU while thyroidectomized rats received no protection from high-iodine diets(2). These findings indicated that the thyroid gland was involved in the protective effect of iodide against ANTU. Iodine determinations were, therefore, made on blood, lung, and liver from normal and thyroidectomized rats fed the high-iodine diet for 5 days. Iodine measurements were also performed on the thyroids of the normal animals receiving increased dietary iodine. The results of iodine measurements on the tissues from normal rats fed a high-iodine diet for 5 days are shown in Table II in which each value is the average for at least 5 animals. It may be noted from a comparison of the data in Table II with that presented in Table I that a pronounced increase in tissue iodine resulted from the feeding of high-iodine diets for 5 days during which time the average daily intake of iodine was 210 mg/kg. The inability of thyroid tissue to increase its iodine content to as great a percentage as other tissues under similar conditions, as shown in Table II, has been explained by Wolff and Chaikoff(13,14) on the basis that increases in blood iodine inhibit the enzymes or other mechanisms concerned with the incorporation of iodine into organic form.

A comparison of the iodine concentrations in tissues from normal and thyroidectomized rats fed the high-iodine diet revealed no significant differences in the quantity of iodine in the lung, blood, or liver of the two groups. These findings indicated that the failure of iodine-fed, thyroidectomized rats to exhibit resistance toward the acute toxicity of ANTU is not due to a difference in the ability of the tissues to retain iodine thus providing further indications that the thyroid gland is responsible for the prophylactic effect of iodine against acute ANTU poisoning in rats. A group of guinea pigs was given iodide at a concentration of 2.85 mg/ml in the drinking water. At the end of a 5-day feeding period, during which time they received an average daily dose of 168 mg/kg of iodide, these animals failed to exhibit any increased resistance toward ANTU. The LD-50 was approximately 200 mg/kg for both normal and iodine-fed groups. Other similarly treated guinea pigs were sacrificed and total thyroid iodine was determined. During the 5-day feeding period the average daily iodide consumption was 182 mg/kg, and, although the total thyroid iodine increased almost 60%, an attendant decrease in thyroid weight caused the total thyroid iodine per animal to remain approximately normal. This differed from the results obtained with rats in which total thyroid iodine doubled after feeding high-iodine diets for 5 days.

Effect of ANTU on the uptake of radioactive iodine by rat and guinea pig tissues. To obtain further information on the influence of ANTU on iodine metabolism measurements on the rate of uptake and the retention of I^{131} were carried out on normal and ANTU-poisoned rats. Two groups containing 5 rats each were given 10 microcuries of I^{131}. Five hours later both groups were sacrificed and it was observed that ANTU strongly inhibited the uptake of I^{131} by the thyroid gland of rats. An average of 9.8% of the injected dose of I^{131} was found in the thyroids of normal rats as compared with only 1% for the ANTU-poisoned group. There was no significant difference in the quantity of I^{131} taken up by the other tissues from normal and ANTU-poisoned rats. Thus, the lungs of both groups

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contained an average of 0.06 microcuries of I^{131}/g while liver contained 0.037 microcuries/g. We also measured the uptake of I^{131} by the tissues of ANTU-poisoned guinea pigs because of the resistance of this species toward ANTU. When guinea pigs which were previously fed an iodine-deficient diet for 1 day were given 10 microcuries of I^{131} an average of only 1.36% of the injected dose was found in the thyroids. The uptake of I^{131} by guinea pig thyroids was therefore only about 0.1 as great as for rats under comparable conditions. Five hours after the intraperitoneal administration of 10 mg/kg of ANTU followed immediately by 10 microcuries of I^{131} the thyroids of guinea pigs contained 0.45% of the injected dose. Administration of 150 mg/kg of ANTU to guinea pigs resulted in only 0.06% of the injected dose being taken up by the thyroids in 5 hours. This dose of 150 mg/kg of ANTU given to guinea pigs is comparable to 10 mg/kg in rats and it produced a similar inhibition of the uptake of I^{131}. Thus, there was a correlation between the toxicity of ANTU and its inhibitory effect on the uptake of iodine by the thyroids of the two species.

Discussion. The results of these studies demonstrated that ANTU reduces the thyroid iodine of rats and guinea pigs with much higher doses being necessary to decrease the thyroid iodine of the latter species which is much more resistant than rats toward ANTU. The normal thyroid iodine value for guinea pigs was 39.2 mg% as compared with 129 mg% for rats. In rats lethal doses of ANTU produced a decrease in thyroid iodine and did not alter the size of the gland while in guinea pigs the thyroid weight decreased to about one-half the normal value after lethal doses of ANTU. The differences in the normal thyroid iodine values for the two species and in the response of the thyroid to ANTU is interesting in view of the marked difference in toxicity of ANTU for the two species.

Our previous experiments had shown(1,2) that rats acquire resistance to several times the lethal dose of ANTU when fed high-iodine diets for short periods of time while guinea pigs do not develop added resistance when fed iodine(5). It appeared from the results of our previous experiments(2) that the thyroid gland was involved in the protective effect of iodine because thyroidectomized rats were not protected by iodide. The iodine determinations presented in this communication seem to offer further evidence in support of the idea that the protective action of iodide is mediated through the thyroid gland of rats. The iodine analysis on tissues from normal and thyroidectomized rats demonstrated that thyroidectomy does not influence the quantity of iodine in the tissues of iodine-fed animals. Therefore, the only apparent difference between normal and thyroidectomized rats fed a high-iodine diet in so far as the iodine concentration of the tissues is concerned was the presence of a high quantity of thyroid iodine in the unthyroidectomized rats. It appears that this increase in thyroid iodine from 129 mg% to 260 mg% is in some manner responsible for the elevation of the LD-50 of ANTU from 6.88 to 76 mg/kg which we have observed in rats(2) after this treatment for 5 days with iodine.

Experiments with radioactive iodine demonstrated that ANTU inhibits the deposition of iodine by the thyroid but does not affect the uptake of iodine by other tissues. Much higher doses of ANTU were required in guinea pigs than in rats to demonstrate this effect which is in agreement with the greater resistance of guinea pigs toward the acute toxic effects of ANTU.

Summary. Total, inorganic, and organic iodine of the tissues of normal and ANTU-poisoned rats was measured. ANTU decreased the thyroid iodine of rats and guinea pigs and caused a small increase in the total iodine of blood and lung when acutely toxic doses of the rodenticide were administered. Feeding a high iodine diet for 5 days caused a marked increase in the total iodine of tissues from normal and thyroidectomized rats. Since pre-treatment of thyroidectomized rats with iodine does not afford protection against ANTU the increase in thyroid iodine of normal iodine-fed rats appears to be involved directly in the prophylactic effect of iodide against ANTU poisoning.

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