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Relation Between Sex Hormones and Changes in Susceptibility of Domestic Norway Rats to Alpha-Naphthyl Thiourea.*

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While investigating the acute toxicity of alpha-naphthyl thiourea (ANTU) for wild Norway rats, Dieke and Richter found that the susceptibility increases sharply at or just prior to the onset of puberty.[1] Thus, it takes about 2.5 times as much ANTU per kg body weight to kill wild Norway rats of 150 g as it does for ones of 200 g, and 6 times as much for those under 100 g. A similar change in susceptibility occurs in domestic Norway rats when thiourea is administered.[2,3] Autopsy of rats dying from thiourea or ANTU revealed massive pleural effusion and pulmonary edema. Apparently these drugs act on the lung capillaries to increase their permeability,[4] but why it should take so much more to kill young rats than adults is not known.

The experiments referred to above indicate but do not prove any direct connection between sexual maturation and susceptibility to poisoning by thiourea and ANTU. The present study, therefore, was undertaken to discover whether actual pubertal changes are responsible for this great difference in susceptibility or whether the coming into puberty at about the same time is purely coincidental. To do this, gonads were removed from one group of young rats to determine if the resistance to ANTU would remain prepubertal even after they had attained the age and weight of adult animals. A second group received sex hormone (testosterone or estrone according to the sex) in order to develop the secondary sex organs at an earlier age than normal and thus to determine whether the response to ANTU poisoning would be that of a young prepubertal rat or that of an adult.

The first part of this experiment included 40 albino rats. As it has been shown that there is no significant sex difference in resistance to acute ANTU poisoning,[1] male litter mates were used as controls for operated females and vice versa. Twenty animals were gonadectomized, the remaining 20 serving as controls. All operations were performed under ether anesthesia on either the 8th or 29th day of life (3 on the 27th). When both experimental and control animals were over 100 days old they were given intraperitoneal injections of ANTU in olive oil. The doses were adjusted so that each animal received 1/10 cc of oil suspension per 100 g body weight. Autopsies performed on all experimental animals revealed no residual gonadal tissue.

Twenty-nine experimental and 33 litter mate control animals were used in the second part of the study. One-half milligram of testosterone propionate (Perandren) was injected daily into the experimental males and 100 I.U. estrone (Theelin) in oil into the females. Injections were started from the 14th to the 22nd day of age and continued for from 10 to 19 days. At the end of the injection period both experimental and control animals were given ANTU in olive oil in the same manner as the first group. At autopsy the condition of the secondary sex organs was checked in all animals to make certain that the injected sex hormone had been effective. Rats used for these experiments came from a colony originally obtained

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1 Dieke, S. H., and Richter, C. P., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 22.

2 Dieke, S. H., and Richter, C. P. *J. Pharmacol.*, 1945, **83**, 195.

3 MacKenzie, J. B., and MacKenzie, C. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 54.

4 Drinker, C. K., *Pulmonary Edema and Inflammation*, Cambridge, Harvard University Press, 1945, p. 39.

TABLE I.
Effect of Castration on Toxicity of ANTU in Domestic Norway Rats.

Dose (mg per kg)	No. rats	Age at castration (days)	Age on receiving ANTU (days)	Avg wt	Survival
10	3	C*	132	172	All died
8	2	8	111-128	224	1 survived
8	4	29	117-130	215	2 "
8	5	C*	106-130	191	1 "
6	3	9	127	289	All died
6	3	27	127	262	All "
6	6	C*	119-125	199	5 survived
4	3	8	128	264	2 "
4	5	29	130	194	All "
4	4	C*	127	215	" "
3	2	C*	118	287	" "

*C = Control.

from the Carworth Farms and were fed a balanced stock diet.

Table I shows the effect of prepubertal gonadectomy on ANTU susceptibility of rats at ages exceeding 100 days. No difference can be seen between those animals castrated on the 8th and those on the 29th day of age. When the data for the controls are combined an LD_{50} value of 6.7 mg/kg is obtained. It will be noted that deaths occurred among the experimental animals following similar doses. Castration therefore did not produce an increased resistance to ANTU poisoning.

Table II shows the results of the animals injected with either testosterone or estrone for a period of 10 to 19 days before administering ANTU. No significant difference was found between rats receiving the poison on the 20th day after birth and those on the 35th to 40th. All rats (with one exception) of both the experimental and control groups survived on doses of ANTU under 60 mg per kg of body weight. Among the females no difference was found between the experimental rats and their controls, animals of both groups surviving a dose of 50 mg and dying at 70 mg or more. A slight decrease in resistance to ANTU is evident in the male group receiving sex hormone, but they were still very much more resistant than normal mature animals. Among the experimental males 1 out of 4 died upon receiving 50 mg of ANTU per kg and with one exception all 12 died on doses over this. The data for the control males correspond to an LD_{50} of approximately 90 mg/kg.

Discussion. Thiourea and its derivatives presumably cause death in both young and adult rats by increasing the permeability of the lung capillaries, thus causing pulmonary edema and pleural effusion. In young animals either it requires a greater quantity of the drug to change the capillary permeability to the same degree or else the lymphatic mechanism for carrying away the fluid is more efficient in young than older rats. A comparison of the weight of the lungs with that of the body in a series of 100 normal domestic rats from our colony shows no sudden increase in the size of the lungs that might account for the formation of more fluid than the lymphatics are able to cope with. Whether or not the size and number of lymphatic channels increase at the same rate as the lung capillary bed is unknown. This paper shows that changes resulting from the presence of increased quantities of sex hormone in the body during the gradual onset of puberty are not responsible for the change in toxicity. Further investigation is in progress to determine whether other changes at this time controlled, either directly or indirectly, by the pituitary are responsible. These include growth of the animal, involution of the thyroid, and changes in metabolic rate.

Summary. In order to determine whether or not actual pubertal changes are responsible for the great decrease in resistance to ANTU observed about the time of puberty, 20 domestic Norway rats were castrated at an early age and given ANTU after reaching an age of 100 days or more. The experimental rats

TABLE II.
Effect of Sex Hormone on the Toxicity of ANTU in Domestic Norway Rats.

Dose (mg/kg)	No. rats	Sex	Age on receiving ANTU (days)	Avg wt (g)	Days of treatment ½ mg perandren per day	Days of treatment 100 I.U. theelin per day	Results
50	1	♀	21	33		10	Survived
50	2	♀	40	88		19	"
50	2	♂	35	55	19		1 "
50	2	♀	39	75		C*	"
50	3	♂	41	85	C*		"
60	2	♀	37	77		10	Died
64	1	♀	33	55		19	Survived
64	2	♀	35	54		C*	"
64	1	♂	35	58	C*		Died
70	2	♀	37	69		19	"
75	1	♀	24	37		10	"
75	2	♀	40	82		10	"
75	2	♂	24	32	10		"
75	1	♂	37	75	10		"
75	2	♂	35	52	19		"
75	1	♀	40	92		C*	"
75	1	♂	26	38	C*		"
75	5	♂	40	80		C*	Survived
100	2	♂	40	94	10		Died
100	2	♂	21	34	19		"
100	4	♂	38	71	19		2 survived
100	1	♀	38	48		19	Died
100	2	♂	26	36	C*		"
100	4	♂	41	78	C*		3 survived
100	1	♀	40	89		C*	Died
125	1	♂	40	69	10		"
125	1	♀	41	48		C*	"
125	1	♂	41	86	C*		"

* Control.

died from doses in the same range as the controls. A second group of 29 suckling rats received daily injections of testosterone or estrone. Both experimental animals and their controls were then given ANTU when they were 35-40 days old. No significant difference in the susceptibility was found. It is, therefore, concluded that the onset of sexual maturity coincides with the decrease in resistance to alpha-naphthyl thiourea but does not produce it.