

became mildly depressed and completely anorexic. They died between the tenth and nineteenth experimental days. The survivors remained normal in appearance and behavior. Three were sacrificed on the twenty-eighth day and the remaining 4 at the end of the experiment. Mild hyperemia of the peritoneum and abdominal viscera was the only gross finding at autopsy in all 10 animals. There was no gross difference in average body weight between the few survivors and the controls.

The third group of rats whose diet contained 0.1% DMC showed a slight, though not significant, weight lag on comparison with the control group at 5 days. Between this time and the ninth week the average weights of the two groups were almost equal. Statistical analysis of the difference of 22 g between the average weights at 9 weeks yielded a critical ratio[†] of 2.2 which borders on significance. One week later the weight difference was 17 g and the ratio was only 1.5. Since the experiment was terminated at this time it is impossible to say whether these differences heralded the beginning of a significant lag in weight gain, and other signs of toxicity, in the treated group. The appearance and be-

havior of the two groups of rats did not differ in any way. Autopsies performed when the animals were sacrificed revealed no gross evidence of pathological changes in any organs or tissues.

Comment. These studies reveal that the toxicity of DMC is of the same order as that demonstrated by Woodard *et al.*⁴ for DDT under grossly similar conditions. However, a more extensive comparison of the two compounds in the same laboratory would be necessary to determine accurately the relative toxicity of the two compounds.

Summary. 1. Rats and mice were intubated with single doses of a new miticidal compound, di-(p-chlorophenyl)methylcarbinol (DMC). The effect of graded doses on ultimate survival is discussed and the ability of large doses to produce signs suggestive of nervous impairment is described.

2. A powdered diet containing 0.1% DMC was well tolerated by growing rats during a ten week period.

3. The results compare favorably with those obtained in a similar limited study of DDT in another laboratory.

⁴ Woodard, G., Nelson, A. A., and Calvery, H. O., *J. Pharm. Exp. Therap.*, 1944, **82**, 152.

Received August 5, 1949. P.S.E.B.M., 1949, **72**.

$$\frac{M_1 - M_2}{\sqrt{SE^2_{M_1} + SE^2_{M_2}}}$$

Effects of Inunction of Alpha-Estradiol on Testes and Thyroids of Albino Rats.* (17417)

JOSEPH H. GARDNER (Introduced by Kermit Christensen)

From the Department of Anatomy, St. Louis University School of Medicine, St. Louis, Mo.

A diverse opinion exists in regard to the effect that the administration of sex hormones has on thyroid activity. Many investigators have noted that the thyroid remains normal or shows a slight degree of hypo-activity after castration or the administration of female

sex hormones.¹⁻¹⁰ In contrast many workers

* This work was carried out at the Department of Anatomy and Physiology, at the University of Kentucky, Lexington, under the direction of Professor R. S. Allen.

¹ Chouke, S., *Endocrinology*, 1930, **14**, 12.

² Aron, M., and Benoit, J., *Compt. rend. de Soc. Biol.*, 1932, **109**, 923.

³ Starr, P., and Bruner, R. C., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 465.

⁴ Shumacker, H. B., and Lamont, A., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 568.

⁵ Uotila, U. U., *Endocrinology*, 1940, **26**, 123.

⁶ Zalesky, M., *Anat. Rec.*, 1935, **62**, 109.

have noted that the administration of female sex hormones cause a hyperactivity of the thyroid.¹¹⁻¹⁷ Investigations at present show that estrogenic substances exert an inhibitory influence on the structure and size of the testes and their accessories.^{5,18-25} Some workers have obtained opposite results.^{10,26}

The present investigation was undertaken because of the conflicting results that have been obtained.

Materials and methods. Three groups of male albino rats were used. Eight male rats of Group 1 (40 days of age) were castrated 2 weeks after the descent of the testes into the scrotum. Treatment began the day following castration and consisted of daily inunction of 270 rat units[†] of alpha-estradiol[‡]

in ointment form to the skin surface of the interscapular region. Group 2 consisted of eight, 15 day old normal non-castrated immature male rats. Treatment was carried out as in Group 1 but was started before the descent of the testes into the scrotum. Six rats in Group 3 served as control animals and received no treatment. On the day following the 24th application of alpha-estradiol on the experimental rats all the animals were killed with chloroform. The thyroids and testes were fixed in Bouin's fluid and the sections were stained with Ehrlich's acid hematoxylin and eosin. Klein's method²⁷ was employed for studying the size of the thyroid follicles and seminiferous tubules. The height of the follicular epithelium was determined by the method of Rawson and Starr.²⁸ An estimated percentage of destruction of the follicular walls was made by comparison with the findings of Morrell and Hart²⁵ who determined in percentage, the area of a section showing destruction. One hundred follicles from a section of the thyroid of each animal were studied for the number of follicles containing wall vacuoles in the colloid as described by Möllendorff.²⁹ The average number of primary and secondary spermatocytes was taken from 20 typical tubules in a section from each animal. The testes were compared as to gross size and as to the interstitial elements of Groups 2 and 3.

Observations. Thyroid follicular size and epithelial height were used to determine an increase or a decrease in thyroid activity. The results are shown in Table I. The long diameter of the thyroid follicles of Groups 1 and 2 averaged respectively 113.8 and 96.1 μ , while in the normal rats (Group 3) it was noticeably less with an average of 66.9 micra. The height of the follicular cells in Groups 1 and 2 averaged respectively 15.3 and 15.6 μ , while group 3 averaged 34.5 μ . Due to the

⁷ Costantini, P., *Rass. di Clin. Ter. e Sc. Aff.*, 1929, **10**, 718.

⁸ Sherwood, T. C., *Endocrinology*, 1940, **26**, 693.

⁹ Sherwood, T. C., *Endocrinology*, 1941, **29**, 215.

¹⁰ Korenchevsky, V., and Dennison, M., *J. Path. and Bact.*, 1935, **12**, 323.

¹¹ Kunde, M. M., *et al.*, *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 122.

¹² Benazzi, M., *Boll. Soc. Ital. Biol. sper.*, 1933, **8**, 790.

¹³ Leiby, G. M., *Proc. Soc. Exp. Biol. and Med.*, 1933, **31**, 15.

¹⁴ Pincus, G., and Werthessen, N., *Am. J. Physiol.*, 1933, **103**, 631.

¹⁵ Amilibis, E., *et al.*, *Klin. Wochen.*, 1936, **15**, 1001.

¹⁶ Nathanson, I. R., *et al.*, *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 737.

¹⁷ Alexin, M., *Arch. f. Gyn.*, 1940, **159**, 432.

¹⁸ Golding, G. L., and Ramirez, F. T., *Endocrinology*, 1939, **12**, 804.

¹⁹ Burrows, H., *J. Path. and Bact.*, 1936, **42**, 161.

²⁰ Greep, R. C., *et al.*, *Anat. Rec.*, 1936, **65**, 261.

²¹ Gardner, W. U., *Anat. Rec.*, 1937, **68**, 339.

²² Ringoen, A. R., *Anat. Rec.*, 1938, **72**, 118.

²³ Pfeiffer, C. A., and Gardner, W. U., *Anat. Rec.*, 1938, **72**, 59.

²⁴ van Wagenen, G., *Anat. Rec.*, 1935, **73**, 387.

²⁵ Morrell, J. A., and Hart, G. W., *Endocrinology*, 1941, **19**, 995.

²⁶ Allanson, M. J., *Exp. Biol.*, 1931, **8**, 389.

[†] The author wishes to thank Dr. W. H. Stoner of the Schering Corporation at Bloomfield, N. J., for supplying the alpha-estradiol (Progynon D-H) used in this experiment.

[‡] Each application consisted of 0.15 g of ointment which contained 0.0225 mg of crystalline estradiol having a rat unit value of 270.

²⁷ Klein, J., *Ann. Int. Med.*, 1935, **8**, 798.

²⁸ Rawson, R. W., and Starr, P., *Arch. Int. Med.*, 1938, **61**, 726.

²⁹ Möllendorff, W., *Handbuch Mikroskop. Anat. des Mensch*, 1939, **6**, 61.

TABLE I.
Histological Changes Occurring in the Thyroid of Groups I and II as Compared to Group III,
Which Was Considered Normal.

Group		Long diameter of follicles, μ	Height of follicular epithelium, μ	Vacuolated colloid per 100 follicles (wall vacuoles)	Estimated % of follicu- lar damage
I Castrated and treated	1	90.7	16.9	16	25
	2	108.7	17.2	31	25
	3	113.9	16.7	28	40
	4	120.5	13.6	35	40
	5	103.1	15.4	15	25
	6	116.4	15.7	43	40
	7	134.6	14.1	23	10
	8	122.6	13.7	39	10
	Avg	113.8	15.3	28.7	26.8
II Non-castrated and treated	1	99.5	17.8	26	0
	2	94.1	17.8	20	0
	3	83.0	13.2	23	10
	4	101.3	15.1	15	10
	5	95.4	13.7	31	25
	6	89.4	13.5	23	25
	7	110.0	21.2	19	10
	8	96.2	13.0	16	10
	Avg	96.1	15.6	21.6	11.2
III Normal control	1	63.0	23.2	7	0
	2	64.5	25.3	6	0
	3	70.5	39.7	4	0
	4	67.1	27.7	2	0
	5	74.7	52.0	8	0
	6	62.0	39.1	3	0
	Avg	66.9	34.5	5	0

increased follicular size and the lower epithelium of Groups 1 and 2 as compared with the normal animals it is apparent that hypoactivity of the thyroid gland was produced by the inunction of alpha-estradiol. The marked difference between Groups 1 and 2 indicates a greater hypothyroid condition in Group 1. This might be explained by the presence of the testes in Group 2, causing a higher androgen concentration.

Because of the greater increase of wall vacuoles in the colloid of the thyroids in Groups 1 and 2 in contrast to the lesser number in Group 3, mention is made of them although no physiological significance is attached to them at present. The wall vacuoles according to Möllendorff are formed by the contraction of the colloid due to fixation. All thyroids of this series were fixed for the same length of time in Bouin's fluid.

Damage to the follicular walls was greater in the castrated and treated rats than in the non-castrated, treated ones and was lacking

in the normal. Morrell and Hart³⁰ report that the thyroids of male and female rats treated with stilbesterol show destruction of the follicles brought about by invasion of leucocytes which may result from the static condition of the gland. The destruction occurred in areas which appeared hyalinized in some cases and fibrous in others. The damage to the thyroid follicles that appeared in Groups 2 and 3 was similar to that of Morrell and Hart. Fibrosis was noticeable in all the damaged thyroids and the hyalinized areas appeared to be due to release of follicular colloid in the tissue spaces.

Alpha-estradiol prevented the descent of the testes into the scrota of the animals of Group 2 (Table II). According to Moore and Price³¹ this is due to the inhibitory effect of estrogens upon the hypophysis by reducing

³⁰ Morrell, J. A., and Hart, G. W., *Endocrinology*, 1941, **29**, 769.

³¹ Moore, C. R., and Price, D., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 38.

TABLE II.
Histological Changes Occurring in the Testes of Group II as Compared to Group III, Which Was Considered Normal.

Group		Location	Size of tubules (Long diam.) in μ	Size of testes compared to controls (Group III)	Sustentacular cells compared to controls (Group III)	Spermatogonia	Mitosis of spermatogonia	Avg No. per 20 tubules		Spermatids	Spermatozoa	Degeneration of tubular walls
								No. of primary spermatocytes	No. of secondary spermatocytes			
Group II Non-castrated treated	1	*	116.4	$\frac{1}{2}$	+	§	§	21.7	18.0	—	—	—
	2	*	130.1	$\frac{1}{2}$	++	§	§	30.3	23.7	—	—	—
	3	*	121.9	$\frac{1}{2}$	+++	§	§	19.0	24.0	—	—	—
	4	*	136.9	$\frac{1}{2}$	+++	§	§	16.9	14.2	—	—	—
	5	*	116.4	$\frac{1}{2}$	+++	§	§	12.0	5.0	—	—	—
	6	*	112.3	$\frac{1}{2}$	+++	§	§	15.6	18.0	—	—	—
	7	*	149.0	$\frac{1}{2}$	+++	§	§	12.9	28.3	—	—	—
	8	*	147.9	$\frac{1}{2}$	++	§	§	11.6	10.5	—	—	—
	Avg		128.8	$\frac{1}{2}$				17.5	17.7			
Group III Normal control	1	†	257.5		+	§	§	58.6	142.4	§	§	—
	2	†	325.3		++	§	§	73.1	169.3	§	§	—
	3	†	282.1		++	§	§	69.0	154.2	§	§	—
	4	†	239.7		++	§	§	87.6	193.4	§	§	—
	5	†	343.8		++	§	§	78.4	181.4	§	§	—
	6	†	304.8		+	§	§	62.0	149.3	§	§	—
	Avg		292.2					71.2	165.0			

* Abdomen. † Scrotum. ‡ Normal. § Present. — Absent.

its gonadotropic secretion. The testes of Group 2 did not remain in the abdomen long enough for all of the germ cells to disappear from the tubules. Spermatogenesis was reduced to the point that spermatid formation was completely absent. Probably these changes in the tubules were due to the alpha-estradiol indirectly, by preventing testicular descent, and permitting cellular changes due to retention in the body cavity. There was no noticeable change in the interstitial elements. The gross size of the testes were half that of the control animals.

of 270 rat units of alpha-estradiol (Progynon D-H) to normal and castrated immature male rats produced noticeable changes in the thyroid glands and in the testes of the non-castrated males. The thyroid glands appeared, histologically, in a hypoactive state. They exhibited an increased colloid content and a low follicular epithelium and destruction to the follicles. The hypoactivity appeared greater in castrated rats than in the non-castrated. Descent of the testes in immature males was prevented by the alpha-estradiol.

Summary. Daily inunction for 24 days

Received August 8, 1949. P.S.E.B.M., 1949, 72.