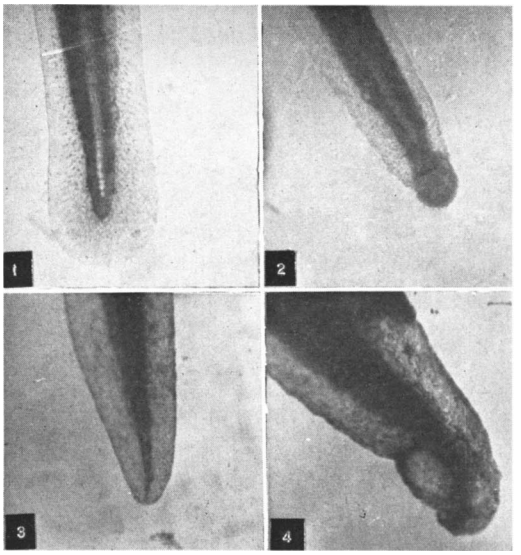

FIG. 1. Normal—fish embryo tail *Brachydanio rerio.*
FIG. 2. Treated, 0.5 ppm—fish embryo tail *Brachydanio rerio.*
FIG. 3. Normal—tadpole tail *Rana pipiens.*
FIG. 4. Treated, 1 ppm—tadpole tail *Rana pipiens.*

and older tadpoles (*Rana,* various species) did not respond to concentrations of 1.0 and 0.5 ppm. One test on tadpoles (newly hatched), *R. pipiens,* at 2.0 ppm resulted in bizarre epithelial growths at the tip of the tail which were eventually absorbed. Older animals and tadpoles that had recovered from treatment would not respond to concentrations of 3.0 and 5.0 ppm.† Kivett(3) tested #742 on chick embryos and obtained evidence of tumor formation.

Discussion. From our studies it appears obvious that #742 produces hyperplasia in some amphibian and teleost embryos when applied during the development of the tail fin. The exact nature of the reaction and the histology involved are not understood. Studies involving tissue cultures and cytological effects will be reported later.

The fact that a delta-16-steroid consistently produces hyperplasia in the same developmental region and stage at relatively low concentrations (0.5 ppm) seems to be significant.

Summary. Embryos of *Brachydanio rerio* and *Rana pipiens* when grown in water containing 0.5-2.0 ppm of 1, 3, 5 (10), 16 estratetraen-3-ol (a Δ^{16} steroid) will consistently develop malformations (hyperplasia) in the region of the tail if exposed before the formation of the tail fin occurs.

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† Appreciation is expressed to members of the classes in Embryology at Oklahoma State Univ. who conducted studies on the effects of #742 on amphibian embryos.

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Anti-Androgenic Activities of A-Nortestosterone and C-Nor-D-Homo-17α-Epitestosterone Acetate. (29080)

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Structural alteration of naturally occurring steroids may lead to compounds with interesting biological properties. Reduction of the 6-membered A-ring of progesterone to the 5-membered A-ring of A-norprogesterone(1) yielded a compound without progestational activity but with the property to antagonize the effects of exogenous and endogenous androgen at specific end organs(2,3,4). The A-nor analogue of testosterone, A-nortestosterone(1), is a very weak androgen and is anti-androgenic. These properties have been

described briefly(5). Recently Kupchan and Levine(6) synthesized C-nor-D-homo-17α-epitestosterone acetate. The androgenic and anti-androgenic properties of this compound will also be described.

Material and methods. Immature male Sprague-Dawley strain rats weighing 55-65 g were castrated and divided into groups of 4 or 8 animals. Compounds were dissolved or suspended in sesame oil. A-nortestosterone, testosterone and testosterone propionate were individually administered. In addition, A-nortestosterone and C-nor-D-homo-17α-epitestosterone acetate were each administered concomitantly with testosterone propionate. Treatment was subcutaneous and injections were administered once daily for 7 days starting on day of castration. On the day following the last injection, the animals were sacrificed. The ventral prostate, seminal vesicles and coagulating glands were removed and weighed on a Roller-Smith torsion balance.

Two-day-old White Leghorn cockerels randomly distributed 5 per cage were also employed to assay the androgenic and anti-androgenic activities of A-nortestosterone and C-nor-D-homo-17α-epitestosterone acetate. These compounds and testosterone were dissolved or suspended in sesame oil and applied in .005 ml volumes to the chick comb for 7 days in a method for androgen assay described elsewhere(7). Anti-androgenic activity was determined by a local method(4). Testosterone in .005 ml sesame oil was applied to one side of the comb and the A-nor or C-nor steroid in .005 ml sesame oil was applied to the other side of the comb for 7 days. On the day following the last application the chicks were sacrificed and comb and body weights were determined.

Results and discussion. A-nortestosterone was found to be weakly androgenic in the chick and rat. It was approximately 1/20 as potent as testosterone in stimulating chick comb growth (Table I) and approximately 1/40 as potent as testosterone in inducing growth of the castrate rat ventral prostate, seminal vesicle or coagulating gland (Table II). C-nor-D-homo-17α-epitestosterone acetate was a much weaker androgen. Compared to testosterone in the chick comb growth assay, this compound was $>1/2500$ and $<1/500$ as potent. Both of these weak androgens, when applied to the chick comb, antagonized the testosterone-induced comb growth (Table I). Total doses of 1000, 200 and 40 μg of A-nortestosterone inhibited the comb growth stimulation of a total dose of 50 μg testosterone by 28, 42 and 63%, respectively. It is interesting that the antagonism was inversely related to the dose of A-nortestosterone. However, at lower doses of testosterone, 10 or 2 μg, A-nortestosterone did not antagonize comb growth stimulation. The 1000 μg dose of A-nortestosterone in combination with 10 μg of testosterone produced a comb growth equal to that resulting from the local application of that dose of the A-norsteroid alone. When this dose of A-nortestosterone was administered concurrently with the lowest total dose of testosterone, 2 μg, the resultant comb growth was less than was produced by that dose of the A-norsteroid alone, but greater than that produced by that dose of testosterone alone. A-nortestosterone in doses of 200 or 40 μg plus 10 μg of testosterone produced comb ratios approximating those of chicks treated with that dose of testosterone alone. Similarly the application of a combination of 200 or 40 μg of A-nortestosterone and 2 μg of testosterone resulted in comb growth which was approximately the same as that obtained by the treatment with the respective doses of the A-norsteroid alone. Total doses of 1000, 200 and 40 μg of C-nor-D-homo-17α-epitestosterone acetate antagonized the comb growth stimulation produced by total doses of 50 μg of testosterone by 49, 48 and 39%, respectively. Again, as in the case of A-nortestosterone, concurrent administration of these same doses of C-nor-D-homo-17α-epitestosterone acetate with 10 or 2 μg of testosterone produced variable results, with little or no antagonism or augmentation of the testosterone-induced comb growth.

Daily doses of 100 or 1000 μg of A-nortestosterone subcutaneously administered did not significantly augment or antagonize the effect of 25 μg of testosterone propionate on

the immature castrate rat ventral prostate or seminal vesicle weight. However, daily subcutaneous doses of 1000 μg of C-nor-D-homo-17α-epitestosterone acetate did inhibit the castrate rat accessory sex gland stimulation induced by 25 μg of testosterone propionate. Resultant ventral prostate weights were 68.0 mg $\pm$ 3.2 and 57.8 mg $\pm$ 2.6 (P = <.05) for the testosterone propionate and testosterone propionate plus C-nor-D-homo-17α-epitestosterone acetate, respectively. Similarly, the resultant seminal vesicle weights were 41.4 mg $\pm$ 5.0 and 26.2 mg $\pm$ 2.3 (P = <.05) for testosterone and for the androgen plus antagonist, respectively.

These studies demonstrate that weak androgens such as A-nortestosterone and C-nor-D-homo-17α-epitestosterone acetate may aug-

TABLE I. Androgenic and Anti-Androgenic Activities of A-Nortestosterone and C-Nor-D-Homo-17α-Epitestosterone Acetate in an Inunction Chick Comb Assay.

Total dose (μg)				
Testosterone	A-nortestosterone	C-Nor-D-homo-17 α-epitestosterone acetate	No. of chicks	Mean comb ratio $\pm$ S.E. Comb-mg/body wt (g)
—	—	—	50	.34 $\pm$ .01
.4	—	—	50	.38 $\pm$ .01
2.0	—	—	50	.52 $\pm$ .02
10.0	—	—	50	.73 $\pm$ .03
50.0	—	—	50	1.44 $\pm$ .04
—	40	—	20	.62 $\pm$ .03
—	200	—	20	.74 $\pm$ .03
—	1000	—	20	.99 $\pm$ .04
50.0	40	—	20	.75 $\pm$ .04
50.0	200	—	20	.98 $\pm$ .05
50.0	1000	—	20	1.13 $\pm$ .07
10.0	40	—	20	.75 $\pm$ .04
10.0	200	—	20	.76 $\pm$ .04
10.0	1000	—	20	1.00 $\pm$ .04
2.0	40	—	10	.65 $\pm$ .03
2.0	200	—	10	.77 $\pm$ .06
2.0	1000	—	10	.79 $\pm$ .06
—	—	1000	5	.46 $\pm$ .05
50.0	—	40	10	1.01 $\pm$ .08
50.0	—	200	10	.87 $\pm$ .08
50.0	—	1000	15	.88 $\pm$ .08
10.0	—	40	10	.64 $\pm$ .04
10.0	—	200	10	.80 $\pm$ .05
10.0	—	1000	10	.66 $\pm$ .04
2.0	—	40	10	.49 $\pm$ .04
2.0	—	200	10	.56 $\pm$ .05
2.0	—	1000	10	.62 $\pm$ .05

TABLE II. Androgenic Activity of Testosterone and A-Nortestosterone in the Immature Castrate Male Rat.

Treatment	Daily dose (mg)	Δ Body wt (g)	Ventral prostate (mg)	Seminal vesicles (mg)	Coagulating gland (mg)
Sesame oil (control)	—	44	13.5 $\pm$ .9	8.5 $\pm$ 1.2	3.9 $\pm$ 1.0
Testosterone (T)	.001	44	16.9 $\pm$ 1.4	8.8 $\pm$ .3	3.3 $\pm$ .6
T	.005	44	22.2 $\pm$ 1.0	10.4 $\pm$ .4	4.5 $\pm$ .3
T	.025	46	38.6 $\pm$ 2.6	16.5 $\pm$ .9	6.3 $\pm$ .7
T	.125	51	60.3 $\pm$ 5.3	34.8 $\pm$ 5.9	11.7 $\pm$ 1.8
T	.625	45	95.9 $\pm$ 10.2	65.8 $\pm$ 8.5	18.5 $\pm$ 1.5
A-Nortestosterone (A)	.04	45	15.4 $\pm$ 1.8	9.6 $\pm$ .4	3.6 $\pm$ .2
A	.2	46	20.3 $\pm$ 1.4	9.0 $\pm$ .4	3.4 $\pm$ .2
A	1.0	44	22.0 $\pm$ 2.5	13.2 $\pm$ 1.2	4.5 $\pm$ .5
A	5.0	48	55.5 $\pm$ 4.7	22.7 $\pm$ .7	7.4 $\pm$ .5
A	25.0	45	93.2 $\pm$ 4.1	72.8 $\pm$ 10.4	21.5 $\pm$ 2.5

ment, antagonize or not alter the chick comb and rat accessory sex organ growth stimulation induced by a more potent androgen such as testosterone. Augmentation or antagonism may result depending on the dose relationship between the weaker and stronger androgens. In the chick comb studies the administration of relatively large amounts of the weak androgens induced an augmentation or additive effect when the quantity of the more potent androgen was relatively small. When the stronger androgen was administered in relatively large dosages the weaker androgens inhibited the growth response, probably by competitive action at the reactive tissue level.

Summary. A-nortestosterone and C-nor-D-homo-17α-epitestosterone acetate were very weak androgens in the chick comb assay. In addition A-nortestosterone was weakly androgenic in the immature castrate rat assay. Both compounds antagonized testosterone-induced chick comb growth, the antagonism being more pronounced when the dose of testosterone was relatively large. At the doses employed C-nor-D-homo-17α-epitestosterone acetate was anti-androgenic in the immature castrate male rat while A-nortestosterone was not.

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Role of Gastric Secretion in Iron Absorption.* (29081)

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The search for the controlling mechanism in iron absorption has been a long one. Iron is poorly excreted and poorly absorbed. Since ionic iron in high concentration is injurious to cells, it seems reasonable to suspect that prior to absorption from the stomach or intestine, the iron might be coupled to some compound. Moreover, since the presence of anemia promotes iron absorption, it might be assumed that increased amounts of such a substance would be found in anemic animals. A study of the kinetics of iron absorption in normal dogs also suggested the possibility of some substance in limited amounts which controls iron absorption(1). There is clinical evidence that gastrectomy reduces the ability to absorb iron. Hobbs has reported that iron deficiency anemia developed in half of the male patients and nearly all of the female patients who underwent gastrectomy, either subtotal or total(2). In only about one-third of the 247 patients studied was there evidence of possible blood loss or low dietary iron intake. Defective iron absorption was presumed to have accounted for anemia in the remaining patients.

The experiments reported here provide evidence that the gastric juice of anemic dogs contains a substance that increases iron absorption.

Methods. A. *In vivo experiments.* Mongrel dogs, both male and female, weighing 10-15 kg were made anemic by repeated phlebotomy (6 dogs), or by repeated injections of acetylphenylhydrazine (one dog). The iron deficient dogs were maintained on an iron

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