various dosage levels. The slope of the curves would be the same in all experiments. However there is no such parallelism between comb-body weight ratios of controls and treated animals, and consequently the slopes of the curves vary. Therefore, corrections for initial bodyweight cannot correct for these variations. For these various reasons the use of a composite standard dose-response curve gives less accurate results than the construction of individual dose-response curves for each assay.

Dose Range. The total dosages used in the 14 experiments ranged from 0.175 γ to 87.5 γ. Not all dosage levels were employed in each experiment. A dosage of 1.75 γ (5 γ per cc) was used in 11, 3.5 γ (10 γ per cc) in 11, 7 γ (20 γ per cc) in 12 and 17.5 γ (50 γ per cc) in 5 experiments. The other dosage levels were employed in less than 5 experiments. Most of the curves are linear within the dosage levels employed, but some show a flattening at the higher dosage levels.

In a number of experiments not reported in this paper, in which testosterone and methyl testosterone were also employed, testosterone was found to give a response of about the same magnitude as androsterone, while methyl testosterone assayed somewhat higher.

Summary and Conclusions. Androgen assay by means of the comb growth response of the immature cockerel was found satisfactory. The method described is based upon that of Frank and his associates, but in addition to minor modifications, differs from the latter in the following respects: (1) male chicks only are used; (2) ether is employed as the vehicle for application of the hormone; (3) individual dose-response curves are constructed with each assay.

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Excretion in the Dog of Androgens and Estrogens in the Bile Following Injections of Androgens.*

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Testis transplants into the mesentery[1,2] and testosterone pellets implanted in the same situation[3] or in the spleen[4,5] fail to exert androgenic activity in castrate male rats, as evidenced by the lack of repair of the prostate and seminal vesicles. It has been concluded from such experiments that testosterone is inactivated by the liver.

We have shown in previous communications[6,7,8,9] that large amounts of estrogenic hormones both of exogenous and endogenous origin are excreted in the bile. Because of these observations it was decided to study the excretion of androgen and estrogen in the bile following injection of androgenic hormones in the dog. In this species administration of

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TABLE I.
Excretion of Androgens and Estrogens in Bile and Urine Following Injection of Androgens.

Exp. No.	Injected mg	Hr	Excreted in bile: Androgens* free	Excreted in bile: Androgens* total	Excreted in bile: Estrogens† free	Excreted in bile: Estrogens† total	Excreted in urine: Androgens free	Excreted in urine: Androgens total	Excreted in urine: Estrogens free	Excreted in urine: Estrogens total
1. Androsterone	50	24	3.2							
		48	1.6							
		72	0.0							
2.	50	24	7.2							
		48	4.0							
3.	80	24	0.25	0.2	0	0	.8	.6	6	20
		48	0.25	0.2	4	0§	.0	.0	0	0
		72	0.08		3	0	.0	.0	0	0
4.	10	24	0.15	0.11						
		48	0.0	0.0						
		72	0.0	0.0						
5. Testosterone	100	24	8.4							
		48	2.0							
		72	0.0							
6.	100	24		0.6		0		.9		40
		48		0.3		20		.2		40
		72						.075		0
7.	100	24	1.5	3.0		0	.8			40
		48	0.9	0.9		0	.6			40
8.	92	24		2.0				.0		
		48		1.6				.0		
9.	83	24	1.1	0.9	40	40	.0	.2	0	0
		48	0.4	0.3	0	4	.0	.1	4	0
		72	0.4		0	0	.0		0	0
10.	50‡	24	0.44	0.48	0	0	.0	.1	24	24
		48	0.0	0.1	20	13	.0	.0	13	13
		72			0	0	.0	.0	0	0
11.	50	24	2.65							
		48	0.75							
12.	10	24								
		48	0.19	0.19	6	6				
		72	0.0	0.0	0	0				
13. Methyl test.	100	24		3.8		0		.65		0
		48		1.5		0		.50		0
		72		1.8		0		.25		0
14.	100	24	0.6	0.6	0	0	.06	.0	100	0
		48	0.06	0.05	20	0	.0	.0	30	0
		72			0	24			0	0
15.	10	24	0.2	0.3	0					
		48	0.2	0.2	0					
		72	0.0	0.0	0					

* Androgens expressed as mg androsterone.
† Estrogen values given in I.U. of estrone.
‡ In this experiment testosterone was given in bile through duodenal fistula.
§ Values for free estrogens higher than those for total may be attributed to destruction in the process of hydrolysis.

androgens is not followed by increased excretion of androgens or 17 ketosteroids in the urine.[10,11] However, in the dog, as in humans, injection of androgens is followed by excretion of estrogens in the urine.[11,12]

Material and Methods. Fifteen experiments were performed in 9 dogs with external bile fistula, the bile draining into a balloon. The dogs were fed purina dog chow and meat, supplemented by cod liver oil and yeast extract. About 100 cc of bile was re-fed daily, either by stomach tube or through a duodenal fistula. During the experimental periods, the

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test animals received bile from untreated dogs, their own bile being removed for assay. Testosterone,‡ methyl testosterone,‡ and androsterone‡ in doses of 10 to 100 mg were injected intravenously in 2 to 3 cc 95% alcohol. In one experiment testosterone was dissolved in 2 cc ether, the ether was mixed with 100 cc bile, and the mixture was fed through a duodenal fistula in 3 divided doses.

For assay of free androgen, the bile collected over a 24-hour period, or an aliquot thereof, was extracted with ether and alcohol.

For total androgen the bile was hydrolized at pH 1 for 10 minutes either with sulfuric or with hydrochloric acid and subsequently extracted with butyl alcohol. After removal of the estrogens with sodium hydroxide the butyl alcohol was evaporated and the residue taken up in ether for assay. With this procedure 70 to 94% of added androsterone and testosterone was recovered from bile. Assays were performed on 3-day-old male chicks by a method described elsewhere.[13] Estrogens were assayed by Fluhmann's method as outlined in a previous paper.[7]

Results and Comment. The data are summarized in Table I. Androgenic substances were present in the bile following a single injection of testosterone, methyl testosterone, or androsterone. Control samples of bile exhibited no androgenic activity. Androgens were excreted in the majority of cases not only during the first 24 hours, but also during the second 24 hours following administration, and in a few experiments, also in the third 24-hour period, all of the bile being removed and only androgen-free bile re-fed. No increase of androgen excretion was demonstrable in the urine during the experimental period.[11] In preliminary experiments we have found that androgens are removed rapidly from the blood following a single intravenous injection, no androgen being demonstrable in the blood at the end of 24 hours. Consequently, androgen excreted in the bile during the second and third 24-hour periods must have its origin in androgen stored in some tissue.

All of the androgen was in the free state in 7 of 8 instances in which both free and conjugated androgens were assayed in the bile.

Androgens were excreted in the bile in every instance, the absolute amounts and percentage recovery varying considerably. The highest excretion occurred in Experiment 2, in which, following injection of 50 mg androsterone, 7.2 mg equivalents of androsterone was excreted in the bile during the first 24 hours and 4 mg during the second 24 hours. If the excreted androgen is androsterone, 22% of the injected material was recovered. On the other hand, a total of only 0.6 mg or 0.75% of the 80 mg injected was recovered in Experiment 3.

One experiment is included in the table in which testosterone was instilled into the duodenum through a duodenal fistula. Androgen was excreted in the bile, indicating that testosterone was resorbed from the intestine in the dog as it is in humans[14] and in capons.[15]

The figures for excretion of androgens probably represent only a fraction of the excreted compounds since part of the compounds excreted in the dog's bile may have little or no biological activity. Injection of testosterone propionate into humans is followed by excretion of androsterone and etiocholanolone in equal amounts in the urine.[16]

Comparison of the biliary excretion of androgens following androgen injection with that of estrogens following estrogen injection in the dog[6-9] reveals qualitative similarities but quantitative differences. Excretion of both androgens and estrogens continues for 2 to 3 days, during much of which time no hormone is found in the blood or in the urine. However, the quantity of androgen recovered

‡ Testosterone and methyl testosterone used in this study was generously supplied by the Schering Corporation through the courtesy of Dr. E. Schwenk and Dr. M. Gilbert, androsterone by the Ciba Corporation through the courtesy of Mr. R. Mautner.

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from the bile is much smaller in terms of bioactivity than that of estrogen.

Excretion of Estrogen in Bile Following Androgen Injection. Normal dog bile exhibits no estrogenic activity.[6] In 6 of 9 experiments estrogenic activity was demonstrated in the bile following injection of androgens. In 5 of these, the bile was examined for both free and conjugated estrogen, all being found to exist in the free state. We have demonstrated estrogens in the urine of dogs following androgen administration,[11] contrary to earlier reports.[12] It is noteworthy that estrogens appear in the urine during the first 24 hours following androgen administration, whereas, except in one case, they first appeared in the bile *after* 24 hours.

The source of estrogen in the bile and urine after administration of androgen is not known. It does not seem to be produced in the testes or adrenals.[11] The bile fistula dogs employed in the present study were females and the ovaries might have been stimulated by the injected androgen to secrete estrogen. However, the fact that estrogen was present in the urine following injection of testosterone in normal and castrate male dogs militates against this assumption. A portion of the injected androgen may have been converted into an estrogenic compound.[17,18] The total amount of excreted estrogen (bile and urine) was 100 i.u. in Experiment 6 (following the administration of 100 mg testosterone), 57 i.u. in Experiment 10 (following the administration of 50 mg testosterone), and 150 i.u. in Experiment 14 (following injection of 100 mg methyl testosterone). If all of the estrogen was estrone this represents transformation of 0.01 to 0.15% of the administered androgen. Hamilton and Dorfman[17] calculate the recovery from urine in humans as 0.04% of the administered androgen. In view of the probable presence of estrogen in the bile of these subjects, not included in this calculation, it would appear that production of estrogen following administration of androgen (conversion of androgens to estrogen?) is greater in humans than in dogs.

Summary and Conclusions. 1. In bile fistula dogs a single injection of androsterone, testosterone, or methyl testosterone is followed by excretion of androgenic material in the bile. Excretion of small amounts of androgenic material may continue for 72 hours in spite of the fact that all of the androgen-containing bile is removed from the body. 2. The quantities excreted vary considerably in different experiments with either of the 3 compounds studied. 3. A single injection of androgen is frequently followed by excretion of estrogen in the bile. 4. In one instance androgen and estrogen were excreted in the bile following intraduodenal instillation of testosterone.

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Effect of Ascorbic Acid on Chick Growth when Added to Purified Rations.*

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It has long been recognized that chicks do not develop symptoms of scurvy when fed rations low or devoid of ascorbic acid.[1-8] For example, Hart, Steenbock, Lepkovsky, and Halpin[7] reported that chicks fed either a natural or a semi-purified ration did not develop scurvy and had an abundant supply of ascorbic acid in their livers. Carrick and Hauge[6] also found that livers of chicks on a scorbutic diet were high in ascorbic acid. In spite of this work the presence of a "scurvy-like" disease in chicks which was prevented by the inclusion of dried skim milk in the ration and cured by the addition of cabbage