day 8 of the cycle and 53.4 μg/g in tissue from day 16 during 2 hr incubation in Krebs Ringer bicarbonate medium. Increases of 20β-ol concentration on incubation were 2.1 and 12.4 μg/g at days 8 and 16 respectively. Mean concentration of progesterone ranged from 4.0 to 18.2 μg/g and 20β-ol from 3.3 to 10.5 μg/g in luteal tissue at seven stages of pregnancy investigated. Blood plasma from pregnant animals contained 0 to 2.13 μg progesterone/100 ml plasma and no detectable 20β-ol. Neither progesterone nor 20β-ol was detected in placental fluids and only progesterone (4.4 μg/kg) was found in one of 6 placentae.

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Response of Chickens to Prolonged Feeding of Crude "Toxic Fat".* (27100)

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"Toxic fat" is the name applied to certain distilled animal fats whose residue, when added to the diet of chickens, produces hydropericardium and ascites(1). The toxic fraction is located in the non-saponifiable portion of the fat(2-4). Its chemical structure is unknown; however, it has recently been crystallized(5).

Hydropericardium and ascites occur only in chickens, whereas, a reduction in growth is produced in ducks and turkeys(6). Retarded sexual development has also been reported in growing pullets when fed "toxic fat"(7). We have been able to produce hydropericardium, ascites and mortality as previously described in acute toxicity studies when proper concentrations of the crude "toxic fat" were fed to chickens one day of age. Most of the birds died within 5 weeks when fed concentrations of "toxic fat" above 1.0%. When levels of 1.0 or less were fed, the birds survived for longer periods of time, and showed better weight gains. With a longer survival time hydropericardial and ascitic effusions were not as great and testicular hypoplasia became apparent. It seemed desirable, therefore, to establish the response of young male chickens to concentrations of crude "toxic fat" which would permit good growth without causing death.

Methods. Thirty-six, Vantress-White Rock

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TABLE I. Effect of "Toxic Fat" Consumption on Development in Male Chickens.

No. birds	% T.F. in diet	Days on trial	No. died	Wt gain per day, g	Avg wt per testis, g	Hydropericardium	Ascites
12	.00	150	0/12	16.5	14.9	0/12	0/12
12	1.00	150	6/12	14.9	3.9	11/12	2/12
12	.50	150	4/12	15.3	4.1	8/12	0/12
6	.00	150	0/6	17.8	12.5	0/6	0/6
12	.25	150	1/12	16.1	6.6	4/12	1/12

male chickens, 4 weeks of age, were fed 1.0% corn oil, 0.5% corn oil and 0.5% "toxic fat", and 1.0% "toxic fat" for 150 days. In the second trial, 12 birds were fed 0.25% "toxic fat" and 6 birds received 0.25% corn oil in the diet.† They, too, were on trial for 150 days. Birds that expired during the course of the experiment and those sacrificed at termination of the trial were autopsied, organ weights recorded and tissues saved for microscopic examination. Body weights were recorded each month. Tissues for microscopic examination were fixed in 10% neutral formalin, dehydrated, embedded in paraffin, sectioned at 7 μ and stained with hematoxylin and eosin.

Results. As shown in Table I, 6 of the 12 birds on the 1.0% "toxic fat" expired during the trial. All of the mortality experienced during the trial occurred after 120 days of feeding. The effect of crude "toxic fat" on growth rate in the test chickens was minimal. Test birds which consumed 1.0% "toxic fat" in the diet gained the least weight. It is of interest that the test birds grew almost as well as the controls. Testicular development was markedly affected by crude "toxic fat" consumption. The control group had an average weight per testis of 14.9 g, the 1.0% group weighed 3.9 g and the 0.5% group averaged 4.1 g.

In these chronic feeding studies ascites did not occur as frequently as hydropericardium or testicular hypoplasia. From these studies it was not possible to anticipate whether concentrations of less than 0.5% crude "toxic fat" would produce any biologic effects. For this reason another assay was conducted on 12 birds receiving 0.25% "toxic fat" to determine whether testicular hypoplasia or hydropericardium would be the more sensitive index of toxicity. The data obtained in this experiment are shown in Table II.

Microscopic examination of the kidneys, pancreas, brain, spleen, adrenals and intestinal tract of experimental birds revealed no specific lesions. Minimal lymphoid hyperplasia was observed in the periportal spaces of the liver and peribronchial areas of the lung. There was focal infiltration of lymphocytes between the muscle fibers of the myocardium in 29 of 36 birds. Edematous fluid separated myocardial fibers in those birds with hydropericardium. Both of these myocardial changes were also observed in acute "toxic fat" feeding experiments. The testes of experimental animals were reduced in size and there was retarded spermatogenesis (Fig. 1, 2, 3). The test birds had normal appearing spermatogonia, primary spermatocytes and Sertoli cells. No spermatids or mature spermatozoa were present in testicles from birds fed 0.5 and 1.0% crude "toxic fat." Mature sperm were encountered in 4 of 12 test birds fed 0.25% "toxic fat."

Discussion. The percentage of "toxic fat" used in these experiments is applicable only for this particular lot of "toxic fat." Since the chemical nature of the toxic fraction is

TABLE II. Effect of 0.25% "Toxic Fat" in the Diet Fed for 150 Days to Chickens.

Bird No.	Bird wt, g	Testis wt, g	Hydropericardium, ml	Ascites, ml
443	2350	6.2	—	—
444	2650	10.0	—	—
445	2000	2.5	—	—
446	2840	13.1	—	—
447	2600	10.0	10.0	—
448	2520	13.5	—	—
449	2900	7.5	—	—
451	2000	.4	5.0	—
452	2320	6.0	—	—
453	2900	7.5	—	—
454	1790	1.5	5.0	75.0*
455	2150	.7	5.0	—

† McMillen Feed Mills, Fort Wayne, Ind.

* Bird expired on 130th day of trial.

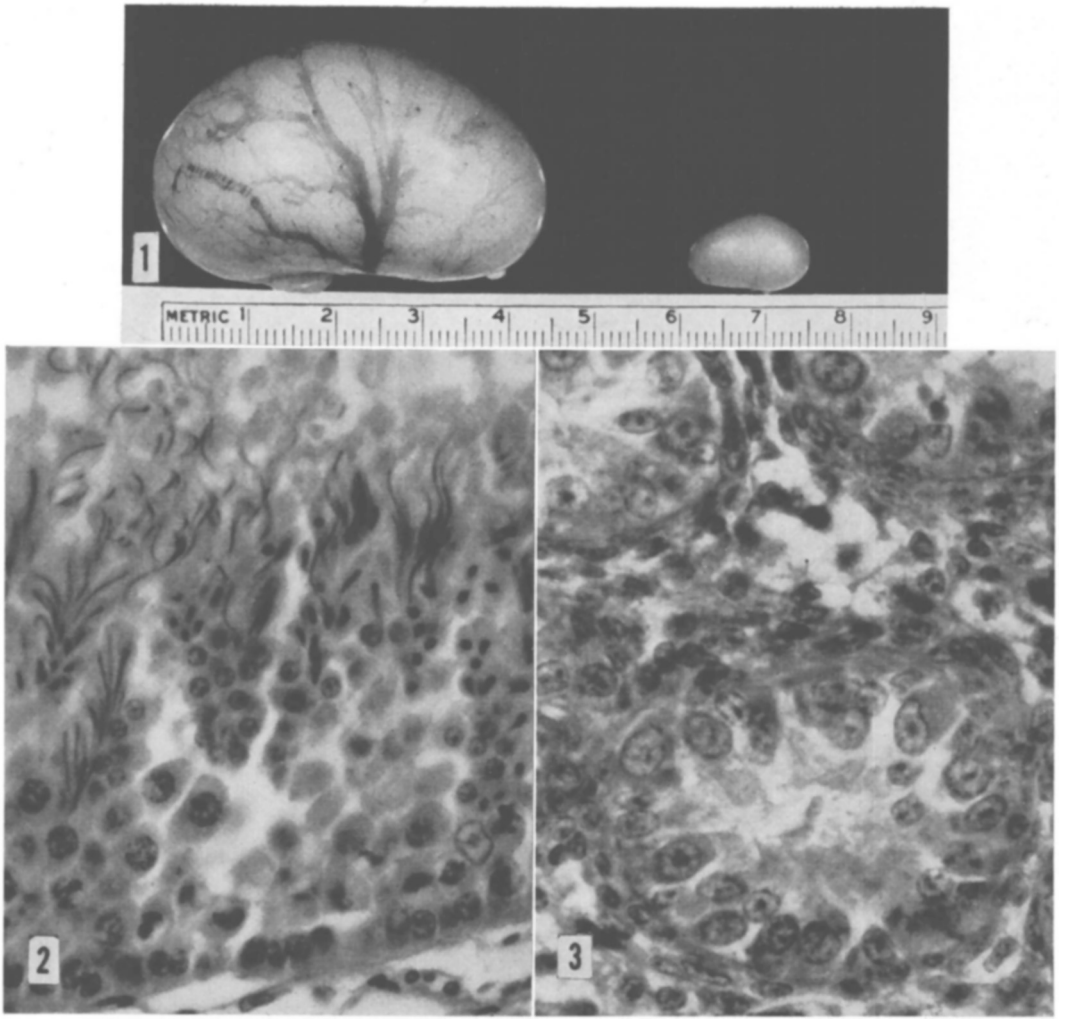

FIG. 1. Testicles from control and test birds of same age and weight. Note testicular hypoplasia developed in experimental birds without significant alteration in comb or body development.

FIG. 2. A segment of a normal seminiferous tubule with active spermatogenesis in testicle of a control bird. H & E stain; ×750.

FIG. 3. Hypoplastic seminiferous tubules with suppression of spermatogenesis in testis from an experimental bird. H & E stain; ×750.

unknown, the toxicity of separate shipments and sources must be determined by biologic assay.

It has been previously demonstrated by other workers that excess "toxic fat" feeding invariably produces hydropericardium, ascites and death within 5 weeks. In this study when the concentration of "toxic fat" was reduced and fed for 150 days, hydropericardium and ascites developed less frequently. Retarded testicular development was a frequent and consistent alteration in the absence of ascites and hydropericardium. The incidence of hydropericardium and ascites was markedly decreased when concentration of "toxic fat" in the diet was reduced to 0.25%. It is of interest to note that growth rate was not appreciably affected by crude "toxic fat" when a concentration of 0.25% was fed. Despite the testicular hypoplasia secondary sex characters such as comb size and body conformation were not altered by these concentrations of "toxic fat."

Summary. These data would indicate that

crude "toxic fat" has a marked effect on testicular development without appreciably affecting the development of secondary sex characters or growth rate. At levels of 0.5 and 1.0% "toxic fat," reduced testicular development usually occurred in conjunction with hydropericardium and ascites. When the level of fat was reduced to 0.25%, hydropericardium and ascites was markedly reduced, whereas, retarded testicular development persisted. It would appear that in immature chickens testicular hypoplasia resulting from consumption of crude "toxic fat" is a more sensitive index of toxicity than is hydropericardium or ascites.

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Changes in Serum Properdin of Mice During Tumor Growth and Following Immunization to Ehrlich Ascites Carcinoma.* (27101)

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Numerous investigators have observed low levels of serum properdin in patients with advanced cancer(1,2,3,4) and leukemia(5). Ishibashi, *et al.*(6) found that patients, following surgical removal of cancer growths, have serum properdin values higher than non-operated cancer patients. Southam and Pillemer(1) and Rottino, Levy and Conte(2) showed that cancer patients with low levels of serum properdin had a low ability to reject cancer cell homografts while persons with normal serum properdin were able readily to reject the cancer homografts. Herbut and Kraemer(7, 8, 9) showed a striking drop in the serum properdin of C3H mice actively growing Gardner lymphosarcoma, 6C3HED; Bradner and Clarke(10) observed a 6-fold decrease in the serum properdin of mice growing sarcoma, S-180; and Orita(11) has determined that serum properdin of Ehrlich carcinoma bearing C6 mice and Brown-Pierce tumor bearing rabbits varied inversely with tumor growth.

This paper presents data showing: (1) diminution of serum properdin in C57/BL, A/He and C3H mice actively growing Ehrlich ascites carcinoma, L No. 2 lymphoma and Gardner 6C3HED lymphosarcoma, respectively; (2) maintenance of control serum properdin levels, following a transient fall, in C57/BL mice given injections of X-irradiated Ehrlich tumor; and (3) maintenance of control serum properdin values, following a transient drop, in C57/BL mice made resistant to Ehrlich carcinoma by injecting X-irradiated Ehrlich tumor followed by 3 to 5 challenges of completely viable Ehrlich cells.

Materials and methods. C57/BL mice were raised in our laboratory from breeding stock obtained from the Roscoe B. Jackson Memorial Labs., Bar Harbor, Me. A/He and C3H mice were obtained from the Cancer Research Genetics Lab., Univ. of California,

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