siblings were given thyroxine, thyrotrophic and somatotrophic hormones or physiological saline intraperitoneally. In the skin the mast cells were counted and concentration of hexosamine and hydroxyproline were determined. The number of mast cells did not show any significant variation in the different groups. Concentration of hexosamine in the skin of thyrotrophin-treated dwarf mice was significantly higher than concentration in control dwarf mice. Hexosamine values in the group of thyroxine-treated mice were higher than in the group of control mice. Hydroxyproline content was significantly higher in the skin of thyrotrophin-treated dwarf mice than in thyroxine-treated dwarf mice.

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Influence of Exercise, Androgen Administration and "Atherogenic" Diet on Hematologic Changes in Pullets.* (25270)

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It is well known that the red blood cell count and hematocrit values of humans, mammals and avian species are much higher in males than in females. Juhn and Domm(1) showed that red blood cell differences between the sexes in chickens occurred after puberty and only in males in association with androgen secretion by testes. Gonadectomy of males prevented red blood cell count from differing significantly from that of females. Despite seasonal variations in the number of erythrocytes it was noted that adult male doves and pigeons had a higher count than adult females in all seasons(2). The present study was undertaken to determine the effects of exercise, androgen and an "atherogenic" diet on the erythrocytes, hematocrit and spleen size of pullets.

Methods. Six-week-old New Hampshire White Leghorn pullets were used in an experiment of approximately 11 weeks. The pullets were divided into 5 groups. Controls were fed plain mash. A second group was fed the same diet plus 2% cholesterol and 5% cottonseed oil. The third group, in addition to the diet of Group II, was exercised for one hour daily. Group IV received the same diet as II, was exercised, and injected with 1.25 mg testosterone propionate ("Oreton")‡ intramuscularly daily. We have found this dose of testosterone to be replacement therapy for castrated males. The last group was treated in the same fashion as the 4th, but no exer-

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TABLE I. Hematologic Changes in Pullets by Exercise, Androgen and "Atherogenic Diet."

Group	Regimen	No. birds	RBC (mill./mm³)	Hematocrit (%)	Spleen wt (g)
I	Control (plain mash)	12	2.79 ± .06	31.0 ± .7	3.39 ± .2
II	Mash + Chol + C.O.	9	2.79 ± .1	31.0 ± .8	3.31 ± .2
III	*Idem* + exercise	7	2.83 ± .1	30.0 ± 1.5	3.04 ± .5
IV	*Idem* + T.P.	8	3.15 ± .1*	35.0 ± 2.0†	1.93 ± .2*
V	Mash + Chol + C.O. + T.P.	10	3.25 ± .1*	37.0 ± .8*	2.56 ± .3*

± S.E. of mean. Chol = Cholesterol; C.O. = Cottonseed oil; T.P. = Testosterone propionate.

* Significant .01. † Significant .02.

cise. The birds were exercised on a treadmill designed for this purpose consisting of a cylindrical structure enclosed in wire mesh. Diameter of the treadmill was 23½ inches, length 33 inches and outside perimeter 5 feet 10 inches. Speed was regulated at 6 ± 1 rpm. The animals were exercised in this apparatus for one hour daily, or approximately 700 yards. Red blood cell and hematocrit determinations were carried out using heparinized blood obtained from wing veins. Erythrocyte counts were made using a Spencer Bright-Line Haemocytometer. Blood was diluted with Wiseman's diluting fluid which contained 50 mg of phloxine, 5 cc of neutral formalin and 75 cc of Ringer's solution(3). Hematocrit values were determined by centrifugation of blood for 30 minutes in Wintrobe hematocrit tubes. At conclusion of the experiment all animals were sacrificed and spleen weights taken.

Results. Those birds treated with testosterone propionate were the only ones significantly different from the controls regarding hematological findings. There was an increase in erythrocyte counts and hematocrits, and a decrease in spleen weights. Addition of cholesterol to the diet, or submitting the birds to exercise, or a combination of these, did not appear to effect the above 3 indices (Table I).

Discussion. These data indicate that the hematologic changes produced probably resulted from the action of testosterone propionate, as the other variables, cholesterol and cottonseed oil as well as exercise, produced no significant changes in red blood cell count, hematocrit or spleen weight. Increase in hematocrit in Groups IV and V is consistent with increased red blood cell count and may represent some degree of hemoconcentration. Normal red blood cell counts in adult male chickens average 3.25 million/mm³(4). In the present experiment it should be noted that pullets receiving testosterone propionate had values similar to adult male birds.

Decrease in spleen weight of the birds in Groups IV and V and the increased number of red blood cells in circulation suggest that the additional erythrocytes have come from spleens. It has been shown that the spleen of fowls is utilized as a storage place for erythrocytes(5).

Since there is evidence that cholesterol is a precursor of steroid hormones of the adrenal gland(5), it may be postulated that an increase in cholesterol in diet might lead to increased production of adrenal androgens. If this should occur, in view of the results obtained in this experiment, one might expect hematological changes also. No such changes were noted in this study. The red blood cell count in those birds receiving cholesterol was the same as in the controls on plain mash.

It appears therefore, that amount of cholesterol in the diet does not influence the hematological picture in pullets. There is reason to believe that excess cholesterol is either deposited on vascular walls and/or is metabolized to cholic acid and excreted in bile. This can be supported by the abundance of bile found at autopsy in all birds fed a diet rich in cholesterol.

Summary. Pullets receiving testosterone propionate demonstrated a significant increase in red blood cell count and hematocrit and a decrease in spleen weight.

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Pancreatic Collagenase, A New Enzyme.* (25271)

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Ziffren and Hosie(1) claimed that pancreatic juice possessed ability to digest collagen fibers. Straumford and Hummel(2) showed that this activity was inhibited by soybean trypsin inhibitor. More recently, Oken and Boucek(3) claimed that impure pancreatic elastase solubilized collagen fibers, whereas the crystalline enzyme did not. Investigations into collagenolytic enzymes have always been questionable because of the difficulty of assaying an enzyme using an insoluble substrate. Recently Gallop(4) has shown that only bacterial collagenase will reduce the viscosity of procollagen derived from swim-bladder tunica of the carp. We studied the effect of pancreatic extracts and enzymes upon viscosity of acid soluble collagen of calf skin.

Material and methods. Purified acid soluble collagen was prepared from 0.5 M acetic acid extracts according to Gross and Kirk(5). This material was lyophilized, and solutions of 0.2 M acetate buffer (pH 5.5) were made to contain 15 mg/ml of procollagen. These solutions were homogeneous with respect to electrophoresis and in amino acid and hexosamine content were as reported by Gross and Kirk (5). Crystalline trypsin, elastase, chymotrypsin and relatively purified hyaluronidase were obtained from Worthington. Human pancreatic juice was obtained from a patient with freely flowing fistula. Aqueous extracts of "Bridase," a whole, desiccated, defatted, activated preparation of swine pancreas provided by Mr. Levin of VioBin, were prepared by extracting 25 to 100 mg of "Bridase" with 5 ml of 0.2 M acetate buffer (pH 5.5) in a glass homogenizer at 4°C. After centrifugation, about 45% of total weight of tissue had been solubilized into the clear supernatant. Concentration of this material was expressed in terms of mg of "Bridase" initially mixed with 1 ml of solvent. Assay of enzyme activity was as follows: 4 ml of substrate solution (15 mg/ml of acetate buffer) was warmed to 20°C, added to an Ubbelohde viscosimeter in which water had a flow time of about 70 seconds. To this viscosimeter was added 1 ml of buffer containing 1 mg of various enzymes described above, or 1 ml of "Bridase" extract. After manual mixing for a few minutes, the solution was incubated for 75 minutes and flow time determined. The difference between relative viscosity of this solution and that of the control previously found, using boiled enzyme solutions, was expressed as percent of initial viscosity reduced by 75 minutes incubation with the enzyme.

Results. The effect of 1 mg each of crystalline trypsin, chymotrypsin and elastase, as well as the effect of 1 mg of hyaluronidase and 1 ml of human pancreatic juice upon relative viscosity of acid soluble collagen was determined. Similarly, the effect of the aqueous extract of 15 mg/ml of "Bridase," with and without addition of 1 mg each of either soybean, ovomucoid and lima bean trypsin inhibitor was also measured, and the results expressed in terms of per cent viscosity reduced is shown in Table I. The pancreatic extract was uniquely able to reduce 50% of the viscosity of acid soluble collagen. This activity was destroyed by boiling and was independent of addition of 3 kinds of trypsin inhibitor. Amount of inhibitor added was in excess as shown by the method of Rhodes *et al.*(6).

Fig. 1 shows that after 75 minutes incuba-

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