EFFECTS OF SEX HORMONES ON ERYTHROCYTE NUMBER AND HEMOGLOBIN CONCENTRATION OF WHITE LEGHORN CHICKENS

by

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INTRODUCTION

For many years biologists have known that male mammals and birds had a consistently higher erythrocyte count and a greater hemoglobin concentration in the blood than female animals of the same species. A thorough search of the available literature failed to disclose the first report of this difference by an experimental biologist. However, an etiological relationship between this difference in the blood picture and the sex glands was reported by several workers. In 1928 Blumel, a German zoologist, found that the erythrocyte count of male rats dropped sharply after castration. Further evidence that the sex glands exercised an influence on the blood picture was reported by Juhn and Domm (1930), who found that the red blood cell counts in sexually immature chickens did not show a sex based difference, but that a sharp divergence between the sexes occurred at puberty when the male birds' counts rose very rapidly. Adult male chickens had an average count about 30 per cent higher than the female average, while castrated male birds approached the female level. Still more definite proof was offered by an Italian, Emmanuele Padoa (1931), who reported that the removal of a hen's ovary, with a resulting proliferation of testicular tissue and elaboration of testicular hormone, caused a sharp rise in the erythrocyte number of the bird. Similar results were obtained in investigations with pigeons (Riddle and Braucher, 1934), (Riddle and Cauthen, 1938), rats (Steinglass et al., 1941), and golden hamsters (Stein

and Jacobsen, 1941).

The purpose of this investigation was not only to check and clarify this relationship between the sex hormones and the blood picture, but also to secure information concerning the method of action of estrogenic hormone on the erythrocyte number and the hemoglobin concentration. Past workers have generally agreed that androgenic hormone increased the erythrocyte and the hemoglobin levels, although the action is not thoroughly understood. Before the era of synthetic testosterone, workers such as Truffi (1927) reported that testicular tissue, grafted into female animals, produced a sudden rise in erythrocyte numbers. More recently, increased erythrocyte counts and hemoglobin concentrations caused by synthetic male sex hormone have been reported by Taber et al. (1941, 1943) in chickens, Domm et al. (1943) in chickens, Vollmer and Gordon (1941), Vollmer et al. (1942) in rats, Finklestein et al. (1944) in rats, and McCullagh and Jones (1942) in eunuchoid male human beings.

Concerning the effect of estrogenic hormone, however, no such widespread agreement exists. Davis and Boynton (1941) found that stilbestrol, a synthetic estrogenic compound, caused an increase in the number of red blood cells. They attributed this increase to a direct stimulation of the hematopoietic system by the estrogen. On the other hand, Tyslowitz and Hartman (1941) observed a decreased erythrocyte count in dogs of both sexes after the administration of estrogen. They concluded, as did Castrodale et al. (1941), that the estrogen in some way inhibited the hematopoietic system. Only a slight anemia was reported by

Feuchinger (1940) after treatment of female rats with estrogen, and the animals soon returned to their normal level although the injections were continued. He also noted a similar drop in erythrocyte number during each period of estrus in rats. Taber et al. (1941, 1943) and Crafts (1941), working with male chickens and rats, reported consistent decreases in both erythrocyte counts and hemoglobin concentrations after injections of estrogenic hormone. Their evidence indicated a possible antagonism between male and female hormone insofar as their relation to the blood picture was concerned. However, using the two hormones together on capons, Domm et al. (1943) reported a resulting increase in erythrocyte number, while Tyslowitz and Dingemanse (1941) observed a decrease in erythrocyte number resulting from the same combination in castrated dogs. In light of such discrepancies, additional research seemed indicated. The writer hoped that this experiment would aid in clarifying both the effect of estrogen on the blood picture and its method of action thereon.

PROCEDURE

This experiment was begun in October, 1947, and continued through June, 1948. All of the work was done in the laboratory of the Department of Zoology of Kansas State College in Manhattan, Kansas. A total of 42 White Leghorn chickens was used as experimental animals. They were selected from two separate shipments, since the experiment was divided into two series; and each bird was marked by attaching a numbered metal clip to its left wing.

All birds were weighed to the nearest gram before the experiment began and again at the conclusion of the work.

The first series consisted of 16 birds, eight capons and eight normal males. All of these birds were received on May 13, 1947, and were about five months old when the experiment began. The capons were castrated by Dr. E. H. Herrick of the Department of Zoology on July 15, 1947. These 16 birds were further subdivided into four groups. Group 1 consisted of four normal males and served as a control group. These birds lived with the others, having an identical environment in every way, but they received no hormones. Group 2 consisted of four capons which received injections of male sex hormone. Four more capons made up Group 3, and this group received injections of both male and female hormones. The fourth group contained four normal males and was treated with estrogenic hormone.

For the second series, a total of 26 birds was used. Thirteen of them were capons, 10 were normal males, and three were normal females. They were received on January 8, 1948, and were about three months old when they were placed on experiment. These capons were castrated on February 17, 1948. The birds in this series were divided into six groups. The control group (Group 5) this time contained five normal males. Group 6 contained five capons which were treated with male sex hormone. Group 7 also contained five capons, but this group was treated with a combination of male and female sex hormones. Female hormone was administered to Group 8, which contained five normal males. Groups 9 and 10, which also received female sex hormone, consisted

of three capons and three normal females respectively.

The experimental birds were injected with the hormone material three times a week for a period of six weeks. "Oreton," prepared by the Schering Corporation, was used as the male sex hormone. It consisted of testosterone propionate in a sesame oil solution, and contained 25 milligrams of active hormone material per cubic centimeter of solution. Each tri-weekly dose was two-tenths of a cubic centimeter of Oreton or five milligrams of testosterone propionate. Each bird thus received a total of 90 milligrams of active hormone.

During the first series, "Theelin," a Parke-Davis product, was used as the female sex hormone. It was used in an aqueous suspension, with either one or two milligrams of active hormone per cubic centimeter of water. Each tri-weekly dose was one milligram of active hormone material and each bird received a total of 18 milligrams during the experimental period. For the groups receiving both hormones the above described dosages were combined and given on the same tri-weekly schedule. All injections were intramuscular.

Prior to the first injection in each series two erythrocyte counts and two hemoglobin determinations were taken on each bird. Thus an individual norm was established for each. During the course of the injections, weekly erythrocyte counts and hemoglobin determinations were made, and after the conclusion of the injections, the weekly determinations were continued until the birds approximated their pre-injection level. Blood for the tests was drawn from a vein on the inner surface of a wing. For erythrocyte

counts, a one to two hundred dilution with Hayem's solution was made, and the red blood cells were counted on a Spencer Bright-line Hemacytometer. A one to two hundred and fifty dilution with one-tenth normal hydrochloric acid was used for the hemoglobin determinations, and the hemoglobin concentration in grams per one hundred cubic centimeters was read from the scale of a Fisher Electro-hemometer.

RESULTS

The recorded results of this investigation consisted of weekly erythrocyte counts, weekly determinations of hemoglobin concentration, and the weight increase of each bird during the 13-week experimental period. No attempt was made to treat these results statistically since the number of individual birds involved was considered too small for such treatment. Interpretation of the results therefore required consideration of the physiological differences between individuals of the same species as well as the differences generally found between any given individual and the so-called norm of the species concerned. In calculating mean data for each group of birds no allowance was made for individual deviation from the mean, but all observed results were included. Mean weekly data for each group were prepared in both graphic and tabular form and included in this report, Figs. 1-10. The maximum variation in erythrocyte count, hemoglobin concentration, and body weight of each individual bird was also tabulated and added to this report, Table 11.

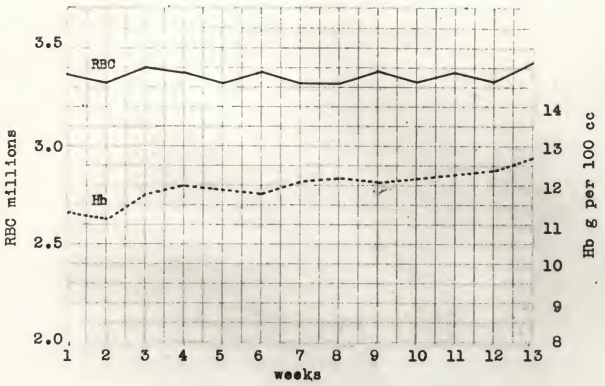

Fig. 1. Group 1; five normal male controls.

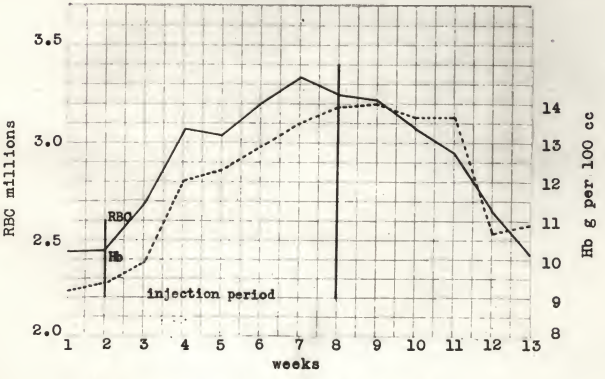

Fig. 2. Group 2; four capons with male hormone.

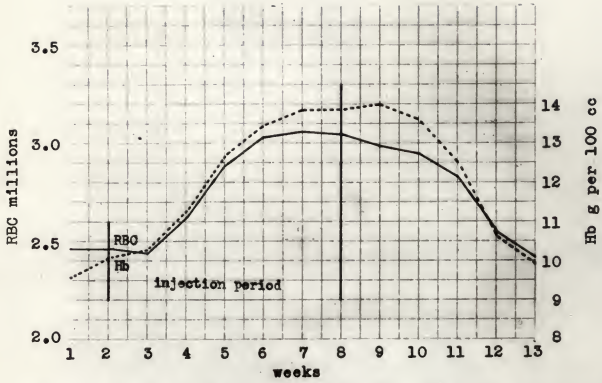

Fig. 3. Group 3; four capons with male and female hormones.

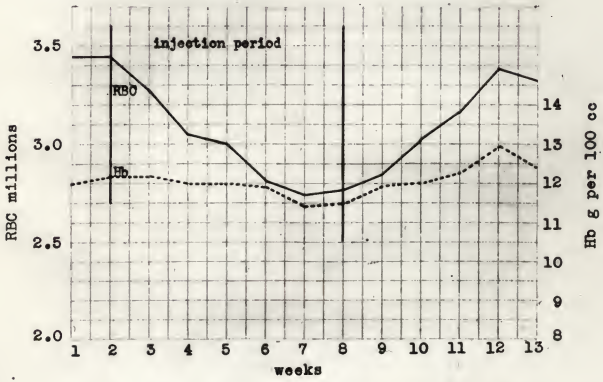

Fig. 4. Group 4; four males with female hormone.

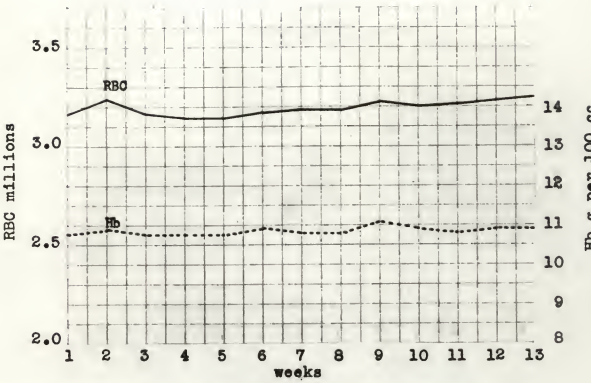

Fig. 5. Group 5; five normal male controls.

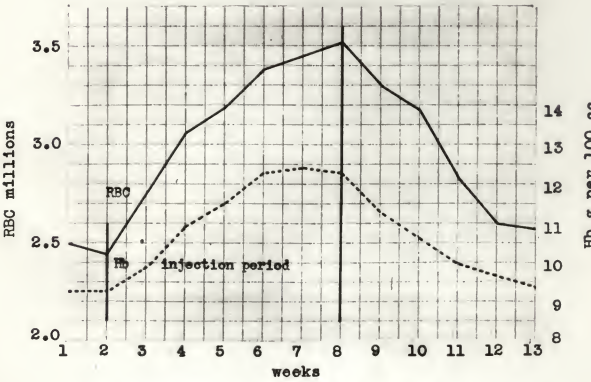

Fig. 6. Group 6; five capons with male hormone.

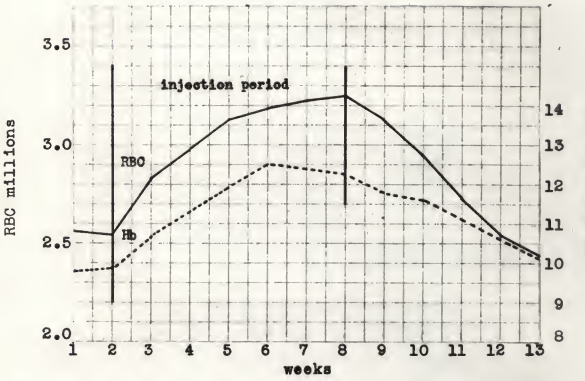

Fig. 7. Group 7; five capons with male and female hormones.

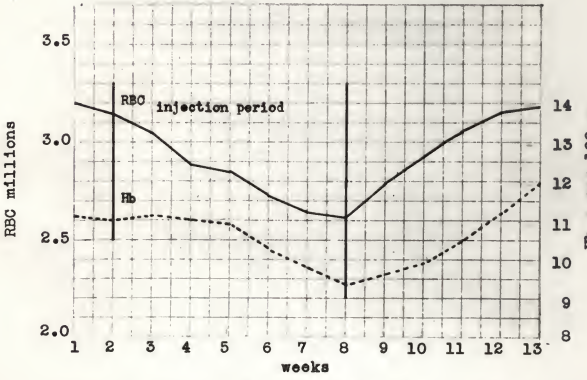

Fig. 8. Group 8; five males with female hormone.

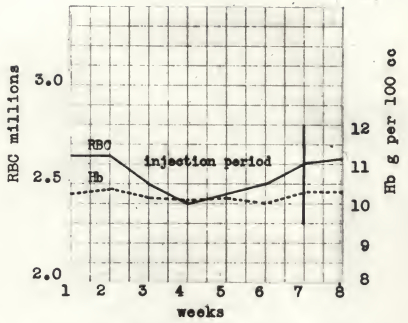

Fig. 9. Group 9; three capons with female hormone.

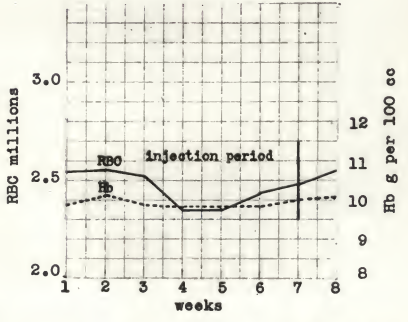

Fig. 10. Group 10; three females with female hormone.

Table 1. Mean weekly data for four normal male controls.

Week	RBC	Hb
1	3 380 000	11.3
2	3 320 000	11.2
3	3 400 000	11.3
4	3 380 000	12.0
5	3 310 000	11.9
6	3 380 000	11.3
7	3 310 000	12.1
8	3 310 000	12.2
9	3 380 000	12.1
10	3 320 000	12.2
11	3 390 000	12.3
12	3 300 000	12.4
13	3 420 000	12.7

Table 2. Mean weekly data for four capons treated with androgenic hormone.

Week	RBC	Hb
1	2 440 000	9.2
2	2 440 000	9.4
3	2 690 000	9.9
4	3 060 000	12.0
5	3 040 000	12.3
6	3 200 000	12.9
7	3 330 000	13.5
8	3 280 000	13.9
9	3 220 000	14.0
10	3 080 000	13.7
11	2 960 000	13.7
12	2 660 000	10.7
13	2 430 000	10.9

Table 3. Mean weekly data for four capons treated with androgenic and estrogenic hormones.

Week	RBC	Hb
1	2 470 000	9.6
2	2 470 000	10.1
3	2 440 000	10.3
4	2 610 000	11.3
5	2 890 000	12.7
6	3 020 000	13.4
7	3 060 000	13.8
8	3 050 000	13.8
9	2 990 000	14.0
10	2 950 000	13.6
11	2 880 000	12.5
12	2 550 000	10.7
13	2 420 000	9.9

Table 4. Mean weekly data for four normal males treated with estrogenic hormone.

Week	RBC	Hb
1	3 450 000	12.0
2	3 450 000	12.2
3	3 270 000	12.2
4	3 050 000	12.0
5	3 000 000	12.0
6	2 810 000	11.9
7	2 750 000	11.4
8	2 770 000	11.5
9	2 850 000	11.9
10	3 010 000	12.0
11	3 170 000	12.3
12	3 390 000	12.9
13	3 320 000	12.4

Table 5. Mean weekly data for five normal male controls.

Week	RBC	Hb
1	3 170 000	10.8
2	3 240 000	10.9
3	3 170 000	10.8
4	3 160 000	10.8
5	3 160 000	10.8
6	3 180 000	10.9
7	3 190 000	10.8
8	3 190 000	10.8
9	3 220 000	11.1
10	3 200 000	10.9
11	3 210 000	10.8
12	3 230 000	10.9
13	3 250 000	10.9

Table 6. Mean weekly data for five capons treated with androgenic hormone.

Week	RBC	Hb
1	2 500 000	9.3
2	2 440 000	9.3
3	2 740 000	9.9
4	3 040 000	10.9
5	3 190 000	11.5
6	3 380 000	12.3
7	3 450 000	12.4
8	3 510 000	12.3
9	3 300 000	11.3
10	3 180 000	10.7
11	2 820 000	10.0
12	2 600 000	9.7
13	2 580 000	9.4

Table 7. Mean weekly data for five capons treated with androgenic and estrogenic hormones.

Week	RBC	Hb
1	2 570 000	9.8
2	2 560 000	9.9
3	2 830 000	10.7
4	2 980 000	11.3
5	3 130 000	11.9
6	3 130 000	12.5
7	3 230 000	12.4
8	3 250 000	12.3
9	3 120 000	11.8
10	2 960 000	11.1
11	2 720 000	10.6
12	2 560 000	10.1
13	2 450 000	9.7

Table 8. Mean weekly data for five normal males treated with estrogenic hormone.

Week	RBC	Hb
1	3 200 000	11.1
2	3 150 000	11.0
3	3 040 000	11.1
4	2 890 000	11.0
5	2 850 000	10.9
6	2 720 000	10.3
7	2 650 000	9.8
8	2 620 000	9.4
9	2 790 000	9.6
10	2 920 000	9.9
11	3 050 000	10.5
12	3 150 000	11.2
13	3 180 000	11.9

Table 9. Mean weekly data for three capons treated with estrogenic hormone.

Week	RBC	Hb
1	2 650 000	10.3
2	2 650 000	10.4
3	2 500 000	10.2
4	2 400 000	10.1
5	2 450 000	10.2
6	2 500 000	10.0
7	2 600 000	10.3
8	2 620 000	10.3

Table 10. Mean weekly data for three females treated with estrogenic hormone.

Week	RBC	Hb
1	2 550 000	9.9
2	2 560 000	10.1
3	2 520 000	9.9
4	2 350 000	9.8
5	2 350 000	9.8
6	2 430 000	9.8
7	2 490 000	10.0
8	2 560 000	10.1

Table 11. Data for individual birds on changes in RBC, Hb, and weight.

Bird	Sex	Hormone	RBC	Hb	Weight
			Per cent		
1296	male	none			died
1297	male	none			+ 2.40
1300	male	none			+10.35
1327	male	none			+ 3.78
1286	capon	androgenic	+39.58	+70.30	+22.85
1290	capon	androgenic	+34.00	+46.39	+21.80
1281	capon	androgenic	+36.00	+68.48	+12.74
1293	capon	androgenic	+59.52	+34.28	+20.83
1294	capon	androgenic and estrogenic	+45.45	+69.69	+ 9.82
1288	capon	androgenic and estrogenic	+23.07	+37.55	+11.90
1299	capon	androgenic and estrogenic	+27.65	+36.63	+14.27
1283	capon	androgenic and estrogenic	+34.78	+42.42	+14.45
1299	male	estrogenic	-12.37	-6.61	+ 9.78
1298	male	estrogenic	-26.53	-8.59	+13.02
1329	male	estrogenic	-26.36	-7.25	+20.00
1328	male	estrogenic	-22.38	-10.56	+13.14
1347	male	none			+25.64
1372	male	none			+37.17
1351	male	none			+42.85
1349	male	none			+31.84
1357	male	none			+21.79
1333	capon	androgenic	+24.13	+31.00	+55.09
1340	capon	androgenic	+47.82	+34.73	+52.53
1343	capon	androgenic	+65.63	+39.32	+44.63
1336	capon	androgenic	died		
1342	capon	androgenic	+46.80	+41.96	+47.70
1339	capon	androgenic and estrogenic	+34.69	+31.25	+32.03
1338	capon	androgenic and estrogenic	+32.65	+29.29	+35.71
1345	capon	androgenic and estrogenic	+32.65	+36.26	+40.80
1335	capon	androgenic and estrogenic	+32.65	+34.78	+36.22
1344	capon	androgenic and estrogenic	+26.52	+16.36	+32.10
1356	male	estrogenic	died		
1355	male	estrogenic	-19.35	-19.31	+42.30
1352	male	estrogenic	-20.58	-17.85	+54.51
1353	male	estrogenic	-20.00	-18.93	+39.63
1373	male	estrogenic	-18.75	-16.36	+50.00
1280	capon	estrogenic			+ 5.71
1289	capon	estrogenic			+ 7.43
1281	capon	estrogenic			+ 3.36
1258	female	estrogenic			+41.42
1259	female	estrogenic			+42.64
1260	female	estrogenic			+42.56

Group 1, consisting of four normal male birds, 1296, 1297, 1300, and 1327, was used as the control group for the first series of experiments. The mean initial erythrocyte count for this group was 3,380,000 and the mean initial hemoglobin concentration was 11.3. This group received no hormones and no bland injections were used. The lowest mean erythrocyte count recorded for this group during the investigation was 3,310,000 while the highest was 3,420,000 observed on the thirteenth and final count. During the 13 weeks, the curve varied slightly from week to week but in no case were the variations significant, Fig. 1. The slight increase in erythrocyte count over the entire period was probably due to the maturing and growth of the birds. Mean hemoglobin concentration reached its high point of 12.7 on the final determination and this curve showed a rather steady gradual increase from week to week. As with the erythrocyte count, the net increase in hemoglobin concentration was attributed to growth and maturity. One individual of this group, bird 1296, died from injuries sustained while fighting during the sixth week of the experiment.

A radically different curve was produced by the four members of Group 2, birds 1286, 1290, 1291, and 1293, Fig. 2. These birds were all capons and received injections of testosterone propionate. Pre-injection counts showed a mean erythrocyte count of 2,440,000 for this group. Immediately after the first injection the mean count began to rise rapidly. In two weeks it reached 3,060,000 and after dropping to 3,040,000 for one week, climbed less steeply to reach 3,330,000 after five weeks of injections. For one week after the last injection it remained at approximately this level

and then began a rapid decrease. At the end of the thirteenth week, it had dropped to 2,430,000 almost identical to the pre-injection level. The average increase in erythrocyte count for the group was 36.47 per cent and three of the individual birds were within three per cent of this average. The fourth bird, 1293, showed a 59.52 per cent increase, but since this individual began the experiment with an extremely low erythrocyte count it was assumed that the relatively large increase was due to a temporary anemia at the beginning of the experiment. This assumption was further supported by the fact that the erythrocyte count of bird 1293 had decreased only to 2,800,000 at the conclusion of the experiment.

Mean weekly hemoglobin concentrations for this group rose steadily from an initial average of 9.2 to a peak of 14.0. The first two weeks of injections caused a steep increase from 9.4 to 12.0 and the subsequent increase was more gradual. The peak of 14.0 was reached one week after the last injection and two weeks later than the erythrocyte peak. The hemoglobin curve formed a plateau between 13.5 and 14.0 beginning during the fifth week of injections and extending for five weeks, or four weeks past the final injection. During the fifth post-injection week the hemoglobin concentration dropped sharply from 13.7 to 10.7 and did not drop any further during the experimental period. The four members of this group varied widely in their percentages of increase in hemoglobin concentration, averaging as a group a 52.17 per cent increase, but as individuals, ranging from a 34.28 per cent increase to a 70.30 per cent increase. There was not a

consistent correlation between erythrocyte count and hemoglobin concentration, since bird 1293 had the greatest increase in erythrocyte number and the smallest increase in hemoglobin concentration.

Birds 1294, 1288, 1289, and 1283, all capons, made up Group 3, which received injections of both male and female hormones. An average erythrocyte count of 2,470,000 was established for this group prior to the first injection. During the first four weeks of injection, the mean count rose to 3,020,000 and added a very small increase the following week to reach a peak of 3,060,000 after five weeks of injections. For this group the curve rose less steeply and to a lower peak than the curve for the preceding group, Fig. 3. A plateau between 3,020,000 and 3,060,000 extended over a period of three weeks, the last three during which the hormones were administered. Immediately after cessation of the injections, the mean erythrocyte count began a gradual decrease which was accelerated during the last three weeks of the experimental period. At the end of the 13-week period, the mean erythrocyte count for the group was 2,420,000, slightly lower than the pre-injection level. As a group these four birds increased their erythrocyte count 26.41 per cent but there was a wide deviation in percentage of increase among the four individuals.

The mean hemoglobin concentration for this group rose steadily from 9.6 to 13.8 during the first five weeks of injections. For three weeks, the last two of the injection period and the first after the injections had ceased, it remained between 13.8 and

14.0, reaching this peak one week after the end of the injection schedule. It then fell rather sharply and steadily until, at the end of the 13-week period, it was 9.9, a slightly higher level than that observed in pre-injection determinations. The mean increase in hemoglobin concentration for this group was 47.82 per cent, but once again the four individuals deviated considerably from the mean.

The four birds in Group 4, 1299, 1298, 1329, and 1328, were normal males and received injections of estrogen. For this group the curve was very nearly an inverted replica of those for Groups 2 and 3, Fig. 4. These birds showed a marked decrease in both erythrocyte count and hemoglobin concentration under the influence of estrogen. A pre-injection mean erythrocyte count of 3,450,000 was established for this group and the mean weekly counts decreased steadily after the injections began. The low mean count of 2,750,000 was reached after five weeks of injections and during the sixth and last week of injections an insignificant increase of 20,000 was recorded. Immediately after the injections ceased the mean erythrocyte count began a steady rise and at the end of 12 weeks stood at 3,390,000. At the end of the 13-week experimental period the mean count had slumped to 3,320,000, a level appreciably lower than the pre-injection level. As a group these birds showed an average decrease of 20.28 per cent.

The mean hemoglobin concentration for these birds was not influenced by the estrogen to the same extent as the erythrocyte count. After four weeks of injections it had dropped only one-tenth of a gram from the pre-injection level of 12.0. During the

last two weeks of the injection period, however, the mean hemoglobin concentration dropped to a minimum of 11.4. Immediately after the injections ceased it began a steady rise and reached 12.9 at the end of the twelfth week. At the end of the thirteenth week, the mean concentration was 12.4, which was four-tenths of a gram higher than the pre-injection level. The slight decrease during the thirteenth week accompanied the previously described slump for the mean erythrocyte count for this group. The decrease in mean hemoglobin concentration for this group was 6.66 per cent but once again there were wide differences in the decreases observed in the individual birds.

Groups 5-10 were used during the second series of experiments. Group 5 included birds 1347, 1351, 1372, 1341, and 1357, all normal males, and was used as the control group for this series. Weekly mean erythrocyte counts for this group ranged from a low of 3,160,000 to a high of 3,250,000. This range was lower than that observed in the other control group because these birds were two months younger than the birds in Group 1. No sudden or significant changes occurred in the curve. Similar results were obtained from weekly hemoglobin determinations. In this case, the mean concentration varied between 10.8 and 11.1 and no sudden or significant changes were observed, Fig. 5.

Five capons, 1333, 1340, 1343, 1336, and 1342, were included in Group 6 and received injections of testosterone propionate. From a pre-injection level of 2,440,000 the mean erythrocyte count rose to 3,450,000 at the end of the six week injection period. The curve of this rise was fairly regular, although slightly

steeper during the first four weeks of injections, Fig. 6. As soon as the injections were stopped the mean erythrocyte count began a sharp decrease, falling to 2,600,000 at the end of the twelfth week and decreasing still further to 2,580,000 at the end of the 13-week experimental period. The increase for the mean erythrocyte count of the group was 45.25 per cent but individual increases ranged from 24.13 per cent to 63.63 per cent.

A pre-injection level of 9.3 was established for the mean hemoglobin concentration of this group. This rose rapidly during the first four weeks of injections, reaching 12.3 at the end of the fourth week. During the fifth week of injections it rose only one-tenth of a gram to reach its peak of 12.4 and during the sixth and last week of injections returned to 12.3. Thus a plateau between 12.3 and 12.4, covering the last three weeks of the injection period, appeared in the curve. After the last injection the mean concentration dropped steadily and at the end of the 13-week period stood at 9.4, only one-tenth of a gram higher than the pre-injection level. As a group, these birds showed an increase in their mean hemoglobin concentration of 33.33 per cent and the individual increases ranged from 31.00 per cent to 41.86 per cent.

Group 7 included birds 1339, 1338, 1345, 1335, and 1344, and as in Group 3, these birds were all capons and were treated with both male and female hormones. In this group the mean erythrocyte count rose rapidly from a pre-injection level of 2,560,000 to 3,130,000 at the end of the third week of injections. During the last three weeks of injections the mean count continued to increase,

but less rapidly, Fig. 7. It reached a peak of 3,250,000 at the end of the six week injection period. Immediately after the injections ceased, the mean count began a steady decrease and reached 2,450,000 at the end of the 13-week injection period. This was definitely lower than the pre-injection level. The increase in the mean erythrocyte count for this group amounted to 26.95 per cent. In this group, some degree of consistency was observed for the only time during the investigation as three birds, 1338, 1345, and 1335, showed identical increases of 32.65 per cent, while bird 1339 increased 34.69 per cent. The fifth member of the group, bird 1344, was the only one varying markedly with an increase of only 26.52 per cent.

A marked increase in hemoglobin concentration was also observed in members of the group. From a pre-injection level of 9.8 the mean hemoglobin concentration rose to a peak of 12.5 after four weeks of injections. It then decreased two-tenths of a gram during the last two weeks of the injection period and greatly accelerated its decrease after the injections had ceased. At the end of the 13-week experimental period it had fallen to 9.7, one-tenth of a gram lower than the pre-injection level. The increase in mean hemoglobin concentration for the group was 27.55 per cent while individual birds varied from 16.36 per cent to 36.26 per cent in their individual increases.

The curve for Group 8 was similar to that for Group 4, Fig. 8. This group, consisting of five normal male birds, 1356, 1355, 1352, 1353, and 1373, received injections of estrogen. The mean erythrocyte count for this group dropped steadily from a pre-

injection level of 3,200,000 to 2,620,000 at the end of the six week course of injections. Its post-injection rise was at almost the same rate as its preceding decrease, and at the end of the 13-week experimental period the mean erythrocyte count was 3,190,000, only 20,000 less than the pre-injection level. The decrease in the mean erythrocyte count for the group was 18.12 per cent and the individual decreases were all in the comparatively narrow range of 16.75 per cent to 20.56 per cent.

At the end of the third week of injections, the mean hemoglobin concentration was 10.9, only two-tenths of a gram lower than the pre-injection level. During the next three weeks, the decrease was more rapid and at the end of the six week injection period the mean concentration was down to 9.4. After the cessation of the injections it rose steadily to reach 11.9 at the end of the 13-week experimental period. As a group, these birds showed a decrease in mean hemoglobin concentration of 16.08 per cent, but individual decreases ranged from 16.36 per cent to 19.31 per cent. One bird, 1356, died during the eighth week of the investigation, apparently from a vitamin deficiency.

Group 9 consisted of three capons, 1280, 1289, and 1291, which received injections of female hormone. The mean erythrocyte count for this group dropped from a pre-injection level of 2,650,000 to 2,400,000 after three weeks of injections, but returned to 2,620,000 before the end of the six week injection period. Mean hemoglobin concentration varied between 10.4 and 10.0 during the investigation, Fig. 9.

Similar results were observed for Group 10, which included

three normal females, 1258, 1259, and 1260. These three birds received injections of estrogen. In this group the mean erythrocyte count dropped to 2,350,000 from a pre-injection level of 2,550,000 during the course of injections, but had returned to 2,560,000 at the end of the six week injection period. The mean hemoglobin concentration ranged between 10.1 and 9.8 for this group. It was considered that these changes were insignificant, Fig. 10.

All of the birds were weighed before the experiment started and again at its conclusion. The percentage of gain of each bird was recorded and included in this paper in Table 11. No evidence was obtained which indicated a correlation between the hormones each bird received and its rate of weight increase.

CONCLUSIONS

From the results obtained in the investigations involving Group 2 and Group 6, the conclusion that testosterone propionate caused an increase in the erythrocyte number and in the hemoglobin concentration was an inevitable derivative. Prior to the first injections these birds differed from the control groups only in lacking testes. All other environmental factors were identical. Yet in every case, the erythrocyte count and the hemoglobin concentration of the capons was definitely lower than in the male birds. Upon the administration of testosterone propionate to these capons their maleness was physiologically restored and their erythrocyte number and hemoglobin concentration rose to

at least approximate, and in many cases, to exceed that of the male birds. When the hormone was withheld, the erythrocyte number and the hemoglobin concentration of the capons returned to pre-injection levels in a few weeks. These changes occurred in every individual belonging to the groups concerned. To summarize, removal of the testes with the resulting decrease in testosterone level, resulted in decreased erythrocyte numbers and hemoglobin concentrations; replacement of the testosterone by injection resulted in the restoration of the erythrocyte numbers and the hemoglobin concentrations to the male level; and withdrawal of the replacement hormone resulted in a return of the erythrocyte count and the hemoglobin concentration to the capon level. Therefore, the testosterone was responsible for the observed increases in erythrocyte numbers and hemoglobin concentration.

Group 4 and Group 8 provided the data from which was derived the conclusion that estrogen caused a decrease in erythrocyte number and hemoglobin concentration of males. The birds in these groups were all normal males and lived under identical conditions with the control groups prior to the course of injections. During the period of injection they differed from the controls only in receiving estrogen. Their pre-injection erythrocyte and hemoglobin levels were very similar to the control birds, but after receiving the estrogen injections they decreased until they approached the capon level. After the injections ceased the erythrocyte numbers and hemoglobin concentrations returned in a few weeks to the pre-injection level. All members of these two groups responded in the same fashion. Since stopping the adminis-

tration of estrogen resulted in a return to the male level, it was concluded that the estrogen was responsible for the observed decrease.

The conclusion that estrogen repressed the erythropoietic action of testosterone propionate on capons was supported by the data secured from Group 3 and Group 7. These two groups, like Group 2 and Group 6, consisted of capons that received testosterone propionate injections. However, these groups differed from Group 2 and Group 6 in that they also received estrogen injections. While the erythrocyte number and hemoglobin concentration of these groups also increased, their mean increase was far less than that of Group 2 and Group 6. Since the only varying factor was the estrogen, it was concluded that it was responsible for the lesser increase. These same groups also supported the conclusion that the estrogen took effect more slowly than the testosterone propionate. When the curve of Group 2 was compared to that of Group 3 the two were found to be very similar for the first three weeks of the injection period. After that point, however, the curve of Group 2 continued to rise at about the same rate while the curve of Group 3 flattened suddenly. A comparison of the curves of Group 6 and Group 7 revealed the same situation. Thus, it was reasonable to hold the flattening to be due to the estrogen becoming effective at that point.

The next conclusion, that is that estrogen did not act upon the blood picture through a direct effect upon the blood elements or blood forming elements were obvious results of the data obtained from Group 9 and Group 10. Group 9 included three capons

and Group 10 three females and all six of these birds received injections of estrogen. Although the erythrocyte count and the hemoglobin concentration decreased slightly during the course of injections, they recovered their pre-injection level before the end of the injection period. Thus the decrease was only temporary and was so small as to be considered insignificant. It was assumed that this temporary depression was probably due to a general endocrine imbalance which was soon corrected by the bird. If the estrogen acted directly on the blood the erythrocyte count and the hemoglobin concentration would have reacted as they did in Group 4 and Group 8. Since no similar decreases were observed in these groups, the conclusion that this was not the method by which estrogen acted was a justifiable one.

An examination of all the data secured seemed to indicate that the estrogen exerted its influence on the blood picture through its repression of the anterior pituitary. The result of estrogen's effect on the anterior pituitary is two-fold. It reduces the amount of gonadotropic hormone produced, indirectly reducing testosterone secretion in a male, and it reduces the erythropoietic secretion of the pituitary. These phenomena occur only when an excess of sex hormone is present. In the case of Group 4 and Group 8, the estrogen reduced the androgen secretion and repressed the erythropoietic secretion, thus acting to reduce the erythrocyte count and the hemoglobin concentration in two ways. In Group 3 and Group 7, both made up of capons, the only action was the reduction of erythropoietic secretion. A comparison of the curves of Groups 3 and 7 with the curves of Groups 4

and 8 showed that the estrogen had a much greater effect on the blood picture of the males where it inhibited both erythropoietic and, indirectly, androgenic secretion than it did upon the blood picture of the capons. Therefore, this hypothesis could satisfactorily explain all cases observed.

DISCUSSION

Almost without exception, the results of endocrinological research in the past have agreed that testosterone causes an increase in erythrocyte number and hemoglobin concentration in mammals and birds. These results have been obtained with a variety of experimental methods as well as with many different experimental animals. Finkelstein et al. (1944) examined the regeneration of erythrocytes and hemoglobin after a hemorrhage-induced anemia in rats and reported that testosterone accelerated the erythrocyte regeneration in normal males and females, hypophysectomized males and females, and castrate males. About the regeneration of hemoglobin, however, their results were not consistent, since in many cases the testosterone injections caused no acceleration of the regeneration process. Examination of the blood picture of eunuchoid men by McCullagh and Jones (1942) revealed that during testosterone therapy both their erythrocyte count and their hemoglobin concentration increased; and that upon cessation of the therapy both returned to their pre-treatment level. Similar results were obtained with rats by Crafts (1946), Vollmer and Gordon (1941), Vollmer et al. (1942), and Steinglass

et al. (1941), with chickens by Taber et al. (1941, 1943), Juhn and Domm (1930), and Domm et al. (1943), and with golden hamsters by Stein and Jacobsen (1944). The results of the writer's investigation were in complete agreement with such conclusions.

The physiology of this phenomenon, however, was not definitely explained by anyone. Each worker advanced one or more possible explanations but none of them were able to secure experimental proof for their hypotheses. McCullagh and Jones (1942) suggested that the explanation might lie either in the effect of androgens on the metabolic rate or in the effect of androgens on the blood forming elements. They supported the latter view, although they advanced an equally strong case for each explanation. Supporting evidence for the direct action theory was found by Vollmer and Gordon (1941) and Vollmer et al. (1942) working with rats. From their examination of the red bone marrow they discovered that testosterone caused a hyperplasia of the erythroblastic elements. They also found, as did Finklestein et al. (1944), that testosterone tended to prevent the hypoplasia of erythroblastic tissue that ordinarily resulted from hypophysectomy. All of these workers suggested that the proliferation of the red marrow might be only a special manifestation of the general stimulation of somatic growth by testosterone as described by Goldzieher (1941) and Rubenstein and Solomon (1941).

Other workers have been inclined to think that the effect of testosterone on the blood picture is a result on an increased basal metabolic rate. Although Meyer and Danow (1942) reported that no change in the basal metabolic rate of rats was caused

either by castration or testosterone injections, most investigators have reported opposite results. A definite increase in basal metabolic rate, caused by testosterone, was reported by Jones et al. (1941), Kenyon et al. (1940), McCullagh and Rossmiller (1941), and Sandiford et al. (1941). On the basis of such reports as these, Crafts (1946) and Steinglass et al. (1941) concluded that testosterone raised the basal metabolic rate, thereby creating an increased oxygen demand; and that the response of the erythropoietic system to this demand resulted in the increased erythrocyte number and hemoglobin concentration so generally observed after testosterone administrations. This theory also covered the known proliferation of the erythroblastic elements, since it assumed that the proliferation was in response to the increased oxygen demand. A few workers, such as Adams and Shevket (1929) and Meyer et al. (1940) suggested that any influence upon the basal metabolic rate was exerted through the thyroid or pituitary, glands which have a known and direct effect upon basal metabolic rate. No data were secured from this investigation which permitted the addition of anything to the discussion concerning the method by which testosterone exerted its influence on the blood picture.

Past workers did not agree completely as to the effect of estrogen on the blood picture. Davis and Boynton (1941) reported that stilbestrol, a synthetic estrogenic compound, had a beneficial effect on the hematopoietic system of human beings of both sexes, but their results were not supported by any other workers. Only a very slight decrease in erythrocyte number and

hemoglobin concentration of male and female dogs after estrogen administration was reported by Tyslowitz and Hartman (1941) but the same workers later (Tyslowitz and Dingemanse, 1941) agreed with Castrodale et al. (1941) that estrogen caused such a severe anemia in dogs of both sexes that it proved fatal. Vollmer and Gordon (1941) and Crafts (1941) reported moderate, but definite, decreases in erythrocyte and hemoglobin levels caused by estrogen in dogs and monkeys of both sexes, as well as castrate animals; and Finklestein et al. (1944) found that estrogen inhibited the regeneration of erythrocytes in anemic rats, male, female, and castrate. A quantitative difference in the reaction of male, female, and castrate chickens to estrogen was reported by Taber et al. (1943) who found that the erythrocyte number of male birds dropped much farther than did the erythrocyte number of female birds or capons receiving the same amount of estrogen. Results of this investigation partly agreed with this conclusion, as estrogen had a greater effect on male birds (Groups 4 and 8) than it did on female birds (Group 10) or on capons (Groups 3, 7, and 9). A German worker, Feuchinger (1940), found that estrogen caused only a temporary decrease in erythrocyte number when given to female rats, rabbits, and human beings; and he further reported that a similar transitory anemia appeared during each period of estrus in rats. With this last result, the previously described experience with Group 10 was in complete agreement.

Conclusions drawn from this investigation were that estrogen depressed the erythrocyte number and the hemoglobin concentration in males and repressed the response to testosterone when both

hormones were given to capons. No such decrease was observed in the case of females and capons without androgen stimulation, but only a small and temporary decrease. Thus, the writer's conclusions agreed with some reached by past workers and disagreed sharply with others.

The probable method of action of estrogen on the blood picture, as suggested by past investigations, has been a very confused matter. Perhaps a majority of the workers assumed that estrogen acted directly on the blood picture. Crafts (1941), Tyslowitz and Hartman (1941), Castrodale et al. (1941), and Tabor et al. (1941) all advanced this assumption. Their conclusions were generally based upon two premises: first, that the estrogen caused a decrease in erythrocyte number of both sexes; and second, that a hypoplasia of the red bone marrow resulted from estrogen injections. One of these workers (Tabor et al. 1941) also thought that an antagonism to testosterone was in part responsible for the effect of estrogen on the blood picture. The fact that the decrease of erythrocyte number due to estrogen was so much greater in males than in females or capons was the basis for this theory. Still a third suggestion was that estrogen reduced the basal metabolic rate and thus the demand by the body for erythrocytes. Steinglass et al. (1941) supported this theory, although the effect of estrogen on the basal metabolic rate is itself a moot point. Sherwood and Bowers (1936) found that ovarian hormones definitely depressed the metabolic rate of dogs while Danforth et al. (1937) concluded that it had no effect on the metabolic rate of rats.

None of the above described theories satisfactorily explained the results obtained in this investigation, with the possible exception of the antagonism theory. Since there is no experimental evidence of a chemical conflict between estrogen and testosterone, the writer doubted that such an antagonism existed. From the data obtained in this investigation, it was concluded that the estrogen probably acted on the blood picture through its effect on the anterior pituitary. The fact that the pituitary was related to the blood picture was demonstrated by Crafts (1941, 1946), Meyer et al. (1940), and Vollmer et al. (1939). They all agreed that hypophysectomy caused a decreased erythrocyte number and a degeneration of the erythroblastic tissues. Hertz and Meyer (1937) and Reilly and Solomon (1940) demonstrated that the sex hormones depressed the activity of the anterior pituitary and Rubenstein and Kurland (1940), Rubenstein and Solomon (1941, 1941) and Eidelsberg and Ornstein (1940) proved that only an excess of sex hormone caused this depression. Reasoning from the data secured from this investigation, in the light of the above listed facts, it was concluded that a workable hypothesis could be constructed that would explain the action of estrogen on the blood picture in terms of its action on the pituitary.

In the case of Group 4 and Group 8 in this experiment, which included normal males receiving estrogen, the hormone, through its action on the pituitary, exerted a dual influence on the blood picture. By reducing the secretion of gonadotropins it indirectly reduced the production of androgen by the testes and so decreased the stimulation of the hematopoietic system. By reducing

the secretion of the erythropoietic fraction of the pituitary, it decreased the hematopoietic stimulation from that source. The lack of similar results in the female birds and in the capons of Group 9 indicated that the dosage used did not constitute an excess of hormone for those particular birds. In the case of the capons in Group 3 and Group 7, in which the androgen induced increase in erythrocyte count repressed by estrogen, the effect was entirely due to the reduction of the erythorpoietic secretion from the pituitary. Naturally, the effect was not as marked in those birds as it was in the normal males, since a single action was not as effective as a two-fold action. The decreases in erythrocyte number observed in female and castrate animals after estrogen administration by previously mentioned workers might very well have been due to the fact that the dosage they used constituted a very definite excess and exerted a strong repressive influence on the pituitary.

SUMMARY

From the procedure and results described in the preceding pages, the following conclusions were derived:

1. Testosterone propionate caused an increase in the erythrocyte number and the hemoglobin concentrations of caponized White Leghorn chickens.

2. Estrogen caused a decrease in the erythrocyte number and the hemoglobin concentration of male White Leghorn chickens.

3. In capons which received both hormones, estrogen repressed

the erythopoietic action of testosterone propionate on caponized White Leghorn chickens.

4. Estrogen took effect more slowly than testosterone propionate when both hormones were given to caponized White Leghorn chickens.

5. Estrogen caused significant changes in erythrocyte count and hemoglobin concentration only in males and in capons who also received testosterone propionate.

6. Estrogen does not act directly upon the blood elements or upon the blood forming elements of White Leghorn chickens.

7. Estrogen may act upon the erythrocyte number and upon the hemoglobin concentration through its effect on the anterior lobe of the pituitary gland of White Leghorn chickens.

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