TEXTURE OF TISSUE AS INFLUENCED BY SEX HORMONES

by

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INTRODUCTION AND REVIEW OF LITERATURE

There has been considerable research done in an attempt to determine the effect of sex hormones on the marketing value of animals. Such factors as "finish", amount of subcutaneous fat, and palatibility have been extensively considered, but only a minimum of research has been done concerning the actual changes within the tissue proper.

Mitchell et al. (1928) in making a comparison of heifer and steer meat found no constant and significant difference in connective tissue content. The age did not appear to have a great influence upon the connective tissue content of meat, nor a significant effect among the different muscles. These latter data were too inconsistent to make a general statement. This work, however, was concerned with relatively young animals.

Lorenz (1944) stated that the use of diethylstilbesterol resulted in an increase in fat and carcass quality of turkeys. Thayer et al. (1945) found, that in cocks over one year of age treated with diethylstilbesterol, there was an improvement in grade and quality of the carcass. Thayer and Davis (1948) fed fattening rations containing estrogens to turkey broilers. Improvement in fatness as measured by fat grade was apparent at the end of two weeks of estrogen feeding, and the difference in favor of the estrogen fed birds was significant by the end of the third week. Sturkie (1946) implanted pellets of diethylstilbesterol (20 to 25 mg) into the shanks or necks of old cocks

18 to 20 months of age. The treatments were effective in improving the grade of the carcass, but they did not cause a significant gain in weight nor improve the eating qualities of the meat appreciably. Old hens two and one-half years of age were hormonized with diethylstilbesterol for a 26 day period without effect.

Lorenz (1945) treated chickens with diethylstilbesterol. There was an increase in deposition of fat which gave the experimental birds a better "finish" than the controls. According to Lorenz the increased muscle fat heightens the flavor of the meat and infiltrates the connective tissue thus improving tenderness. This was indicated in 14 month old cocks. The controls had the usual darkened tough stringy meat, but the meat of the treated birds was lighter in color, though perhaps not equal to the quality of young cockeral flesh, nevertheless relatively tender and juicy.

Papanicolau and Falk (1938), in a survey of the various muscles of female guinea pigs treated with gonadotropic hormone (follutin), found there was considerable hypertrophy. Comparative measurements of individual muscles and total amount of muscular tissue indicated that the effect is on the muscular system in general. The temporal muscles of the adult guinea pigs castrated before sexual maturity, remain flat and small in the adult females. The muscles of such castrated males did not respond to treatment with gonadotropic hormone. This treatment was likewise ineffective in spayed females. Papanicolau found

that androgenic hormone (testerone propionate) has a stimulating effect upon the muscles, producing enlargement after prolonged administration.

Wainman and Shipinovnoff (1941) found that castration of rats resulted in a marked decrease in the size of the striated perineal muscles (bulbo cavernosus, ischio cavernosus, and levator ani). The decrease in the muscle fiber width was the most marked change. Testosterone propionate prevented the effects of castration on these muscles. In normal animals, the administration of testosterone propionate caused an increase in bulk in these muscles. The perineal musculature was more responsive to castration and treatment with testosterone propionate than other striated muscles. The treatment of immature spayed females with testosterone propionate produced a great size increase in their perineal musculature.

Herrick (1945) found the average breaking strength of the gastrocnemius muscle of White Leghorn pullets was increased approximately 41 percent by testosterone propionate administered intramuscularly, while the strength of the gastrocnemius muscle of White Leghorn capons was increased approximately 23 percent. He found that male sex hormone doubled the tensile strength of the skin of both female and caponized fowls. Chemical analysis showed that there was also an increase in collagen nitrogen of the same general order as that of the increase in tensile strength. Histological differences were observed in skin from fowls treated with testosterone propionate and untreated fowls. Skin from the

treated birds was dense with collagenous fibers formed extensively. Skin from untreated birds was much less dense with collagenous fibers interspersed with many undifferentiated cells. It was concluded that the greater tensile strength of skin in young treated birds is due largely to the greater differentiation of the skin cells in the formation of collagenous fibers. The more abundant and more completely differentiated fibers give greater strength.

It is evident from the forgoing discussion that treatment with testosterone propionate results in an increase in collagenous connective tissue and subsequently in an increase in the toughness of the tissue. Herrick found this to be true of both skin and muscle tissues as shown by the increase in collagen nitrogen. However, the effect of discontinuation of testosterone propionate, after the tissues have acquired this toughened condition is not known. It has been postulated that the discontinuation of male sex hormone will result in muscle and skin tissues of birds losing their acquired toughness and that these tissues will return to their previous tender condition. It is the purpose of this study to investigate the validity of this postulate.

MATERIALS AND METHODS

Two groups of White Rock cocks were used. Group I consisted of six cocks approximately two years of age. Three birds were retained as controls and three birds were used as experimental

animals. One cock was caponized and the other birds had three pellets (15 mg per pellet) of diethylstilbesterol implanted subcutaneously in the neck. Twenty-eight days later one of the experimental animals was given an additional two pellets. All of the experimental animals acquired the usual capon characteristics.

Forty-five days after the initial implantation with diethylstilbesterol all six of the birds were killed. The gastrocnemius muscle and the skin around the leg were tested for tensile strength. Samples of the pectoralis major muscle, gastrocnemius muscle, and skin were taken. These samples were frozen and stored in a deep freeze unit until a later date when they were analyzed for collagen nitrogen.

The gastrocnemius muscle was prepared for testing by removing the feathers and disarticulating the leg at the upper end of the femur. The skin was then inverted back from the proximal portion of the femur to the heel. The gastrocnemius muscle was then separated out leaving it attached at its origin and insertion. The tibia was severed between the intact ends of the gastrocnemius muscle. The muscle, with original attachments intact, was exposed to a breaking pull by attaching the femur solidly at one end and the foot to a cable, which led over a pully to a weight container at the other end. Sand was poured into the weight container until the muscle broke (Plate I).

After the tensile strength of the gastrocnemius muscle had been tested the cylinder of skin around the leg was tested. The

skin was left attached to the leg at the distal end and the other end was held in a clamp. Thus the skin around the leg was subjected to a breaking strength in the same manner as the gastrocnemius muscle (Plate II).

Methods which have been used for determining the tenderness of meat other than tensile strength are: (1) rating by palatability committee, (2) histological examination, (3) measuring the shearing and penetration force, and (4) chemical determination of collagen content. Because of the apparent relationship which has been found to exist between the amount of collagen and the degree of tenderness it was desirable to use this method in testing the tissues for toughness.

The methods for the determination of collagen are based on the fact that collagen is hydrolysed to gelatin upon sustained heating at high temperatures. Muscles are essentially fibers that are held together by connective tissue (elastin and collagen). The percentage of elastin in muscles is, in most cases, only an insignificant portion of the total connective tissue. Thus collagen comprises the bulk of the connective tissue. Elastin and collagen may be separated from each other because of their individual properties.

Hall et al. (1944) made preliminary studies on methods for the separation of collagen. The centrifuge method as developed by Meisner (1947) was used in this study for the separation of collagen. More specifically the actual procedure was taken from a publication by Hartly and Hall (1949).

EXPLANATION OF PLATE I

Apparatus for testing the tensile strength of the gastrocnemius muscle.

PLATE I

Fig. 1

EXPLANATION OF PLATE II

Apparatus for testing the tensile strength of the skin around the leg.

PLATE II

Fig. 2

The procedure is essentially as follows. The sample was prepared by removing the external fat and visible cartilage. The tissue was put through a hand grinder, first with a coarse blade and then with a fine blade. Five gram portions of the ground sample were weighed into small jars. Forty ml of ice water was added along with enough 0.1 normal sulfuric acid to bring the suspension to the desired isoelectric point (PH 5.1 for muscle and PH 5.2 for skin). The sample was then homogenized for five minutes in a Waring Blender.

The water soluble proteins were extracted first. To do this the homogenate was rinsed into a 250 ml beaker and allowed to stand for 10 minutes. The liquid was decanted into 50 ml centrifuge tubes and centrifuged at 3000 rpm. for five minutes. The supernatant was discarded. The remaining suspension was rinsed into the tubes and centrifuged. The residue was mixed with approximately 25 ml of water (45 to 50 degrees C.) and centrifuged. The residue was washed in this manner five times, all of the supernatants being discarded.

Following the removal of the water soluble proteins the collagen was hydrolysed to gelatin. This was accomplished by washing the residue into 125 ml Erlenmeyer flasks and dispersing well making the total volume approximately 80 ml. The flasks were stoppered with a cotton plug and autoclaved at 15 pounds pressure for two hours.

Since gelatin is readily soluble in hot water it is easily separated from other proteins such as elastin and keratin which

are not soluble in hot water. The liquid was decanted into centrifuge tubes and centrifuged three minutes at 3000 rpm. The residue was mixed with approximately 25 ml of boiling water and placed in a hot water bath for two minutes, being stirred continuously. The residue was centrifuged and washed in this manner five times. All of the supernatants were combined in a Kjeldahl flask with 25 ml of concentrated sulfuric acid. The supernatants were analysed for collagen nitrogen by the Kjeldahl method.

Total nitrogen was determined on approximately two grams of original material. This material was digested with 37.5 ml of concentrated sulfuric acid and analysed for total nitrogen by the Kjeldahl method. The collagen nitrogen was expressed as a percentage of the total nitrogen.

Group II consisted of seven capons approximately five months of age. Six of the capons were injected intramuscularly with five mg of testosterone propionate in oil twice weekly (10 mg per week) for a period of six weeks. One capon was not injected with male sex hormone. At the end of the six week injection period, the capon and one treated bird, were killed and tested in the same manner for tensile strength and for collagen nitrogen as the birds in group I. Two weeks later another treated bird was killed, four weeks after the last injection of male sex hormone two more birds were killed and at six weeks the last two treated birds were killed and tested. This experiment was conducted to determine the time after the source of male sex hormone

was removed or suppressed that changes in the tissues occur.

RESULTS AND CONCLUSIONS

Tables 1 and 2 summarize the data obtained from group I. Because of the small sample size and the variation in treatment of the experimental animals no attempt was made to treat these data statistically. However, by making the assumption that all of the experimental animals were treated essentially the same it can be readily seen that the arithmetic means of the experimental animals are significantly lower than the arithmetic means of the control animals.

The average tensile strength of the skin around the leg of the controls (normal cocks) was 151 pounds as compared to an average of 93 pounds in the experimental (caponized or treated with estrogen) group. This was an average reduction in tensile strength of 31.8 percent. The average tensile strength of the gastrocnemius muscle in the control group was 115 pounds as compared to 87 pounds in the experimental animals. This was an average reduction of 24.4 percent.

Collagen nitrogen was expressed as a percent of the total nitrogen. The average percentage figure for collagen nitrogen in the skin of the control group was 63.8 percent as compared with 49.2 percent in the experimental group. This was an average reduction in collagen nitrogen of 22.9 percent (63.8 percent minus 49.3 percent / 63.8 percent). The average percentage

figure for collagen nitrogen in the gastrocnemius muscle of the controls was 24.0 percent as compared to 18.1 percent in the experimental group. This was an average reduction of 24.2 percent. The average percentage figure for collagen nitrogen in the pectoralis major muscle in the controls was 8.8 percent as compared with 6.53 percent in the experimental group. This was an average reduction of 25.8 percent.

The average reduction in collagen nitrogen of the skin (22.9 percent), gastrocnemius muscle (25.4 percent), and pectoralis major muscle (24.2 percent) are all nearly the same. There is a correlation between the reduction in tensile strength of the gastrocnemius (24.4 percent) and the reduction in the collagen nitrogen of the gastrocnemius muscle (25.4 percent). The correlation between the reduction in the tensile strength of skin (31.8 percent) and the reduction in the collagen nitrogen in the skin (22.9 percent) was not as close as that for the gastrocnemius muscle, however the same general trend is true for both.

The reduction in tensile strength and collagen nitrogen are both correlated with the amount of diethylstilbesterol implanted. (Table 1). In general the tensile strength and the collagen nitrogen were greater for fowl 271, treated with three pellets of diethylstilbesterol, than for fowl 260, treated with five pellets of diethylstilbesterol. Fowl 260 had reached a degree of tenderness at the end of 45 days which was comparable to the tenderness of the capon. In all of the experimental animals, regardless of the treatment, the tensile strengths and collagen

Table 1. Summary of data for experimental animals in group I.

Treatment of animal	Tensile strength expressed in pounds		Chemical analysis, collagen nitrogen expressed as percent of total nitrogen		
	Skin	Gastrocnemius muscle	Skin	Gastrocnemius muscle	Pectoralis major muscle
5 pellets diethylstilbesterol	86	81	49.7	13.5	7.9
3 pellets diethylstilbesterol	130	100	58.1	20.4	6.0
Capon	62	81	39.7	20.5	5.8
Arithmetic mean	93	87	49.2	18.1	6.53

Table 2. Summary of data from control animals in group I.

Fowl no.	Tensile strength expressed in pounds		Chemical analysis, collagen nitrogen expressed as a percent of total nitrogen		
	Skin	Gastrocnemius muscle	Skin	Gastrocnemius muscle	Pectoralis major muscle
5	172	117	60.6	25.1	9.1
249	144	126	63.6	22.4	10.6
273	137	103	67.1	19.9	6.8
Arithmetic mean	151	115	63.8	24.0	8.8

Table 3. Summary of data for group II. Five month old cocks were injected with 10 mg testosterone propionate weekly for a period of six weeks. Injections were then stopped and animals were killed and tested at two week intervals.

Time after injections were stopped : animals were killed	Tensile strength expressed in pounds		Chemical analysis, collagen nitrogen expressed as a percent of total nitrogen		
	Gastrocnemius muscle	Skin	Skin	Gastrocnemius muscle	Pectoralis muscle
0 weeks	54	60	73.96	17.13	8.76
Two weeks	54	60	73.16	19.83	6.44
Four weeks	53	55	70.74	17.58	5.78
Six weeks	46	44	65.13	17.31	6.30
Capon	48	43	53.88	16.65	5.21

nitrogen were lower than the average tensile strengths and collagen nitrogen of the control group.

It was concluded that there is on the average a significant reduction in toughness of the tissues in the group of experimental animals tested.

The data for group II are summarized in Table 3. Each verticle column of this table was graphed (Plates III, IV, and V). The time after injections with male sex hormone were stopped was graphed against the percentage decrease in collagen nitrogen and tensile strength. The graphs all show that there was no appreciable decrease in tensile strength or collagen nitrogen for at least two weeks after injections with testosterone propionate were stopped. The drop in tensile strength and collagen nitrogen was quite rapid from two weeks to six weeks after injections were stopped. In all cases at the end of the six week period the reduction in both tensile strength and collagen nitrogen was approaching that for the capon. It appears that a little more than six weeks is required for the complete reduction to the capon condition.

SUMMARY

There was on the average a significant reduction in toughness of the tissues in cockerels that had been caponized or had been injected with female sex hormone.

There was a definite correlation between the reduction in

tensile strength and collagen nitrogen.

After capon tissues were toughened by injections of male sex hormone, there was no reduction in toughness during the first two weeks after injections with testosterone propionate were stopped. The reduction in toughness was quite rapid from two to six weeks after injections were stopped and in all instances the tenderness was approaching that of the capon at the end of the six week period. However, it appears that a little more than six weeks are required for them to return to their previous tender condition.

EXPLANATION OF PLATE III

Fig. 3. Graph showing the drop in the tensile strength of the gastrocnemius muscle in the experimental animals in group II.

Fig. 4. Graph showing the drop in collagen nitrogen in the gastrocnemius muscle in the experimental animals in group II.

PLATE III

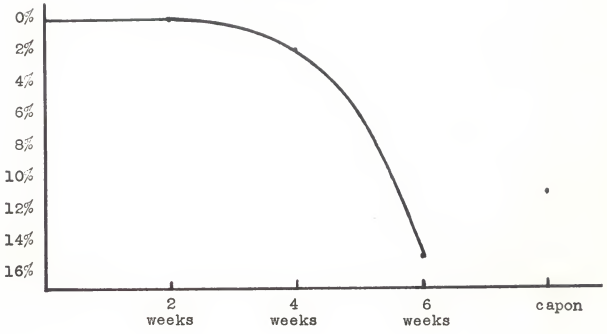


Fig. 3

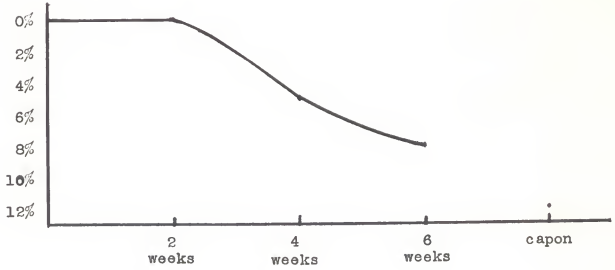


Fig. 4

EXPLANATION OF PLATE IV

Fig. 5. Graph showing the drop in tensile strength of the skin around the leg in the experimental animals in group II.

Fig. 6. Graph showing the drop in collagen nitrogen in the skin of the experimental animals in group II.

PLATE IV

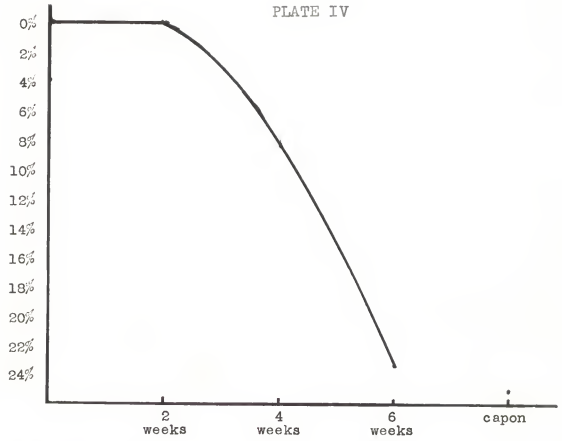


Fig. 5

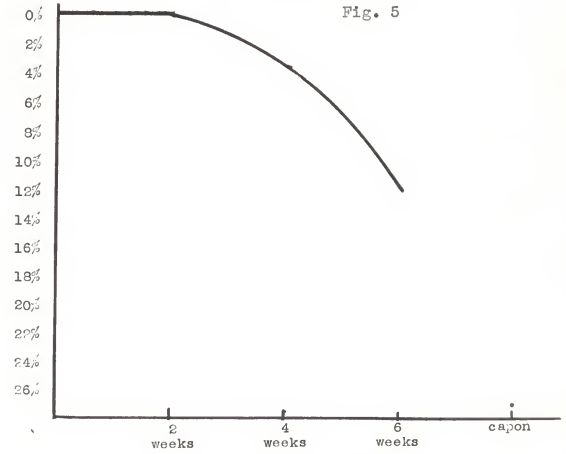
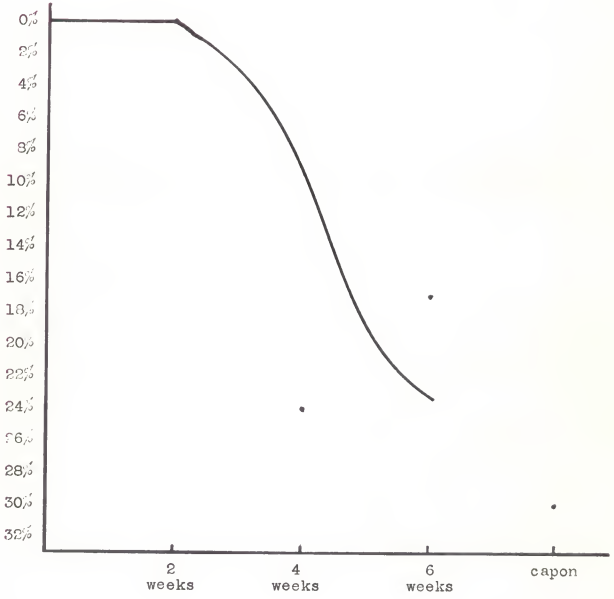


Fig. 6

EXPLANATION OF PLATE V

Fig. 7. Graph showing the drop in the collagen nitrogen in the pectoralis major muscle in the experimental animals in group II.

PLATE V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2 weeks
4 weeks
6 weeks
capon

Fig. 7

ACKNOWLEDGMENT

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