REGULATION OF FUNCTION AND ACTOMYOSIN CONTENT OF CARDIAC MUSCLE BY ESTROGEN

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REGULATION OF FUNCTION AND ACTOMYOSIN CONTENT OF CARDIAC MUSCLE BY ESTROGEN

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ABSTRACT

Little information is available on the influence of estrogen on heart muscle. This situation exists in spite of the known difference in sex incidence of at least some types of heart disease, the use of gonadal steroids in treatment of heart disease, and the recognized greater longevity of females. This, along with the need for knowledge concerning the mechanism of action of such steroids on the cellular level, stimulated this study.

The effects of estrogen on properties of the myocardium were noted in albino rats treated as follows: ovariectomized; sham operated females; ovariectomized given 0.1 gamma alpha estradiol daily; orchiectomized; sham operated males; and orchiectomized given 1.0 gamma alpha estradiol daily. On the 30th day of treatment the animals were weighed and killed. Heart, adrenal, uterus or seminal vesicles were weighed and dried. Refractory periods, excitability to square wave stimuli, and active and passive tension curves were determined in vitro on left ventricular columnae carneae.

No significant differences existed in heart weight, heart-body weight ratios or water content of the myocardium. Expected changes existed in organ-body weight ratios for adrenal, uterus, and seminal vesicles. No differences in excitability or refractory periods were noted. Mean-developed tension at a given length was significantly

greater in intact females than in castrate females, while developed tension in estradiol-treated castrates approached normal values. In the male groups no significant difference in developed tension was found. Mean-developed tensions of intact females and males were not dissimilar. No differences in passive tension curves existed in any groups.

In an attempt to investigate the mechanisms involved in the reduced contractility of isolated surviving left ventricular columnae carneae of ovariectomized rats, further studies were completed. Glycerol-extracted columnae were prepared, and ventricular actomyosin was quantitatively and qualitatively studied. These studies were completed after 30 days of treatment in sham operated females, ovariectomized females, and the following ovariectomized alpha estradiol-treated groups: 0.1 gamma, 1.0 gamma, and 10 gamma. A 100 gamma alpha estradiol group of sham operated animals was also studied.

Maximal A.T.P. induced tension was significantly reduced in glycerol-extracted fibers of hearts of the untreated ovariectomized group. Tension developed by glycerol-extracted fibers of estrogen-treated groups was significantly greater than the untreated ovariectomized group. Nitrogen content of the glycerol-extracted columnae carneae was not altered in any of the groups.

Actomyosin was quantitatively determined by change in viscosity in response to A.T.P. and by micro-Kjeldahl technique. Actomyosin concentration was significantly reduced in the ventricle of the ovariectomized groups as compared to ventricles of the untreated intact group. Concentration of actomyosin was significantly increased in ovariectomized groups by treatment with estrogen. No qualitative change in contractile protein was found.

It can be concluded that alterations in developed tension in isolated surviving columnae carneae and glycerinated columnae carneae are related directly to quantitative changes in actomyosin concentration. These results indicate a dependence of the cardiac contractile system on estrogen similar to that of the uterus. Further, it suggests a general effect of this hormone on contractile protein synthesis in all muscular tissue. The importance of the gonadal steroids and the gonadal-pituitary axis on muscle development and maintenance should be investigated further.

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INTRODUCTION

This work is concerned with the physiologic effects of estrogen on rat myocardium. In Selye's Textbook of Endocrinology published in 1947 he states, "heart action is not significantly influenced by ovarian hormones"; however, no references are given to support this statement. In a literary survey, only one reference was found in which sex steroids were administered to animals for a period of time and the functions of the heart studied. A number of papers may be found in which these steroids have been added to cardiac muscle baths and effects on tension studied. One certainly cannot relate such results to in vivo functions for a number of reasons, outstanding among which is the fact that the heart is not exposed to such steroids in a one-shot manner, but rather is continuously exposed to varying concentrations of hormones.

Biologists know well that the entire female reproductive tract is significantly influenced by estrogen, as well as certain general characteristics including body shape, character of the voice, and pubic hair distribution. Knowledge concerning the less obvious actions of estrogen is lacking. A need certainly exists for eliciting more information on the mechanism of action of these steroids at the cellular level. A second and probably more urgent reason for knowing the various effects of estrogen, particularly on the cardiovascular system, is the sex

difference in mortality from cardiovascular disease. Heart-disease statistics compiled by the Metropolitan Life Insurance Company for the years 1951-1953 are impressive. In the age range from 40-74 years, heart disease kills twice as many men as women in the United States: the incidence is 872 per 100,000 men versus 437 women. In all countries heart disease kills more men than women at all ages. Regardless of diet and standard of living, the ratio is never reversed. A third reason is the widespread use of estrogen and its derivatives in clinical medicine. This use includes treatment of adolescent acne, inhibition of metastasis of breast and prostatic cander, relief of pruritus associated with advanced liver disease, and attempts to decrease the incidence of myocardial infarction. If estrogen is effective in these listed clinical conditions, it must indeed have diversified and subtle effects.

Review of the literature will include the relation of estrogen to muscle function, including skeletal, smooth and cardiac, and to protein anabolism and specific enzyme systems. This writer found that a review of these areas was necessary for understanding the possible effects of estrogen on cardiac muscle. Such a knowledge gives one indications of possible mechanisms of estrogen action. If one compares the literature of skeletal muscle to that of cardiac muscle, he will immediately be aware of the

lack of knowledge available on steroid action in cardiac muscle. A second reason of importance to have knowledge of estrogen's action on skeletal and smooth muscle is the possibility of arriving at a unified concept of steroid action on all muscle at a cellular level. No attempt will be made to include a review of the voluminous literature on the well known reproductive action of estrogen and its relation to the pituitary-ovarian axis.

LITERATURE REVIEW

GONADAL STEROID EFFECTS ON THE MYOCARDIUM

The literature concerning gonadal hormone influences on the myocardium may be divided into observations dealing with effects on energy-generating systems and observations dealing with effects on functional activity and contractile protein systems.

In the first group, one may include effects on glycogen, phosphocreatine (P.C.) and adenosinediphosphate (A.D.P.) content of the myocardium.

Schumann (1939) determined the glycogen content of the rat myocardium. He found a mean value of 478 mg.% in intact male rats. Determinations completed 21 days and 54 days following castration gave values of 402 and 318 mg.% respectively. The mean concentration 54 days following castration was significantly reduced as compared to the intact animal. Glycogen content of 484 mg.%, 215 days following castration, was not unlike the average for intact animals. He suggested that castration resulted in a defect in energy metabolism of the male heart related to the absence of testosterone. This function is then taken over by some other anabolic principle. No comment was made on the specific nature of this physiologic adjustment.

Schumann (1940) published investigations on effects of castration on A.D.P. and P.C. concentration. Fifty-four days after castration, P.C. had decreased to 63% of the normal value, while glycogen decreased to 66% of control values. Adenosinediphosphate showed a significant decrease from 19.6 $\pm$ 0.5 mg.%. Administration of 5 mg. of testosterone daily from the 54th to the 60th day resulted in a return of P.C. and A.D.P. to normal values. Glycogen showed a more striking response in that it increased to 777 mg.% as compared to 478 mg.% for intact animals.

Schumann (1942) found similar effects in hearts of ovariectomized and estrogen-treated female rats.

It has therefore been shown that castration of either females or males alters the energy metabolism of the myocardium and, further, that the changes induced are corrected by the specific hormone in question. It is of interest that in adrenalectomized rats no changes are observed in A.D.P. or P.C. concentration in the myocardium (Schumann, 1942) and that administration of testosterone does not increase these substances above normal (Schumann, 1942). Myocardial glycogen content is markedly reduced in adrenalectomized male rats, and testosterone is effective in returning this to normal intact values (Schumann, 1940). Further, testosterone administered to intact male rats results in an increase

in myocardial glycogen content to 819 mg.% as compared to control values of 478 mg.%. It appears that carbohydrate metabolism in the myocardium is markedly influenced by the presence of testosterone, even in the absence of castration.

In summary, testosterone and estrogen have a role in maintaining glycogen and high-energy phosphate metabolites of the myocardium. Since these phosphate compounds, along with glycogen, provide energy for muscle contraction, one would expect alterations in functional capacity in gonadectomized rat hearts.

In the second area of investigation, namely functional activity and protein systems, one finds the observations of Blasius, Kafer, and Seitz (1956) on the effect of testosterone on contractile proteins of the heart. They isolated proteins of the myocardium and fractionated them by micro-electrophoresis. Three large fractions were found: myoalbumin; a myosin fraction including alpha, beta, and gamma groups; and a third fraction including myogen, myoglobin, and water soluble protein elements. No quantitative or qualitative difference was observed between hearts of intact males, intact females, or castrate male rabbits. In hearts of castrate male rabbits, treatment with 1 mg., 25 mg., and 50 mg. of testosterone propionate for 90 days following castration resulted in an increase in the myosin fraction. This

increase was proportional to the quantity of testosterone given, and the increase was most marked in the alpha myosin curve.

Blasius et al. (1957) reported on the efficacy of various synthetic steroids, including testosterone-phenylpropionate, a combination of different testosterone esters with prolonged depot action (propionate, phenyl-propionate, isocapronate, caprinate); nor-androstenolon-phenylpropionate; and one synthetic steroid, methyl-estrenolone, that combines the protein anabolic effect with a so-called "gestative-luteinizing" effect. As in their original experiment these androgenic steroids resulted in an increase in the myosin fraction of heart from castrate rabbits; however, the response of alpha, beta, and gamma components of myosin varied. Methyl-estrenolone resulted in a marked response in all three components of the myosin fraction. They concluded that this protein anabolic effect is not necessarily limited to androgenic steroids, but may also be mediated by a steroid with estrogenic activity.

This discussion has demonstrated that estrogen and testosterone have a role in maintaining the energetics of the myocardium and further, that testosterone and possibly estrogen mediate an increase in myosin content of the myocardium. This demonstrates that both the energy supply mechanism and the contractile protein system may be altered by the gonadal steroids. One would

thus expect altered myocardial activity when these steroids are absent or administered.

One paper was found in which steroids were administered for a period of time and then myocardial function was studied. Korenchevsky, Hall, Burbank and Cohen (1941) completed a long-term experiment noting gonadal steroid relation to capacity of the myocardium for work. They investigated the action of androsterone and testosterone propionate on hearts from castrate male rats. These steroids were administered for periods up to two months following castration. Work measurements were made by attaching 10 and 15 gram weights to a lever attached to the heart. All contractions were recorded on a kymograph. Amplitudes of contraction were measured and their number counted before, during, and after suspension of the weights. They found on calculation of work performed that hearts of uninjected castrates were significantly weaker than either those of the uninjected normal controls or hearts of castrated rats treated with androsterone or testosterone. These men made no conclusion as to the significance of their work. Increased work capacity of treated castrate males could be explained by known increases that occur in energy supply (Schumann, 1939, 1940) and in the contractile proteins (Blasius et al. 1956) on testosterone administration. However, the decreased capacity of castrate animals cannot be related to

a decrease in "myosin" content (Blasius et al. 1956), but rather only to a defect in the energy-supplying system. No results were reported for female rats, and no further work by these authors could be found.

A difference in response of male and female hearts to stress of treadmill exercise has been reported. Van Liere and Northup (1957) noted a significantly greater increase in heart-body weight ratio in exercised albino female rats than in males. No significant difference between the ratios of male and female albino control hearts was observed. They concluded that females show a more efficient response to severe exercise, suggesting that female sex hormone might be responsible for the observed difference. No such difference was observed in similar experiments on hooded rats. It should be observed that the albino females lost weight on exercise while hooded females did not.

In addition to changes in mechanical activity, electrical activity has been reported to be influenced by altered gonadal function. Electrocardiogram (E.C.G.) changes have been reported in response to deficiencies of estrogen. Ovarian insufficiency, as observed in young women with underdevelopment of the ovaries and in women at the menopause has been reported to be associated with typical changes in the E.C.G. (Scherf and Boyd, 1940). These changes include a depression of the S-T segment in

all leads, being maximal in lead 2. In addition, the T wave is frequently reduced. These changes are reversed by treatment with synthetic or natural estrogens.

One area of functional activity not yet mentioned is the *in vitro* cardiac muscle studies in which steroids were added to the perfusing fluid of a bath chamber. This work, in the opinion of the author, has limited application to the understanding of the *in vivo* role of gonadal hormones on the myocardium. This opinion is based on: (1) the difficulties in solubilizing the involved steroid, (2) the exposure of muscle for relatively short periods of time to fixed quantities of one hormone, (3) the question of state of circulating hormones; that is, the importance of protein binding of *in vivo* circulating estrogen for its activity (Szego, 1953).

In vitro cardiac muscle studies include the use of testosterone, progesterone, and their metabolites, as well as estrogen. Gowdey, Loynes and Ward (1950) investigated effects of a large number of steroids on isolated frog and rabbit heart. Estrone, estradiol, and testosterone were found to augment contractility but showed little effect upon heart rate. Progesterone, however, caused a definite reduction in cardiac contractility. Rubin (1950) made similar observations on the action of progesterone on isolated papillary muscle of the cat. Nahum, Geller, Levine, and Sikand (1951) studied effects of progesterone

and pregnolene on isolated cat papillary muscle and hearts of intact dogs. Pregnolene was found to decrease contractility and lowered the threshold of isolated papillary muscle. In intact dogs the threshold was found to be unchanged with 100 mg.% of pregnolene. Progesterone was found, as reported before, to decrease contractility of isolated preparations, and to increase the threshold in intact animals. It is of interest that neither of these steroids produced an effect until after 15 minutes of infusion.

Szent-Györgyi (1953) observed that progesterone was one of the steroids that abolished the treppe response in frog ventricles. The ventricle regained its former strength more rapidly. Utilizing his concept of "favorable state", he assumes this to mean that progesterone regulated the change in K^+ from one contraction to the next, such that the outflow of K^+ was kept in balance with its inward flux, within the muscle fiber.

In reviewing the literature on estrogenic effects on the myocardium, past work has been limited to effects on glycogen and other energy-supplying substances. No study on tension production in ovariectomized or ovariectomized, treated females was found.

ESTROGEN EFFECTS ON SMOOTH MUSCLE

In considering muscle that is obviously regulated by endocrine factors, one thinks first of uterus.

G. W. Corner (1923) was one of the early workers who demonstrated that uterine activity was mediated by hormones of the ovary. Reynolds (1931) noted in rabbits that estrogen affects motility of the uterus only *in vivo* and only after a definite latent period. He observed, following ovariectomy and before regressive changes in histology occurred, that motility was markedly decreased. Frank *et al.* (1925) observed that with administration of estrogen to ovariectomized rats, the excised uterus, which is ordinarily inactive as a result of castration, underwent rhythmic contractions.

It was only a question of time before the techniques of Szent-Györgyi (1949) were utilized to determine the presence of actomyosin in uterus, and further to attempt to find the mechanism by which estrogen affects motility of uterus.

Csapo (1948), a student in the laboratory of Szent-Györgyi, demonstrated the presence of actomyosin in the uterus. With a viscosimetric technique for determination of actomyosin, he demonstrated that uterine muscle has a lower actomyosin concentration than skeletal muscle.

Csapo (1950 a) employed actomyosin threads prepared from uterine muscle and observed a difference in their contraction as compared to skeletal muscle extracted actomyosin threads. Threads extracted from uterus were characterized by a longer "latency period" and slower and

smaller contractions, similar in behavior to the differences in contraction observed for the whole muscle.

Csapo (1948) determined uterine actomyosin in advancing pregnancy and found that actomyosin increased as pregnancy advanced. He then attempted to find the effect of ovariectomy on actomyosin concentrations (Csapo, 1950b). Using virgin female rabbits, he found that ovariectomy produced a decrease in uterine weight. On determining uterine actomyosin concentration, he observed, two months following ovariectomy, an 80% decrease from control levels. Adenosine triphosphate activity was 15 times less than that for tissues of estrous rabbits. Administration of estrogen caused a marked increase in actomyosin formation within twelve hours. Actomyosin continued to increase for the duration of estrogen treatment. Adenosine triphosphate activity increased 10 times within 12 hours and estrus values were obtained within 4 days of estrogen treatment. This work was substantiated by Blasius and Schuck (1955) and Cretius (1957).

Blasius and Schuck (1955) investigated the uterine muscle protein electrophoretically, employing uteri of immature rabbits before and after estrogen administration. They demonstrated four components in uterine muscle proteins: myogen, B myosin, actomyosin, and myoalbumin. The only pattern change observed after estrogen treatment was an increase in the actomyosin component. This work has demonstrated that the concentration of uterine

actomyosin, the contractile protein of the myometrium, is under control of ovarian estrogenic hormone. Actomyosin concentration changes during physiological conditions such as pregnancy and the menopause, which involve alterations in estrogen output of the ovaries. It would therefore appear that the quantity of actomyosin present at any time is consistent with the work required of the uterus in a given physiological condition.

Cretius (1957) determined the contractile proteins separately in the corpus and in the cervix of human uteri. These uteri included non-pregnant, pregnant, and puerperal uteri. His method of actomyosin determination was that of Szent-Györgyi. Pregnant uteri contained more contractile protein per weight than non-pregnant ones, and these contained more during sexual maturity than during the menopause. The cervix was found to contain less contractile protein than the fundus. Cretius postulated that decrease of contractile proteins in the fundus causes, clinically, a primary inertia. Finally Cretius demonstrated that administrations of estrogen produced an increase in contractile proteins in the uterine musculature of climacteric women.

Csapo and Corner (1953) demonstrated that the concentration of contractile substance would determine the maximal force which the muscle is able to develop. They measured maximal isometric tension developed by uterine strips. These were removed from virgin mature, female

white rabbits under controlled hormonal conditions in which the concentration of uterine actomyosin had previously been determined, i.e., in natural estrus, after ovariectomy, and after ovariectomy followed by estrogen treatment. Average maximal tension of uterine strips from estrous rabbits was 9.6 gm. per $mm.^2$ cross-sectional area. The muscle contained 7.6 mg. actomyosin per gram of tissue. Values for uterine muscle three weeks after bilateral ovariectomy decreased to an average of 2.3 gm. of tension per $mm.^2$ and 1.7 mg. of actomyosin per gram of tissue. Comparing estrus and the castrate state as extremes, the ratio of tension in the two contrasting states is $9.6/2.3 \pm 4.2$, and that of actomyosin $7.6/1.7 \pm 4.5$. The figures are therefore in agreement. On administering estrogen to ovariectomized animals, maximal tension returned to the estrus value of 9.55 gm., with a proportionate increase in actomyosin.

Administration of progesterone to castrate animals (Csapo and Corner, 1953) produced no significant changes in tension developed by their uteri. When estrogen and progesterone were combined, maximal tension of the uterus was not significantly different from that occurring with estrogen alone. It would appear that progesterone does not significantly alter actomyosin content of uterus.

Without question, it has been demonstrated that the contractile system of the uterus is subject to the

influence of estrogen. With increasing estrogen levels, actomyosin increases and the magnitude of developed tension increases.

Of particular interest is progesterone's role in membrane excitability. Csapo et al. (1953) observed that progesterone had no function in maintaining or increasing the actomyosin-contractile system or the high-energy phosphate supply. Rather, progesterone's role in motility is related to holding in abeyance a chemical structure that is capable of maximal contractile capacity. He found when the uterus is dominated by progesterone that normal electrical or pharmacological stimuli are relatively ineffective. However, if estrogen alone is present the uterus is readily and effectively stimulated electrically. It would appear that progesterone acts only to block conduction within the muscle and prevent the typical all-or-none response. This work is the foundation for Csapo's theory (1956) of control of onset of labor; that is, the prime cause of labor is withdrawal of progesterone.

Effects on Other Smooth Muscle.

A question of interest to this writer is whether the uterine myometrium is a unique form of smooth muscle or whether it is a question of sensitivity of anabolic reactions to endocrine stimuli. Could the same observation be made on smooth muscle of blood vessels?

In discussing effects of steroids on circulatory status, one is instantly reminded of vasomotor instability observed at the natural menopause and with surgical castration. This syndrome involves a combination of a variety of symptoms and signs that include both cardiovascular and autonomic nerve manifestations. The signs and symptoms of "change of life" include: "hot flushes", palpitations, cardiac pain, sighing-dyspnea, sweating, vertigo, and "splitting headache" (Scherf, 1940).

One cannot explain the presence of such difficulties on the basis of an altered cardiovascular system alone. It is obvious that these changes are observed in men as well as in women who are in anxiety-producing situations. In addition, frequency of these symptoms is greatest in those women who experience clinical difficulty at menstrual periods. For this reason, it is difficult to evaluate effectiveness of estrogen therapy in this condition. However, it has been universally reported that estrogen is effective. The only objective finding on estrogen treatment is the correction of the S-T segment depression in the E.C.G. (Scherf and Boyd, 1940). Subjective findings include disappearance of "hot flushes", palpitations, and paresthesias. Even though estrogen is effective, one must question the site of action and whether this effect is a direct one or merely serves to correct some hormone imbalance including the pituitary-adrenal and pituitary-thyroid axis.

Reynolds (1941) contributed much toward the study of the effect of estrogen on smooth muscle by his observations on local effects of estrogen on arteriole tone. In man, he found that intramuscular estrogen was capable of inducing appreciable increases in finger volume for as long as two hours. In addition to this work, Reynolds et al. (1940) applied estrogen intradermally to ears of ovariectomized rabbits. A substantial vasodilation occurred when the results were quantitated according to percent of light transmission; a decrease of 15-20% was noted following estrogen injections. Congestion of mucosal surfaces, especially the nasal mucosa, during estrus in animals is well-known. Mortimer (1936) found he could induce vasodilation with local application of estrogen to nasal mucosa of monkeys.

It would appear that estrogen has an effect on the functional status of arterioles, at least in skin and mucosal surfaces. The physiological significance in vascular homeostasis is yet to be established. The literature is not consistent; Liebhart (1934) and Crainicianu (1932) observed a prolonged lowering of blood pressure in women with estrogen therapy, while Steinkamm and Giesen (1936) and Wallis (1936) found no depression of blood pressure in women with even larger dosages of estrogen. In rats, Oster (1950) reported a significant effect induced by castration. With a tail plethysmography method for blood pressure determination, he

found an increase in mean systolic pressure in castrate males of 16 mm. Hg, and a 13 mm. Hg decrease in castrate females. He did not, however, indicate his variation.

Depressant action of estrogen on blood pressure has been observed in one other species, the chicken. Weiss et al. (1957) found systolic pressure of adult male Leghorns to be 15-25% higher than that of females. No significant difference existed between sexes up to 8 weeks of age. However, between the 8th and 13th week, pressures began to diverge with a resultant 26-30 mm. Hg sex difference. This was due to a rise in male systolic pressure, whereas the female systolic pressure remained relatively stable. It is of interest that the diastolic pressure rose while heart rate decreased, suggesting that an increase in cardiac output occurred. These changes occurred at the same time as sexual maturation.

In summary, estrogen has little, if any, effect on blood pressure in man. There is indication that it may have a hypotensive effect in rats and chickens, as suggested by its vasodilating action on cutaneous and mucosal blood vessels.

Pertinent to the blood pressure problem is the role of estrogen in prevention of coronary and aortic atherosclerosis (Katz et al. 1953). This work has been done primarily in cholesterol-fed rabbits and chickens. Hyperlipemia and aortic atheromata of cholesterol-fed

intact female rabbits can be markedly inhibited by administration of estradiol (Ludden et al. 1942). Atheromatosis of coronary arteries of cholesterol-fed chickens can be prevented, and a regression induced, by use of estrogen (Pick, 1952). This has been related to alterations in circulating B-lipoproteins and, hence, to degenerative changes of the intima. It is known that atherosclerosis with hypertension forms a vicious cycle terminating in arteriosclerotic heart disease. Although estrogen appears to have a definite vascular morphogenic effect, this author will make no attempt to review the voluminous literature concerning estrogen's relation to experimental atherosclerosis.

GONADAL STEROID EFFECTS ON SKELETAL MUSCLE

A. Androgenic Effects

Investigation of the action of androgenic steroids on striated muscle was stimulated by the report of Papanicolaou and Falk (1938) on the hypertrophy of skeletal muscle induced by androgenic hormones. In adult females the temporal muscles are comparatively small and flat, while in the adult male they are much larger. They found, during the course of an experiment with gonadotrophic hormones, that the temporal muscles of adult females underwent considerable hypertrophy. In addition, they reported that growth was not limited to the temporal muscle, but other muscles of the body were

similarly affected, indicating that the effect is on the muscular system in general. They further observed, in adult male guinea pigs castrated before sexual maturity, that the temporal muscles remain small as in the adult female. On treatment with gonadotrophic hormones the muscles of such castrated males did not respond. In castrated females such treatment was also ineffective, indicating that the presence of gonads is necessary for the production of muscular hypertrophy. On administering testosterone propionate to castrate immature males and spayed, as well as normal, adult females, a definite hypertrophy of the temporal and somatic muscles occurred. They therefore assumed that the trophic action of gonadotrophic hormone in intact females was mediated indirectly through androgenic hormones released from interstitial cells of the ovary. Ovaries from these treated animals showed hyperplasia and hypertrophy of interstitial tissue. No data on muscle weights were given in this publication. Dosages of gonadotrophic hormone, testosterone, estradiol, and progesterone were not given. The question of purity of gonadotrophic hormone seems rather academic in the absence of any reported data. Kochakian, Humm and Bartlett (1948), as well as Scow and Roe (1953) were successful in confirming atrophy of temporal muscle in adult male guinea pigs following castration and its correction by androgen administration.

Kochakian and Tillotsen (1955) investigated effect of castration on 49 individual skeletal muscles of guinea pigs and substantiated Papanicolaou's and Falk's (1938) findings of a generalized effect on the muscular system. In general, degree of weight loss induced by castration was most marked in cephalad muscle groups and least in caudal muscle groups.

In noting the effect of age and castration on total skeletal muscle mass, Kochakian and Tillotsen (1955) found that the total muscle mass increased progressively with age in the normal guinea pig. In this experiment the two groups of animals were not pair-fed and no mention of any change in food intake was noted. In addition, the spontaneous activity of the castrate animals might well be decreased, which would effect the growth increment. Nevertheless, it is apparent that the muscular system has varying degrees of sensitivity to the castrate state.

Scow and Hagan (1955) determined the effects of testosterone on myosin, collagen, and other protein fractions of the temporal and the rectus femoris muscle in castrate guinea pigs. It was found that all nitrogenous fractions of the temporal muscle were reduced by castration; this includes myosin, collagen, non-protein nitrogen, a "water-soluble" protein (corresponds to the sarcoplasmic protein fraction and includes myoalbumin, most of the glycolytic cycle enzymes, and all particulates)

and an alkali-soluble stroma fraction. The greatest change was in myosin formation, which was reduced to 7% of that of intact animals. In the rectus femoris muscle, myosin, "water-soluble" protein, and non-protein nitrogen were not affected by castration, whereas formation of alkali-soluble stroma and collagen fractions were reduced about 50%. Testosterone administration to castrate guinea pigs returned the altered nitrogenous fractions of both muscles to normal.

The difference between temporal and rectus femoris muscle in the response of myosin and actomyosin to testosterone is quite striking. Scow and Hagan (1955) suggest two possibilities; the first is that these fractions in the two muscles are not identical and such identification would require electrophoretic or ultracentrifugation studies. The second possibility suggested is that formation of the major part of proteins in the temporal muscle of normal male guinea pigs is under direct control of testosterone. He suggests that this muscle should be classified as a secondary sex characteristic of the male guinea pig. To support this, Scow and Hagan (1957) observed that fierce fighting occurs between males when placed with estrous females. Hypertrophy of head and neck muscles is an important factor in this sexual behavior pattern in the guinea pig.

Collagen formation in the rectus femoris and temporal muscle of the guinea pig was decreased by castration

and restored to normal by testosterone. Scow and Hagan (1957) repeated their studies on thigh muscles in castrate rats and found no effect on formation of various protein fractions or on weight gain. Their observation of collagen reduction in thigh muscles of guinea pigs was not confirmed in the rat.

Wainman and Shipounoff (1941) observed, in male rats, a stimulating effect of testosterone propionate on the perineal complex: bulbocavernosus, ischiocavernosus, and levator ani muscles. The profound atrophy of castration was prevented by the administration of testosterone. Comparable changes were not seen in other striated muscles. Scow (1952) substantiated this work. He reported a weight increase of 6-7 times in the levator ani muscle of castrate rats given testosterone. Eisenberg and Gordan (1950) noted effects of various hormones on the levator ani. They observed that the levator weighed 17 ± 1.0 mg. in the 30-day-old male rat. The castrate male rat levator weighed 33 ± 2.9 mg. at 60 days, while intact normal muscle weighed 101 ± 6.3 mg. Thus, even in the castrate animal there is growth of the levator ani. They observed that pituitary growth hormone, unesterified testosterone, testosterone propionate, and methyltestosterone are potent myotrophic agents, all restoring the weight from 23% to 49% of normal levator ani weight.

Stewart (1955) induced the levator ani muscle of the rat to hypertrophy by subcutaneous implantation of

testosterone pellets 30 days prior to sacrificing the animals. Determinations of total protein nitrogen and protein fractionation were made. Analysis of cytoplasm, connective tissue, and contractile protein showed no significant alteration in relative distribution of proteins. He concluded that the hypertrophy induced was a true growth.

Leonard (1952) determined glycogen levels in the rectus femoris, abdominal and cremaster muscles on the seventh day following castration of male rats. Castration was found to decrease glycogen content of all three muscles, but only in the cremaster muscle was this difference statistically significant. On determination of glycogen 32 days after castration, all three muscles showed a significant decrease. Testosterone administration to castrate males resulted in a significant increase in glycogen in all three muscles within 6 days. Spayed females showed the same response to testosterone in abdominal and thigh muscle. This work demonstrates the importance of the time-interval between castration and observation of expected changes.

The growth effects of androgenic hormones have been extensively investigated in the male rat and guinea pig. It has been successfully demonstrated that growth of the masticatory muscles of the male guinea pig is under the primary influence of the male sex hormone. In male rats the analogous muscle is the levator ani group and the

cremaster. This effect has been localized to an action on nitrogen metabolism, such as affecting the quantity of contractile or non-contractile proteins including collagen. In addition, glycogen content of cremaster, rectus femoris, and abdominal muscles of the male rat can be increased with testosterone administration.

The obvious question after observing alterations in growth of these muscles is: What is the effect of androgens upon tension production? To the writer's knowledge, such work has not been done in the male rat or male guinea pig. Simonson, Kearns, and Enzer (1941) found in four men, two castrates and two eunuchoids, that methyltestosterone was capable of inducing marked improvement in work capacity. However, no adequate controls were provided and possible effects of learning and training make the results difficult to evaluate. Samuels, Henschel, and Keys (1942), studying male medical students, found no improvement in physical ability on administering 50 mg. of methyltestosterone daily for a two-week period. This was a double blind study with each man serving as his own control and receiving first a placebo for two weeks, then receiving testosterone. Physical ability was measured by determining grip strength. This finding in itself hardly negates the results in castrate males mentioned above.

The effect of testosterone on growth of muscle and tension production might well be similar to effects of

somatotrophin on skeletal muscle. Bartoli, Reed and Struck (1937) found a small, but definite, increase in weight of the quadriceps muscle of male rats treated with pituitary extracts. Greenbaum and Young (1950) confirmed this observation with growth hormone. They found that the rate of growth of tissues was not uniform throughout the body. Differences in rates of growth of muscles were noted. Plattener and Reed (1939) studied the twitch tension of the gastrocnemius muscle during long-continued stimulation and found that this hypertrophied muscle did not develop more tension than did muscles from control rats. All of their experiments were performed on the gastrocnemius, a muscle in which increase in weight under growth hormone treatment is relatively small. Bigland and Jehring (1952) employed the quadriceps muscle of female hooded Norway rats, after 21 days of treatment with 0.5 mg. of "pure" growth hormone. They completed their studies by using *in situ* a femoral-nerve-quadriceps preparation, in which the nerve was stimulated through electrodes. The animals were pair-fed with untreated controls. Isometric records of single twitches, summated twitches, tetanus, and fatigue were obtained. In spite of differences in weight of up to 40% between the two groups of muscles, the treated muscle gave less tension per gram of muscle weight than the controls. Histological comparison of treated muscle with untreated controls showed an increase of fiber

cross-sectional area of 6-12% associated with an increase in muscle weight of 20-30%. Because of size of the quadriceps, contractions could not be measured when the muscle was stimulated directly. The possibility of defects in transmission at the neuromuscular junction or of changes in the propagation of the excitation wave might account for these findings. Nevertheless, there is a possibility that with increased growth of muscle there is not a direct 1:1 ratio with the ability to develop tension on stimulation. For this reason, the response to stimulation of the masticatory muscles of guinea pigs and of the levator ani of rats should be investigated.

B. Estrogen Effects

Effects of estrogen on skeletal muscle growth or tension production have not yielded the consistently trophic effects noted in testosterone investigations. Papanicolaou and Falk (1938) in their original work found no definite effect on any voluntary muscles on administering progesterone or estradiol for periods of 6-8 weeks. They attributed the response of intact females to gonadotrophic hormones to the release of androgenic substances from interstitial tissue of the ovary.

As a result of this early work, most investigations of estrogens are limited to application of estrogenic preparations to muscles of males being investigated for their response to androgens. Wainman and Shipounoff (1941) found that progesterone increased the weight of

the levator ani of castrate male rats 15% above untreated castrates, while estradiol dipropionate and desoxycorticosterone acetate produced no significant increase in the weight of the muscle.

A recent paper was found in which estrogen effects on striated muscle were investigated. Bajusz (1959) noted effects of various hormones on regression of muscle atrophy following denervation. He used female rats and crushed the obturator, sciatic, and femoral nerves intrapelvically. Such a procedure resulted in a marked atrophy of the triceps surae muscle. Methyltestosterone and alpha estradiol were most effective in preventing this denervation atrophy, maintaining 89.8% and 85.4% of preoperative weights respectively. No tension studies were done.

No study is known in which tension production per gram of skeletal muscle is compared for males and females. As already mentioned, the response to growth is not always directly related to tension production. Even though estrogens are incapable of inducing skeletal muscle growth in ovariectomized females, there may be an effect on work capacity of specific muscles.

GONADAL STEROID EFFECTS ON NITROGEN AND PROTEIN METABOLISM

A. Androgenic Effects

The role of androgens in metabolism of protein was first investigated by Kochakian and Merlin (1935). They found that "male hormone" extracted from urine of male

medical students produced a marked reduction in urinary nitrogen excretion of "thin" and "fat" castrate dogs maintained on a constant diet. The maximal rate of nitrogen retention was attained in two to three days, and continued injections or increased dosages did not increase the rate of retention. The maximal rate of retention was 5-6 mg. per kg. of body weight per day. On cessation of injections some nitrogen was lost, and it was assumed that this had not been incorporated into proteins. The "male hormone" employed in this experiment was identified as androsterone. Following this experiment testosterone was employed in hypophysectomized-castrate dogs (Kochakian, 1937), castrate rats (Kochakian, 1944), hypophysectomized male rats (Rupp and Paschkis, 1953), and eunuchoids (Knowlton et al., 1942) with the same results. It was observed that treatment of normal dogs in the same manner as castrate dogs resulted in no nitrogen retention (Kochakian and Merlin, 1936). In none of these experiments was fecal nitrogen decreased.

The fate of retained nitrogen has been the subject of investigation by Kochakian and Merlin (1935). In their original experiment, they found urine urea decreased, likewise blood N.P.N. and urea were also decreased. It was suggested by these workers that the retained nitrogen was utilized in skeletal muscles and

secondary sex organs. No alteration was noted in plasma protein concentration.

Kochakian, Robertson and Bartlett (1950) investigated the site and nature of protein synthesis in castrate rats treated with testosterone. Castrate adult male rats brought into body weight equilibrium and injected with testosterone propionate deposited protein in the carcass, seminal vesicles, prostates, liver, and kidney, in decreasing order. On continued treatment for 46 days, loss of carcass fat and protein occurred. This protein apparently was diverted to accessory sex organs and to the kidney, since these organs continued to grow. Appetite changes were observed with testosterone administration to castrate males, whereas no effect was noted in intact male rats. Determination of amino acids of the gastrocnemius, liver, kidney, seminal vesicles, and prostate indicated no qualitative or quantitative change per unit weight of tissue. They assumed, therefore, that true growth of tissue occurred. Total protein, non-protein nitrogen, and amino acid nitrogen of plasma from treated castrate rats were identical to those of control rats.

In summary, androgens have an anabolic action in castrate animals but not in intact animals. This is manifested by retention of urinary nitrogen and reversal of a negative nitrogen balance, but unassociated with any elevation of plasma nitrogen. The retained nitrogen is

utilized in specific organ-tissue growth. The anabolic effect of androgens on skeletal muscle has been described in detail in the skeletal muscle section of this literature review.

The mechanism of the anabolic effect remains to be determined. It is effective in the hypophysectomized animal (Rupp and Paschkis, 1953), but it is known that androgens lose their nitrogen-retaining ability in the absence of insulin (Gaebler and Tarnowski, 1943). The exact cellular mechanisms remain to be ascertained.

B. Estrogenic Effects

The action of estrogen was first investigated by Thorn and Engel (1938). They found a marked decrease in nitrogen excretion of five normal dogs after a single subcutaneous injection of 5 mg. of alpha estradiol or 15 mg. of estrone, but no effect was noted after 20 mg. of progesterone. This implies that estrogen is active in nitrogen metabolism in a different manner or at a site different from testosterone, since testosterone or androsterone is not effective in intact males but only in castrate animals while estrogen is effective in non-operated animals. Gaebler and Tarnowski (1943) found that administration of estrone to normal dogs did not induce elevation of N.P.N. or blood urea, in the presence of reduced urinary nitrogen. Knowlton et al. (1942) administered 5 mg. per day of alpha estradiol benzoate to two eunuchoids and one hypogonadal woman for 4-20 days

and noted decreased nitrogen excretion comparable to that obtained with 5 mg. per day of testosterone propionate. In studies of a 19-year-old normal girl, they found the characteristic decrease in urine nitrogen on administering 5 mg. per day of alpha estradiol; but as reported above in dogs, progesterone did not decrease urine nitrogen.

Workers have found increases in plasma protein of birds on estrogen administration. Common et al. (1948) demonstrated that estrogen administration to chickens increased serum calcium, phosphoproteins, lipids, and liver proteins. Mandel, Clavert and Mandel (1947) demonstrated in pigeons that the increase in total plasma proteins following estrogen administration was mainly in the albumin fraction. Sturkie (1951) studied the effect of estrogen on plasma protein and plasma volume in chickens. Using 20 mg. of dienestrol per day in their feed for 32 days, he found no significant difference in total plasma protein. Continuing injections for 14 more days at 40 mg. per day per bird, plasma proteins increased from 5.14 gm.% to 8.50 gm.%. Globulins increased from 2.11 gm.% to 3.20 gm.%, with no change in the A/G ratio. Thus, in chickens, plasma proteins are significantly increased by estrogen as much as 63% within 14 days. After ceasing treatment, plasma proteins returned to pretreatment levels within two weeks. No significant change in plasma

volume or hematocrit was found by Sturkie with either dosage of estrogen.

It may therefore be concluded that estrogens, like androgens, are active in preparing a milieu favorable for protein synthesis. Further, it has been demonstrated in birds that one may induce synthesis of plasma proteins. This has not been found to be true in mammals (Gaebler and Tarnowski, 1943) (Knowlton et al., 1942), even though nitrogen retention has been demonstrated.

Estrogens have not been found to be uniformly anabolic hormones. Glasser (1954) studied the influence of stilbestrol on nitrogen metabolism in adult male rats. Injection of 0.1 mg. of stilbestrol daily for 20 days resulted in a body weight loss and a negative nitrogen balance. The latter was not due to an increased urinary nitrogen, but rather to a reduction in food intake without conserving body nitrogen. Hormone withdrawal resulted in an immediate recovery of body weight and a return to positive nitrogen balance.

Telfer (1953) noted the influence of estradiol on nucleic acids, respiratory enzymes, and nitrogen distribution in rat uterus. Her work demonstrates a metabolic system which is exceedingly sensitive to estrogen. Nitrogen content of the uterus determined within 48 hours of the first estrogen injection was markedly increased. This increase was primarily in the mitochondria fraction with the expected increase in ribonucleic acids.

Progesterone administration to ovariectomized rats did not increase uterine nitrogen.

In summary, estrogen administration induces the retention of urinary nitrogen in mammals in the absence of any blood nitrogen change. Since testosterone results in nitrogen deposition in the carcass, one might expect estrogens to induce similar changes. As discussed in the skeletal muscle section of this review, estrogen has no effect on skeletal muscle weight. The most logical remaining site of nitrogen utilization is in the uterus. As already discussed, there is a rapid and marked response of the uterus to estrogen administration. This reminds one of the negative nitrogen balance and marked urinary nitrogen loss in post-partum women associated with involution of the uterus in the presence of low post-partum estrogen.

The changes in plasma proteins and minerals in birds is consistent with requirements of these animals for egg laying, a process directly influenced by estrogen.

ESTROGEN EFFECTS ON ENZYME SYSTEMS

The ultimate explanation of biological actions of estrogens will probably be found in an investigation in the realm of enzymatic phenomena. The essential specific enzyme systems in which estrogen is active as a substrate or as a coenzyme must be elucidated. A number of enzyme systems have been investigated.

These include: B-glucuronidase, alkaline phosphatase, cholinesterase, and succinic dehydrogenase.

B-glucuronidase was one of the first to be investigated, primarily because of the finding of the conjugation of steroids with glucuronic acid. A relationship between estrogen activity and B-glucuronidase was first established by Fishman and Fishman (1944). They examined influences on the enzyme of repeated injections of ovariectomized mice with estrone, 17-B estradiol, estriol glucuronide, stilbestrol, grogesterone, and pregnanediol. The estrogens, natural or synthetic, caused a rise in B-glucuronidase activity in the uterus, but not liver, kidney, and spleen. Progesterone and pregnanediol had no effect on uterine enzyme activity. Ovariectomy was found to cause a fall in enzyme activity in the uterus compared with intact controls. They explained these results in terms of adaptation of enzyme concentration to quantity of substrate present, estrogen being the substrate. Lack of response of non-reproductive organs such as the liver, kidney, and spleen to estrogen was explained by Fishman (1947) in one of two ways. Either a specific glucuronidase for estrogens exists in the uterus; or physicochemical factors, such as permeability of tissue to estrogens, were responsible. The findings for non-reproductive organs have not always been consistent with Fishman's results. Kerr et al. (1950) found stimulation of liver B-glucuronidase activity by

estrone in ovariectomized mice; and this effect was antagonized by testosterone and progesterone, although neither of the latter had any effect by themselves. This action of estrone was also seen in normal and castrate males, but was absent in intact female mice. Estriol and estradiol did not affect liver B-glucuronidase activity. No changes in the kidney enzyme were found. They related these results to the stimulated mitotic activity of estrone in these livers; this was not seen in intact females.

Other tissues known to have alterations in B-glucuronidase include proliferated human breast tissue in pregnancy, with even higher values in carcinoma of breast (Fishman and Anlyan, 1947). Blood plasma B-glucuronidase activity increases during pregnancy and drops to prepartum levels after parturition (Fishman et al., 1950). This fall is prevented by administering stilbestrol (Fishman et al., 1950). There is no sex difference in human serum B-glucuronidase activity, suggesting that the change found in the plasma activity during pregnancy is related to spillage from tissue such as endometrium, which is one of the richest sources of B-glucuronidase in humans (Odell and Fishman, 1950). This work suggests that B-glucuronidase activity is under endocrine control. Whether it has the nature of an adaptive response to the growth stimulation by estrogens remains to be determined.

Alkaline phosphatase of the uterus has been the object of interest. Atkinson and Elftman (1946) found that in castrated female mice large amounts of alkaline phosphatase are present in the longitudinal muscle of the uterus, whereas much smaller amounts are found in the uterine glands and epithelium. On injection of estradiol benzoate into these animals, a marked increase in alkaline phosphatase was observed in uterine glands, epithelium, and in circular muscle. In monkeys, Atkinson and Engle (1947) found phosphatase increased in endometrial glands following estrogen administration, and subsequent reduction was noted on injecting progesterone. In addition, these men found alkaline phosphatase activity was highest in endometrium of women during the proliferative phase, reduced during the secretory phase, and absent several days before the menses. Atkinson and Elftman (1946) proposed that increase in alkaline phosphatase of the uterus after estradiol administration could be related either to appearance of glycogen or to disappearance of lipids. Stafford et al. (1947) observed increases in acid and alkaline phosphatase in the corpora lutea as pregnancy advanced and also during lactation. In support of Atkinson's work, Li et al. (1946) found a decrease in alkaline phosphatase of rat plasma in hypophysectomy.

Birkhause and Zeller (1940) found that the livers of mature female rats possessed 3-5 times as much capacity

for hydrolyzing acetylcholine as male rat livers. Sawyer and Everett (1946) observed that serum cholinesterase levels parallel estrogen activity, but not that of progesterone. The enzyme levels were low in estrogen-poor conditions, such as the first half of pregnancy and the post-partum state; whereas they were high in conditions characterized by high estrogen activity, such as estrus and the last half of pregnancy. On castration of female rats, the nonspecific cholinesterase of serum dropped to a level of about that found in males. On castration of male rats, this enzyme level became greater than control male values. In the castrate female, administration of estradiol restored blood cholinesterase to normal while testosterone administeration antagonized this estrogen action. Progesterone was found by these men to have no effect on blood cholinesterase in castrate female rats.

Succinic dehydrogenase and succinoxidase systems have been investigated by the Meyer, McShan group. Meyer et al. (1945) found lutein tissue of the ovaries of pregnant and pseudopregnant rats rich in succinic dehydrogenase. They observed a return to normal values in corpora lutea following parturition (Meyer et al., 1947). In addition, cytochrome oxidase concentration was much higher in corpora lutea of pregnancy than in diestrus. Investigation in vitro of this enzyme system isolated from liver and pituitary tissue of rats revealed that the

synthetic estrogen, stilbestrol, and estrone both produced inhibition (McShan and Meyer, 1946). In a study of *in vivo* effects of estrogen, liver and uterine tissues were assayed for succinic dehydrogenase and cytochrome oxidase, following treatment of adult female rats with various estrogens daily for 10 days. They found inhibition in these tissues when the animals had been treated with estrogen containing the phenolic group, whereas no inhibition was found associated with the alcoholic group of androgens. The inhibition was shown to be through the cytochrome oxidase. They suggested that phenolic groups of estrogenic compounds combine with the active centers and remain attached to the enzyme.

In this review of estrogen's action on enzymes, the papers so far discussed have been mostly limited to observations on concentration of enzymes in tissue following estrogen administration. This is far from determining the primary site of estrogen activity, since this is a manifestation of the general growth-promoting action of estrogen. The findings that estrogen inhibits certain enzyme systems both *in vitro* and *in vivo* contributes little to the answer of the basic mechanism of activity, since it is highly unlikely that estrogen produces its marked changes in metabolism by inhibiting synthesizing reactions.

In contrast to the papers previously discussed is the work of Hagerman and Villee (1953). They investigated

the mode of action of estrogen on human endometrium. They found that small amounts of estradiol added *in vitro* increased the rate at which oxygen and pyruvic acid are utilized. They found that in addition to endometrium, placenta contained an estrogen-sensitive system, and this was later extended to include the mammary gland, ventral prostate gland, and the pituitary gland (Villee and Gordan, 1955). Liver and kidney did not contain such an estrogen-sensitive enzyme system (Villee, 1955). On the basis of this work, Villee postulated the existence of a specific DPN-linked isocitric dehydrogenase that was activated by minute quantities of estradiol.

Talalay and Williams-Ashman (1958) confirmed existence of a soluble enzyme from human placenta that promotes transfer of hydrogen between two pyridine nucleotide coenzymes, and that this reaction is activated by minute quantities of certain steroid hormones. The overall reaction that is catalyzed:

$$TPNH + DPN^{+} \longrightarrow TPN^{+} + DPNH$$

TPN and DPN being the oxidized form of tri- and diphosphopyridine nucleotides, with DPNH and TPNH being the reduced forms. It was found by Talalay that the response of crude placental extracts to steroids was variable and that stimulation of reduction of DPN by certain hormones disappeared upon fractionation of the extracts. They observed that addition of catalytic quantities of

TPN to the reaction mixture increased markedly the ability of different placental preparations to respond to steroids, and even restored the effect in inactive preparations. Apparent stimulation of a DPN-linked isocitric dehydrogenase can be accounted for in terms of coupling of TPN-specific isocitric dehydrogenase of placenta with action of a soluble trans-hydrogenating system described here:

$$\text{Isocitrate} + \text{TPN}^+ \longrightarrow \text{alpha-keto glutarate} + CO_2 + \text{TPNH} + \text{H} \qquad \text{(A)}$$

$$\text{TPNH} + \text{DPN}^+ \rightleftharpoons \text{TPN}^+ + \text{DPNH} \qquad \text{(B)}$$

$$\text{Isocitrate} \quad \text{DPN} \rightleftharpoons \text{alpha-keto glutarate} + CO_2 + \text{DPNH} + H^+$$

In step B, the transhydrogenase system is activated by steroids. Partially purified fractions from placenta which catalyze hydrogen transfer between the two forms of pyridine nucleotide also catalyze oxidation of steroids by DPN and TPN. Of the steroids examined, only those which can undergo oxido-reduction by pyridine nucleotide-linked hydorxysteroid dehydrogenase are active. This is consistent with the idea that proteins catalyzing dehydrogenation are identical.

In further studies, Talalay et al. (1958) demonstrated the estrogen sensitive transhydrogenase to be the same enzyme as the 17-B estradiol dehydrogenase. Evidence for this included the following findings: the two enzymatic activities are not separated by purifying

fractionation procedures; and secondly, only those steroids which can undergo enzymatic oxidation can be active in transhydrogenation. Talalay's work led to the following conclusions: (1) The enzymatic basis of steroid-mediated transhydrogenation is ascribed to the reversible oxidation of steroid by hydroxysteroid dehydrogenase which react at comparable rates with both DPN and TPN. (2) Hydroxysteroid dehydrogenase with dual nucleotide specificity may function as a pyridine nucleotide transhydrogenase. Thus, it is suggested that steroids may be more properly regarded as hydrogen carriers and pyridine nucleotides as substrates in these reactions. As an example of such a coupled reaction:

$$\text{Estradiol} + \text{DPN}^+ \rightleftharpoons \text{Estrone} + \text{DPNH} + \text{H}^+$$

$$\text{H}^+ + \text{Estrone} + \text{TPNH} \rightleftharpoons \text{Estradiol} + \text{TPN}^+$$

$$\text{Sum: TPNH} + \text{DPN}^+ \rightleftharpoons \text{DPNH} + \text{TPN}^+$$

Hurlock and Talalay (1958) found that purified rat liver 3-alpha hydroxysteroid dehydrogenase in the presence of minute amounts of androsterone or other 3-alpha hydroxy or 3-ketosteroids catalyze the transfer of hydrogen between DPN and TPN. They have therefore succeeded in extending their original observations in support of the idea of steroids being coenzymes of hydrogen transfer between pyridine nucleotides.

The importance of these findings in explaining the observations of other workers is easily seen. There is evidence that DPN and TPN serve different metabolic functions and that the natural occurrence of these two different nucleotides is of great functional significance. TPNH is active as a reducing agent in synthetic reactions that take place outside the mitochondria and in which DPNH cannot participate. An example of such a reaction is the entry of 1-C fragments into serine and into purines catalyzed by a series of folic acid-dependent enzyme systems which utilized TPNH as a specific hydrogen donor. Mueller and Herranen (1956) found that shortly after administering 17-B estradiol to ovariectomized rats, the incorporation of 1-C fragments into serine and purines of nucleic acids in the uterus was greatly increased.

In accessory organs of reproduction in the male, a number of biochemical changes induced by testosterone could have as their basis a change in TPN or DPN. These biochemical changes include the synthesis of fatty acids from acetate in the prostate gland, which is most sensitive to the presence of testosterone (Nyden et al., 1953). Androgenic steroids initiate and support the accumulation and secretion of fructose and citric acid in some male accessory sex tissue (Williams-Ashman et al., 1954). Synthesis of fructose by these organs involves reduction of glucose to sorbitol by TPNH, followed by DPN-linked

oxidation of sorbitol to fructose (Hers, 1956). Overall conversion of glucose to fructose simulates the action of pyridine nucleotide transhydrogenase, in that there is a stoichiometric transfer of hydrogen from TPNH to DPN.

There appears to be ample evidence that many of the observed biochemical changes induced by the presence of estrogens may well be explained on the basis that estradiol is acting as a coenzyme in transhydrogenation. There are, however, many compounds with high estrogenic activity that are structurally incapable of participating in hydrogen transfer systems. The second consideration of importance is that certain compounds that are highly estrogenic in one species are inactive or, at best, weakly active in another; it remains to be found where their biochemical action resides.

ESTROGEN METABOLISM

A thorough discussion of the metabolism of estrogen has not been included in the literature review. However, a few statements for completeness can be made here. The exact chemical precursor of natural estrogens is not known. Three possibilities exist: the first two concern cholesterol. Levin and Jailer (1948) observed a decrease in ovarian cholesterol following gonadotrophic stimulation. Direct evidence of transformation of cholesterol to 17-B estradiol, the true ovarian estrogen (Heard and O'Donnell, 1954), is lacking. Cholesterol could be

converted first to progesterone, then through estrone to 17-B estradiol or directly to 17-B estradiol. The third possibility is based on finding labeled estradiol and estrone in the perfusate of ovaries of the sow following addition of labeled acetate (Werthessen et al., 1953). It would appear to this writer that active 2-C fragments are serving as the precursor of cholesterol and that cholesterol could either be converted to progesterone or directly to 17-B estradiol, depending on the presence of the specific gonadotrophin. This point in the steroid cycle might well be the metabolic site of action of follicle-stimulating hormone and luteotrophin.

The site of production of 17-B estradiol is believed to be cells of the theca interna (McKay and Robinson, 1947). The question of interconversion of estrogens is a complex one. Ryan and Engel (1953) demonstrated that 17-B estradiol and estrone are readily inter-converted in human tissues including placenta, liver, adrenal cortex, pregnancy breast, and hyperplastic endometrium. The importance of this conversion in terms of transhydrogenation (Talalay et al., 1958) has been discussed in the enzyme section of the literature review. While both estradiol and estrone are quite active estrogens, estriol is the least potent and is considered a degradation product (Gallagher, 1944).

The metabolism of estrogens appears to center, at least in part, in the liver. The liver appears to be

the major site of estrogen inactivation (Biskind, 1941). The appearance of hyper-estrinism has been well documented in partially hepatectomized rats (Schiller, 1944) and in human cirrhotics (Glass, 1950). It appears that the liver carries out this function in two ways. Heller (1940) found that incubation of 17-B estradiol or estrone with liver slices induces a marked loss of estrogenic activity. A second method in contrast to inactivation is the existence of an entero-hepatic circulation, in which estrogens are excreted into the biliary tract (Cantarow et al., 1942). The kidney also plays a role in estrogen metabolism by conjugating estrogen with glucuronic acid and sulfuric acid (Fishman, 1951).

This is a superficial review of the metabolism of estrogens and this writer is not attempting to discuss all areas of estrogen metabolism nor to infer that all the questions are answered in any of the areas discussed.

MATERIALS AND METHODS

In these experiments the influence of gonadectomy and steroid administration on male and female albino rat hearts were investigated. Myocardial function was studied in isolated surviving columnae carneae (trabeculae carneae) of the left ventricle in preparations of glycerol-extracted columnae carneae and further, by extraction of ventricular actomyosin. Although the bulk of the experiments were on female animals a few preliminary studies were made on castrate and estrogen-treated males.

Experiment I. Determination of Properties of Isolated Left Ventricular Carneae

Sixty-six albino rats of the Sprague-Dawley strain weighing between 75 and 100 grams at the start of the experiment were employed in two series of experiments. The animals were divided into the following groups:

	No.
Series A.	
1) Intact females.	15
2) Intact males.	13
Series B.	
1) Sham operated females given sesame oil injections.	5
2) Ovariectomized females given sesame oil injections.	6
3) Ovariectomized females given 0.1 gamma alpha estradiol/day	8

	No.
4) Sham operated males given sesame oil injections.	4
5) Orchiectomized males given sesame oil injections.	7
6) Orchiectomized males given 1.0 gamma alpha estradiol/day.	8

In series B, treatment was initiated one day post-operatively and continued for a thirty-day period. Injections were made daily in varying subcutaneous areas.

On the final day of treatment, the animals were weighed and killed by decapitation. The hearts were immediately excised and placed in bicarbonate buffered Ringer's solution described by Feigen et al. (1952). The composition of the solution was as follows: In 1 liter of distilled water:NaCl, 9.0 grams, KCl, 0.42 grams, $CaCl_2$, 0.62 grams, $NaHCO_3$, 0.60 grams and glucose, 1.0 gram.

In addition to the heart, adrenals and uterus, or instead of uterus,seminal vesicles, were removed and weighed wet on a torsion balance. After removal of the columnae carneae, the heart with the above-mentioned organs was placed in an oven at 100°C. and dry weights determined after 24-48 hours.

Properties of left ventricular columnae carneae were determined according to the method of Ullrick and Whitehorn (1956). The heart was removed from the buffered Ringer's solution and the right ventricle was cut away, exposing the interventricular septum. The septum

was excised, exposing the chamber of the left ventricle. On the posterior wall of this chamber, the paired columnae carneae were found. These consist of rounded muscle columns extending from the apex of the left ventricle to the atrio-ventricular junction (Figure 1). On dissection, these muscle columns are cylindrical and in a 100-gram rat have a diameter of approximately one millimeter and a length of six to eight millimeters. The length of the columnae carneae nearest the interventricular septum was measured to the nearest 0.25 mm. with a millimeter rule. This length is termed the "in situ length".

Figure 2 is a diagram of the experimental apparatus; numbers in parenthesis refer to this figure.

The bundle was dissected free and the atrial end (10) fastened in a stainless steel muscle spring (9). The apical end (10) was placed in a plastic clamp (7) which contained within it a pair of chlorided silver electrodes (11). The electrodes were freshly cleaned and chlorided for each experiment. The stainless-steel spring (9) was attached by means of a connecting wire (4) to a rigid spring arm (5) and a Statham strain gauge (3), model 0.7-0.3-8000. This spring arm in turn was connected to a micrometer screw (1) so that the arm could be lowered or raised in relation to the muscle. In this manner the muscle length could be adjusted to the measured in situ length. The resistance to stretch, or resting

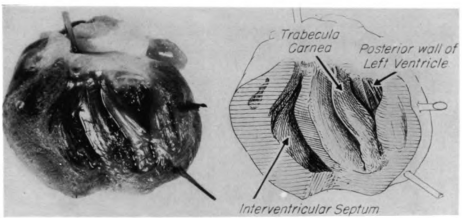

Figure 1. Photograph and diagram of rat heart muscle preparation.

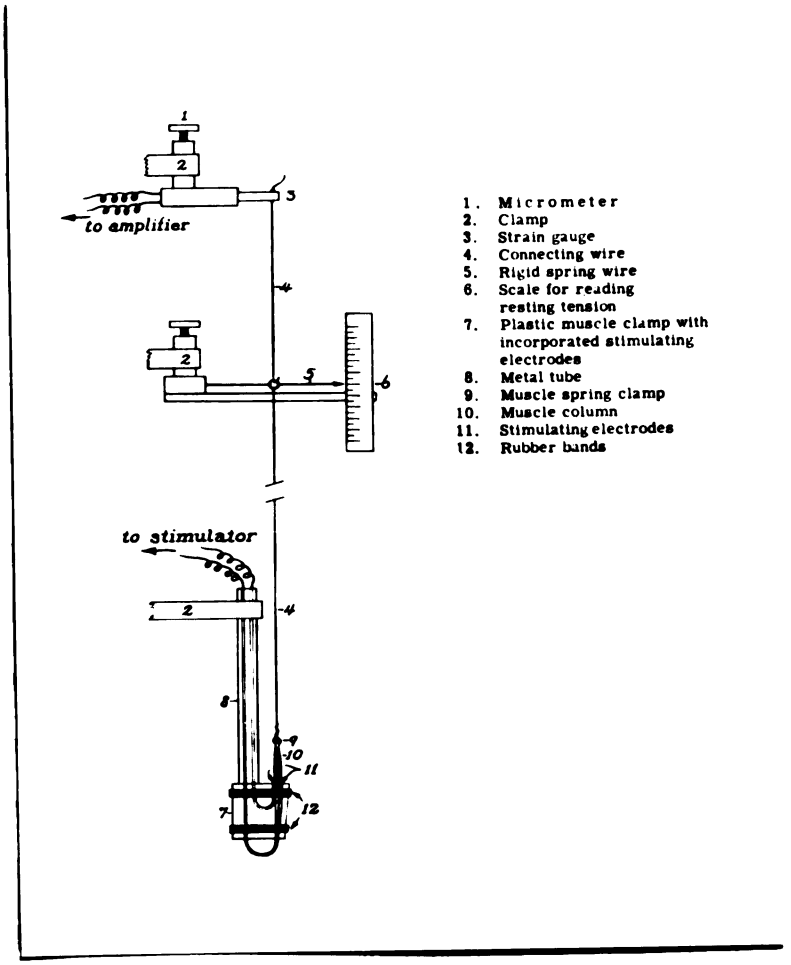

Figure 2. Drawing of the experimental apparatus.

tension, of the muscle could be read in grams from a calibrated scale (6), indicated by noting the position of the arm of the spring (5) at the point of connection to the muscle. The muscle connection was in alignment with the spring arm and the strain gauge. Contractions of the muscle on stimulation were essentially isometric.

Output of the strain gauge was amplified and recorded on one channel of a Grass ink-writing oscillograph. The system was calibrated by placing known weights on the stainless steel spring clamp (9) and measuring the pen deflection from the baseline on the oscillograph; thus, changes from the baseline represent changes in tension of the isometric contracting muscle. In this way the scale for passive tension could be calibrated.

The strain gauge and muscle clamp assembly was mounted on a vertical adjustable carriage which allowed the unit to be lowered into a glass muscle bath. This inner chamber was fixed in a Sargent constant temperature water bath, so that the temperature of the muscle bath was kept constant at 37.7° $\pm$ 0.25°C.

The inner chamber, which held the muscle, contained 45 ml. of the buffered Ringer's previously described. Fresh Ringer's solution was constantly added to the bath at a rate of 5-6 ml. per minute by a steady drip. The solution in the bath was oxygenated by means of oxygen entering the bottom of the bath through a gas dispenser

tube. Volume of the bath was maintained by means of an automatic overflow siphon.

After the muscle bundle was placed in the bath, it was immediately stretched to *in situ* length and allowed to equilibrate to the length adjustment (setting) for 45 minutes. Threshold to a square wave stimulus of 6 milliseconds' duration was determined, and active contractions were induced at a frequency of 1 per second. The strength of the stimulus used was read from the calibrated screen of a cathode ray oscilloscope, which was placed in series with the output of a square wave generator. Stimulus strengths were recorded in milliamperes.

A second channel of the Grass oscillograph received a separate output from the stimulator which signalled the generation of each pulse. A Franz timer was attached to the oscillograph. In this way stimulus, muscle tension, and time were simultaneously recorded. A sample record of an isometric contraction, stimulus, and time line are shown in Figure 3.

At the end of the 45 minute equilibration period at *in situ* length, refractory period, strength-duration curve, and active and passive length-tension relationships were determined.

Refractory period determinations were carried out by applying a square wave stimulus of 6 milliseconds' duration immediately following the driving stimulus. The strength of both stimuli was approximately 10 times threshold

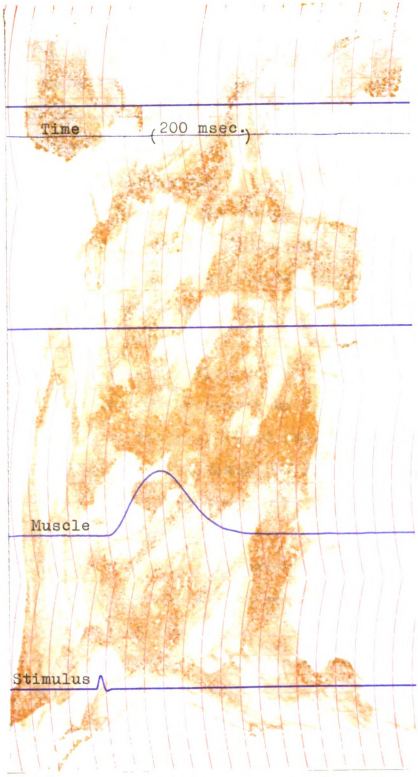

Figure 3. Record of an isometric contraction, stimulus and time line.

intensity. The Grass oscillograph was set to run at its fastest speed while the interval between the driving and second stimulus was gradually increased. This period between stimuli was gradually increased until a second response of the muscle was first seen. The time interval between the two stimuli was then measured on the oscillograph paper with the aid of the Franz timer and recorded in milliseconds as the absolute refractory period. Figure 4 is a typical refractory period determination.

Strength-duration curves were obtained by applying a stimulus of varying duration 500 milliseconds after the driving stimulus. The strength of the second stimulus was obtained from the calibrated oscilloscope. This procedure was repeated for stimuli of 10, 6, 4, 2, 1, 0.8, 0.6, 0.4, and 0.2 milliseconds' duration. Figure 6 is a strength-duration curve.

Following this group of determinations, the strain gauge was disconnected; and by means of the micrometer screw on the spring arm mount, all resting tension was removed from the muscle. The micrometer was then adjusted to the point where resting tension just began to develop, and the strain gauge was reconnected to the system. The muscle was then allowed to equilibrate for 30 minutes. At the end of the 30 minute equilibration period, that length, at which resting tension just began to develop, was recorded as the equilibration length. A record was then taken of the tension developed by the

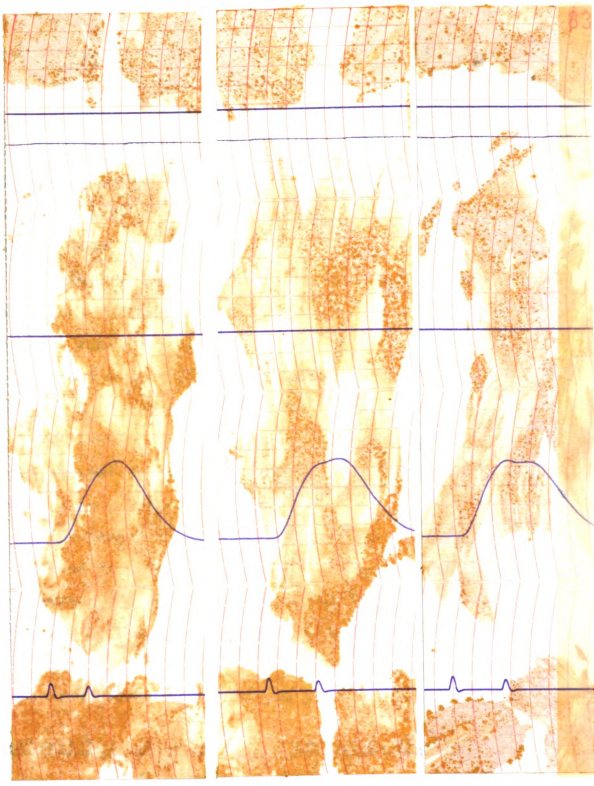

Figure 4. A typical refractory period determination.

muscle. Muscle length was measured with a calibrated binocular dissecting microscope, set beside the transparent bath. The muscle throughout this procedure, as before, was stimulated at a 1 per second frequency with 4-6 milliampere stimuli of 6 milliseconds' duration. The muscle was then stretched in 10% increments of equilibration length, by increasing the tension on the muscle by adjustment of the micrometer screw. At each new length, and therefore new resting tension, a ten-minute period of equilibration was allowed in order that the length would become constant before a record of active tension was obtained. Length-tension diagrams were prepared by plotting the resting and active tension of the muscle against the percent increase of equilibrium length. Figure 10 is a length-tension diagram.

After length-tension data were obtained, the muscle was removed from the bath and the active contracting portion between the clamps was cut free, weighed, dried at 100°C. for 24 hours, and then reweighed. The portions of the muscle within the clamps were added to the remainder of the ventricles and weighed wet, dried, and reweighed.

Experiment II: Extraction of Ventricular Actomyosin and Studies on Glycerol-Extracted Columnae Carneae

Forty-six albino rats weighing between 80-100 grams at the start of the experiment were used. The animals

were divided into the following groups:

	No.
1) Sham operated, given sesame oil injections.	8
2) Sham operated, given 100 gamma alpha estradiol/day.	8
3) Ovariectomized, given 0.1 gamma alpha estradiol/day.	8
4) Ovariectomized, given 1.0 gamma alpha estradiol/day.	8
5) Ovariectomized, given 10.0 gamma alpha estradiol/day.	6
6) Ovariectomized, given sesame oil injections.	8

Treatment was initiated one day postoperatively and continued for a thirty-day period.

On the final day of treatment, the rats were killed as described in the first experiment. The hearts were excised, opened, and placed in ice-cold de-ionized water. The columnae carneae were tied to applicator sticks at *in situ* length and glycerinated according to the technique described by Szent-Györgyi (1949) and Benson *et al.* (1958). This consists of placing the bundles, tied to applicator sticks, in 50% ice-cold glycerol for 72 hours. At the end of this time the bundles were transferred to fresh 50% glycerol and placed in a freezer at 0°C. for a period of 2 weeks before use.

The remainder of the ventricles were weighed on a Roller-Smith torsion balance and placed on a glass plate chilled with ice. This tissue was minced fine with a

razor blade and transferred to a glass homogenizing vessel surrounded by ice. Five ml. of cold 0.6 M KCl buffered with 0.4 M $NaHCO_3$ and 0.01 M Na_2CO_3 were added and homogenation carried out for 6 minutes, using a tissue grinder of the Potter-Elvehjem type driven by an electric motor at high speed.

The homogenate was transferred to a 50 ml. centrifuge tube. The homogenizing vessel was washed thoroughly with 2 ml. of ice-cold 0.6 M KCl and this was added to the homogenate. Two-hundredths ml. of a 10% solution of the disodium salt of A.T.P. was then added to the homogenate. Following thorough mixing, the homogenate was placed in a refrigerator at 6 $\pm$ 2°C.

After 24 hours the homogenate was centrifuged for 10 minutes at 2,000 g. in a refrigerated centrifuge at 0°C. The supernatant was decanted. It appeared viscous red-brown and moderately turbid. The volume of supernatant was measured and sufficient amounts of ice-cold glass-distilled water were added to bring the molarity of the 0.6 M KCl solution to 0.1 M. Actomyosin appeared as a fine slightly flocculant precipitate. This solution was allowed to stand in ice for 1 hour to allow maximal actomyosin precipitation. It was then centrifuged at 0°C. at 2,000 g. for 10 minutes and the supernatant was discarded. The sediment was then redissolved in 7 ml. of buffered 0.6 M KCl.

One ml. of solution containing the redissolved protein was withdrawn for nitrogen determination by the micro-Kjeldahl technique. The remainder of the solution was used for determination of relative viscosity (Ostwald viscosimeters at 20°C.) before and after addition of A.T.P. Determination of relative viscosity was performed by measuring the outflow time of solvent without protein and then determining the outflow time of the solution containing protein before and after the addition of 0.02 ml. of a 5.0% solution of the disodium salt of A.T.P.

$$n\ rel = \frac{t}{t_0}$$

where t = the measured outflow time of the protein-containing solution.

t_0 = the outflow time of the medium without protein.

n rel = relative viscosity.

Change in viscosity in response to A.T.P. has a direct relationship to the concentration of actomyosin in solution. This is seen in Figure 5 and is another means of quantitatively measuring actomyosin. This relationship was first reported by Mommaerts (1950) and by Balenovic and Straub (1942) for actomyosin of rabbit striated muscle.

Viscosity data may also be expressed in terms of "viscosity number" and "A.T.P. sensitivity" of Portzehl

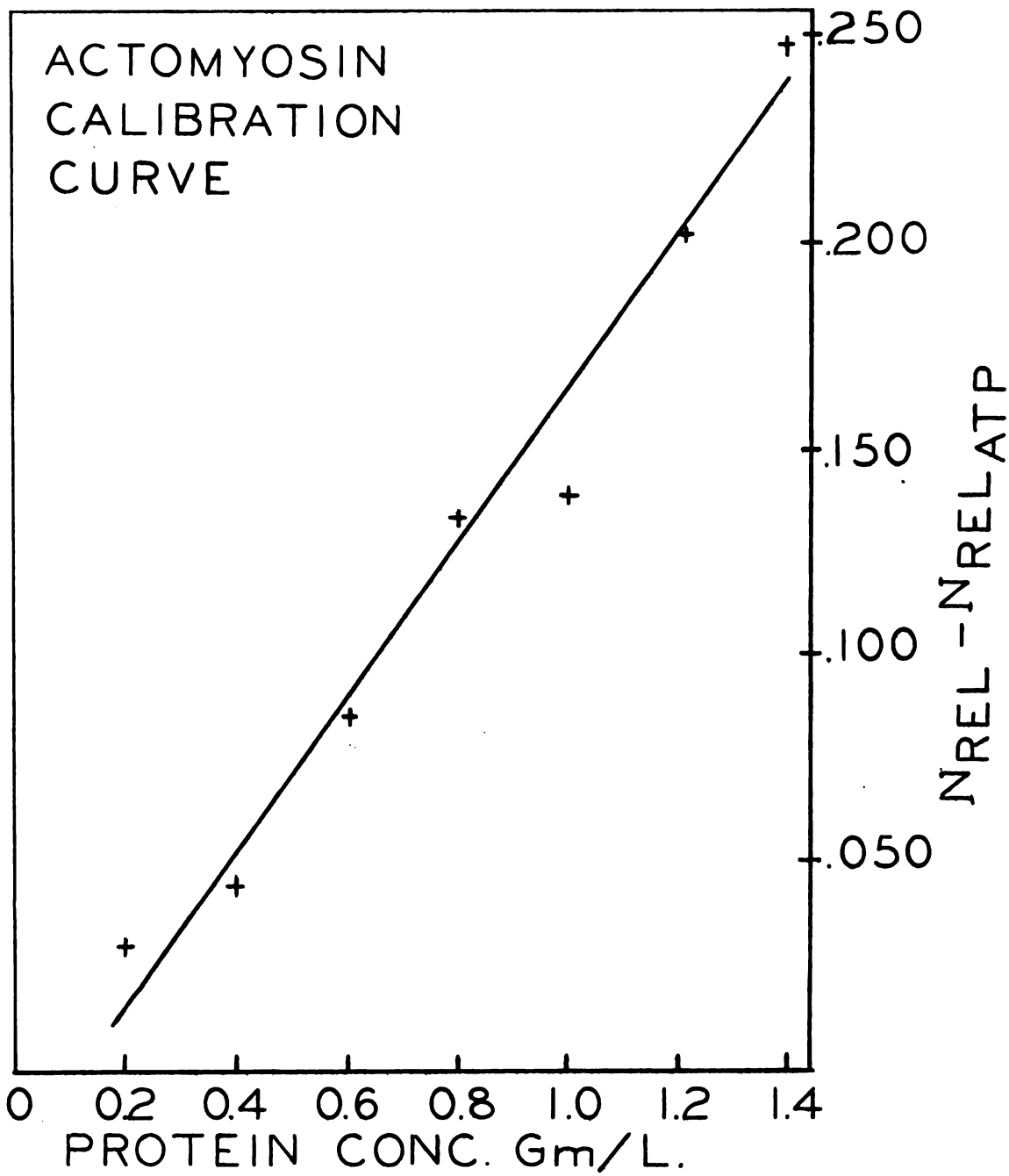

Figure 5. Actomyosin calibration curve.

et al. (1950). "Viscosity number" relates viscosity to protein concentration and is defined by the equation:

$$\text{Viscosity number} = Z_n = \frac{2.3 \log n \text{ rel}}{C}$$

Where C = concentration of protein in mg./ml. "A.T.P. sensitivity" is a percentage and is used to characterize actomyosin qualitatively as to actin content. This is defined as:

$$\text{A.T.P. sensitivity} = \frac{Z_n - Z_n\text{A.T.P.}}{Z_n\text{A.T.P.}} \times 100$$

Where Z_n = viscosity number before addition of A.T.P.

Z_nA.T.P. = viscosity number after addition of A.T.P.

In summary, data obtained by this procedure allow a quantitative and qualitative characterization of isolated actomyosin. Isolation and purification of actomyosin was completed by the utilization of Benson's et al. (1955) modification of Szent-Györgyi's (1949) procedure.

Development of tension by A.T.P.-treated glycerinated strips was determined as follows. After a two-week period of storage in a freezer at 0°C., the glycerinated bundles were transferred to 15% glycerol for one hour. Each bundle was teased into two or more strips 0.2-0.3 mm. in diameter and loops of fine silk thread were tied to the ends of each strip. Each strip was then placed between two hooks. The top hook was fixed to a Statham

strain gauge (G-7-0.3-800): the bottom hook was adjusted by means of a micrometer so that 50 mg. of tension was placed on the strip. The strip was then lowered into a muscle bath containing a perfusion solution of 0.05 M KCl, 0.005 M $MgCl_2$ and 0.0008 M $CaCl_2$ buffered at ph 8.2 with tris (hydroxymethyl) amino methane. Temperature of the bath was maintained at 25°C. After equilibration for ten minutes, the perfusion fluid was removed and replaced with the same fluid, but with added disodium salt of A.T.P. (5×10^{-3}) at ph 7.0. The isometric tension developed by the fiber was recorded by the Statham strain gauge and suitable amplifiers on a Grass recorder. The bundle was then removed, dried at 100°C., and nitrogen was determined by a micro-Kjeldahl technique.

RESULTS

In the 1956-1957 year a preliminary investigation was completed. This consisted of a comparison of myocardial function of intact male and female rats. This preliminary work will be referred to as series A in Experiment I. Series B of Experiment I consists of a study of the influence of castration in both males and females, including groups treated with two different dosages of alpha estradiol. This work was completed in the summer of 1957. Experiment II, completed in the summer of 1958, involved studies on glycerol-extracted columnae carneae and total ventricular actomyosin extraction of intact, ovariectomized and estradiol-treated ovariectomized and intact rats.

General Data

Terminal body weights

The mean terminal body weights for Experiment I and II are listed in Table 1. In series A the intact males have continued to grow while the intact females reached a plateau. Series B is consistent with series A, intact females weigh significantly less than intact males. Ovariectomy or ovariectomy with 0.1 gamma alpha estradiol does not produce a significant difference in terminal body weights from those of the intact female. Growth appears to be decreased by castration of male rats, but the difference is not statistically significant.

Table 1.

TERMINAL BODY WEIGHTS. MEANS ± S.D.

Exper. I		Groups	No.	Body Weight in Grams
Series A	1.	Intact females	15	206.4 ± 5.2
	2.	Intact males	13	247.2 ± 33.0
Series B	1.	Intact females	5	207 ± 25.2
	2.	Castrate females	6	222 ± 24.7
	3.	Castrate females 0.1 γ estradiol	8	216 ± 21.7
	4.	Intact males	4	301 ± 44.2
	5.	Castrate males	7	254 ± 68.1
	6.	Castrate males 1.0 γ estradiol	8	227 ± 28.8**
Exper. II				
	1.	Intact females	8	213.2 ± 12.8
	2.	Castrate females	8	213.1 ± 25.1
	3.	Castrate females 0.1 γ estradiol	8	208.2 ± 17.1
	4.	Castrate females 1.0 γ estradiol	8	187.7 ± 7.6*
	5.	Castrate females 10.0 γ estradiol	6	172.7 ± 12.4*
	6.	Intact females 100.0 γ estradiol	8	159.6 ± 13.5*

*Significant at < 0.01 level from intact females of Experiment II.
**Significant at < 0.01 level from intact males of Series B, Experiment I.

(Analysis of data by Unpaired Rank Analysis of F. Wilcoxon (1949)).

The mean of the castrate male groups with 1.0 gamma alpha estradiol is significantly less than that of the intact male group.

In Experiment II the intact group and the ovariectomized groups do not have different terminal body weights. It would appear that treatment with alpha-estradiol in sufficient dosage in ovariectomized rats results in a decrease in mean body weights. This decrease in body weight is statistically significant in 1.0, 10.0, and 100 gamma-treated groups as compared to the intact group.

Organ Weights: Heart, Adrenal, Uterine, and Seminal Vesicles

With the observed changes in body weights, absolute organ weights would give misleading impressions. In an effort to avoid this, wet organ weight-terminal body weight ratios have been calculated. Table 2 lists these data for Experiments I and II.

The wet heart-body weight ratios are not different in Series A or Series B of Experiment I or of Experiment II. The hearts of animals in Experiment II are larger than those of Experiment I, but no significant difference exists between groups in Experiment II.

Adrenal-body weight ratios of the intact male and intact female of Series A and Series B are significantly different. Castration of the male rat does not result in a significant increase in adrenal weight.

Table 2

WET ORGAN-BODY WEIGHT RATIOS. MEANS ± S.D.

	Groups*	Heart	Adrenal	Uterus	Seminal Vesicle
		grams per 100 grams body weight			
Exper. I					
Series A	1	2.59 ± 0.21	0.258 ± 0.023	1.70 ± 0.30	--
	2	2.63 ± 0.15	0.142 ± 0.015	--	2.39 ± 0.51
Series B	1	2.50 ± 0.13	0.271 ± 0.024	1.80 ± 0.40	--
	2	2.58 ± 0.13	0.280 ± 0.041	0.33 ± 0.04	--
	3	2.53 ± 0.26	0.320 ± 0.040	1.58 ± 0.32	--
	4	2.53 ± 0.18	0.160 ± 0.050	--	1.79 ± 0.64
	5	2.52 ± 0.13	0.225 ± 0.045	--	0.144 ± 0.065***
	6	2.58 ± 0.14	0.261 ± 0.054***	--	0.167 ± 0.050***
Exper. II					
	1	2.86 ± 0.17	0.269 ± 0.065	1.88 ± 0.50	--
	2	2.71 ± 0.12	0.300 ± 0.020	0.39 ± 0.026***	--
	3	2.78 ± 0.18	0.315 ± 0.041	1.17 ± 0.22**	--
	4	2.98 ± 0.12	0.379 ± 0.047***	1.58 ± 0.16	--
	5	3.01 ± 0.10	0.355 ± 0.040**	2.88 ± 0.87**	--
	6	2.97 ± 0.22	0.328 ± 0.058	8.01 ± 3.84***	--

*Group No. same as Table 1.
**Significant at 0.05 level.
***Significant at <0.01 level.

(Analysis of Data by Unpaired Rank Analysis of F. Wilcoxon (1949))

Castrate male rats treated with alpha-estradiol are significantly different from intact males. Ovariectomy does not result in significant adrenal weight changes. One or 10 gamma of estradiol per day in ovariectomized rats results in a significant adrenal weight increase as compared to intact females, whereas 0.1 gamma or 100 gamma-treated females are not significantly different from untreated intact females.

Uterine weight was significantly reduced in the ovariectomized groups of Series B and Experiment II. Estradiol administration significantly increased uterine weights in the ovariectomized groups. This increase is directly proportional to the quantity of estrogen administered, up to a limit.

Seminal vesicle weights of the castrate male rats are significantly reduced as compared to the intact males. Seminal vesicles of castrate males receiving estrogen were not significantly different from the non-treated castrate rats.

Water content of the heart

Water content of the myocardium was not altered in any of the groups in Series A or Series B. Table 3 lists mean group values for Series A and B of Experiment I.

Table 3.

WATER CONTENT OF VENTRICULAR MYOCARDIUM. MEANS ± S.D.

	Groups	No.	Percent Water
Exper. I			
Series A	1. Intact females	15	76.40 ± 0.62
	2. Intact males	13	77.64 ± 0.75
Series B	1. Intact females	5	78.05 ± 0.52
	2. Castrate females	6	77.89 ± 0.54
	3. Castrate females 0.1 γ ∝estradiol	8	77.89 ± 0.41
	4. Intact males	4	77.80 ± 0.68
	5. Castrate males	7	78.76 ± 1.20
	6. Castrate males 1.0 γ ∝estradiol	8	77.97 ± 0.20

(Analysis of data by Unpaired Rank Analysis of F. Wilcoxon (1949)).

Electrical Properties of Isolated Surviving Columnae Carneae

Refractory period

Table 4 shows the results of the refractory period determinations for series A and B of Experiment I. There was no significant difference between intact groups and experimental groups.

Strength-duration curve

The data for the strength-duration curves for series A are found in Table 5. No difference between the male and female groups exists. Figure 6 confirms this, since the curves are exceedingly close. Table 6 lists the chronaxie and rheobase determinations for series A and the female groups of series B. No difference was found in any of the groups. Chronaxie and rheobase determinations were not completed for the male groups of series B.

Mechanical Properties of Isolated Surviving Columnae Carneae

Passive-tension curves of isolated surviving columnae Carneae

Figures 7, 8, and 9 show passive-tensions of the groups of series A and B of Experiment I, plotted against percent of equilibrium length. The passive-tensions developed for intact male and females of series A are not different. Comparison of the intact

Table 4.

REFRACTORY PERIODS OF ISOLATED SURVIVING COLUMNAE CARNEAE

Means ± S.D.

Experiment I.

Series	Groups	No.	Milliseconds
A	1. Intact females	15	58.2 ± 5.6
	2. Intact males	13	61.4 ± 7.0
B	1. Intact females	5	56.5 ± 11.2
	2. Castrate females	6	61.6 ± 8.6
	3. Castrate females 0.1 γ estradiol	8	54.3 ± 8.5
	4. Intact males	4	57.6 ± 8.0
	5. Castrate males	7	54.6 ± 7.4
	6. Castrate males 1.0 γ estradiol	8	56.7 ± 3.8

(Analysis of data by Unpaired Rank Analysis of F. Wilcoxon (1949)).

Table 5.

STRENGTH-DURATION VALUES FOR SERIES A

Means ± S.D.

Experiment I.

Duration of Stimulus in Milliseconds	No.	Threshold in Microamperes	
		Females	Males
10	14	152 ± 19.4	121 ± 20.0
6	14	158 ± 20.8	135 ± 22.2
4	14	183 ± 22.2	159 ± 28.0
2	14	216 ± 43.3	184 ± 34.4
1	14	274 ± 52.2	221 ± 40.8
0.8	14	319 ± 69.0	273 ± 43.6
0.6	14	365 ± 69.4	335 ± 56.1
0.4	14	478 ± 92.7	456 ± 88.8
0.2	14	723 ± 110.0	843 ± 155.5

(Analysis of data by Unpaired Rank Analysis of F. Wilcoxon (1949)).

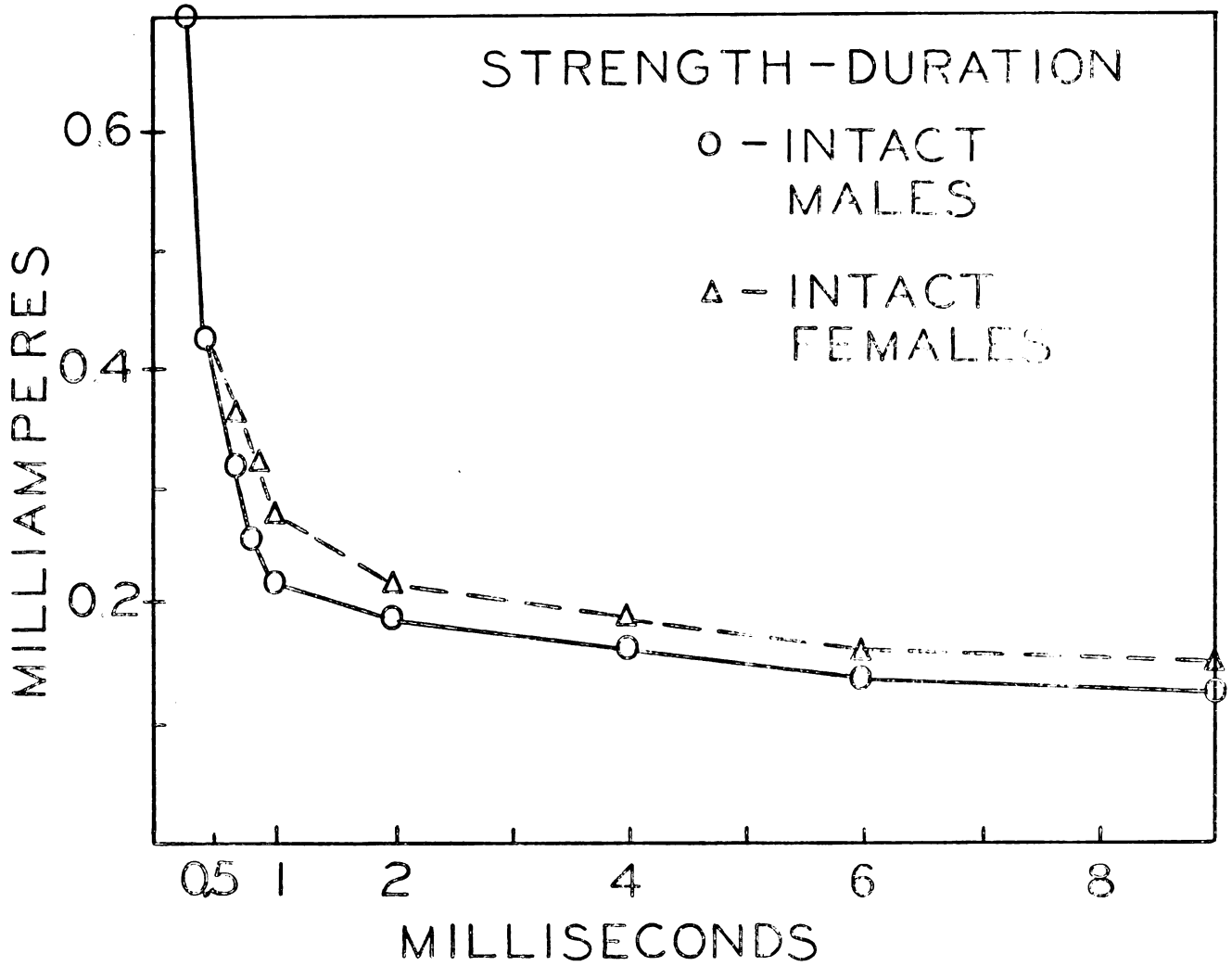

Figure 6. Strength-duration curves for intact males and females of Series A, Experiment I.

Table 6.

CHRONAXIE AND RHEOBASE FOR EXPERIMENT I. MEANS ± S.D.

	Groups	Chronaxie in Milliseconds	Rheobase in Microamperes
Series A	Intact females	0.80 ± 0.14	152 ± 17
	Intact males	0.90 ± 0.19	121 ± 19
Series B	Intact females	0.86 ± 0.16	136 ± 16
	Castrate females	0.92 ± 0.18	142 ± 20
	Castrate females 0.1 γ estradiol	1.10 ± 0.20	128 ± 19

(Analysis of data by Unpaired Rank Analysis of F. Wilcoxon (1949)).

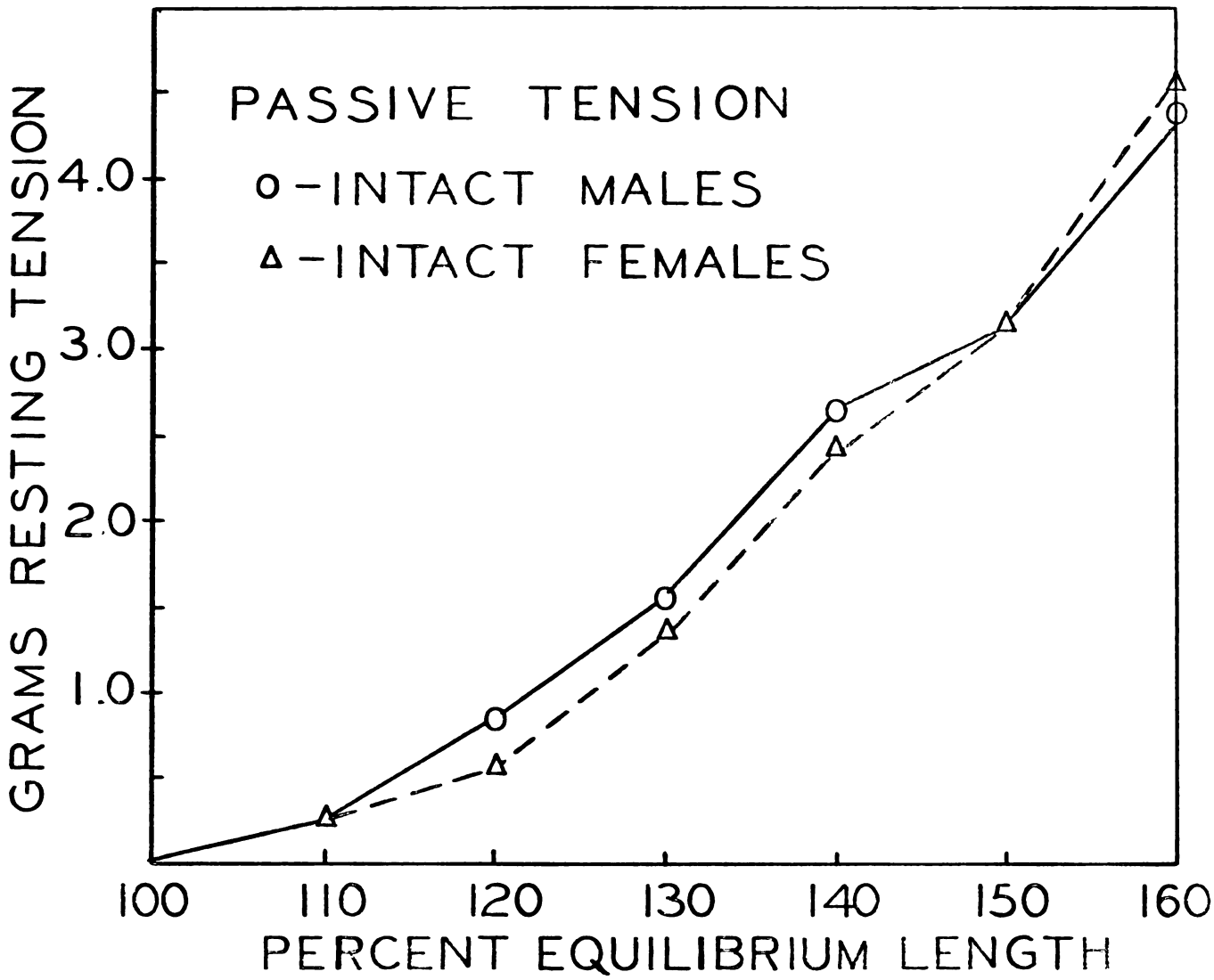

Figure 7. Passive-tension curves for intact males and females of Series A, Experiment I.

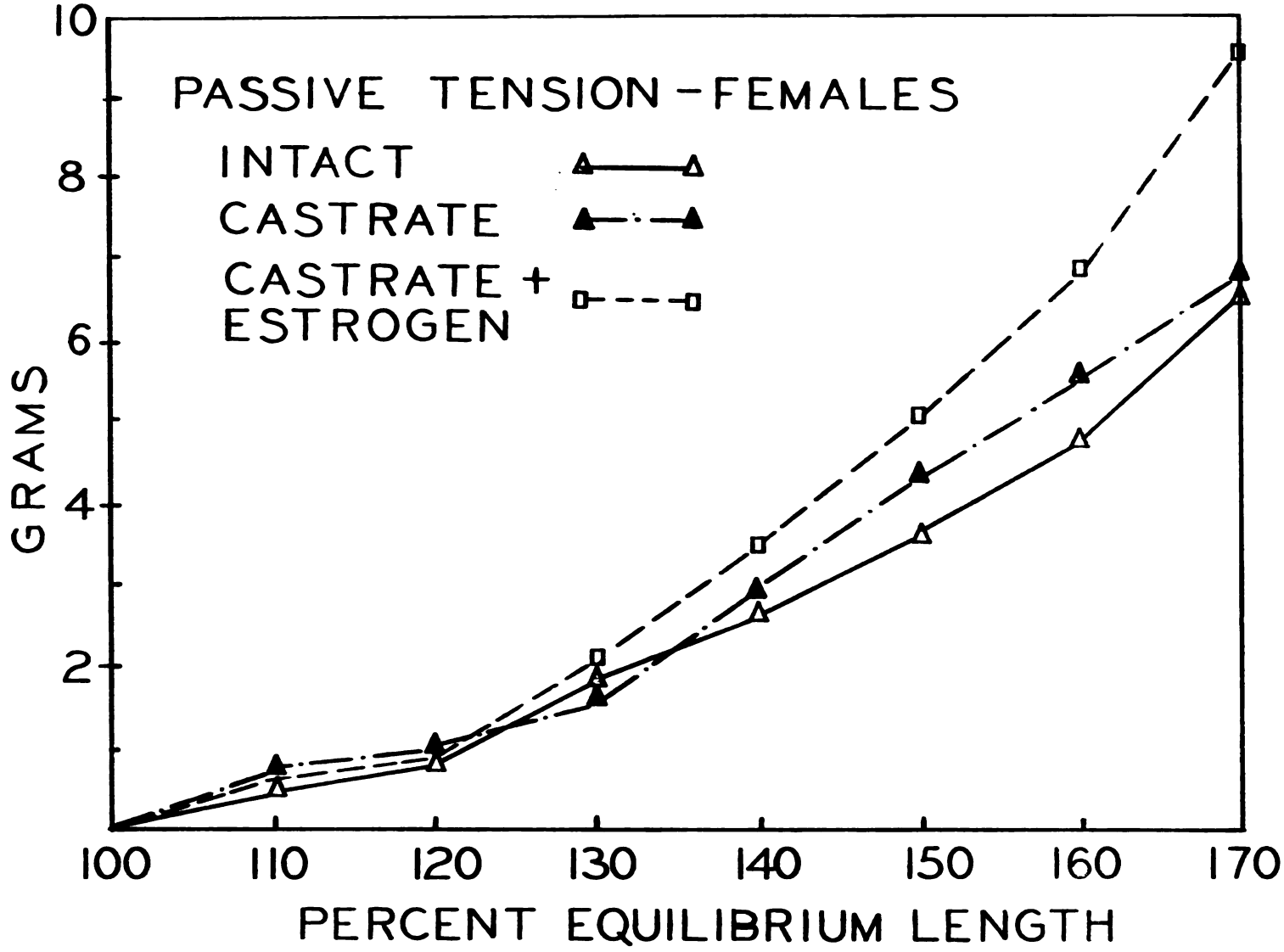

Figure 8. Passive-tension curves for female groups of Series B, Experiment I.

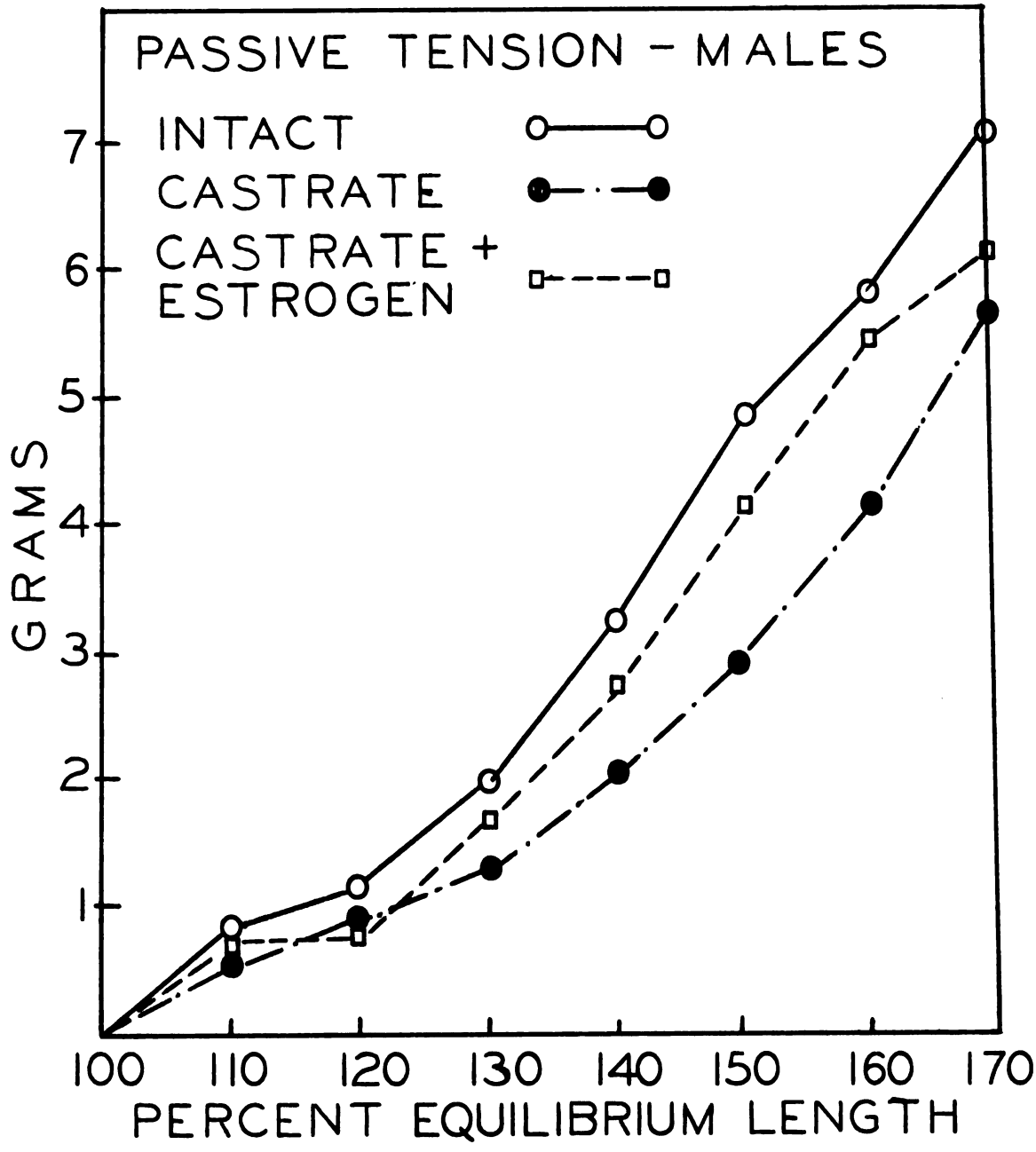

Figure 9. Passive-tension curves for male groups of Series B, Experiment I.

and experimental curves for females and males show similar shapes and slopes in all six groups of series B.

Developed-Tension of isolated surviving columnae carneae

Figures 10 and 11 show tension developed as a function of equilibrium length for the female and male groups of series B. It is clear that muscles from ovariectomized untreated animals develop less tension at a given length than do control muscles. Values for mean maximal tension development shown in Table 7 also indicate a reduction in females' due to castration. The ovariectomized treated group is not significantly different from the intact group. The male rat curves (Figure 11) show no difference between intact and experimental groups. Curves for intact male and female rats are likewise similar. Table 7 lists the maximal tension in grams per milligram of wet weight for series A and B of Experiment I. The results of series A show no difference between intact males and females. Maximal tension developed by the male experimental and control groups are not different.

Developed-Tension of Glycerol-Extracted Columnae Carneae

Table 8 lists the maximal isometric tension developed in the presence of A.T.P. Tension is expressed in milligrams per milligram of protein per millimeter of fiber length. This is an attempt to relate tension to cross-sectional area. Tension developed was significantly reduced in fibers of the untreated ovariectomized group. Treatment with increasing levels of estradiol resulted in

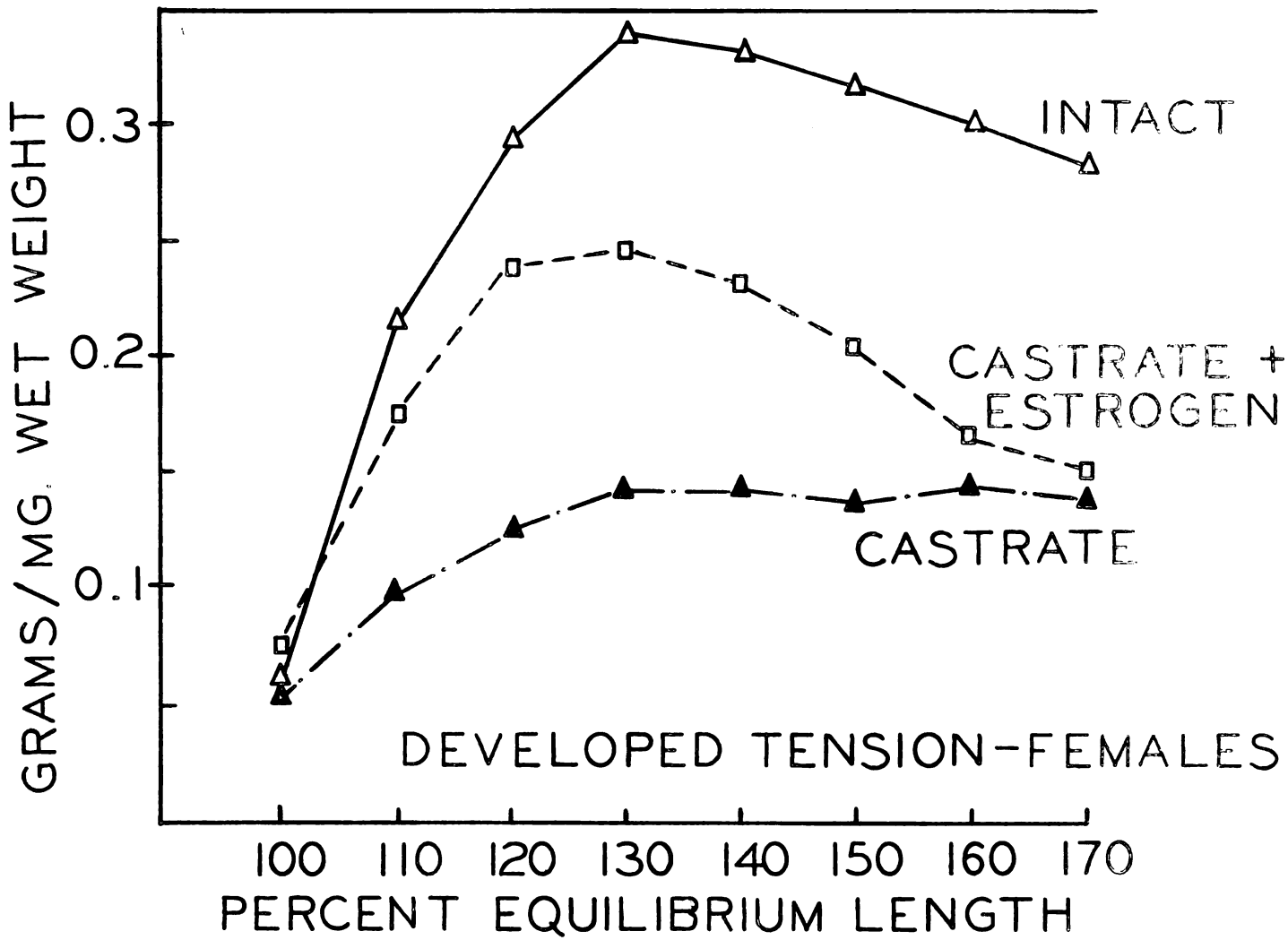

Figure 10. Developed-tension curves for female groups of Series B, Experiment I.

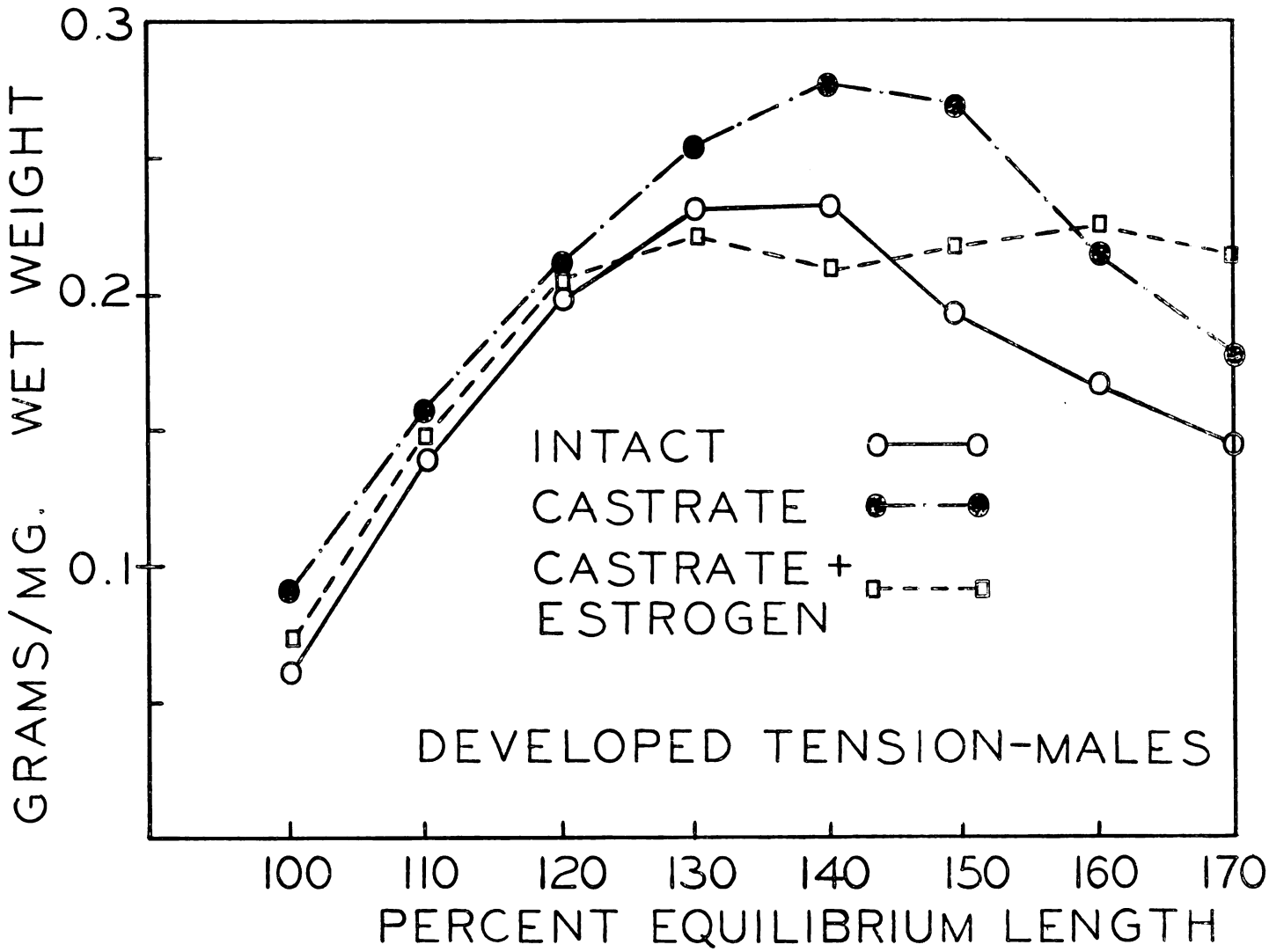

Figure 11. Developed-tension curves for male groups of Series B, Experiment I.

Table 7.

MAXIMUM DEVELOPED TENSION OF ISOLATED SURVIVING COLUMNAE CARNEAE

Means ± S.D.

	Groups	Maximum Tension grams/mg. wet weight
Series A		
	1. Intact females	0.256 ± 0.096
	2. Intact males	0.253 ± 0.080
Series B		
	1. Intact females	0.355 ± 0.081
	2. Castrate females	0.156 ± 0.056*
	3. Castrate females 0.1 γ estradiol	0.253 ± 0.034
	4. Intact males	0.238 ± 0.091
	5. Castrate males	0.294 ± 0.117
	6. Castrate males 1.0 γ estradiol	0.284 ± 0.058

*t test = $\dfrac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{\dfrac{S_1^2}{n_1} + \dfrac{S_2^2}{n_2}}}$

where $\bar{Y}_1$ and $\bar{Y}_2$, and S_1^2 and S_2^2 are the respective means and variances of the two series of n_1 and n_2 individuals.

$t_{(9)} = 2.35$ -- significant at < 0.05 level.

Table 8. MAXIMAL DEVELOPED TENSION AND NITROGEN CONTENT OF GLYCEROL-EXTRACTED COLUMNAE CARNEAE. MEANS ± S.D. EXPERIMENT II.

Groups	No.	Maximum Tension in mg./mg. of protein/mm. of length	Percent Nitrogen***
Intact females	8	747.0 ± 157*	65.33 ± 6.9
Castrate females	8	274.0 ± 43*,**	69.20 ± 13.4
Castrate females 0.1 γ estradiol	8	355.0 ± 120*	64.04 ± 11.2
Castrate females 1.0 γ estradiol	8	574.1 ± 141	65.71 ± 15.0
Castrate females 10.0 γ estradiol	6	692.5 ± 209	69.26 ± 9.1
Intact females 100.0 γ estradiol	8	815.9 ± 212**	62.78 ± 11.2

*$F_{2,21}$ = 4.44, 5% - 3.47, 1% - 5.78, significant at < 0.05 level.

**$t_{(14)}$ = 2.44, 5% - 2.14, 1% - 2.97, significant at < 0.05 level.

*** (Analysis of data by Unpaired Rank Analysis of F. Wilcoxon (1949)).

increasing the mean maximal developed tension; however, there was greater variability in these groups. The untreated ovariectomized group and the 100 gamma treated intact group are significantly different. The intact treated group is not different from the untreated intact groups.

Nitrogen Content of Glycerol-Extracted Columnae Carneae

Total nitrogen of glycerol-extracted bundles was determined. These data are listed in Table 8. No significant difference in total nitrogen content was observed for the control or the experimental groups.

Quantitative and Qualitative Determinations of Total Ventricular Actomyosin

Actomyosin content of ventricular muscle was determined by precipitation with determination of nitrogen content and by change in viscosity on addition of A.T.P. Values found by these two methods are listed in Table 9. The mean values for each group obtained by the two methods agree well. The quantity of contractile protein isolated is markedly reduced in the ovariectomized group. The mean actomyosin isolated increased as the quantity of estrogen administered increased; however, the variability also increased. There is no significant difference between the estrogen-treated groups.

Qualitative information about actomyosin was also derived from viscosity studies. These data are also listed in Table 9.

Table 9. QUANTITATIVE AND QUALITATIVE STUDY OF VENTRICULAR ACTOMYOSIN. MEANS ± S.D.

Groups	No.	Actomyosin Content of Ventricles mg./gm. wet weight by ppt. N_2	Actomyosin Content of Ventricles mg./gm. wet weight by viscosity change	Intrinsic *** Viscosity	"[illegible] *** [illegible]"
Intact females	8	9.76 ± 1.02*	11.50 ± 1.93	0.268 ± 0.054	[illegible]
Castrate females	8	5.00 ± 1.20*,**	4.27 ± 1.52	0.243 ± 0.049	[illegible]
Castrate females 0.1 γ estradiol	8	8.22 ± 1.08*	6.98 ± 1.04	0.291 ± 0.086	[illegible]
Castrate females 1.0 γ estradiol	8	9.51 ± 3.37**	8.45 ± 2.26	0.240 ± 0.130	[illegible]
Castrate females 10.0 γ estradiol	6	10.76 ± 4.83**	10.54 ± 3.49	0.273 ± 0.070	[illegible]
Intact females 100.0 γ estradiol	8	14.42 ± 3.37	9.66 ± 3.57	0.273 ± 0.090	[illegible]

*$F_{2,\ 21}$ = 3.78, 5% - 3.47, 1% - 5.78, significant at < 0.05 level.

**$F_{2,\ 19}$ = 4.15, 5% - 3.52, 1% - 5.93, significant at < 0.05 level.

***(Analysis of data by Unpaired Rank Analysis of F. Wilcoxon (1949)).

Intrinsic viscosity ($\frac{\eta\ rel-1}{mg\ of\ protein\ /\ ml.}$) corrects relative viscosity for protein content. No difference between groups was found when this correction was made. As an index of actin content, expressed in viscosity response to A.T.P., "A.T.P. sensitivity" was calculated. The mean values for the ovariectomized group are somewhat lower than for the intact group, and there was some increase on estrogen administration; however, variability is large and differences are not statistically significant.

DISCUSSION

Study of isolated surviving columnae carneae of ovariectomized rats demonstrated impaired ability to develop tension upon stimulation. Developed tension of columnae carneae of estrogen-treated ovariectomized females was not unlike control females. Changes in ability to develop tension on stimulation was unaccompanied by any alteration in heart-body weight ratios, indicating that no change in work requirements was induced by the experimental conditions. Explanation of such an observation should be found in a consideration of physiology of muscle contraction. Szent-Györgyi (1953) states: "contraction in muscle is essentially a reaction of actomyosin, A.T.P., and ions". Since columnae carneae studies were completed in vitro in a controlled milieu, free of extrinsic nerves and humoral agents, an intrinsic change in cardiac muscle must have occurred. This alteration can be placed in one or more of three areas: First, changes in membrane and electrolyte composition of cells; second, changes in the energy-supplying systems; and lastly, changes in the contractile protein, actomyosin.

There were no changes in percent water of the myocardium and electrical properties of columnae carneae in experimental and control groups, suggesting that significant change in cellular electrolytes did not occur. However, determinations of transmembrane potentials and

intracellular and extracellular electrolyte concentrations were not done and there is the possibility that there were changes. The likelihood of steroids acting on membranes has been noted by many workers. Works of importance are those of Szent-Györgyi (1953) and Csapo (1959).

Szent-Györgyi, investigating the "staircase" phenomenon in frog heart, surveyed the action of a number of steroids including: estrone, testosterone, androsterone, estradiol, cortisone, progesterone, and 11-desoxycorticosterone. It was found that desoxycorticosterone, progesterone, cortisone, estrone, and testosterone abolished the "staircase" phenomenon; whereas androsterone and estradiol prolonged it. Szent-Györgyi believes the "staircase" reflects intracellular ionic atmosphere, and that when "staircase" is abolished, it means there was no change in intracellular potassium from one contraction to another. Outward flow of potassium was in balance with its influx. Going further, he concludes that decreased potassium will favor formation of actomyosin and development of tension, while increased potassium will have opposite effects. In light of his observations, one would suspect that the presence or absence of steroids, such as that seen in estrogen treatment or in the ovariectomized animal, would have a significant role in tension production.

Csapo (1959) demonstrated that uteri dominated by progesterone are relatively inactive when subjected to normal electrical or pharmacological stimuli. Maximal

tension can be obtained only by stimulating the membrane simultaneously at several points, suggesting that with progesterone, propagation of depolarization fails to occur. Such a phenomenon has been observed by Csapo (1959) when an estrogen-dominated uterus was exposed in vitro to a modified Krebs solution containing excess potassium and low sodium. The inference is that progesterone reduces both potassium and sodium gradients across excitable membranes. Marshall (1959) demonstrated existence of foci in estrogen-dominated uteri whose electrical potentials are analogous to those of the sinus node of hearts, i.e., slow membrane depolarization culminating in the upstroke of an action potential. These observations suggest an important functional site of steroid activity on cell membranes with a direct role on electrolyte transfer.

It was thought necessary, because of the work of Szent-Györgyi and Csapo, to evaluate the role of the cell membrane and electrolyte metabolism to the altered response of the experimental female groups. This was accomplished by use of a model of muscle, namely, glycerol-extracted muscle. Szent-Györgyi (1949) reported on extraction of rabbit psoas with 50% glycerol. Such a preparation represents a contractile system in which membrane effects have been eliminated. Small molecular weight compounds and electrolytes are eluted. Contraction is then induced by perfusion of glycerinated fibers in an electrolyte solution in the presence of A.T.P. Observations on such a

preparation therefore immediately implicate or rule out the importance of cell membrane and electrolyte change. The results of glycerol-extracted fiber studies completed by this writer paralleled those of isolated surviving columnae carneae studies. Glycerinated fibers of hearts of ovariectomized animals developed significantly less tension than those of intact females. Groups receiving estrogen were intermediate in their response, with increasing tension production as estrogen administration increased. One can conclude that altered ability to develop tension in these experiments must be due to changes other than at the cell membrane or in electrolyte concentration.

In the preparation of glycerol-extracted fibers, small molecular weight compounds are eluted, including high-energy phosphate compounds (Szent-Györgyi, 1949). Among these, A.T.P. or some nuclectide is required to induce contractions. Lacking this, contraction fails. Schumann (1940) demonstrated reduced quantities of A.D.P. and P.C. in hearts of castrate male rats. One would expect altered concentrations of such compounds in hearts of ovariectomized rats; however, quantitative determinations of these compounds were not performed. Since glycerol-extracted fibers in the presence of A.T.P. sufficient to induce contraction failed to achieve control tension levels, one would question the functional importance of reductions in these high-energy phosphate compounds.

The finding that reduced developed tension in relation to presence or absence of estrogen in the glycerol-extracted preparation infers there is either a change in configuration of actomyosin and/or a change in quantity of actomyosin present. The literature furnishes several examples of functional and endocrine states in which actomyosin is altered either quantitatively or qualitatively. Benson (1955) reported that dogs with experimental heart failure showed a decrease in concentration of ventricular actomyosin, reduced viscosity per unit of actomyosin, and decreased viscosity response to the addition of A.T.P. Benson suggested that a defect exists in longitudinal orientation of actomyosin produced by increased resting tension characteristic of hearts in failure. Ellenbogen et al. (1959) found a molecular weight change in actomyosin isolated from dog myocardium in failure; molecular weight increased from 223,000 to 740,000. Blasius et al. (1956) observed no change in quantity of actomyosin isolated from hearts of castrate male rabbits as compared to hearts of intact males. However, the concentration of actomyosin in cardiac muscle of castrate male rabbits was increased by testosterone treatment. Endocrine effects on actomyosin of skeletal and uterine muscle have been reported. Testosterone is known to play a regulatory role on concentration of actomyosin of head and neck muscles of guinea pigs (Scow et al., 1955), and on the levator ani group of muscles of male rats (Stewart, 1955).

Estrogen has been said to have such a function in only one muscle, namely, uterus. Csapo (1948, 1950 a, 1950 b) demonstrated that the primary hormone involved in metabolism of contractile proteins of the uterus is estrogen.

In this writer's work, actomyosin of the myocardium was studied both quantitatively and qualitatively in intact, ovariectomized, and estrogen-treated groups. The results demonstrate that estrogen has a fundamental role in function of the myocardia of female rats. Alterations in tension in isolated surviving columnae carneae and glycerinated columnae carneae are related directly to quantitative changes in actomyosin concentration.

Qualitative changes in the actomyosin molecule may be ascertained by determination of "A.T.P. sensitivity" (as described in "Materials and Methods") and for intrinsic viscosity. Measurement of viscosity response of isolated actomyosin in the presence of A.T.P., calculated as "A.T.P. sensitivity", has been considered to be a measure of the actin content (Portzehl *et al.*, 1950). "A.T.P. sensitivity" values for the ovariectomized group are somewhat lower than for the intact group; and there was some increase on estrogen administration, but variability was large and differences are not statistically significant. Intrinsic viscosity ($\frac{\text{n rel-1}}{\text{mg. of protein/ml.}}$) relates relative viscosity (see page 61) to protein concentration. When thus expressed in terms of protein concentration, no differences in viscosity were observed in any group.

The results reported here parallel those obtained for uterus by Chaps et al. (1953), who found the lowest quantities of actomyosin when circulating estrogen was low or absent. In pregnancy or with increasing quantities of estrogen, actomyosin increased. This was also substantiated by Blasius et al. (1955) and Cretius (1957). With respect to actomyosin content, cardiac and uterine muscle respond similarly to estrogen. However, estrogen results in a marked increase in uterine-body weight ratios, whereas heart-body weight ratios are not influenced by the presence or absence of estrogen. The conclusion is that estrogen has, in addition to the classical growth-promoting action on uterus, a prominent role in specific contractile protein synthesis.

Several questions immediately arise if one postulates an estrogen function in maintenance of myocardial actomyosin. First, what maintains or regulates actomyosin concentration in the myocardium of male rats? In Experiment I, three male groups were investigated. No differences in tension production of isolated surviving columnae carneae of intact, orchiectomized, or estrogen treated orchiectomized males were found. The results in orchiectomized and intact male groups are consistent with Blasius' et al. (1956) findings. Lack of effect in hearts of males receiving 1.0 gamma alpha estradiol could be interpreted in a number of ways. Since Blasius et al. (1956) found that increasing quantities of testosterone induced increasing

quantities of actomyosin in hearts of castrate male rabbits, it could be postulated that estrogen's influence on actomyosin is limited to hearts of females, while testosterone's activity would be limited to hearts of males. Such an idea is not consistent with our knowledge of hormone action. Rather, one could speculate and state that there is a difference in the sensitivity of the involved metabolic systems; that is, to produce an effect in males, the quantity of estrogen needed is many times greater than that required to produce similar effects in females.

The lack of effect of castration on developed tension of male hearts does not rule out the effectiveness or importance of estrogen in males. The adrenal glands could well be altering their output of estrogenic principles, in response to elevated gonadotrophins. The most likely reason for a difference in response to orchiectomy and ovariectomy on tension production is that they do not produce equivalent alterations in endocrine balance.

The question as to whether the increased actomyosin found with estrogen administration is mediated directly by estrogen or indirectly through the pituitary should be considered. Experiments reported in this thesis have not taken into account effects of the pituitary. No data are reported on hypophysectomized rats. The literature shows that hypophysectomized rats have a reduced heart-body weight ratio, and that administration of growth hormone results in an increase in heart weight (Beznak, 1952).

Growth hormone, as well as specific trophic hormones, thyroid-stimulating hormone, and the adrenocorticotrophic hormone may well be involved in cardiac muscle metabolism. As in all endocrine studies, ablation of one gland results in changes in the entire endocrine constellation. In comparison of intact females to intact male rats, no difference in tension production of isolated surviving columnae carneae was found. This is consistent with Blasius' et al. (1956) work, where no difference was found in actomyosin content in hearts of intact males and females.

The next question of importance is: If ovariectomy produces such pronounced changes in actomyosin concentration, does it also produce in vivo alterations in cardiac function? None of the ovariectomized animals gave any indication of cardiac failure, i.e., edema, pulmonary congestion, or excessive weight gains. A possible explanation for the failure to observe a reduction in cardiac reserve is the reduced physical activity reported for ovariectomized females (Pincus, 1950). Operated animals were not exercised or stressed. If they had been, it is possible that there would have been a demonstration of reduction in cardiac reserve.

Finally, if cardiac actomyosin increase is a direct effect of estrogen, how is this mediated? A search of the literature reveals no studies of estrogen effects upon cardiac enzymes. Villee (1955) observed an estrogen-sensitive enzyme system which increased utilization of

oxygen and pyruvate. This observation was first made in the endometrium but was later extended to the mammary glands, ventral-prostate gland, and pituitary; while liver and kidney did not contain such an estrogen-sensitive system. No statement of investigation of heart muscle was made. The possible regulatory role of estrogens or androgens upon enzyme systems of heart needs investigation.

Certain aspects of the studies in this thesis require special comment. This will be limited to the glycerol-extracted fibers and actomyosin studies.

Variability of glycerol-extracted fiber data was great. In part, it is believed to be due to the intrinsic nature of the preparation. Briggs' (1958) data shows a variability in the standard deviation of the means as high as 35%, while Benson's et al. (1958) work has a variability in standard deviation of 16%. Furthermore, in the present study, fibers were not tied prior to removal from the hearts but rather in situ measurements were made; and after removal, the bundles were stretched to in situ length and then tied. Some bundles could not be stretched to in situ length and some were stretched to lengths greater than in situ length. Since the magnitude of contraction is dependent on initial length of the preparation, one may see an obvious reason for variability.

With respect to the actomyosin study, one should mention literature reports on quantities of actomyosin isolated from different muscles. It has been demonstrated

that actomyosin is found in decreasing quantities in skeletal, cardiac, and uterine muscle (Csapo, 1950 a). The quantity of actomyosin isolated per unit weight of the heart depends on the chamber from which the sample is taken. Left ventricle has more than right ventricle (Kovats, 1949). Similarly higher concentrations of actomyosin are found in the fundus of the uterus as compared to the cervix (Cretius, 1957). Values reported for dog (Benson et al., 1955), pig (Kovats, 1949), and rabbit (Kovats, 1949) hearts expressed in percent wet weight of tissue are, 5.4 - 5.7%; 2.9 - 3.5%; and 2.0 - 2.7%, respectively. Rat heart actomyosin concentration first reported here was 0.5 - 1.0%. This value is approximately half that reported for a closely related species, the rabbit. Total protein determinations on ventricular myocardium were not done; but rather, total protein was determined on the extracted fibers. No change in total protein in any of the female groups was found. It is easily seen that actomyosin could be greatly reduced before any change in total protein would be noted.

The difficulty in extracting actomyosin from cardiac muscle should be mentioned. Szent-Györgyi (1949) first attempted this, and encountered such problems that he selected the rabbit psoas as his source of actomyosin. The first investigator to isolate and quantitate cardiac actomyosin was Kovats (1949).

This writer realizes that the study reported here is incomplete. Data on hypophysectomized rats and an extensive study of hearts of male rats is necessary. In addition to tension and actomyosin studies on the above groups, females with higher dosages of estrogen should also be investigated. It would be interesting to note the effects of these steroids on smooth muscle other than the uterus and on skeletal muscle. If consistent results could be found for the mentioned groups and smooth muscle, such as the taenia coli, a more unified theory of muscle development and maintenance by steroids would be possible.

SUMMARY AND CONCLUSIONS

1. The influence of castration and estrogen administration on the properties of the myocardium of the albino rat was studied.

2. Heart weight, heart-body weight ratios, and water content of the myocardium were not altered by ovariectomy, orchiectomy, or estrogen treatment of castrate males and females. No differences in these parameters existed between intact male and female groups.

3. Expected changes in organ-body weight ratios for adrenal, uterus, and seminal vesicles were found in the experimental groups.

4. *In vitro* studies of isolated surviving columnae carneae revealed no differences in excitability curves or refractory periods between intact females, intact males, and experimental female groups. Study of mechanical properties revealed a significant reduction in mean maximal developed tensions in castrate females, as compared to intact females. Developed tension in estrogen-treated castrate females was not different from intact females. No significant difference in developed tension was observed in any of the male groups. Passive tension curves were not altered in any of the male or female groups.

5. Maximal A.T.P.-induced tension of glycerol-extracted fibers of hearts of untreated castrate females

was significantly reduced from that of the intact females. Tensions developed by fibers of estrogen treated castrate groups were not different from intact controls. Total nitrogen content of the glycerol-extracted columnae carneae was unaltered by ovariectomy or estrogen treatment.

6. Total ventricular actomyosin content was significantly reduced in ventricles of castrate female groups, as compared to ventricles of intact females. Actomyosin content increased in the estrogen treated castrate groups. No qualitative change in actomyosin was found.

7. It is concluded that there exists a dependence of the cardiac contractile system on estrogen, similar to that known for the uterus. This suggests a general effect of estrogen on contractile protein of all muscle.

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