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CUTANEOUS COLLAGEN AND HEXOSAMINE AND FEMUR COLLAGEN OF TESTOSTERONE PROPIONATE-TREATED RATS OF VARIOUS AGES*

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Abstract—Body weight, collagen per total skin, hexosamine per total skin, hexosamine-to-collagen ratios of the total skin, and collagen per femur were determined in normal and in testosterone propionate-treated (1 to 21 daily injections) weanling, young adult, and adult male and female rats. Differences with age and sex in the response of the substances measured to testosterone propionate were observed. The body weight of the testosterone propionate-treated male rats was not significantly greater than that of the control rats at any experimental period, but in each age group of female rats, the testosterone propionate treatment resulted in significantly increased body weights at certain experimental periods. An anabolic effect from testosterone propionate on collagen was observed only in the skin and femur collagen of the weanling female rats. The response of skin and femur collagen to testosterone propionate was different in some experimental groups. The hexosamine-to-collagen ratios did not indicate a consistent and significant change in the amount of hexosamine-containing substances of the ground substance (acid mucopolysaccharides and mucoproteins) relative to fibers (collagen) in the skin of either male or female testosterone propionate-treated rats.

ALTHOUGH the anabolic and androgenic effects upon some tissues of testosterone and certain of its derivatives have been extensively investigated, only a few studies have been concerned with the possible action of testosterone and related steroids on connective tissue. Administration of testosterone to young male rats[1] resulted in longer, thicker collagen fibers, but a decrease in the ground substance of the surviving mesentery. Testosterone has been shown to increase collagen formation in the skin of capons.[2] An increase in neutral salt-soluble skin collagen, but no change in the quantity of citrate-soluble skin collagen, was observed in testosterone-treated young male rats.[3] Collagen formation in carrageenin granulomas in the guinea pig was found to be unaltered by testosterone administration.[4] Healing wounds of testosterone-treated rats had increased tensile strength[5, 6] but no significant change in hexosamine (a component of the mucopolysaccharides and mucoproteins of the ground substance) or water content.[5] Jorgenson and Schmidt[5] also reported that testosterone treatment did not alter hydroxyproline (and thus collagen) or hexosamine content of granulation tissue in the rat. Other workers[7] have concluded that in male rats testosterone stimulates the growth of granulation tissue and accelerates its maturation. Various testosterone derivatives have also been investigated relative to their effects on granulation tissue and on wound healing.[8–10] The results of these later studies are not uniform

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and are in disagreement with some of the above results. The variability of the conclusions reached from the later studies may be due to basic differences in steroid action or quantity of steroid administered.

In most of the studies mentioned, 5–10 mg of testosterone or testosterone propionate/kg body weight was administered daily. The anabolic and androgenic responses of various tissues in normal rats, which received quantities of testosterone propionate similar to those administered in the studies upon connective tissue, have been reported. The administration of 300 and 1,000 μg of testosterone propionate daily to male rats (average initial weight, 48 g) with free access to food and water resulted in significant increases ($P < 0{\cdot}01$) in the weight of the levator ani muscle, the ventral prostate, and the seminal vesicles.[11] The 300-μg daily dosage failed to change body weight significantly, while the 1,000-μg daily dosage resulted in a body-weight increase significant at $P < 0{\cdot}05$. Kochakian[12] administered 2·5 mg testosterone propionate daily for 136 days to male rats fasted for 1 week prior to the start of injections (275–300 g body weight before fasting). At the termination of the experiment, the body-weight gain of the treated rats was 14·8% less than that of the controls, but the seminal vesicles and prostate of the treated animals weighed 2·5 times those of the control rats. Kochakian and Webster[13] treated adult male rats (450 g) with 2·5 mg testosterone propionate daily for 28 days. The observed loss in body weight was due primarily to loss of fat from the subcutaneous and abdominal areas. Although the protein content of the skin was decreased, the weight and protein content of the seminal vesicles and prostate were more than doubled. Female rats which received daily 6 mg testosterone propionate/kg from weanling to 70 days of age had definite enlargement of the clitoris and marked changes in the hair texture not observed at lower doses.[14] The testosterone propionate treatment produced marked maturation of the skeleton. The authors concluded that at this level of testosterone propionate administration, the androgenic effect of the hormone was greater than the anabolic effect.

The endogenous production of at least certain hormones varies with age and sex. In studying the effects of hormones on animals, this fact is infrequently considered and may account for much of the variability of conclusions reached in studies in which hormones are administered to animals. This paper gives the body weight, collagen per total skin, hexosamine per total skin, and hexosamine-to-collagen ratios of the total skin and collagen per total femur in male and female rats of different ages treated with an equal amount (based on animal weight, 10 mg/kg) of testosterone propionate. Analytical results are expressed as quantities per total skin, since the testosterone treatment may result in changes in concentration of the substances analyzed in dry fat-free skin (by effects on other components of the skin) without having any effects on the total quantity of the components analyzed and since total quantities better describe any effects of the hormone on the substances quantitated which may occur when animal growth rates are altered. The analytical results are compared in relation to possible differences in testosterone action upon rats of different ages and sex under the conditions of the present investigation.

EXPERIMENTAL PROCEDURE

Separate experiments were performed with 60 weanling, 60 young adult, and 60 adult male Holtzman-strain rats (initial age and average weight 27 days, 70 days,

and 1 year; 68, 271, and 575 g respectively) and 60 weanling, 60 young adult, and 60 adult female Holtzman-strain rats (initial age and average body weight 29 days, 71 days, and 1 year; 87, 218, and 360 g respectively). The animals of each age and sex classification were divided into 10 weight-matched groups of 6 rats per group. Five groups of each age and sex received 10 mg testosterone propionate (50 mg/cc in cottonseed oil) per kg initial body weight i.m. daily. Control animals did not receive injections. Previous studies demonstrated that injections of similar volumes of oil did not affect the body weight or quantities of substances measured in the present study. All animals were fed a standard lab chow and tap water *ad libitum*.

A group of untreated (control) and a group of testosterone propionate-treated rats of each age and sex were sacrificed 1, 4, 7, 14, and 21 days after the start of testosterone injections. The rats were shaved with electric clippers and the entire skins except for the face, paws, and tail were removed. Each skin was freed from adhering fat and muscle and finely minced. The minced-skin samples from the 6 weanling animals in each experimental group were pooled prior to analysis in order to obtain adequate tissue for all analytical procedures utilized (the tissues were subjected to additional studies not contained in the present report). The pooled minced-skin samples from the weanling rats and each minced-skin sample from the other animals were transferred to weighing bottles, and 0·5-g aliquots of each were removed for analyses of hydroxyproline and hexosamine and extracted for 48 hr with ether: alcohol (1:1 by volume) in a Soxhlet extractor. Hydroxyproline analyses were performed by the method of Prockop and Udenfriend.[15] Collagen content was calculated by multiplying hydroxyproline content by 7·46.[16] Hexosamine was determined by the Boas modification[17] of the Elson-Morgan reaction after purification of the hydrolysates on ion-exchange columns (Dowex AG 50W-X8). Collagen per total skin and hexosamine per total skin were calculated from the total weight of the skin after mincing and the weight of the aliquots analyzed.

One femur was removed from each rat, dissected free from soft tissue, defatted by extraction for 48 hr in a Soxhlet extractor with 1:1 (by volume) alcohol-ether, and dried. The hydroxyproline content of each bone was determined by analysis of the hydroxyproline in hydrolysates of the whole bones by the Leach modification[18] of the Neuman-Logan procedure. Collagen content was calculated by multiplying hydroxyproline content by 7·46.[16] Statistical evaluation of all data was made by comparison of the 6 testosterone-treated rats in each experimental group with the corresponding group of 6 control animals.

RESULTS

The testosterone propionate treatment affected the body weight of the male and female rats differently (Table 1). The body weight of the testosterone-treated male rats was not significantly greater than that of the control rats at any experimental period of any of the three age groups. The only experimental period at which the body weight of the testosterone-treated male rats was significantly less ($P < 0·01$) than the corresponding control rats was that of the young adult rats after 21 days of testosterone treatment. In contrast, testosterone treatment of the female rats resulted in significant ($P < 0·01$) increases in the body weight, at certain experimental periods, of each of the three age classifications of rats (Table 1). After 14 and 21 days of testosterone treatment, the body weight of the testosterone-treated weanling female rats was significantly greater ($P < 0·01$) than that of the control animals. Through 14 days

TABLE 1. BODY WEIGHT OF CONTROL AND TESTOSTERONE-TREATED RATS

Days on experiment	Body weight (g)								
	Weanling			Young adult			Adult		
	C*	X†	X/C × 100	C	X	X/C × 100	C	X	X/C × 100
Male									
1	72 ± 2‡	72 ± 3	100	266 ± 4	267 ± 3	100	557 ± 13	569 ± 8	102
4	91 ± 3	96 ± 4	105	284 ± 5	289 ± 5	102	563 ± 16	559 ± 17	99
7	116 ± 3	118 ± 6	102	307 ± 4	295 ± 5	96	568 ± 15	561 ± 14	99
14	158 ± 4	160 ± 4	101	335 ± 6	324 ± 5	97	603 ± 31	544 ± 8	90
21	188 ± 11	179 ± 5	95	366 ± 8	319 ± 2§	87	588 ± 23	565 ± 5	96
Female									
1	89 ± 3	89 ± 2	100	214 ± 4	212 ± 4	99	361 ± 7	367 ± 7	102
4	111 ± 2	112 ± 3	101	221 ± 5	225 ± 4	102	360 ± 7	366 ± 5	102
7	128 ± 4	136 ± 4	106	226 ± 4	240 ± 5	106	369 ± 6	381 ± 7	103
14	153 ± 2	173 ± 5§	113	238 ± 4	259 ± 5§	109	374 ± 6	392 ± 10	105
21	171 ± 5	192 ± 5§	112	280 ± 9	267 ± 5	95	370 ± 6	397 ± 7§	107

* Control.

† Testosterone-treated.

‡ Mean ± standard error of the mean.

§ Differs significantly ($P < 0.01$) from corresponding control values.

on experiment, the body weight of the testosterone-treated young adult female rats increased more rapidly and after 14 days on experiment was significantly greater ($P < 0.01$) than that of the control animals. From 14 to 21 days on experiment, the growth rate of the testosterone-treated young adult female rats decreased to less than that of the control rats, and after 21 days on experiment, the body weight of the testosterone-treated young adult female rats was somewhat less than that of the control animals (difference not statistically significant). The body weight of the control adult female rats did not vary significantly with experimental period. However, after 21 days of testosterone treatment, the body weight of the testosterone-treated adult female rats had increased to a value significantly greater ($P < 0.01$) than that of the control rats. All of the testosterone-treated rats remained vigorous and in apparently good health throughout the experiment.

The response of dermal collagen to testosterone propionate was found to vary with age and sex. There was no significant increase from control values in collagen per total skin in any group of the three ages of testosterone-treated male rats. At 7 days and at subsequent experimental periods, the collagen per total skin was decreased from control values in each age group of testosterone-treated male rats, but the only significant decreases ($P < 0.01$) were those observed from 7 to 21 days in the young adult rats. The larger quantity of collagen per total skin (and also hexosamine per total skin) observed at 4 days in both control and testosterone-treated adult male rats was due to a greater amount of skin obtained from the 4-day experimental groups. The body weight and collagen per total skin of the testosterone-treated rats expressed as a per cent of control values are given in Tables 1 and 2. From 7 to 21 days on experiment these calculations indicate that in each age group of male rats the collagen per total skin of the testosterone-treated rats decreased from control values relatively more than did the body weight.

In contrast to the indications of an unfavorable effect of testosterone propionate on the cutaneous collagen of male rats, in the dosage used in the present experiments, no significant decreases from control values or trend toward lower collagen per total skin were observed in any group of the testosterone-treated female rats (Table 2). If the coefficient of variation of the collagen per total skin from the pooled samples of the weanling female rats is assumed to be the same as the average coefficient of variation of the collagen per total skin of the control young adult female rats (4·0%), the collagen per total skin of the testosterone-treated weanling female rats is significantly greater ($P < 0.01$) than that of the control rats after 21 days on experiment. In contrast to the male rats, when the body weight and collagen per total skin of the testosterone-treated female rats were expressed as a per cent of control values (Tables 1 and 2), it was found that neither body weight nor collagen per total skin of the testosterone-treated female rats was more consistently increased or decreased relative to control values. The values for collagen per total skin (and also hexosamine per total skin) of both the control and testosterone-treated young adult female rats at 7 days and the adult female rats at 1 day are low, probably because of mechanical loss of skin during mincing. The 4- and 14-day values for collagen per total skin (and also hexosamine per total skin) of the adult female rats were not determined because of the lack of positive results at the other experimental periods.

The hexosamine per total skin for the various experimental groups is given in Table 3. No significant differences at $P < 0.01$ were found between the hexosamine per

TABLE 2. COLLAGEN PER TOTAL SKIN OF CONTROL AND TESTOSTERONE-TREATED RATS

Days on experiment	Collagen per total skin (g)								
	Weanling			Young adult			Adult		
	C*	X†	X/C × 100	C	X	X/C × 100	C	X	X/C × 100
Male									
1	0·44	0·42	95	3·38 ± 0·14‡	3·35 ± 0·15	99	9·5 ± 0·4	9·5 ± 0·3	100
4	0·54	0·59	109	3·39 ± 0·09	3·50 ± 0·12	103	10·7 ± 0·8	10·8 ± 0·5	101
7	0·91	0·84	92	3·78 ± 0·11	3·26 ± 0·18§	86	10·0 ± 0·5	9·3 ± 0·5	93
14	1·39	1·32	95	4·43 ± 0·09	3·44 ± 0·06§	78	9·5 ± 0·6	8·6 ± 0·3	91
21	2·29	2·02	88	4·89 ± 0·24	3·55 ± 0·12§	73	10·0 ± 0·5	8·9 ± 0·2	89
Female									
1	0·55	0·56	102	2·32 ± 0·09	2·59 ± 0·06	112	4·48 ± 0·12	4·54 ± 0·10	101
4	0·75	0·80	107	2·24 ± 0·04	2·29 ± 0·10	102			
7	0·98	0·95	97	1·94 ± 0·07	2·09 ± 0·06	108	5·39 ± 0·23	4·84 ± 0·21	90
14	1·45	1·63	112	2·46 ± 0·07	2·21 ± 0·03	90			
21	1·77	2·23§	126	2·83 ± 0·16	2·72 ± 0·11	96	5·20 ± 0·12	4·89 ± 0·21	94

* See footnotes to Table 1.

TABLE 3. HEXOSAMINE PER TOTAL SKIN OF CONTROL AND TESTOSTERONE-TREATED RATS

Days on experiment	Hexosamine per total skin (mg)								
	Weanling			Young adult			Adult		
	C*	X†	X/C × 100	C	X	X/C × 100	C	X	X/C × 100
Male									
1	3·9	3·6	94	27·7 ± 1·3‡	29·4 ± 0·7	106	51·5 ± 2·4	55·6 ± 3·4	108
4	4·8	5·9	124	26·7 ± 1·6	26·7 ± 1·5	100	58·0 ± 1·9	63·0 ± 4·8	109
7	7·6	8·1	106	32·1 ± 0·4	30·5 ± 1·0	95	57·0 ± 1·9	53·0 ± 3·5	93
14	12·2	10·6	87	32·5 ± 0·7	30·0 ± 0·7	92	49·5 ± 2·1	42·4 ± 1·2	86
21	13·6	11·9	87				53·7 ± 3·9	48·0 ± 2·9	89
Female									
1	4·3	4·2	99	14·7 ± 0·4	15·7 ± 0·6	107	20·1 ± 0·8	20·7 ± 1·1	103
4	5·6	5·8	104	15·1 ± 0·9	14·7 ± 0·6	97			
7	8·5	8·1	95	12·8 ± 0·5	13·4 ± 0·7	104	22·8 ± 1·2	23·9 ± 1·0	105
14	11·4	12·3	108	14·9 ± 0·7	14·7 ± 0·8	99			
21	12·2	12·8	105	16·6 ± 1·0	15·8 ± 0·7	96	24·5 ± 0·6	23·9 ± 0·5	98

* See footnotes to Table 1.

total skin of the control and testosterone propionate-treated male rats, although in each age class the quantity of hexosamine per total skin was less in the testosterone-treated animals subsequent to 7 days on experiment. At the 14-day experimental period, the hexosamine per total skin of both young adult and adult testosterone-treated male rats was significantly less ($P < 0.05$) than that of the control rats. There were no indications of either an increased or decreased quantity of hexosamine per total skin in any of the three age groups of testosterone-treated female rats. The body weight and hexosamine per total skin of the testosterone-treated male rats, expressed as a per cent of control values (Tables 1 and 3), indicate that, subsequent to 7 days on experiment, hexosamine per total skin was decreased from control values relatively more than was body weight in each age group of male rats. In contrast, similar calculations with data from the female rats indicate that body weight of the testosterone-treated rats was changed from control values more than was hexosamine per total skin.

TABLE 4. HEXOSAMINE-TO-COLLAGEN RATIOS* OF CONTROL AND TESTOSTERONE-TREATED RATS

Days on Experiment	Hexosamine-to-collagen ratio								
	Weanling			Young adult			Adult		
	C†	X‡	X/C × 100	C	X	X/C × 100	C	X	X/C × 100
Male									
1	0·89	0·86	97	0·82	0·88	107	0·54	0·59	109
4	0·89	1·00	112	0·79	0·76	96	0·54	0·58	107
7	0·84	0·96	114	0·85	0·94	111	0·57	0·57	100
14	0·88	0·80	91	0·73	0·87	119	0·52	0·49	94
21	0·59	0·59	100				0·54	0·54	100
Female									
1	0·78	0·75	96	0·63	0·61	97	0·44	0·46	105
4	0·75	0·73	97	0·67	0·64	96			
7	0·87	0·85	98	0·66	0·64	97	0·47	0·44	94
14	0·79	0·76	96	0·61	0·67	110			
21	0·69	0·57	81	0·59	0·58	98	0·50	0·46	92

* Milligrams hexosamine per total skin × 100/mg collagen per total skin.

† Control.

‡ Testosterone-treated.

The hexosamine-to-collagen ratios of the various experimental groups are given in Table 4. A change in the hexosamine-to-collagen ratios of the testosterone propionate-treated rats from those observed in the control animals would indicate a probable change in the relative amount of acid mucopolysaccharides and mucoproteins of the ground substance and fibers (collagen) in the skin of the testosterone-treated rats. A consistent and significant change in the cutaneous hexosamine-to-collagen ratios of the testosterone-treated rats was not evident in rats of either sex. The hexosamine-to-collagen ratios indicate that the analytical values for hexosamine per total skin, for the weanling male rats at 21 days on experiment are low.

The response of femur collagen (Table 5) to testosterone propionate was not identical with that of skin collagen. There was no significant difference between, nor any indication of a change in, the quantity of collagen per total femur of any group of

TABLE 5. COLLAGEN PER TOTAL FEMUR OF CONTROL AND TESTOSTERONE-TREATED RATS

Days on experiment	Collagen per femur (mg)								
	Weanling			Young adult			Adult		
	C*	X†	X/C × 100	C	X	X/C × 100	C	X	X/C × 100
Male									
1	13·3 ± 0·6‡	13·3 ± 0·5	100·0	63·7 ± 1·1	62·6 ± 2·0	98·3	129·5 ± 3·0	123·0 ± 5·1	95·0
4	20·0 ± 1·3	19·9 ± 0·9	99·5	63·1 ± 1·7	64·2 ± 1·9	101·7	116·6 ± 3·6	119·6 ± 2·6	102·5
7	22·6 ± 0·8	21·6 ± 0·9	95·6				125·7 ± 4·7	121·0 ± 3·3	96·3
14	34·7 ± 0·7	35·9 ± 0·7	103·5	76·5 ± 0·9	80·8 ± 2·2	105·6	125·0 ± 1·0	114·7 ± 3·1	91·8
21	42·8 ± 1·7	41·0 ± 2·0	95·8	83·6 ± 0·9	81·4 ± 2·0	97·4	116·4 ± 2·7	121·6 ± 2·1	104·5
Female									
1	18·9 ± 0·4	18·9 ± 0·6	100·0	61·9 ± 1·0	57·3 ± 1·0	92·6	98·2 ± 3·3	95·0 ± 2·2	96·7
4	25·0 ± 0·6	23·1 ± 0·1	92·4	61·8 ± 0·6	63·0 ± 1·6	101·9	94·3 ± 2·9	100·1 ± 3·4	106·2
7	24·2 ± 0·9	24·5 ± 0·3	101·2	67·0 ± 1·1	71·7 ± 1·6	107·0			
14	37·1 ± 0·9	43·8 ± 0·9§	118·1	70·6 ± 1·1	76·6 ± 2·8	108·5			
21	40·8 ± 1·2	48·1 ± 1·2§	117·9	78·1 ± 1·8	78·1 ± 0·6	100·0	109·9 ± 3·9	109·7 ± 1·9	99·8

* See footnotes to Table 1.

testosterone-treated male rats and the corresponding control animals. This result is in contrast to the data for collagen per total skin in which a significant decrease in collagen per total skin was observed at the 14- and 21-day experimental periods in young adult male rats and in which a trend toward decreased collagen per total skin was observed in weanling and adult male rats.

The collagen per total femur of the testosterone propionate-treated weanling female rats was significantly greater ($P < 0{\cdot}01$) than that of the control animals at the 14- and 21-day experimental periods. This observation is similar to that obtained for total skin collagen of weanling animals. No significant differences between collagen per total femur of control and testosterone-treated young adult and adult female rats were observed. After 14 and 21 days of testosterone treatment the dry fat-free weight, and after 14 days the per cent collagen, were significantly greater ($P < 0{\cdot}01$) in the testosterone-treated weanling female rats. No other significant differences were observed between the dry fat-free weight and per cent collagen of the testosterone-treated rats and the corresponding control animals.

DISCUSSION

Previous reports have demonstrated that testosterone propionate administered to normal rats of various ages in quantities similar to that utilized in the present experiments produces anabolic and androgenic effects upon tissues used as standard indices of androgenic activity.[11–14] The experiments described in this paper have given data on three additional aspects of the administration of testosterone propionate to normal rats: (1) the possible anabolic effect of testosterone propionate on a specific protein, collagen; (2) the comparative effect of testosterone propionate on cutaneous collagen and hexosamine and the femur collagen of rats of different ages and sex; and (3) the comparative effect of testosterone propionate on collagen from two sources, the skin and the femur.

Although testosterone has been shown to be active physiologically under many conditions in stimulating protein synthesis (anabolic activity) which is reflected in body-weight increase (skeletal muscle) and by a decrease in nitrogen excretion in the urine, at the dosage used in the present experiments testosterone propionate administration failed to increase the cutaneous or femur collagen in any of the three age groups of male rats. However, the increases observed for skin and femur collagen of weanling female rats demonstrates that in some physiological conditions testosterone propionate does have an anabolic effect on collagen. It has been observed[8] that the daily administration of 0·5 mg 4-hydroxy-17-methyltestosterone to mice promoted wound healing, but that 1·0 mg had a pronounced adverse effect. It is possible that the endogenous production of testosterone and related steroids varies with age and sex in a manner such that when a standard quantity of testosterone propionate is administered, a situation similar to the above may result.

In this series of experiments, as in most others dealing with hormonal effects on connective tissue or wound healing, the dosages of testosterone propionate are higher in relation to body weight than is usually considered therapeutic or physiological in the human. The extent to which this fact affects the results obtained can be determined only by further study. The ratio of anabolic to androgenic potency of testerone propionate is low in comparison to many structurally modificed androgenic steroids.[11, 19] These structurally modified steroids, because of their relatively greater anabolic

potency, might have anabolic effects on collagen, without undesirable side effects, not observed with testosterone propionate.

Kochakian and Webster[13] have investigated the effect of testosterone propionate on the appetite, body weight, and composition of the normal growing rat. Under conditions similar to the present investigation, an approximately 5% decrease in food consumption was noted. A decrease in body fat calorically equivalent to the change in food consumption was observed. A significant loss of protein from the skin was found, but the protein loss of the whole animal was slight. The results of the present experiments indicate that at least a portion of the previously noted loss of protein from the skin was probably from loss of collagen and also that skin collagen is not very sensitive to testosterone stimulation.

It has been observed previously that body-weight response to androgen administration is affected by the age of the rat.[20, 21] This observation may be related to the amount and composition of depot fat.[20] The differences in response to testosterone propionate with age and sex observed in the present study may similarly be at least partly related to differences in available body fat with age and sex and consequently to differences in energy and substances available for anabolic reactions. No explanation is available for the observed differences in the action of testosterone propionate on collagen from the skin and femurs.

Pearce and co-workers[6] studied the effect on wound healing of 2 mg testosterone propionate/day administered to male rats (200–300 g). In normal testosterone-treated rats acceleration of wound healing was observed 5 days after wounding. This effect was not sustained, since microscopic appearance of collagen and tensile-strength measurements of the wounds were equal 10 days after wounding in testosterone-treated and control animals. Kochakian and associates[20, 22] had previously reported that increased and accelerated assimilation of protein in testosterone-treated rats is of short duration. In the age group of the present investigation, similar to that studied by Pearce *et al.*, not even short-duration stimulation of collagen synthesis was noted. It is thus possible that testosterone propionate has a greater effect on collagen synthesis in an area of intense synthetic activity. This point deserves further study.

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