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A. KIISKINEN

PHYSICAL TRAINING AND CONNECTIVE TISSUES IN YOUNG MICE

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ABSTRACT:

The effect of physical training on the growth of Achilles tendons and long bones was studied in male mice of NMRI-strain. The mice to be trained and their controls were about 2 weeks old at the beginning of the training, which took place on a 5^{o} inclined treadmill 5 days a week for 3 to 22 weeks. The duration of daily exercise was increased progressively over 3 weeks. The final daily exercise bouts were 50 and 80 minutes for moderate programs and 180 minutes for the intensive program at a speed of 30 cm/s. At the end of each experiment the Achilles tendons, the long bones of hind and fore limbs, the heart and a blood sample were taken for analyses.

A transient increase in the dry weights of the Achilles tendons and the long bones was observed after 4 - 7 weeks' of moderate training. The intensive training increased the density and decreased the volume and length of long bones. Some programs tended to increase the breaking load of the femur. Moderate or intensive training continued to maturity brought about shorther femoral bones whereas the dry weight of the Achilles tendons and the long bones was not changed significantly.

I conclude that the physical training of growing mice accelerates the maturation process, evokes permanent changes in the length of the femoral bones and causes transient changes in the dry weight, density, and volume of the long bones. The size of the effect depends on the age of the experimental animals as well as on the intensity and duration of the training program.

INDEX WORDS: Physical training, growth, physical properties, long bones, tendons

INTRODUCTION

Growing animals exposed to substantial physical exercise develop hypertrophy of heart and skeletal muscles, tendons, cartilage and long bones (11, 10, 27). Mature animals show only muscle hypertrophy after corresponding exercise (12). Contradictory results have been published as to the exercise-induced density and longitudinal growth of bones and the height growth of men (12, 15, 18, 22, 9, 27, 21). Several publications report an increased breaking load of bone-ligament and bone-tendon preparates (36, 30). No change in the breaking load of long bones taken from trained rats, however, was observed by Saville and Whyte (27).

The inconsistency of the results may be due to variations in age, species and strain of animals, experimental methods, and expressing data in ways not comparable to each other. Therefore, no larger generalization of the results is possible; more systematic research is needed. For reviews of the subject see references: Rarick (23), Malina (20), Larson (19), Viidik (37), Booth and Gould (2), and Tipton et al. (33).

The experiments on which this report is based were designed to determine the effects of physical exercise on growing male mice. The breaking load, dry weight and longitudinal growth of long bones were measured as well as the dry weight and breaking load of tendons. The experiments were also focused on the question, whether the state of maturation has any influence on the above-mentioned parameters or on the development of the volume and density of long bones in growing mice. We also investigated and will report separately on changes arising from training in the chemical composition of long bones, Achilles tendons, skin and heart muscle.

MATERIAL AND METHODS

Animals and training procedures. Male Wistar mice of NMRI-strain (Ylä-Mankkaa, Finland), two weeks old (14 ± 2 days), were randomly assigned to test and control groups. Groups of animals trained at different times over a period of 3 years had their own control groups.

The mice to be trained were taught within a few minutes to run on a treadmill at a low speed by means of high voltage electric stimulation (Fig. 1). After learning electric stimulation was used only occasionally. The duration of daily exercise as well as running speed were progressively increased during the first 3 weeks of training, and thereafter kept constant until the end of the experiment (Table 1). The training was performed on a 5^{o} inclined treadmill operated by an electric motor at a speed of 30 cm/s 5 days a week for 3, 5, 7 and 12 weeks for training programs II and III and for 22 weeks for training program I (Fig 2). The daily exercise was divided into two sessions, one in the morning and the other in the afternoon. The operating speed of the treadmill was not changed during sessions, but the 70 cm long moving rubber belt allowed a maximum of 2 seconds standing between bouts of running. Physical activity outside the training sessions recorded by an activity meter (Animex, type DSE, Farad Electronics, Sweden) over 2 weeks (3rd - 5th weeks of training) showed no differences between the trained and control groups.

Since there were significant differences in the initial body weights between some experimental groups of mice a control experiment was carried out in which lighter (group A) and heavier (group B) mice were trained for 7 weeks by moderate training program II and by intensive training program III simultaneously (Table 6).

The death rate during 3 to 7 weeks training was about the same in all experimental groups, but after 12 weeks' training mortality in the intensive training group was markedly higher vis a vis the group in moderate training (program III: trained 63 % and control 30 %; program II: 17 % and 14 % respectively). Therefore only moderate program I was continued up to 22 weeks (death rate 23 % for trained mice, and 18 % for control mice). The trained as well as the control mice were kept in normal laboratory conditions each plastic cage (35 x 20 x 17 cm)

housing about 10 mice and a pelleted mice diet (Ylä-Mankkaa, Finland) and tap water were given ad libitum, and a day to night cycle of 12 hours was employed.

Preparation of samples and breaking load measurements. The mice were killed under ether anesthesia by decapitation. A blood sample was taken in a heparinized or EDTA tube and blood hemoglobin concentration was determined during the same day by the cyanmethemoglobin method. The hind and fore limbs and the heart were cut out using scissors at room temperature and immediately chilled in ice in sealed plastic tubes. The tissues were stored deep-frozen (at -20°C) for later preparations. For the preparations and measurements the tissues were allowed to thaw at room temperature. The Achilles tendons were carefully separated from muscle and bone tissue, dried (at 95°C overnight) and weighed (Mettler H2OT, Switzerland). The long bones were freed off from muscle and soft connective tissue as carefully as possible using scissors, scalpel and forceps and weighed. For determining volume and density the bones were weighed in distilled water at room temperature, and thereafter reweighed in air. The volume and density of the bones were calculated by the Archimedes principle. The length of the femur was measured using calipers (Mauser) and the breaking load by electric tensiometer (Märkälujuusmittari, Keskuslaboratorio, Finland, Fig. 3 a-c) bending the bone at a speed of 1 cm/min until it broke in the middle shaft; the required force was recorded automatically (variation coefficient for breaking load measurements of bones was 15.4 % and for patella tendons 14.4 %). Thereafter, the bone marrow was removed by means of compressing air through an injection needle fitted to a syringe into each half of the femur broken in the middle of the shaft and the pieces of femur and humerus were dried (at 95°C, for 2 days), and weighed.

The patella tendon was carefully isolated from muscle and bone and fastened at each end to a pair of needle holders (Shur-Hold 92, Wester Bros, USA) held at constant pressure at a calibrated distance of 1 mm and breaking load was measured pulling the tendon apart at a speed of 1 cm/min until it broke (Fig 3 d-f). The breaking occurred in most cases in the middle of the specimen and no systematic error due to the fixing of the tendon was

observed. Care was taken to avoid drying out of the specimen during preparation and measuring, and a drop of physiologic saline solution was applied if necessary.

Statistical methods. The results were statistically evaluated by Student's t-test for noncorrelating means (two sided test)

RESULTS

The pattern of body and skeletal maturation for control mice is shown (Fig. 4) as levelling off after the initial period of rapid growth. The adaptation responses of mice and their connective tissues to moderate and intensive training appear in Tables 2 to 5 and Figure 5. Although there were differences in the initial body weights no difference was observed in the final body weights and blood hemoglobin concentration between the trained and control mice after moderate training programs (Table 2). The moderate training program produced a transient increase in the dry weight of long bones and Achilles tendons at the end of the most intensive growth period after 4, 5 and 7 weeks of training (Table 3). The effect disappeared when the animals reached maturity even though training was continued up to 12 and 22 weeks. On the other hand intensive training even had an adverse influence on the heart weight and the dry weights of Achilles tendons and long bones in the animals being lighter at the beginning of training(Table 4). Particularly after 7 weeks of intensive training the weight development of tissues as well as the gain in body weight were markedly retarded. The femoral bones of trained mice remained shorter after all training programs compared to the controls (Table 5). In order to find out whether the inconsistency of results was due to overloading or to variation in the initial body weights of the experimental mice a control experiment was carried out.

In this experiment, mice from the lighter group (A) and the heavier group (B) were trained simultaneously on both training intensities for 7 weeks (Table 6). The development of body weight was retarded by both training programs in group A. The retarding of body weight development in the lighter group (A) was obviously the result of overloading caused

by excessively heavy training. The moderate training program retarded the longitudinal growth of the femur and increased the density of the femur and humerus in group A. Furthermore, the intensive training of group A retarded the dry weight gain of long bones and Achilles tendons; it also slightly reduced the increase in volume of femur and humerus. No corresponding changes were observed in group B. The results indicate that besides the intensity of the training program the age and/or weight of animals at the out-set of training also has influence on the results.

The total breaking load of the patella tendon was not changed by any training program although in relating the results to body weight an increase in the breaking load after 3 weeks' intensive training was observed (Tables 6, 7 and 8). On the other hand the total breaking load of the femur was increased after 7 weeks' moderate training, as well as after 3 and 5 weeks' intensive training where the results are related to body weight.

DISCUSSION

Several investigators have proposed the existence of critical periods during which the growing body or a part of the body is most sensitive to the growth stimulating effects of exercise. Those periods, if they exist at all, are not yet identified (20). Our results suggest that sensitivity of connective tissues to exercise in mice is at a maximum during the period of most intensive growth. This implies that special attention should be paid to the intensity of training of prepubertal animals. The age differences of 2-3 days of young (about 2 weeks' old) mice already caused differences in the effects brought about by intensive training (Table 6).

Long bones. The increase and decrease in the dry weight of long bones induced by moderate and intensive training programs respectively turned out to be transient where training was continued up to and after maturity. These changes can therefore be regarded as indications of accelerated and retarded growth (Fig. 5, Tables 2 and 4). Premature maturation in relation to chronological age was found by radiographic measurements of skeletal age in young swimmers (4) as well as in the bone volume of trained growing rats (27).

Both moderate and intensive physical training also had permanent effects on long bones. The retarded longitudinal growth of femoral bones could be due to mechanical compressive forces acting on the epiphyseal zones. At the same time, however, the increased release of the growth hormone induced by physical exercise (e.g. 25) or some other mechanisms can accelerate the maturation of bones, thus allowing the earlier closing of epiphyses. The growth of childrens' lower extremities was observed to be retarded by strenuous physical work (15). Lamb et al. (18) found shorter tibias in the prepubertal rats after swimming training and Tipton et al. (31) after 10 weeks' running program in rats. On the other hand many authors of earlier studies stated that physical exercise stimulates the growth of long bones (29, 7, 8, 12). The opinion is supported by the findings showing a longer and larger dominative upper extremity in pitchers and tennis players compared to the nondominating extremity (5, 14, 1). The increased length of upper extremities caused by exercise may also be evoked by an increased production of the growth hormone in the absence of growth impairing compressive pressure.

Besides the above-mentioned dimensional changes, other structural alterations occured in the bones of intensively trained mice: increases in density and decreases in volume (Table 6). The changes were larger in the lighter group A than in the heavier (2-3 days older) group B mice. Saville and Whyte (27) reported, after respective studies with rats an increase in bone volume but no change in bone density. In bipedal rats the increased weight bearing on the hind limbs increased the density of the bones compared to the bones of quadruped rats (26). King and Pengelly (17) observed an increased density in rat tibias after running training. Nilsson and Westlin (21) found the femoral bones of weight lifters to be densest, followed by those of runners and thereafter by those of swimmers. The results give further support to the assumption that compressive forces influence bone density. Prolonged intensive dynamic training, however, also seems to play a role in the question (6).

I found significant changes in the total breaking load of femur only after one training program (Table 7). Saville and Whyte (27), failed to observe any change in 'breaking force' (compression) in their

corresponding studies with growing rats, whereas Smith and Saville (28) found increased breaking force in the bones of bipedal rats compared to quadrupeds. Our experiments as well as those of Saville and Whyte (27) would probably have shown more convincing changes in breaking load if more accurate methods were available. Furthermore it should be noted that the transverse loading used in my measurements stresses bones in a direction basically different from the functional loads during training. According to Wolff's law one could expect more specific and greather changes after training in the compressive breaking load of bone.

Tendons. Moderate training accelerated the gain in the dry weight of Achilles tendons in growing mice (Table 2), but equalled the total dry weight of Achilles tendons in untrained mice at the end of the growth period. Viidik (34) similarly found no differences in the dry weight of tendons between trained and untrained rabbits after prolonged exercise. The results support the assumption that exercise induced transient increases in the dry weights of tendons during growth could be considered as an indication of accelerated maturation.

Trained and untrained mice had the same total breaking load resistance in patella tendons and only a slight increase where the results are related to body weight (Table 8). In most of the earlier experiments an increase in breaking load was observed with bone-ligament and/or bone-tendon preparates. Only two studies failed to confirm this finding (3, 24). The breaking load values of those studies, however, are not comparable with those of mine, because the rupture occurs almost always at the insertion point of ligament or tendon to bone. So far only Viidik (34) has reported experiments with isolated tendons. The results were interpreted to show an increase in the 'maximal and linear load' values of tendons in the trained rabbits. This statement was made after reducing absolute results to units of fresh weight per unit length (according to Viidik, 35). Expressing data in this way is comparable to making calculations per cross section of the sample where there are no differences in the density of the tissues. If the results had been given, however, in relation to the collagen content of the sample per length unit of the sample (Viidik's recent method) the result from the same original data

would have shown a decrease in the maximal breaking load of the tendons assuming the tendons which where tested mechanically (tendo m. tibialis posterioris, tendo m. peronei longi, and tendo m. peronei quarti) and those analysed for hydroxyproline (peroneus brevis tendon) reacted identically to the stimulus of training. We also have reported increased tensile strength values for isolated patella tendons based on the data related to the body weight of experimental animals (16). This data presentation, however, does not reveal real difference in the breaking load values.

Additional research with more accurate methods is needed to clarify whether training affects the breaking load of isolated tendons and bones. Changes in other rheological variables of soft connective tissues such as elastic stiffness, elongation, and viscosity also await further research.

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TABLE 1. Training programs

Training week	Training intensity (min/day)		
	Moderate		Intensive
	I	II	III
1	15	20	60
2	25	40	120
3 - 22	50	80	180

TABLE 2. Effects of 3 to 22 weeks' moderate training (I+II) on the gain of body and heart weight and the blood hemoglobin concentration

Variable	Training time (weeks)	Animals: Trained			Animals: Control			p
Initial body weight (g)	(3)	11.1 ±	1.6	(33)	11.0 ±	1.6	(43)	N.S.
	(4)	12.4 ±	1.6	(42)	12.5 ±	2.3	(36)	N.S.
	(5)	10.8 ±	1.6	(10)	12.1 ±	0.9	(10)	<.05
	(7)	12.7 ±	2.2	(28)	9.9 ±	1.8	(24)	<.001
	(12)	10.6 ±	3.2	(51)	10.4 ±	1.9	(22)	N.S.
	(22)	11.9 ±	5.5	(85)	12.4 ±	5.4	(63)	N.S.
Final body weight (g)	3	28.7 ±	2.8	(30)	28.8 ±	3.1	(28)	N.S.
	4	32.4 ±	3.9	(27)	31.7 ±	3.9	(29)	N.S.
	5	35.1 ±	3.8	(6)	34.3 ±	3.3	(6)	N.S.
	7	34.5 ±	2.8	(21)	33.9 ±	2.9	(11)	N.S.
	12	37.1 ±	3.8	(19)	37.9 ±	2.3	(18)	N.S.
	22	41.8 ±	4.6	(20)	42.1 ±	5.2	(22)	N.S.
Wet weight of heart (mg)	3	132.5 ±	20.0	(30)	128.7 ±	16.8	(28)	N.S.
	4	153.5 ±	25.5	(27)	142.4 ±	21.8	(29)	N.S.
	5	155.7 ±	22.4	(6)	142.4 ±	9.8	(6)	N.S.
	7	150.9 ±	12.6	(21)	146.4 ±	10.7	(11)	N.S.
	12	183.0 ±	29.2	(19)	162.7 ±	18.1	(18)	<.025
	22	203.5 ±	29.1	(20)	192.7 ±	41.8	(22)	N.S.
Blood hemoglobin concentrat. (g/l)	3	105.3	20.5	(25)	109.7	22.4	(21)	N.S.
	5	133.2	4.4	(6)	133.5	6.3	(6)	N.S.
	7	121.5	18.7	(21)	115.7	8.9	(11)	N.S.
	22	127.9	12.5	(11)	125.3	12.6	(14)	N.S.

Note Mean ± SD and number of observations are given together with Student's t-test

TABLE 3. Effects of 3 to 22 weeks' moderate training (I+II) on the dry weight of Achilles tendon and long bones and the length of femur

Variable	Training time (weeks)	Animals: Trained		Control		p
Dry weight of Achilles tendon (mg)	3	1.54 ± 0.25	(21)	1.39 ± 0.22	(24)	<.05
	5	1.79 ± 0.30	(5)	1.56 ± 0.16	(6)	N.S.
	7	1.83 ± 0.23	(21)	1.73 ± 0.27	(10)	N.S.
	12	1.83 ± 0.19	(19)	1.84 ± 0.16	(18)	N.S.
	22	1.73 ± 0.39	(20)	1.72 ± 0.32	(22)	N.S.
Dry weight of long bones (femur + humerus) (mg)	3	45.17 ± 5.05	(28)	45.39 ± 5.23	(28)	N.S.
	4	57.29 ± 8.34	(27)	51.35 ± 6.58	(29)	<.01
	5	62.72 ± 9.54	(6)	52.87 ± 10.51	(5)	N.S.
	7	63.75 ± 7.18	(21)	53.54 ± 6.32	(10)	<.001
	12	73.22 ± 10.20	(19)	72.43 ± 6.10	(18)	N.S.
	22	69.57 ± 8.16	(19)	75.58 ± 12.09	(22)	N.S.
Length of femur (mm)	3	14.61 ± 0.56	(29)	14.74 ± 0.55	(28)	N.S.
	4	14.92 ± 0.56	(27)	14.77 ± 0.60	(29)	N.S.
	5	16.27 ± 0.75	(6)	15.92 ± 0.57	(6)	N.S.
	7	15.92 ± 0.39	(20)	15.62 ± 0.54	(10)	N.S.
	12	15.83 ± 0.58	(19)	16.33 ± 0.45	(18)	<.01
	22	16.65 ± 0.45	(11)	16.98 ± 0.48	(13)	N.S.

Note Mean ± SD and number of observations are given together with Student's t-test

TABLE 4. Effects of 3 to 12 weeks' intensive training (III) on the gain of body and heart weight and the blood hemoglobin concentration

Variable	Training time (weeks)	Animals: Trained	Control	p
Initial body weight (g)	(3)	9.3 ± 2.3 (45)	9.6 ± 2.4 (31)	N.S.
	(5)	12.0 ± 1.7 (10)	12.1 ± 0.9 (10)	N.S.
	(7)	7.0 ± 1.2 (24)	7.8 ± 0.9 (22)	<.05
	(12)	11.5 ± 1.1 (30)	11.8 ± 0.9 (20)	N.S.
Final body weight (g)	3	25.7 ± 4.2 (33)	28.5 ± 4.0 (24)	<.025
	5	33.7 ± 3.7 (6)	34.3 ± 3.3 (6)	N.S.
	7	30.7 ± 2.9 (14)	35.3 ± 3.8 (17)	<.005
	12	40.7 ± 1.7 (11)	42.0 ± 2.7 (14)	N.S.
Wet weight of heart (mg)	3	116.2 ± 20.7 (33)	125.9 ± 19.7 (24)	N.S.
	5	152.8 ± 18.7 (6)	142.4 ± 9.6 (6)	N.S.
	7	144.9 ± 17.5 (14)	150.1 ± 15.1 (17)	N.S.
	12	178.1 ± 14.7 (11)	167.1 ± 13.7 (14)	N.S.
Blood hemoglobin concentration (g/l)	3	121.5 ± 23.3 (33)	128.2 ± 20.0 (24)	N.S.
	5	128.8 ± 7.5 (6)	133.5 ± 6.3 (6)	N.S.
	7	113.7 ± 21.1 (12)	129.5 ± 10.1 (17)	<.02
	12	111.3 ± 16.0 (19)	127.8 ± 15.4 (21)	<.005

Note Mean ± SD and number of observations are given together with Student's t-test

TABLE 5. Effects of 3 to 12 weeks' intensive training (III) on the dry weight of Achilles tendon and long bones and the length of femur

Variable	Training time (weeks)	Animals		
		Trained	Control	p
Dry weight of Achilles tendon (mg)	3	1.09 ± 0.37 (27)	1.17 ± 0.35 (19)	N.S.
	5	1.62 ± 0.14 (6)	1.56 ± 0.16 (6)	N.S.
	7	1.03 ± 0.11 (14)	1.23 ± 0.18 (17)	<.05
	12	2.16 ± 0.37 (14)	2.29 ± 0.26 (14)	N.S.
Dry weight of long bones (femur + humerus) (mg)	3	42.83 ± 7.35 (33)	43.29 ± 6.50 (24)	N.S.
	5	55.85 ± 9.24 (6)	52.87 ± 10.51 (5)	N.S.
	7	52.96 ± 7.32 (14)	56.72 ± 8.03 (17)	N.S.
	12	72.38 ± 7.44 (11)	75.61 ± 6.31 (13)	N.S.
Length of femur (mm)	3	14.37 ± 0.79 (33)	14.67 ± 0.86 (18)	N.S.
	5	15.77 ± 0.73 (6)	15.92 ± 0.57 (6)	N.S.
	7	15.44 ± 0.42 (14)	16.02 ± 0.37 (17)	<.001
	12	16.90 ± 0.41 (11)	17.42 ± 0.32 (13)	<.001

Note Mean ± SD and number of observations are given together with Student's t-test

TABLE 6. Effects of 2-3 days' age difference at the beginning of training on the growth and development of long bones and Achilles tendons after 7 weeks' moderate and intensive training. Means ± SD and number of observations together with Student's t-test are given. (A-group= 11-12-day-old; B-group=13-14-day-old mice)

Variable	Age group	Training program and animals: Moderate (II)	Intensive (III)	Control	p II/C	p III/C	p II/III
Initial body weight (g)	A	8.6 ± 0.6 (20)[2]	8.5 ± 0.7 (24)	8.5 ± 0.7 (43)	N.S.	N.S.	N.S.
	B	10.8 ± 1.1 (26)	11.8 ± 1.2 (25)	11.1 ± 1.2 (48)	N.S.	<.05	<.01
Final body weight (g)	A	34.9 ± 2.9 (11)	32.1 ± 1.4 (11)	38.0 ± 1.7 (24)	<.001	<.001	<.02
	B	36.0 ± 2.2 (12)	36.7 ± 3.2 (11)	38.7 ± 3.4 (23)	<.05	N.S.	N.S.
Dry weight femur and humerus (mg)	A	68.69 ± 6.16(11)	63.35 ± 4.06(11)	66.77 ± 5.81(24)	N.S.	<.05	<.05
	B	67.69 ± 5.04(12)	67.14 ± 5.25(11)	65.76 ± 5.19(23)	N.S.	N.S.	N.S.
Length of femur (mm)	A	15.70 ± 0.40(11)	15.40 ± 0.10(8)	16.10 ± 0.40(24)	<.02	<.0001	<.05
	B	15.80 ± 0.30(12)	15.40 ± 0.60(11)	15.80 ± 0.50(23)	N.S.	<.05	<.05
Volume of femur and (cmm)	A	63.61 ± 6.28(11)	59.67 ± 2.76(11)	64.43 ± 5.58(24)	N.S.	<.02	<.1
	B	65.73 ± 4.27(12)	64.68 ± 5.10(10)	65.78 ± 6.18(20)	N.S.	N.S.	N.S.
Density of femur and (mg/cmm)	A	1.65 ± 0.03(11)	1.70 ± 0.07(11)	1.61 ± 0.06(24)	<.05	<.001	<.05
	B	1.60 ± 0.04(12)	1.68 ± 0.05(11)	1.64 ± 0.06(23)	N.S.	N.S.	<.001
Dry weight of Achilles tendon (mg)	A	1.74 ± 0.18(11)	1.55 ± 0.09(11)	1.73 ± 0.12(21)	N.S.	<.001	<.01
	B	1.80 ± 0.24(12)	1.78 ± 0.23(11)	1.74 ± 0.18(23)	N.S.	N.S.	N.S.
Breaking load of femur(TOT) (N)	A	8.81 ± 1.27(11)	8.02 ± 1.08(11)	8.57 ± 0.94(23)	N.S.	N.S.	N.S.
	B	8.71 ± 0.80(12)	8.16 ± 2.54(11)	8.70 ± 1.24(22)	N.S.	N.S.	N.S.
Breaking load of femur (TOT/BW) (N/g)	A	0.27 ± 0.02(11)	0.25 ± 0.03(11)	0.23 ± 0.02(23)	<.001	<.05	N.S.
	B	2.42 ± 0.01(12)	0.24 ± 0.03(11)	0.23 ± 0.03(22)	N.S.	N.S.	N.S.

TABLE 7. Effects of 3 to 7 weeks' moderate training (II) on the breaking load of femur and patella tendon. Mean ± SD and number of observations are given together with Student's t-test.

Variable	Training time (weeks)	Animals		p
		Trained	Control	
Breaking load of patella tendon (TOT) (N)	3	11.58 ± 1.43 (21)	11.17 ± 2.37 (31)	N.S.
	7	11.14 ± 1.55 (21)	10.45 ± 1.13 (10)	N.S.
Breaking load of patella tendon (TOT/BW) (N/g)	3	0.41 ± 0.09 (21)	0.40 ± 0.08 (21)	N.S.
	7	0.32 ± 0.04 (21)	0.31 ± 0.05 (10)	N.S.
Breaking load of femur (TOT) (N)	3	3.54 ± 0.55 (28)	3.80 ± 0.80 (26)	N.S.
	5	6.29 ± 1.26 (6)	5.69 ± 0.70 (6)	N.S.
	7	5.14 ± 0.78 (19)	4.46 ± 0.83 (10)	<.05
Breaking load of femur (TOT/BW) (N/g)	3	0.12 ± 0.02 (28)	0.12 ± 0.02 (23)	N.S.
	5	0.18 ± 0.02 (6)	0.17 ± 0.01 (6)	N.S.
	7	0.15 ± 0.02 (19)	0.14 ± 0.02 (10)	N.S.

Note The same animals as in Table 2.

TABLE 8. Effects of 3 to 7 weeks' intensive training (III) on the breaking load of femur and patella tendon. Mean ± SD and number of observations are given together with Student's t-test.

Variable	Training time (weeks)	Animals		
		Trained	Control	p
Breaking load of patella tendon (TOT) (N)	3	10.96 ± 2.78 (19)	10.79 ± 2.29 (8)	N.S.
	7	11.98 ± 2.20 (14)	12.50 ± 2.16 (17)	N.S.
Breaking load of patella tendon (TOT/BW) (N/g)	3	0.47 ± 0.01 (19)	0.41 ± 0.01 (8)	<.005
	7	0.37 ± 0.08 (14)	0.36 ± 0.05 (17)	N.S.
Breaking load of femur (TOT) (N)	3	5.94 ± 1.74 (32)	5.18 ± 1.80 (17)	N.S.
	5	5.78 ± 1.01 (6)	5.69 ± 0.70 (6)	N.S.
	7	5.32 ± 1.05 (14)	5.49 ± 1.10 (17)	N.S.
Breaking load of femur (TOT/BW) (N/g)	3	0.26 ± 0.09 (27)	0.19 ± 0.07 (14)	<.02
	5	0.24 ± 0.02 (6)	0.17 ± 0.01 (6)	<.005
	7	0.17 ± 0.03 (14)	0.16 ± 0.02 (8)	N.S.

Note The same animals as in Table 4.

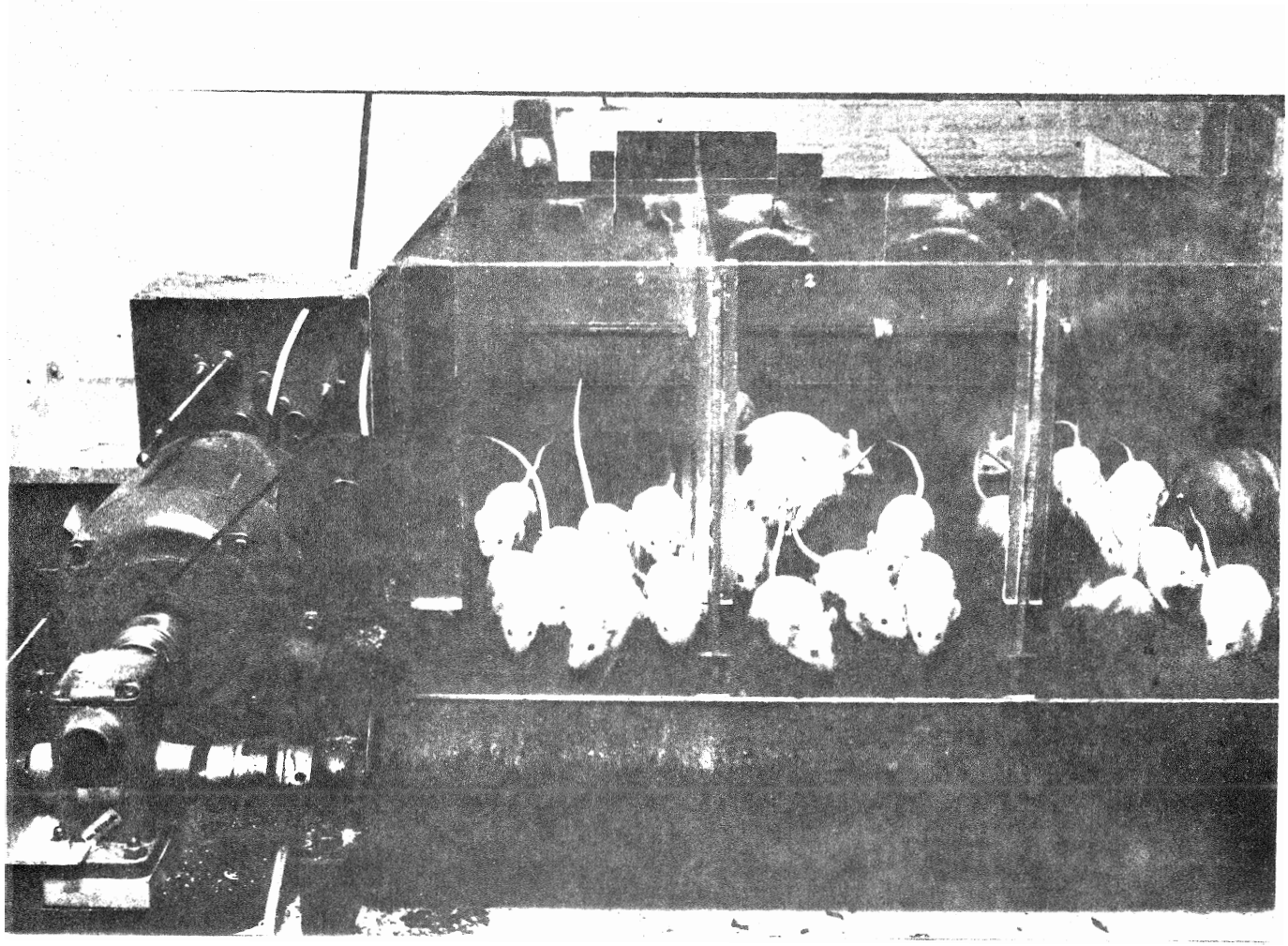

FIGURE 1. The treadmill was 70 cm long and 55 cm wide divided longitudinally into 4 plexy-glass running compartments. The operating speed of the electric motor driving the rubber belt could be adjusted continously as well as the angle of inclination. A high voltage electric current was connected to a network of wiring in the plexy-glass fence at the back, which gave an electric shock when touched. About 40 mice could run on the treadmill at any one time.

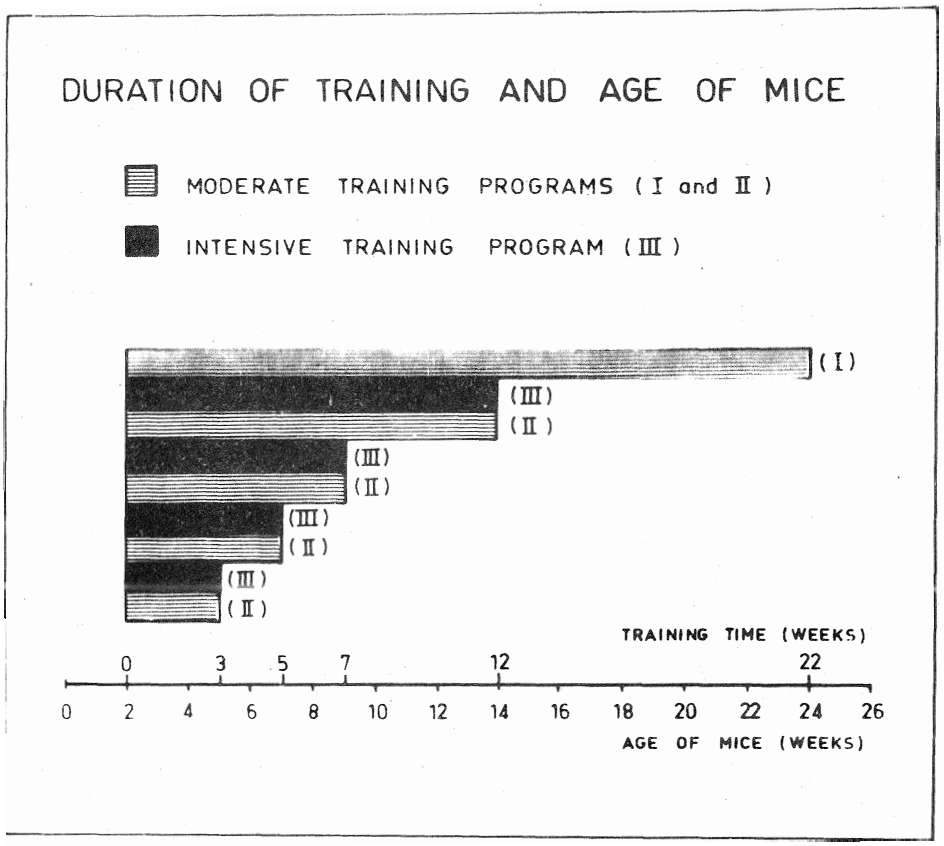

FIGURE 2. Age of mice and duration of the different training programs (see Table 1).

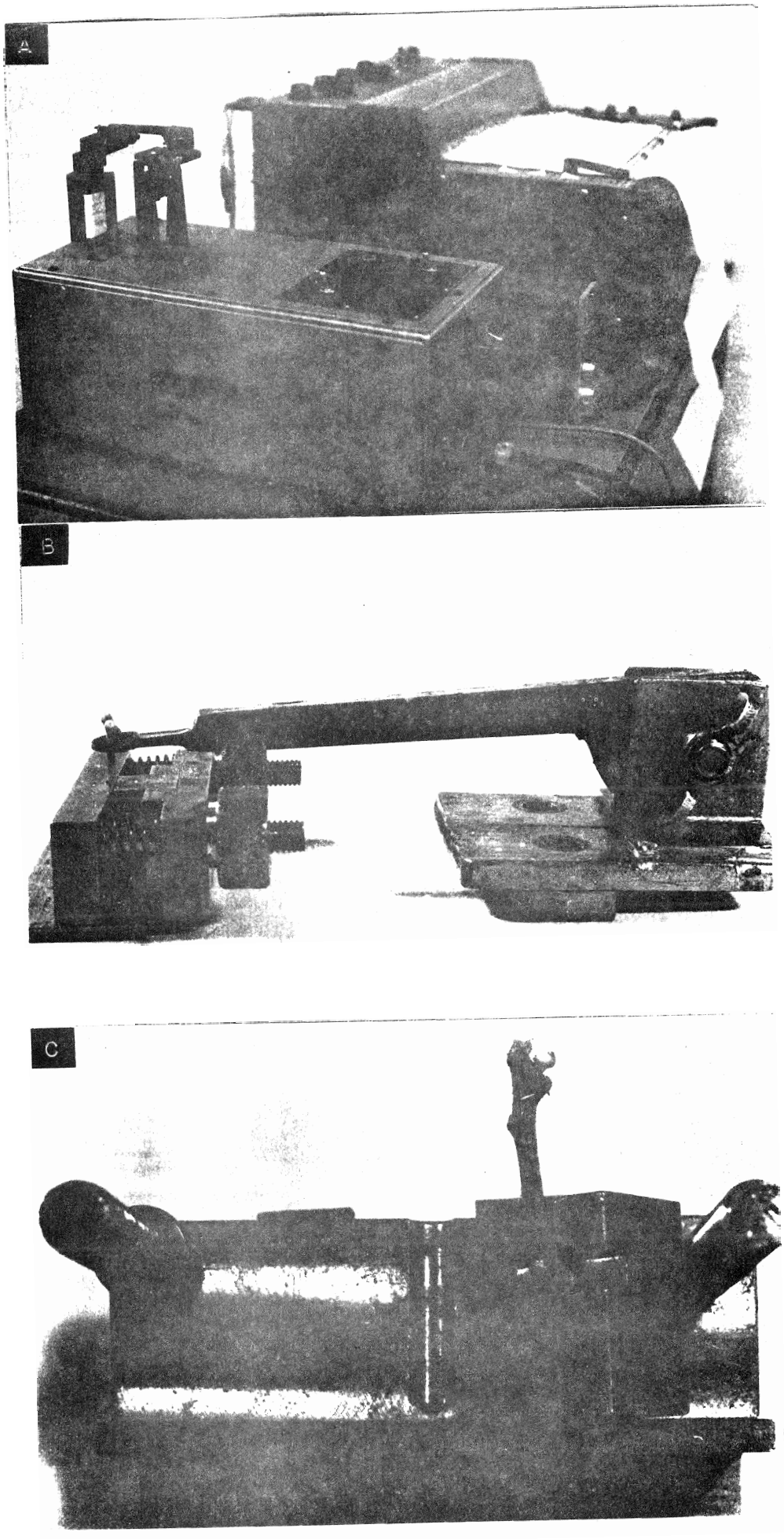

FIGURE 3. MEASURING THE BREAKING LOAD OF FEMUR (A-C).

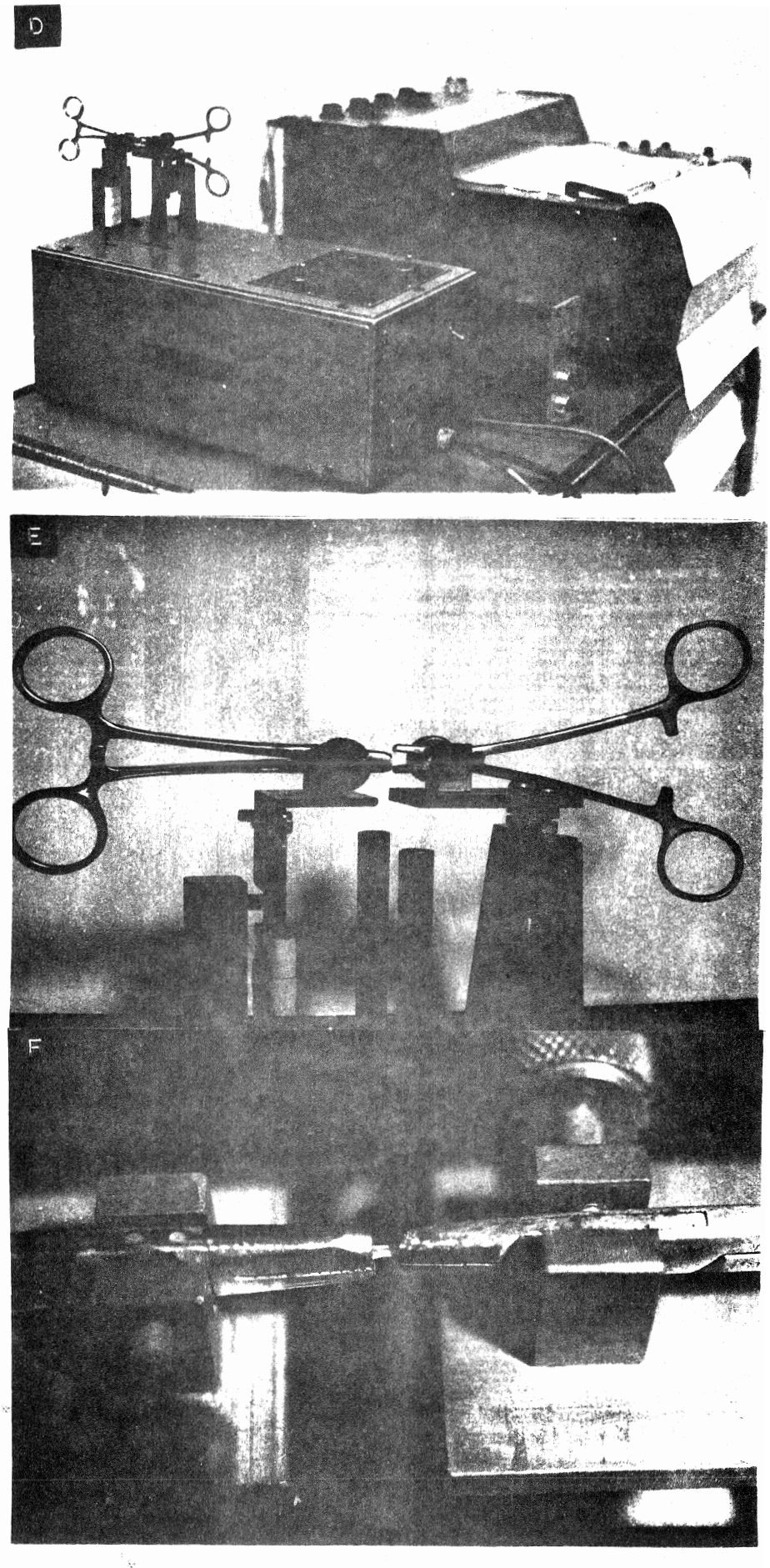

FIGURE 3. MEASURING THE BREAKING LOAD OF PATELLA TENDON (D-F).

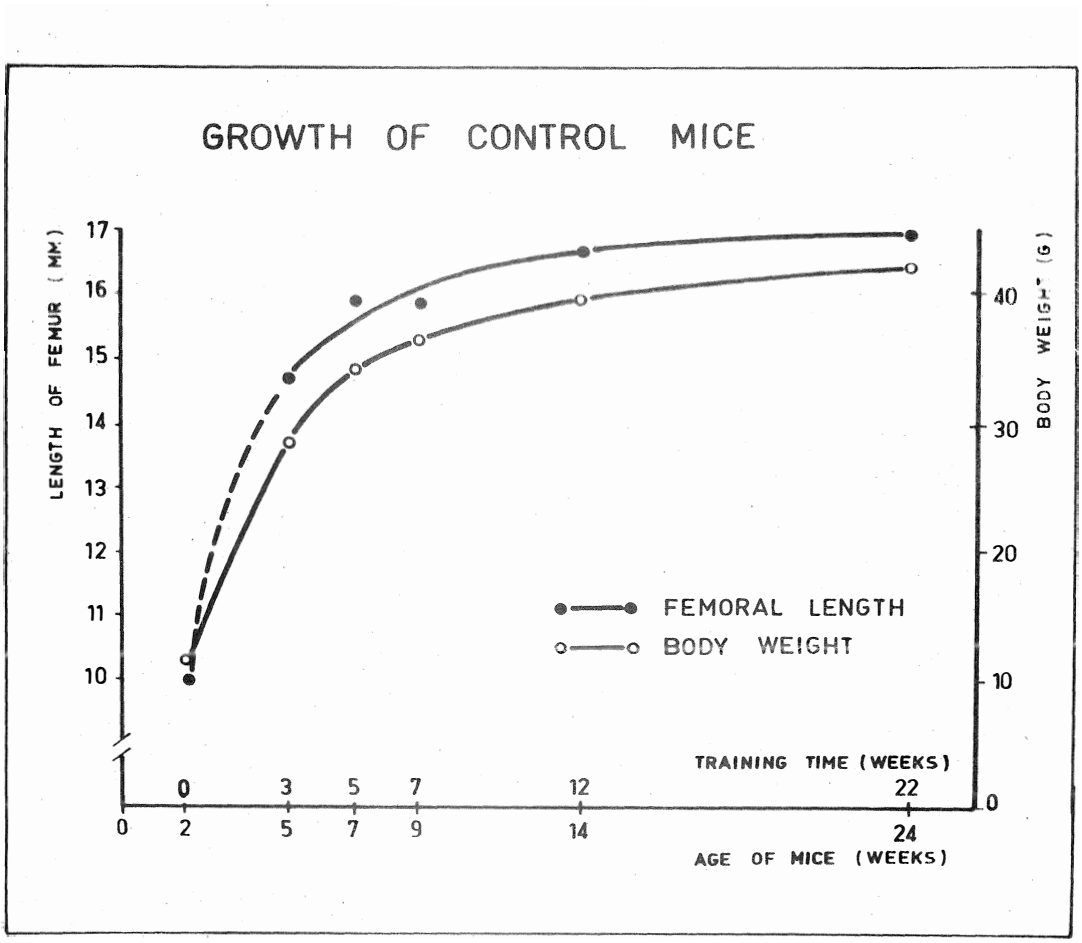

FIGURE 4. The growth of femoral length and body weight for control mice during the experimental period.

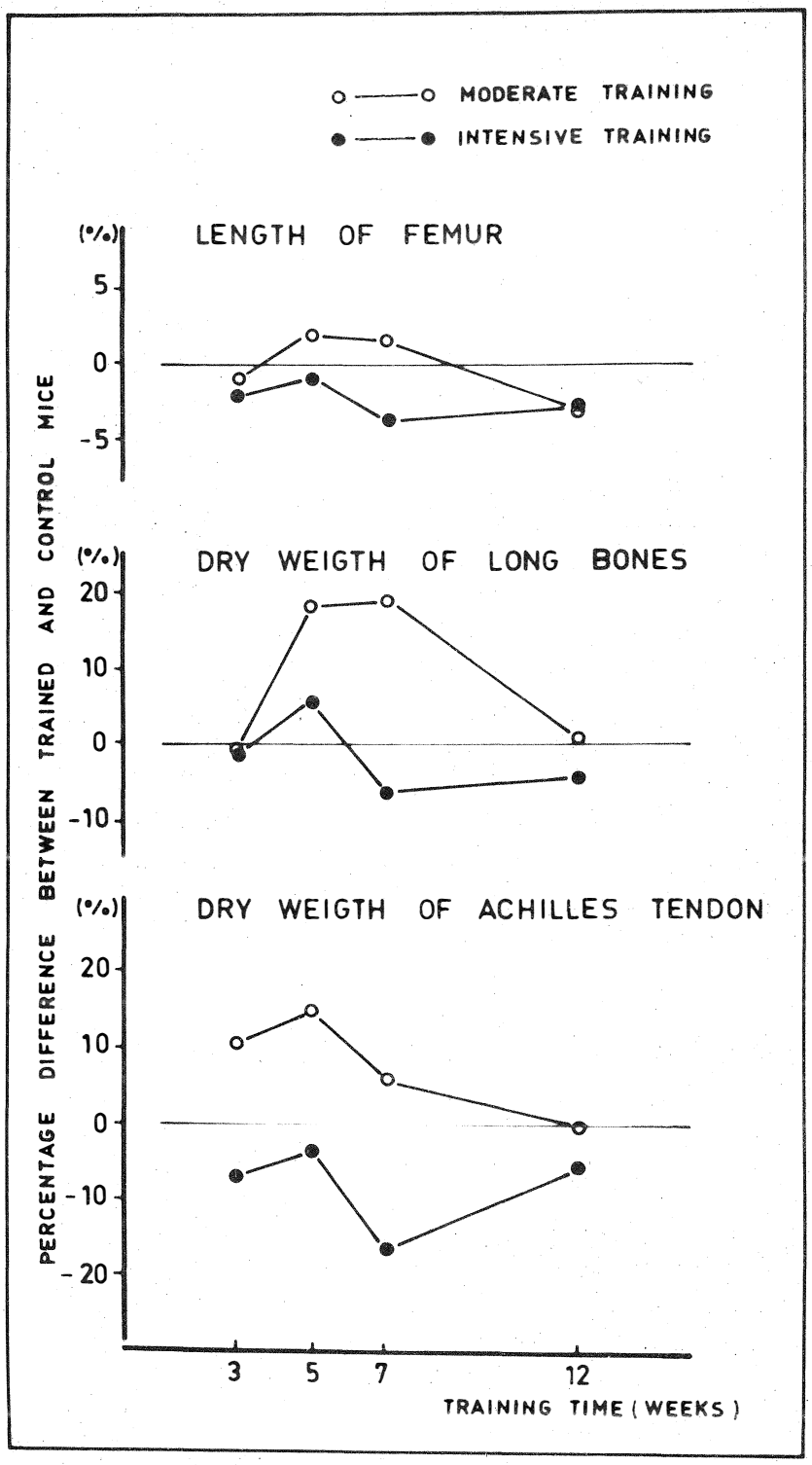

FIGURE 5. Percentage differences between trained and control mice in the dry weights of Achilles tendons and long bones as well as the length of femur after 3 to 12 weeks' moderate and intensive training.

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