FATIGUE AND WORK CAPACITY OF MUSCLES FROM FROGS TREATED WITH MALE SEX HORMONE

by

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INTRODUCTION AND LITERATURE

There has long been a common belief that male sex hormone is responsible in no small way for the muscular strength and endurance associated with male animals. One of the most constant effects of testosterone treatment in patients with lowered testis hormone production (eunuchoids, castrates) is the feeling of increased working capacity and endurance. Thus, it would appear that, in order to maintain the muscle condition for higher working capacity and strength, there must be an adequate level of male sex hormone. In an attempt to gain some factual knowledge as to the effect of endocrine factors, including the androgenic hormones, on vigor of an individual, the Department of Physiology of Ohio State University, in 1924 launched a series of investigations on the various factors that serve to modify the vigor of the individual. The method used was to confine albino rats in small housing cages having uninterrupted access to revolving cylinders in which they could exercise at will. Hoskins (1925) in the third of these investigations compared the voluntary activity of 16 castrate male rats with 16 normal male rats. The animals were placed in a revolving cage and the number of revolutions recorded. It was estimated that about 95 percent of the total activity of the animal was registered by this method. Hoskins found that castration diminished the voluntary activity of the

male rats by about 60 percent. Gans and Hoskins (1926) continuing the studies on vigor measured the strength and performance of the gastrocnemius muscle in male rats. The sciatic nerve was stimulated by induction shocks until complete fatigue occurred. The castrate animals were found to do approximately 25 percent less work than the normal animals. Hoskins (1927) attempted to restore the activity of castrate animals by testis grafts but the results were negative. Gans and Hoskins (1926) also determined that the weights of individual muscles of castrate animals were greater than those of normal animals. In contrast to this, Papanicolaou and Falk (1938) found that after prolonged administration of androgenic hormone (testosterone propionate) there was a definite hypertrophy of the temporal and other muscles of the body of castrate immature male guinea pigs, and spayed as well as normal adult female guinea pigs. They stated that the androgenic hormone has a stimulating effect upon muscles, producing enlargement after prolonged administration. Wainman and Shipnounoff (1941) found that castration caused a marked decrease in size of the striated perineal muscles. Testosterone propionate prevented the effect of castration on these muscles. In the normal animal an increase in bulk of these muscles was brought about by the administration of testosterone propionate. In this laboratory, Herrick (1945) found that the average breaking strength of the gastrocnemius muscle of white leghorn pullets was increased approximately 41 percent by testosterone administered

intramuscularly while the strength of the gastrocnemius muscle of white leghorn capons was increased approximately 23 percent. He also found that male sex hormone doubled the tensile strength of the skin of both female and caponized fowls.

The effect of male sex hormone on the muscle strength of the human male has been demonstrated by some workers. In experiments such as those performed by Gans and Hoskins (1926) where fatigue was produced by electrical stimulation of the sciatic nerve, the cause of the fatigue is located in the motor end-plates. Such fatigue cannot be reproduced in man by voluntary action. According to Simonson, Kearns and Enzer (1941) Mosso has shown that electrical stimulation applied to the muscle through the skin immediately after complete fatigue of voluntary finger movements in the finger ergograph produced contraction. Thus, fatigue of man in this type of work must be localized in the central nervous system. Simonson, Kearns and Enzer (1941) investigated the effect of testosterone on the endurance of man for muscular work. Their subjects were four men with diminished production of the male sex hormones (two eunuchoids, two castrates). Methyl testosterone in tablet form was administered orally at a dosage of 25 milligrams four times daily for three weeks. Results obtained showed that testosterone therapy has a definite influence on the endurance and performance in different types of work. They stated there were indications of increased resistance of the central nervous

system against fatigue. The same authors corroborated their first findings by testing six additional subjects in 1944, using similar methods and testing procedures to obtain similar results. Samuels, Henschel and Keys (1942) attempted to determine whether the responses to fatiguing exercises might be altered in normal man by the administration of androgens. Their subjects were four healthy male medical students, whose ages were between 21 and 30 years. They concluded after three to four weeks treatment with methyl testosterone that the androgens did not significantly change the physical vigor of normal men.

How androgenic hormones influence muscle activity is still unexplained. Hesser, Langworthy and Vest (1940) believe that in the greater muscular development and capacity of the adult male, credit must be given to general muscular as well as tonic and psychomotor stimulation produced by androgenic therapy. They believe, in view of the actual structural changes induced in the striated musculature by androgenic hormones, that the effect is predominately a mechanical one. They believe testosterone acts as a metabolic synergist in much the same manner as adrenal cortical substance, causing electrolyte, nitrogen and water retention. Williamson and Gulick (1941) found that when male rabbits were given testosterone, with or without the administration of creatine, the testosterone diverted creatine into the muscles, or caused its cumulative retention in the muscles. They believe that this may be a part of the mech-

anism for the improvement in dynamometric response which Hesser, Langworthy and Vest (1940) reported they found with the administration of testosterone in cases of myotonia atrophica. Herrick (1945) found that the tissues of fowls treated with testosterone contained more collagen nitrogen and a larger number of connective tissue fibers than in normal birds.

Since the objective data are somewhat limited on the subject of androgen effect on actual work capacity of muscles, the data contained in this paper are offered as evidence of the effect of testosterone on work capacity and fatigue of muscles.

MATERIAL AND METHODS

The test animal used in all experiments was the common leopard frog, Rana pipiens Schreber. The hormones used were the commercial products of the Schering Corporation, ORETON (25 milligrams of crystalline testosterone propionate in each cubic centimeter of peanut oil) and ORETON-M (10 milligrams of methyl testosterone in each tablet). The animals in all cases were paired according to body weight with one control and one experimental animal for each pair. The maximum difference in each pair was held to four grams. Most of the pairs had a difference of at least two grams (see Table 1). Two groups were evaluated during the experiment. Group I was evaluated

without sexing the animals, while Group II was evaluated after the sex of each animal had been determined. In all cases the animals were out of breeding season. All experimental animals to which testosterone propionate was administered were given 0.2 cubic centimeter of peanut oil containing five milligrams testosterone propionate on alternate days until seven doses had been given. Then on the day prior to sacrificing the animals 0.1 cubic centimeter was administered. The total dosage was 1.5 cubic centimeters or an equivalent of 37.5 milligrams of crystalline testosterone. Group I animals were injected intraperitoneally; Group II animals were injected intramuscularly. Those experimental animals which received methyl testosterone were given one tablet orally, each tablet containing ten milligrams of methyl testosterone, on alternate days until seven tablets had been given; then on the day prior to sacrificing the animal one-half tablet was given. This was a total dosage of 75 milligrams of testosterone. All animals were kept in glass biological specimen dishes, 200 millimeters outside diameter, and covered with a glass plate. A small amount of water was added to the dishes and changed on alternate days.

The apparatus used to record the fatigue and work data consisted of the following pieces of equipment arranged as shown in Plate I, Fig. 1.

1. Kymograph with smoked glazed paper on the drum.
2. Muscle lever with writing point attached.

3. Muscle clamp.
4. Ring stand with two extension clamps.
5. Weight pan and set of ten gram weights.
6. Key switch, normally open.
7. Adjustable induction coil.
8. Dry cell battery.
9. Assorted wire.
10. Ringer's solution.

To prepare the muscle for use the following procedure was followed throughout all experiments.

The frog was killed by removing the brain and destroying the spinal cord. The hind leg was cut off close to the body. The skin of the leg near the ankle was held with a forceps while an incision was made in it. The incision was continued completely around the ankle and, when loosened, the skin was pulled back to the knee exposing the calf muscles. The gastrocnemius muscle was then separated and the tibo-fibula was snipped through with a scissors below the knee. The skin was then pulled back to cover the gastrocnemius muscle to keep it moist. The tendon of Achilles was freed from the bones of the foot and when approximately one-fourth inch of the tendon was free, it was cut off. The preparation now consisted of the bone of the upper leg (the femur) attached to the gastrocnemius muscle with its tendon. The preparation was placed in Ringer's solution until attached to the recording apparatus.

When the recording apparatus was ready, the muscle was

removed from the Ringer's solution with a forceps and was placed in the apparatus in the following manner. One electrode consisting of a hooked pin attached to a wire from the secondary coil of the inductorium was inserted through the tendon of Achilles. This pin was in turn attached to the muscle lever by a thread. The femur of the preparation was then inserted in the muscle clamp and securely fastened. The muscle clamp was adjusted so that a small amount of tension was supplied to the muscle by the weight pan containing one ten gram weight. The weight pan was attached to the muscle lever at a predetermined distance from the fulcrum. To standardize the tension as nearly as possible for all preparations, the after-weighting screw was tightened until it just made contact with the muscle lever. It was then given an additional one-fourth turn. Thus the muscle carried no weight except during the period of contraction. The second wire from the secondary coil of the inductorium was attached to the binding post provided on the muscle clamp. The muscle was moistened with Ringer's solution at intervals during the recording period. The induction coil was adjusted to give a maximum "break" stimulus but no "make" stimulus. When this point was found, the kymograph drum with its smoked glazed paper covering was moved into position, care being taken to avoid undue friction between the writing point and the drum, and recording of contractions started. Two types of records were made from the Group I animals. One record of fatigue was made from one gastrocnemius muscle and one of work

was made from the other gastrocnemius muscle of each animal.

The fatigue record was made in the following manner.

A ten gram weight was placed in the weight pan. The drum of the kymograph was started revolving at slow speed. Using a metronome to determine the time interval, a Faradic stimulus was sent to the muscle at one second intervals until complete fatigue occurred. Since the speed of the revolving drum and the time interval between stimuli were constant, the method of evaluation used was to determine the area of the recorded area in square centimeters and to make comparison between experimental animals and controls (see Plate I, Fig. 2). These comparisons will be discussed in subsequent paragraphs.

In recording the work records for Group I the following method was used.

Starting with a ten gram weight in the weight pan, three contractions were recorded on the smoked drum. Another ten gram weight was added and three more contractions were recorded. This procedure was repeated using additional weights until a recorded contraction of one millimeter or less was noted. At this point the record was considered complete.

In computing work from the records a proportion was evolved using similar triangles as shown in Plate II, Fig. 3. By similar triangles $\frac{a}{b} = \frac{c}{d}$; then solving for the unknown $a = \frac{bc}{d}$.

In all experiments the values for "c" and "d" were kept

constant. "b" was determined by measuring the three contractions for each weight and averaging the sum. Then by a simple algebraic problem the value for "a" could be computed. To determine work the actual contraction "a" was multiplied by the weight. This was repeated for each weight. With the values thus obtained, a graph was developed plotting the work in gram-millimeters against the weight in grams.

Recordings for Group II were combined work-fatigue records. The same procedure was used as in Group I to prepare the muscles and mount them in the apparatus. The apparatus was modified from that used for Group I by adding another kymograph with drum, muscle clamp, muscle lever, and ring stand with extension clamps. The gastrocnemius muscle from the left leg was mounted in the left hand apparatus and the right gastrocnemius muscle was mounted in the right hand apparatus. This permitted taking records from both muscles simultaneously using only one key and inductorium (Plate III, Fig. 5).

The procedure was modified from the work procedure of Group I in that, after the first record was carried out to complete fatigue, the muscles were allowed to rest for five minutes and were again stimulated and weights added until complete fatigue occurred. This procedure of alternately fatiguing and resting the muscles was carried on until five records, or as many as could be obtained less than five, were made. The computation procedure for determining the work for each period was identical to that used for Group I.

DISCUSSION AND CONCLUSIONS

Group I. The animals injected intraperitoneally with testosterone propionate showed no significant difference in results from those which were given methyl testosterone orally. All results were grouped together. Animals were weighed before treatment and before being sacrificed, but neither control nor experimental animals showed any significant loss or gain in body weight. Fifteen pairs of records were used for computing work data and 12 pairs of records were used for computing fatigue data.

In the experimental animals the fatigue records showed an average of 58.12 square centimeters while the control animals showed an average of 52.78 square centimeters. This is a difference of 5.34 square centimeters in favor of the experimental animals or an increase of approximately ten percent over the control group (see Table 2).

The work records of the experimental animals when computed and expressed in gram-millimeters of work showed the experimental group to have done an average of 1061.14 gram-millimeters of work compared to the control animals work average of 700.15 gram-millimeters. This was a difference of 360.99 gram-millimeters in favor of the experimental group. There was an increase in work of approximately 51 percent over the control group. This percentage value approaches the 60 percent value which Hoskins (1925) found in the difference in the voluntary

activity between castrated and normal rats. The graphical picture of the averages for each group of animals is shown in Plate II, Fig. 4. For both groups the data when plotted gave a modified sine curve. It should be noted that the control group reached its maximum work at a weight of 40 grams doing 106.5 gram-millimeters of work at that point. The experimental group reached its peak of work at 50 grams doing 141 gram-millimeters of work at that point. However, at 40 grams, the average work done by the experimental group was 140 gram-millimeters of work. Thus, it should be noted that there was a plateau of values between 40 and 50 grams for the experimental animals but in the case of the control animals the peak was reached at 40 grams and immediately began to fall. The experimental group did more work at every weight as shown by the comparison of the curves in Plate II, Fig. 3. In only two cases did the experimental animals do less work than their controls (see Table 1).

Group II. Twelve pairs of records were obtained from this group. One-half the experimental animals received methyl testosterone orally in the same dosage used for Group I animals. The other half of the experimental animals received testosterone propionate injected intramuscularly into the thigh muscles. The dosage was the same as for Group I animals receiving testosterone propionate. Only one animal of the entire Group II was a male animal.

In evaluating the records obtained the following method was used. The total amount of work for each period was computed. The total work done in the initial period was taken as 100 percent and the other four periods expressed in percent of the initial work. The average of the experimental group percentages compared to the control group percentages gave the following results. After the first five minute rest period, the experimental animals averaged 63.12 percent as much work as in the initial period while the control animals averaged 59.48 percent as much. After the second five minute rest period, the experimental animals averaged 25.44 percent as much as in the initial period compared to 17.43 percent as much in the control animals. After the third rest period, the experimental animals did 10.87 percent of the initial work while the control animals did 5.41 percent. After the last rest period, the experimental group averaged 5.08 percent compared to 2.02 percent by the control group. These results are tabulated below.

	After first period	After second period	After third period	After fourth period
Experimental	63.12	25.44	10.87	5.08
Control	59.48	17.43	5.41	2.02

From the results of Group I and Group II, it is concluded that male sex hormone reduces the rate of fatigue and increases

the ability to do work in the gastrocnemius muscle of the frog, Rana pipiens.

SUMMARY

1. It was found that male sex hormone increased the resistance to fatigue by approximately ten percent in the gastrocnemius muscle of the frog, Rana pipiens.

2. Male sex hormone increased the work ability of the gastrocnemius muscle of the frog, Rana pipiens, approximately 51 percent.

3. The combined work-fatigue records from Group II animals show that male sex hormone reduced the rate of fatigue after the initial fatigue period in the experimental animals as compared to the control animals.

4. Similar results were obtained with both testosterone propionate and methyl testosterone, no significant difference being noted.

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APPENDIX

Table 1. A comparison of the body weight, work in gram-millimeters, and the type of male sex hormone administered.

Frog*	Type of hormone**	Body weight (in gm.)	Gm-mm of work
X-3C	M	26	648.1
C-4C		30	808.8
X-1D	M	28	693.3
C-8D		28	1232.3
X-3A	M	28	792.7
C-6A		30	327.7
X-8	P	30	1206.7
C-1		32	662.6
X-1A	M	32	1164.6
C-4A		36	461.7
X-2D	M	36	1223.0
C-6D		34	641.9
X-7	P	38	187.6
C-5		36	126.2
X-3B	M	36	1383.9
C-8A		40	820.6
X-4B	M	36	1453.8
C-8B		40	1426.4
X-7C	M	38	1390.5
C-8C		38	824.7
X-3	P	43	597.8
C-0		40	167.1
X-5B	M	40	1241.5
C-1B		44	1007.6
X-7A	M	42	873.1
C-2A		42	402.8
X-7B	M	54	2000.4
C-6B		50	891.7

*X - Denotes experimental animal.
C - Denotes control animal.

**P - Denotes testosterone propionate in oil.
M - Denotes methyl testosterone.

Table 2. A comparison of fatigue in control and experimental animals.

Frog*	Type of hormone**	Body weight (in gm.)	Fatigue (sq. cm.)
X-3C	M	26	39.26
C-4C		30	54.20
X-3A	M	28	39.42
C-6A		30	46.17
X-8	P	30	78.44
C-1		32	56.56
X-1A	M	32	43.94
C-4A		36	60.34
X-2D	M	36	68.99
C-6D		34	71.26
X-3B	M	36	80.43
C-8A		40	51.60
X-4B	M	36	49.86
C-8B		40	61.85
X-7C	M	38	52.41
C-8C		38	23.55
X-3	P	43	49.00
C-0		40	39.61
X-5B	M	40	43.40
C-1B		44	30.30
X-7A	M	42	48.53
C-2A		42	79.12
X-7B	M	54	97.63
C-6B		50	90.73

*X - Denotes experimental animals.
C - Denotes control animals.

**P - Denotes testosterone propionate in oil.
M - Denotes methyl testosterone.

EXPLANATION OF PLATE I

Fig. 1. Photograph of the recording apparatus used for Group I records.

Fig. 2. Photograph showing the comparison of areas for Group I fatigue records.

PLATE I

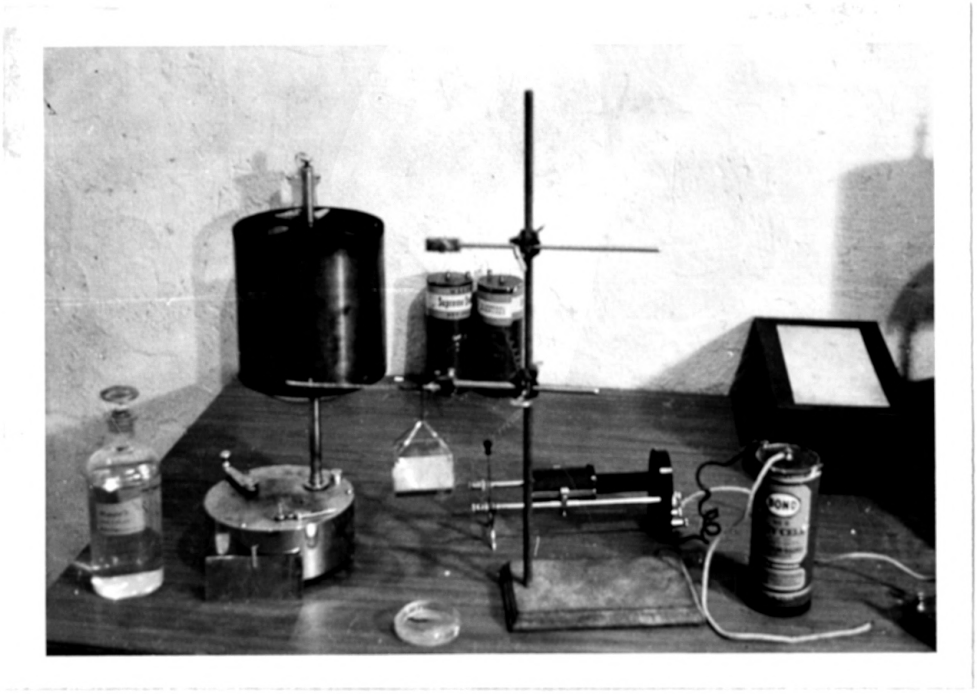


Fig. 1

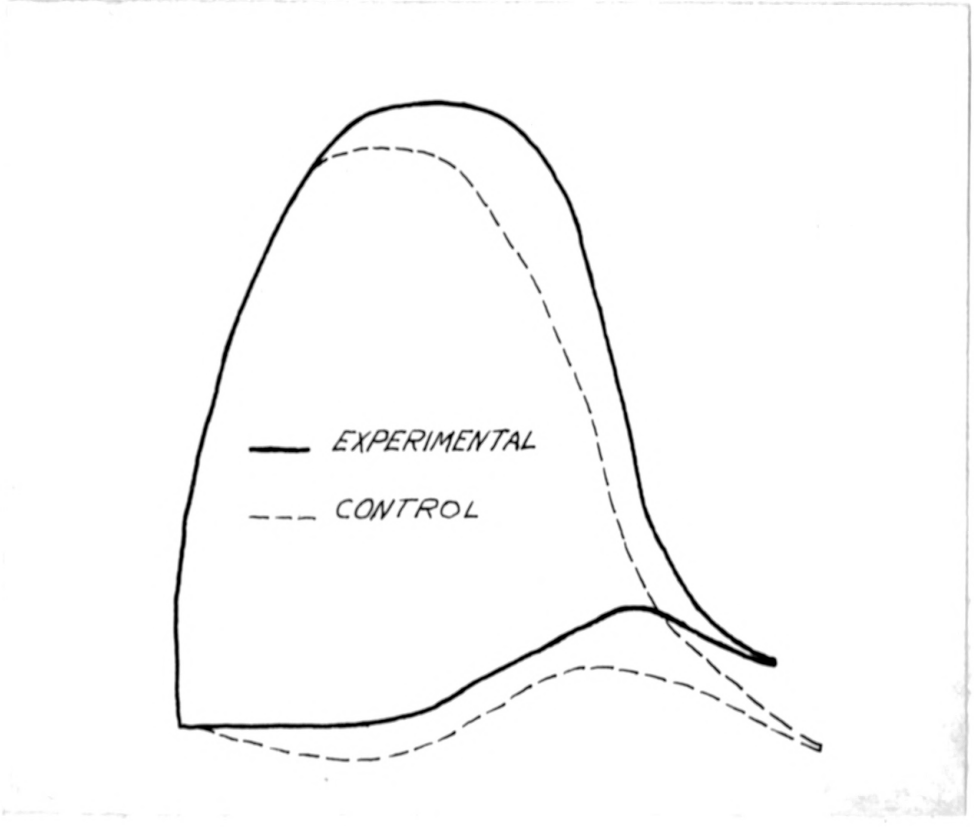


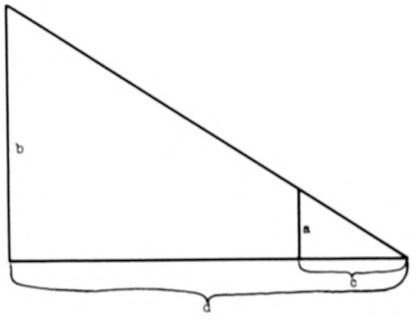
Fig. 2

EXPLANATION OF PLATE II

Fig. 3. Photograph showing similar triangles used to compute actual muscle contraction.

Fig. 4. Graph showing the comparison of work between experimental and control animals.

PLATE II



a - actual contraction
b - recorded contraction
c - distance from fulcrum to muscle
d - distance from fulcrum to writing point

Fig. 3

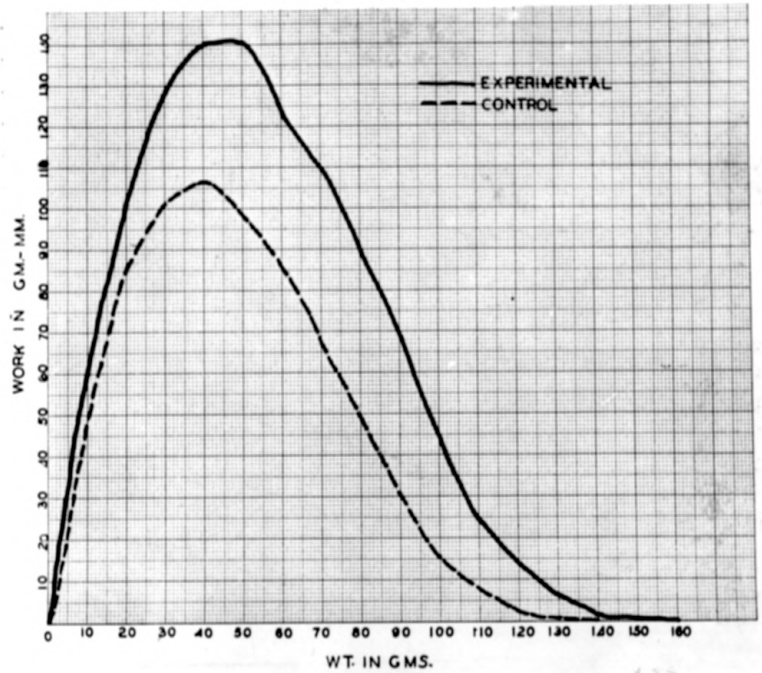


Fig. 4

EXPLANATION OF PLATE III

Fig. 5. Photograph of the recording apparatus used for Group II records.

PLATE III

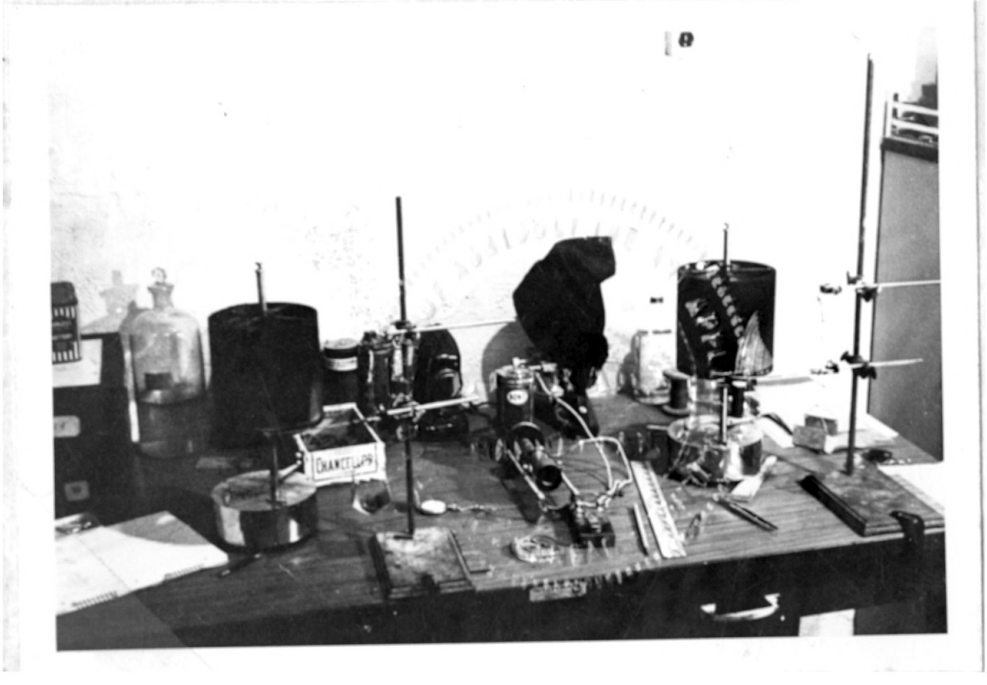


Fig. 5