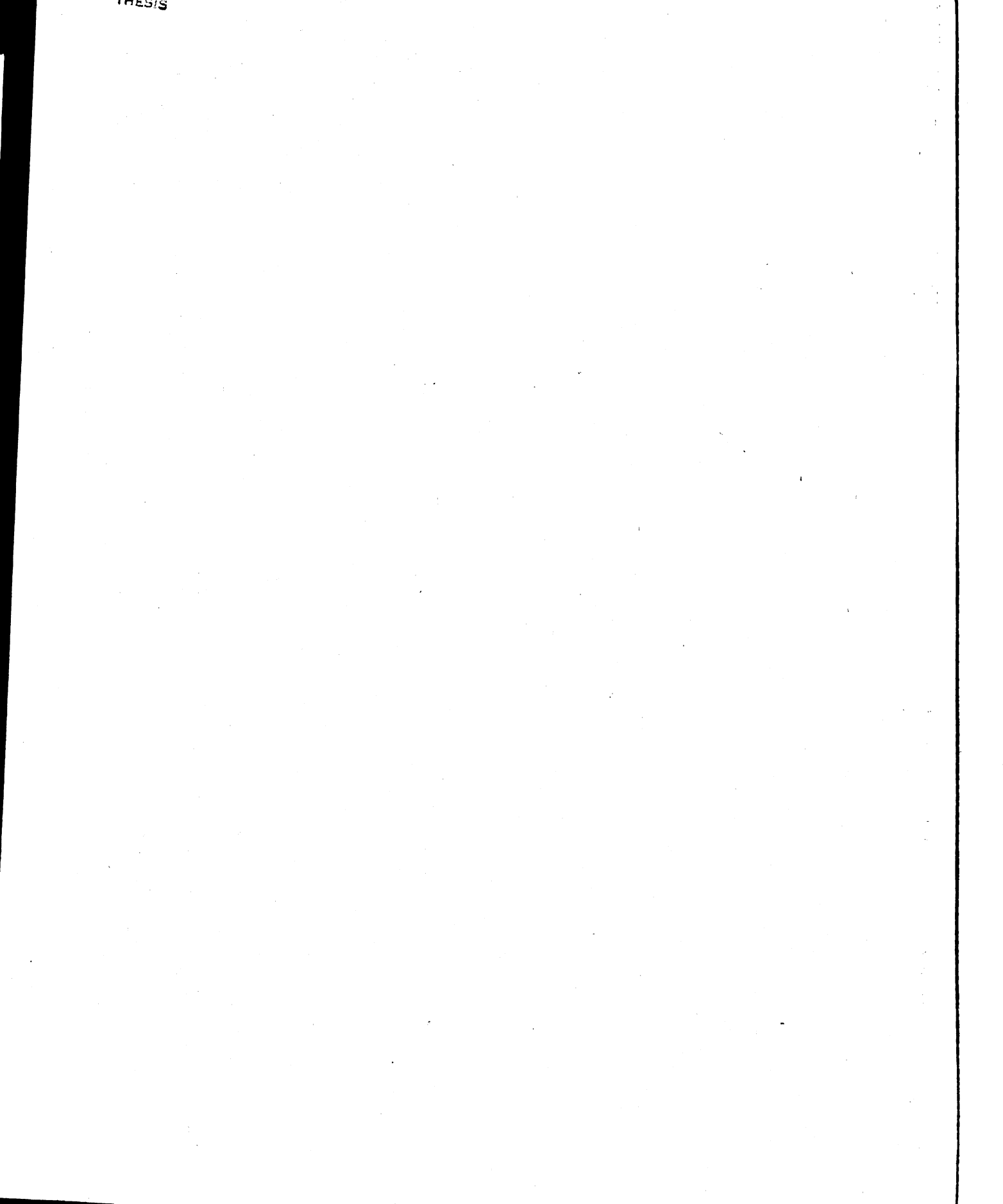




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OVERWINTERING OF THE NEMATODE
PARASITES OF SHEEP WITH NOTES
ON THE COURSE OF THE INFECTION

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Moacyr Gomes de Freitas
1947



This is to certify that the
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Overwintering of the Nematode Parasites of
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Moacyr Gomes Freitas

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OVERWINTERING OF THE INTERNAL PARASITES OF SHEEP
WITH NOTES ON THE COURSE OF THE INFECTION.

By
Moacyr Gomes de Freitas

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INTRODUCTION

The economic importance of the parasitic diseases in the domestic animals has been demonstrated by numerous investigators. It has been estimated that the parasites of domestic animals cause an annual loss of \$500,000,000 in the United States. Among the domestic animals, sheep are considered most susceptible to attack by parasites, chiefly by nematodes. The nematodes parasitizing sheep are too large in number to enumerate in this paper. About sixty-three species are known to infect sheep.

The losses caused by these parasites have justified the numerous investigations related to biology and bionomics of the nematodes infecting sheep. Only the rational control of the nematode parasites, based on data of life history and resistance of the free-living stages under natural climatic conditions, will be able to give satisfactory results.

Despite the great advances which have been made in the last forty years concerning many species of nematodes parasitizing sheep, the control of these worms remains one of the great problems to be solved by the veterinarians and animal husbandrymen.

According to these investigations the roundworms occurring in the digestive tract of sheep produce a large number of eggs that are discharged with the feces to the exterior. Under favorable climatic conditions, mainly temperature and humidity, the eggs develop to vermiform larvae in a short time, depending on the

species of nematode. In the soil the larvae undergo two molts of the cuticle, before reaching the infective larval stage which usually retains the last sheath.

Control of these nematode parasites depends on treatment to remove the adult roundworms by anthelmintics or on prevention of infection through rational management.

The work reported in this paper was carried out to determine if sheep could acquire the infective larvae from their bedding during a normal winter. One group of sheep receiving no treatment and a second group receiving phenothiazine and salt were studied.

REVIEW OF THE LITERATURE

Ransom (1906) found that eggs and preinfective larvae of Haemonchus contortus possess little resistance. When the infective larva was exposed on soil to natural conditions of the winter, in Maryland, it was able to survive for twelve weeks.

In subsequent work the same author, Ransom (1906), reported that in the latitude of Washington, D. C., infested pasture became free of hookworms and modular worms but not from Haemonchus contortus when it was not used from October 25th to June 26th.

Later, Ransom (1910) found pastures remaining contaminated by nematodes after a lapse of nearly eight months from October through June in Maryland. He also found that pre-infective larvae and eggs were killed within a few hours when exposed to temperatures below freezing.

Veglia (1915) working out the life history of Haemonchus contortus, reported that the infective larvae were unable to survive the natural outdoor conditions of South Africa. The author reported that the eggs did not survive at a temperature of 32°F., after forty-eight hours.

Guberlet (1921) reported in Oklahoma that infective larvae of Haemonchus contortus were found surviving after five months in soil.

Veglia (1923) did not find any marked difference between the resistance to external conditions, in South Africa, of infective larvae of Oesophagostomum columbianum and Haemonchus contortus.

Turner and Moon (1929) working in Pennsylvania reported that the number of eggs of trioststrongylid worms passed by sheep reached the minimum during the winter months and rose to a peak in summer.

Bozevich (1930) noted that some larvae of Haemonchus contortus exposed on soil to natural climatic conditions survived seven months. Eggs were killed after six to eight hours at temperature of 28°F.. At 32°F., the first stage larvae were dead after twenty-eight hours, while the second stage larvae were killed after one week.

Mönnig (1930) in South Africa, found that pre-infective larvae of Trichostrongylus sp. were killed by freezing but the infective larvae were able to infect a lamb after being exposed to freezing for ten days.

Zavadowsky and Zvjagintzeo (1931) found that the number of eggs of Nematodirus sp., passed in the feces, reached a minimum during the winter and rose to a peak in the summer.

Schmid (1933) obtained results identical to those of Turner and Moon in 1929.

Rebressier (1933) found in Chic that modular worms larvae (Cesophagostomum columbianum) survived at least one winter on pasture.

Dikmans and Andrews (1933) found that the larvae of Haemonchus contortus, Nematodirus spathiger and Ostertagia circumcincta, survived over the winter on pasture, in Beltsville, Maryland.

Zavadovskii and Vorobeva (1934) observed that 97 to 99 percent of the larvae of Trichostrongylidae kept in the open in Moscow died, and the survivors were so inactive that their infective capacity was practically gone.

Taylor (1935) confirmed the results found by Turner and Moon (1929), and Schmid (1933).

March (1936) recorded that the number of eggs of Ostertagia circumcincta reached a summer peak, and that there was a sharp drop during the months following the peak.

Kammlade, Graham and Boley (1936) found that a pasture in Illinois was able to maintain infection of Haemonchus contortus over winter, but as a source of parasitism it was of much less importance than the animal parasitized.

Griffiths (1937) demonstrated the ability of Ostertagia circumcincta, Nematodirus filicollis, and Trichostrongylus colubriformis

to survive a normal Canadian winter at temperature ranging from 15°F. to 52.5°F..

Taylor (1936), working with an unspecified mixture of Trichostrongylidae in England, found that most of the larvae were killed during the first week of exposure on the pasture, and that only a small proportion survived for more than sixty-three days.

Baker (1939) reported that in the vicinity of Ithaca, New York, the infective stages of various species of nematodes remain viable for periods of at least twenty-one months.

Swales (1940) reported that the free living stages of Ostertagia circumcincta were able to survive the winter on a Canadian pasture but the same was not true regarding Haemonchus contortus and Oesophagostomum columbianum.

Shorb (1942) found that no stages of the common sheep nematodes developed to infectivity during the winter (1938-39) in Beltsville, Maryland. Small numbers of pre-infective larvae of Ostertagia sp., Nematodirus sp. and Trichostrongylus sp. survived the winter.

Later, Shorb (1943) reported that no larvae of Haemonchus contortus were recovered from grass plots in the spring following the applications of feces containing eggs during the previous winter.

Charles (1943) found that a pasture at Beltsville, Maryland, contaminated with sheep feces containing a great number of eggs of Oesophagostomum columbianum, failed to remain infested with nodular

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worms, after a period of rest from November through May.

Kates (1948) reported, for the conditions existing at Beltsville, Maryland, over fall, winter and spring of 1941-42, that there was no evidence of survival on pasture of the pre-infective larvae of Oesophagostomum columbianum, Cooperia curticei and Bunostomum trisinocephalum. Trichostrongylus spp. and Haemonchus contortus were reported to survive in small numbers while Hematodius spp. and Ostertagia spp. were capable of survival in relatively high numbers.

Serles (1948) also demonstrated at Beltsville, Maryland, that no larvae of Haemonchus contortus could be isolated from the soil of a pasture that was kept free of animals from November 1, 1940 to June 2, 1941.

Dineburg (1944) reported that at Beltsville, Maryland, a lamb that grazed nine days on two plots contaminated with infective larvae of Haemonchus contortus and that was exposed to outdoor conditions during two hundred sixty-six days, failed to become parasitized.

In another paper, Dineburg (1944) could not obtain larvae from plots contaminated with eggs of Haemonchus contortus, after three days of exposure at temperatures below 35°F. (23° to 65°), at Beltsville, Maryland. Shorb (1944) reported that a few larvae of Haemonchus contortus survived from October until the middle of April but all the larvae were sluggish and vacuolated, and probably incapable of infecting the sheep.

Short (1944) found that at temperatures below 55°F. no development to infectivity of Haemonchus contortus took place, and death resulted if the exposure was sufficiently long. A small number of eggs were able to survive as long as two weeks at temperatures from 12°F. to 28°F.

Furman (1944) found that nonembryonated and embryonated eggs of Ostertagia circumcincta survived from ten to twenty-two days at a temperature of 21.2°F., when placed under 5 mm. of water. He reported also that the pre-infective larvae survived between one and two months at a temperature of 41°F. At a temperature of 21.2°F. he found that the infective larvae are killed in about two weeks.

In later work, Furman (1944) under outdoor conditions in California, found a much greater number of infective larvae of Ostertagia circumcincta during the fall and winter months than in the summer.

Hawkins, Cole, Kline and Drudge (1944), studying the course of untreated nematode infections of sheep in Michigan, found that the number of eggs of trichostrongylid worms passed by the ewes reached a minimum during the winter months and rose to a peak in the next spring. The rise and fall in egg counts was accompanied by inverse changes in hemoglobin.

Hawkins, Cole and Kline (1944) reported in Michigan that the pasture was apparently free of Haemonchus contortus in two months in the late summer and early fall, and that Oesophagostomum columbianum and Chabertia ovina were able to maintain infection until three and

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one-half months. Also, they found that Trichostrongylus colubriformis, Haematolius sp. and Trichuris ovis larvae were still viable after four and one-half months.

Dinsburg (1943) reported that at Beltsville, Maryland, eggs and larvae of Cybaea, Trichostrongylus and Cesostomatum were killed, when placed outdoors for fourteen days at a near maximum temperature of exposure, 42°F.. He found also that the preinfective larvae of Ostertagia survived at the same temperatures which killed the other three parasites.

Seghetti and Marsh (1943) studying the parasitism in lambs in eastern Montana obtained larvae of Ostertagia, Trichostrongylus and Haematolius from a pasture that did not contain sheep from November 26, 1943 to April 8, 1944, but their number was small as compared with free living soil nematodes also encountered.

MATERIAL AND METHODS

The experiments reported in this paper were conducted in the sheep barn of the Experimental Station at Michigan State College. Three groups of sheep were used in these experiments. One of the groups received one part of phenothiazine and fourteen parts of salt during the course of this work, while other groups received no medication.

For the purpose of this experiment, names were given to the group: Group A, to the ewes that did not receive phenothiazine; Group B, to the yearlings, also that did not receive the drug; Group

C, to the ewes that received phenothiazine, as described above. These groups of sheep were brought from the pasture to the barn in November, 1946 and groups A and B were kept together while the group C was held in a separate pen.

It was known that the sheep of the flock used in this experiment were parasitized by the following species of nematodes: Chabertia ovina, Cooperia curticei, Ostertagia circumcincta, Ostertagia marshalli, Trichostrongylus sp., Haemonchus contortus, Monostomum sp., Oesophagostomum columbianum, Dictyocaulus filaria, Muellorius capillaris, Trichuris ovis and Strongyloides papillosus.

Before the removal of the animals from the pasture to the barn, fecal examinations were made to check the presence of the worms. Grass of the pasture was checked three times for the presence of infective larvae, by means of a Baermann apparatus.

During the experiment, sheep 100, 193 and 200 died. At necropsy the following worms were picked out: Haemonchus contortus, Monostomum sp., Ostertagia circumcincta, Trichostrongylus sp., Dictyocaulus filaria, Trichuris ovis and Strongyloides papillosus.

Throughout the experiments, temperatures in the barn were recorded with a recording thermometer.

Every two weeks from November to March 17, and every week from March until the end of these experiments, samples of the straw were picked up from the pens, and examined for the presence of the infective larvae of parasitic nematodes. The infective larvae could be obtained

from the straw by using the following method that was adapted from Taylor's modification of Baermann's method. Samples of straw were picked from different points of the sheep barn, at random, in such a way that every spot was represented. On reaching the laboratory, the straw was thoroughly mixed until a homogenous mixture was obtained. About one hundred fifty grams of such a mixture was placed in a large bucket and water was added until it almost reached the top. Then the whole mixture was stirred in order to obtain a complete suspension. After that the straw was allowed to stand for three hours or more in the bucket, after which it was removed. The suspension was then strained through a coarse mesh and again through a fine screen of forty meshes and allowed to settle over night. Next morning, the supernatant fluid was carefully syphoned off leaving a sediment in the bottom of the bucket. This sediment was poured off into large centrifuge tubes which were centrifuged from three or five minutes at 1,000 R.P.M. The supernatant fluid was again carefully syphoned off and the sediment containing the larvae was poured out around filter paper, 15 cms. in diameter, standing over several sheets of filter paper. Care had to be taken in order that the sediment spread over the whole surface of the filter paper. The preparation was watched and before the moisture of the sediment had completely disappeared, it was picked up and placed, sediment side down, into a large Baermann apparatus, previously prepared. The material was allowed to remain in the funnel for forty-eight hours, and then 15 ml. of

liquid were withdrawn and centrifuged for one minute. The supernatant liquid was pipetted off and the sediment examined for the presence of infective larvae.

The feces were obtained directly from the rectum of the sheep and put in small bottles which were placed in the ice box until the egg counts were made. The technique used to count the nematode eggs was the Stoll method, slightly modified by Hawkins, Cole, Kline and Drudge as described below. Four grams of feces were placed in the Stoll flask and distilled water was added to make 60 ml. Some glass beads were put into the flask, and after being shaken for a while, the flask was put into the ice box over night. The next day, 1.5 ml. or 0.15 ml. of the contents of the Stoll flask was placed in a small centrifuge tube which was then filled with concentrated sugar solution. A cover slip was placed on top of the centrifuge tube in contact with the liquid so that only a small bubble remained and centrifuged at 1,000 RPM for about one minute. After this operation, the cover slip was removed, placed on a slide, and the eggs counted. To establish the actual number of eggs per gram of feces, the number of eggs encountered in the slide examined was multiplied by 10 or 100, according to the amount of material previously taken from the Stoll flask. In other words, if the quantity of the Stoll flask contents taken was 1.5 ml., the actual number was obtained by multiplying the number counted on the slide by 10. If 0.15 ml. was used, the result was obtained by multiplying by 100. Generally two or three slides were counted to establish a mean.

In this work, no attempt was made to separate the eggs of the different species.

For hemoglobin determination, the Sanford and Sheard photometric method was used (Hoffmann, 1941). The blood was drawn from the jugular vein and collected in tubes containing potassium oxalate.

The sheep were weighed at two week intervals.

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RESULTS AND DISCUSSION

The first infective larvae from the pens of groups A and B were observed on April 14, in very small numbers, as compared with pre-infective larvae and free-living nematodes. No infective larvae were observed from the pens of group C during these observations.

These infective larvae were identified as Haematodirus sp., Haemonchus contortus, and other short tail larvae, probably Trichostrongylus sp.. No attempt was made to determine the pre-infective larvae.

The results obtained, using the technique described, are recorded in Table I. Free-living nematodes were obtained in great number at all times, not only from the pen of sheep not receiving phenothiazine but also from the pen of sheep receiving that drug. First stage larvae of Dictyocaulus filaria were picked up on February 3, and subsequently. Second stage larvae of this worm were observed on April 1, from both pens. One infective larva of this lung worm was seen on April 7.

On April 14, a number of pre-infective and infective larvae of Trichostrongylidae and pre-infective larvae of Dictyocaulus filaria were recovered from the pen of the sheep not receiving phenothiazine. Only free-living and Dictyocaulus larvae were obtained from the pen of ewes receiving phenothiazine.

A summary of the temperatures prevailing in the barn during the period in which these trials were in progress are recorded in Figure I which shows the maxima and minimum weekly means and the

weekly mean. Examination of this graph make it possible to conclude, in agreement with various authors that have worked on the bionomics of the nematodes parasitizing sheep, that the temperatures recorded during the winter months were too low for the development of these worms. Up to the present time, investigations on the overwintering of nematodes parasitizing sheep have been carried out almost exclusively on pasture or in simulated pasture conditions. We understand that these results may not apply in whole, to the conditions in the barn. Probably other factors interfere with the development of these parasites in the barn. It is known that the temperature under the straw was higher than that recorded in the graphs. Eggs of some species are able to survive at low temperatures for a relatively long time, such as the temperature observed during these experiments. As new layers of straw were added the environment of the nematode eggs became warmer. On the other hand, we must consider the unfavorable conditions to the normal development of the larvae such as the natural fermentations that are able to kill a great number of larvae. Despite adverse temperatures it was evidenced as will be demonstrated below that some eggs developed to infective larvae in late February when the temperature rose to 40°F., and remained near this level. During this period of time there was a peak of 50°F. and a low of 32°F. Therefore, the number of infective larvae must have been very small as was demonstrated by the failure of the Baermann apparatus in recovering them. On March 7, two lambs were born in group A, one of which died on April 10. At post-mortem examination ten

Nematodirus sp. and one Trichostrongylus sp. were recovered. On February 31, yearling 100 died, and at post-mortem examination, Haemonchus contortus, Trichostrongylus sp. Ostertagia circumcincta, Nematodirus sp., Dictyocephalus filaria and Trichuris ovis were found in great number. A few nodules of Oesophagostomum and some immature forms of Haemonchus contortus were obtained. Previously, on February 31 and 24, yearling 200 and 193 died and the following nematodes were recorded: Nematodirus sp., Trichostrongylus sp., Haemonchus contortus, Dictyocephalus filaria and Trichuris ovis. No immature forms were found. The number of nematodes encountered at necropsy of the sheep above cited are recorded in Table II.

We are unable to explain why other species of nematodes, very frequently encountered parasitizing sheep at Michigan State College, could not be found at post mortem examination. It is possible that these worms may have been passed out of the body as their biological cycle had finished.

Between early December, 1946, and late February, 1947, the temperature remained below freezing most of the time. The maximum was 40°F. except for two days in December, the 27th and 28th, when the temperature rose to 46°F. and 43°F. respectively, and on January 25 and 26, when it reached 42°F. The minimum temperature registered was 16°F., on February 5 and 6.

From late March to the end of these experiments, the temperatures recorded were never below 40°F., except on April 1, when it reached 33°F. During this period the temperature rose to a maximum of 67°F. At these

temperatures the development of the free-living stages of parasitic nematodes was possible as was demonstrated by the recovery of the infective larvae from the straw on April 14 and again on April 21. The number of infective larvae was very small as compared with free-living nematodes and Dictyocaulus filaria larvae.

Egg counts of the three groups of sheep in the experiment were made at regular intervals and the results are given in Figures II, III and IV.

Figure II shows the variation observed in the egg counts of the yearlings during this period. On examination of this Figure, we observe that there was a drop in the number of eggs during the winter months. On November 29, 1946, egg counts in this group showed an average of 3,000 per gram of feces. In February, the average fell to 1,100. Fifteen days later, and in subsequent counts, the number of eggs increased sharply to reach 17,500 on March 29. As three animals of this group died, no average was calculated after that date. The two sheep still alive had a great increase in the number of eggs per gram of feces. Yearling 194, from 3,500 on March 29 to 6,000 on April 12; and yearling 195, from 12,500 went to 20,000.

Identical results were obtained from the ewes not receiving pherothiazine, except that there was a decline on March 29, probably because no feces could be collected from ewe 47. On April 12, the rise was observed again. The number of eggs of ewe 47 rose from 8,100 on March 15 to 41,000 on April 25.

Regarding the group of cows receiving a salt-phenothiazine mixture, the results were completely different. Practically no marked difference was observed among the egg counts of this group of sheep. The average ranged from 110 to 357 eggs per gram of feces which can be considered insignificant.

The results observed in groups A and B are in accordance with those observed by Turner and Moon (1929), Zavadowsky and Zvjagintzeo (1932) in Russia, Schmidt (1933) in Germany, Taylor (1935) in England, Marsh (1936), and Hawkins, Cole, Kline and Brudge (1944) in Michigan.

The increase in egg counts may be partially due to ingestion of infective larvae during the period that the sheep remained in the barn. This increase may be partly due to ingestion of infective larvae which survived from the previous fall. Also, this increase may be due to a certain natural periodicity of the worms. We agree with Taylor (1935) who suggests the fluctuation in the egg counts are referable to variation in the rate of egg production of the adult worms rather than the fluctuation in the rate of intake of infective larvae.

The results observed in egg counts of group C confirms those of Habermann and Shorb (1942). They found that phenothiazine-salt mixtures offered to sheep for daily consumption at the rate of 1:14 were effective in stopping the development of parasitic larvae. It is assumed that there was no development of eggs to infective larvae in this pen during the time the animals received that mixture. Thorp and Keith (1943) found that phenothiazine-salt mixture reduced the number of nematode eggs in the feces.

The results of hemoglobin determination during the course of these experiments are summarized in Figures II, III and IV, together with those of egg counts. As will be evidenced by an examination of figures II and III of the sheep not receiving phenothiazine, the rise and fall in egg counts were accompanied by inverse changes in hemoglobin, as already noted by Hawkins, Cole, Kling and Brudge (1944). Regarding the ewes receiving phenothiazine, in examining Figure IV, any conclusions could be obtained. The variations observed are considered normal in sheep.

In order to appreciate the gains in weights of the sheep in these experiments, Figures V, VI and VII were organized which compare the yearlings and ewes not receiving phenothiazine and the ewes receiving this treatment. In Figure V we can see that from November 29, until March 29, the mean weight increase of the yearlings was 13.7 pounds.

Figure VI, shows an average increase of 3.7 pounds per animal. On February 10, there was a big increase that is attributed to some mistake considering the conditions of pregnancy of these animals.

The results found for the group of ewes receiving phenothiazine are shown in Figure VI. There was an average increase of 13.9 pounds per animal.

SUMMARY AND CONCLUSIONS

Experiments were conducted from November, 1946 to April, 1947, at the sheep barn of the Michigan State College Experimental Station, to obtain data on the survival of nematode parasites in the barn during the winter months with notes on hemoglobin, weight and nematode egg counts of sheep.

One group of yearlings and one group of ewes not receiving any treatment and one group of ewes receiving a 1:14 phenothiazine salt mixture during this time were used as test animals in these experiments.

By means of a modified Taylor Baermann technique, samples of straw were picked up at regular intervals to check the presence of infective larvae of nematode parasites. The first infective larvae were observed on April 14, in very small numbers from the pen of the animals not receiving phenothiazine. No infective larvae were observed from the pen of animals receiving a salt-phenothiazine mixture. These larvae were identified as Haematostirus sp., Haemonchus contortus, and other short tail larvae. Dictyocaulus filaria, first and second stage larvae were picked up before April 1, from both groups. From late March to the end of these experiments the temperatures recorded were never below 40°F., except on April 1, when it reached 38°F. During this period the temperature rose to a maximum of 37°F.

Despite low temperatures it was evidenced that some eggs developed to infective larvae in late February when the temperature rose to 40°F. and remained near this level until late in March, with a peak of 50°F. and a low of 33°F. The number of infective larvae

must have been very small as was demonstrated by the failure of the Baermann apparatus to recover them.

There was no evidence of infective larvae from December, 1946 until late February.

There was a drop in the number of nematode eggs during the winter months followed by a rise in the groups of sheep not receiving a salt-phenothiazine mixture.

The fall and rise in egg counts does not appear to correspond with the increase and decrease of parasites.

Practically, there was no marked difference among the egg counts of sheep receiving a salt-phenothiazine mixture.

An inverse fall and rise in hemoglobin was found in comparison with the nematode egg counts.

Between November 29 and March 29, the yearlings gained an average of 13.7 pounds in weight. The ewes not receiving phenothiazine an average gain of 8.7 pounds and the ewes receiving phenothiazine an average of 13.9 pounds.

TABLE I
RECOVERY OF INFECTIVE LARVAE FROM THE BARN

	12/18	1/3	1/13	1/17	2/3	2/17	3/3	3/17	3/19	4/1	4/7	4/14	4/21
Groups A & B	No infective larvae. Free-living nematodes Nematode eggs				No infective larvae Free-living nematodes Nematode eggs Pre-infective larvae of <u>D. filaria</u>				Infective larvae of Trichostrongylidae and <u>D. filaria</u> . Free-living nematodes				
Group C	No infective larvae Free-living nematodes Nematode eggs				No infective larvae Free-living nematodes Nematode eggs Pre-infective larvae of <u>D. filaria</u>				No infective larvae <u>D. filaria</u> , 1st & 2nd stage larvae Free-living nematodes				

TABLE II
NUMBER OF WORMS FOUND IN POST MORTEM EXAMINATION

Sheep	Abomasum	Small Intestine
100	7,100	11,200
198	1,300	3,400
200	3,300	14,300

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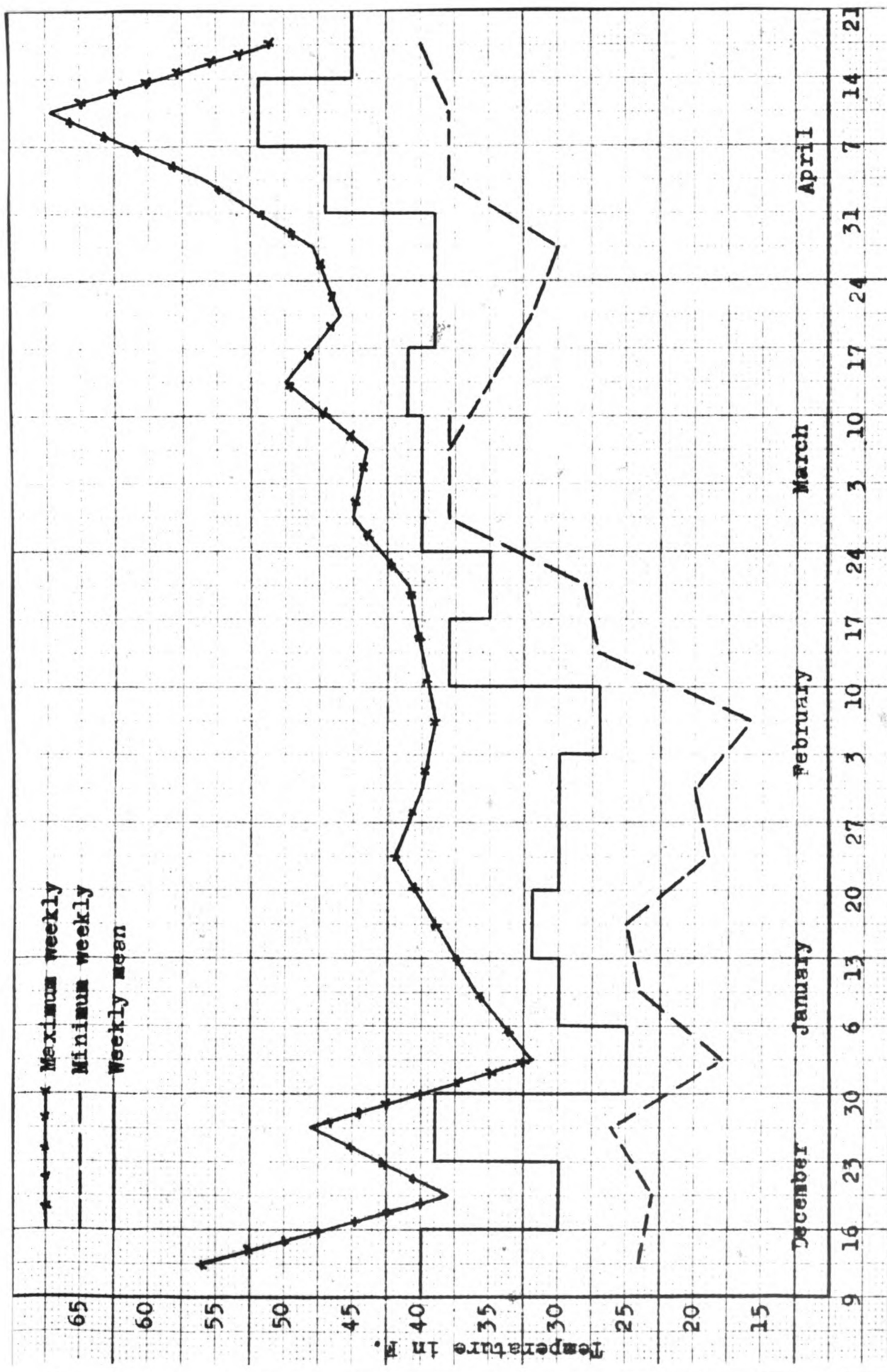


FIG. 1. TEMPERATURES IN THE BARN

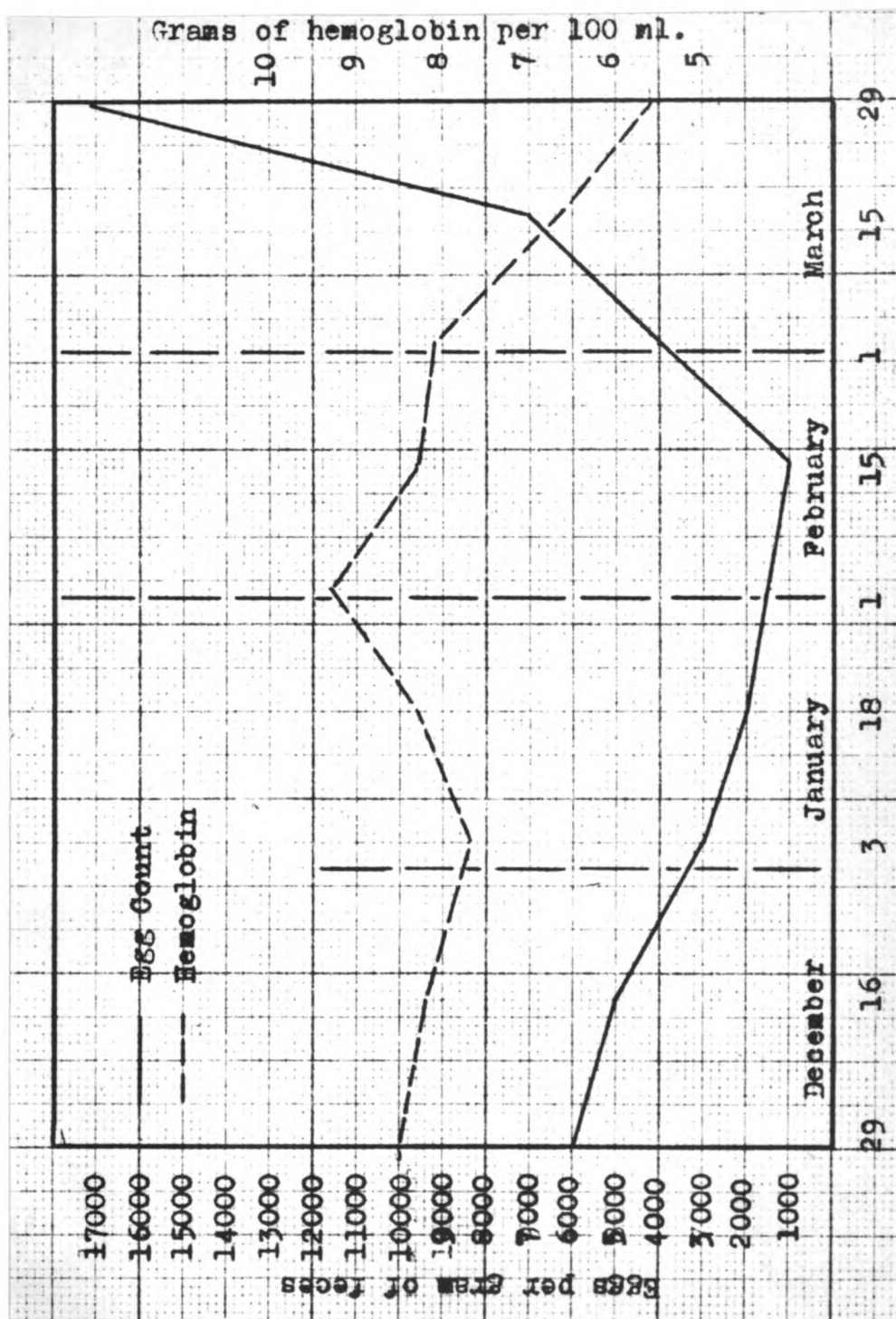


FIG. 11. HEMOGLOBIN AND EGG COUNTS OF YEARLING

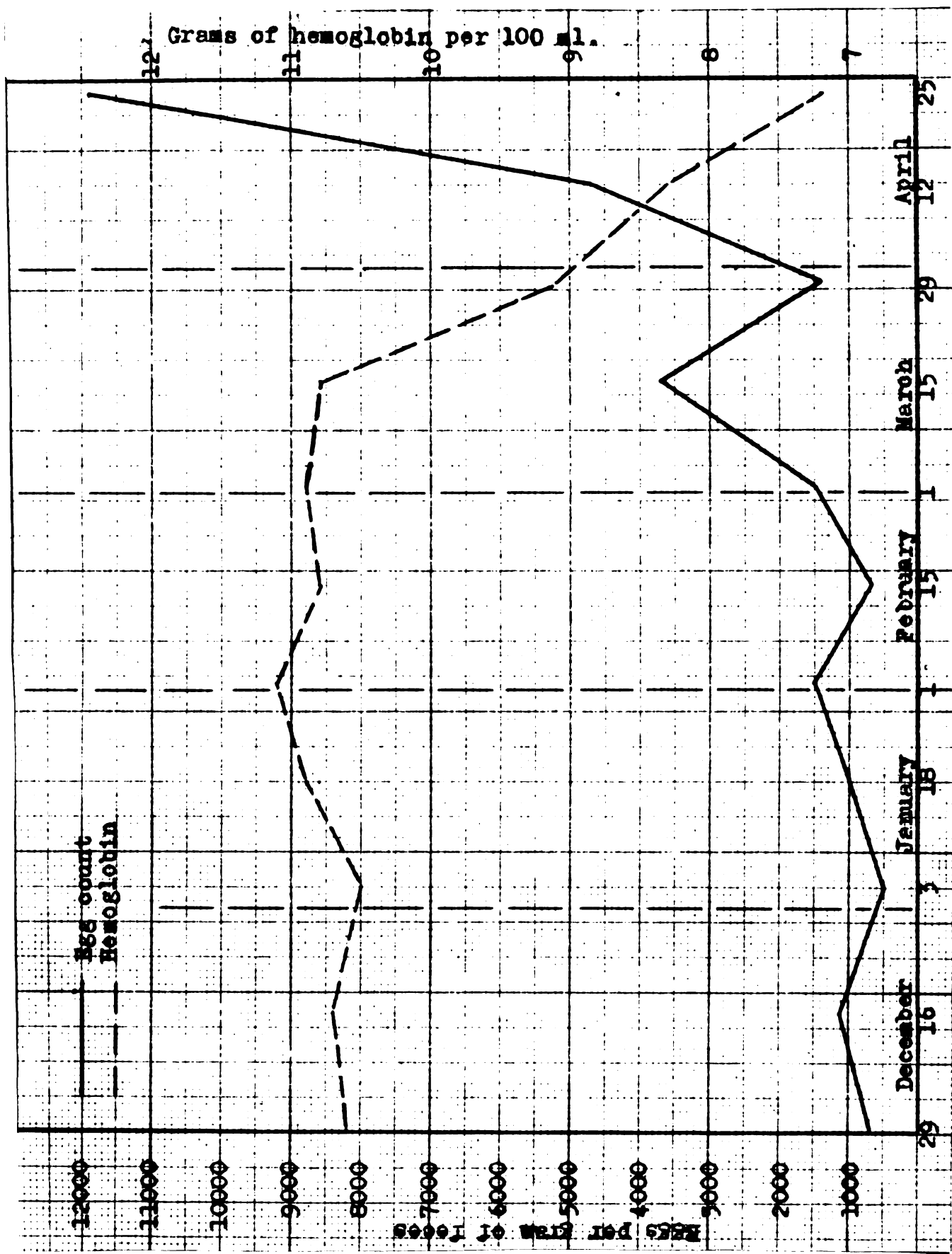


Fig. III. HEMOGLOBIN AND EGG COUNTS OF EWES NOT RECEIVING PHENOTHIAZINE

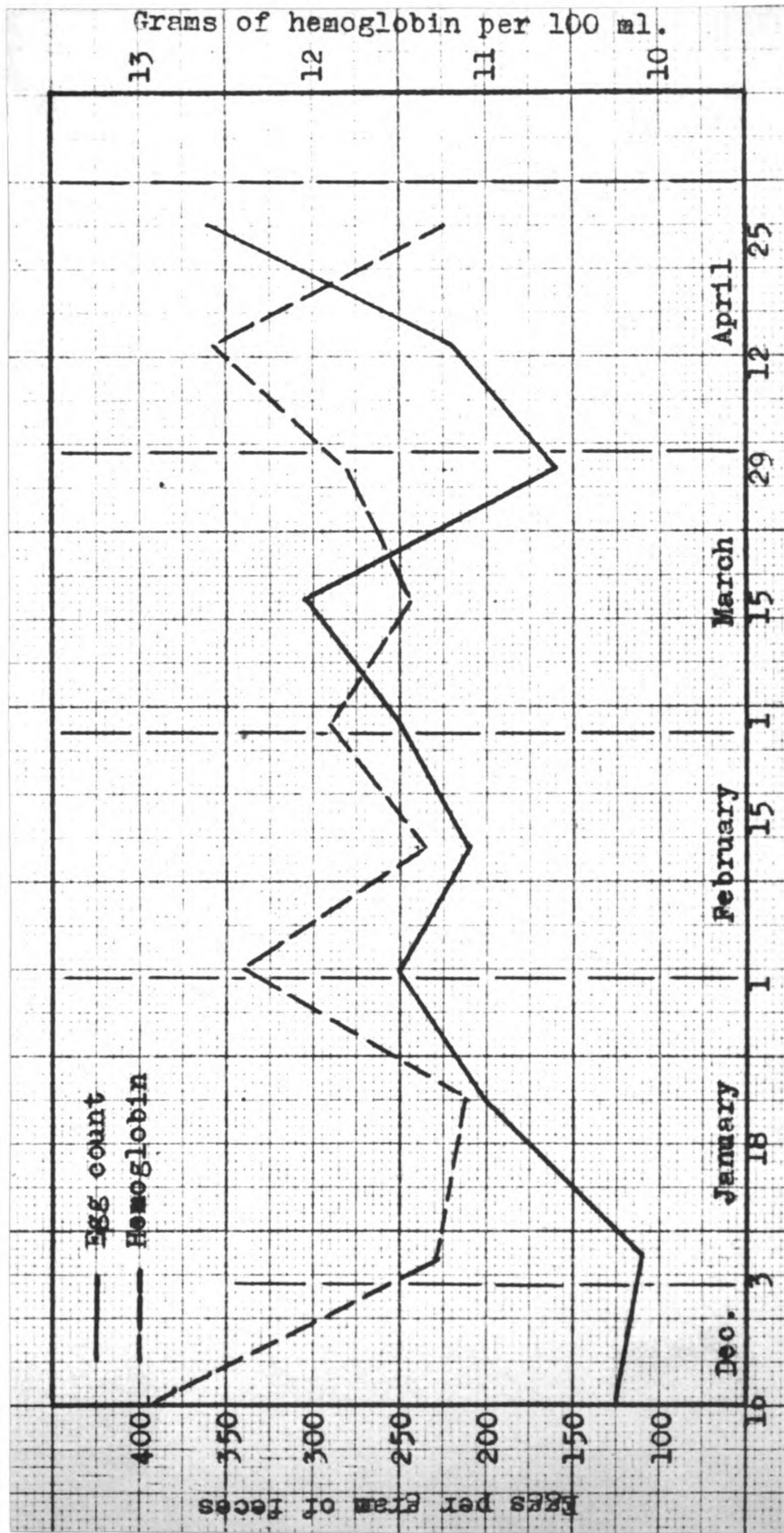


FIG. IV. HEMOGLOBIN AND EGG COUNTS OF EWES RECEIVING PHENOTHIAZINE

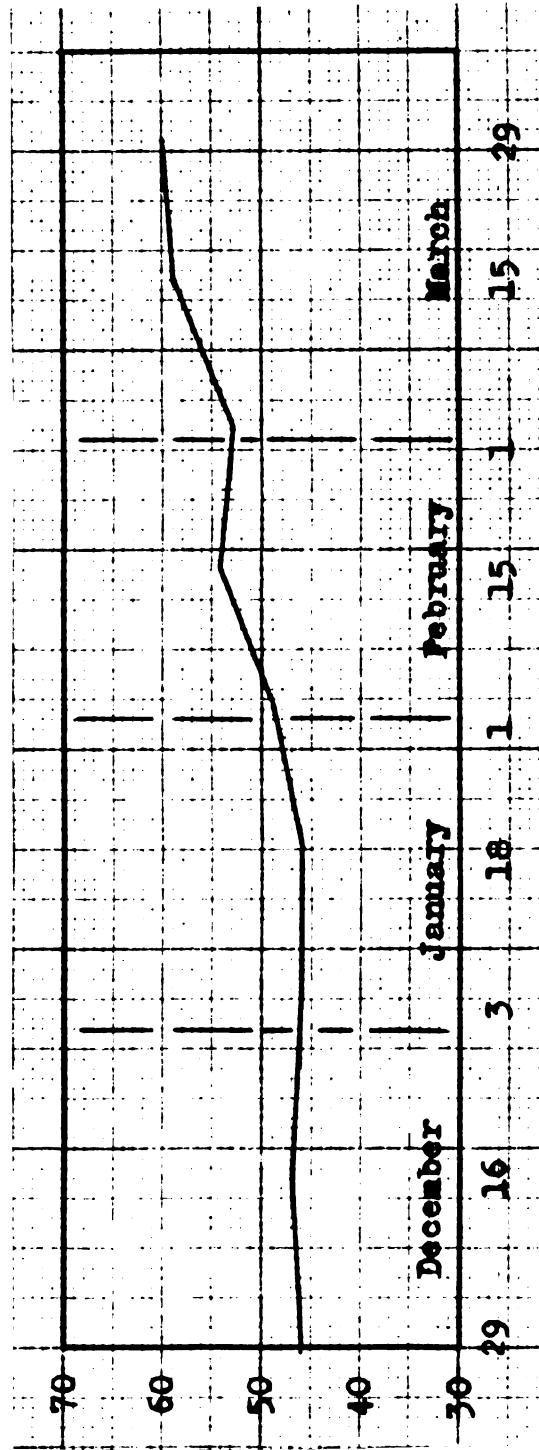


FIG. V. WEIGHTS OF YEARLINGS

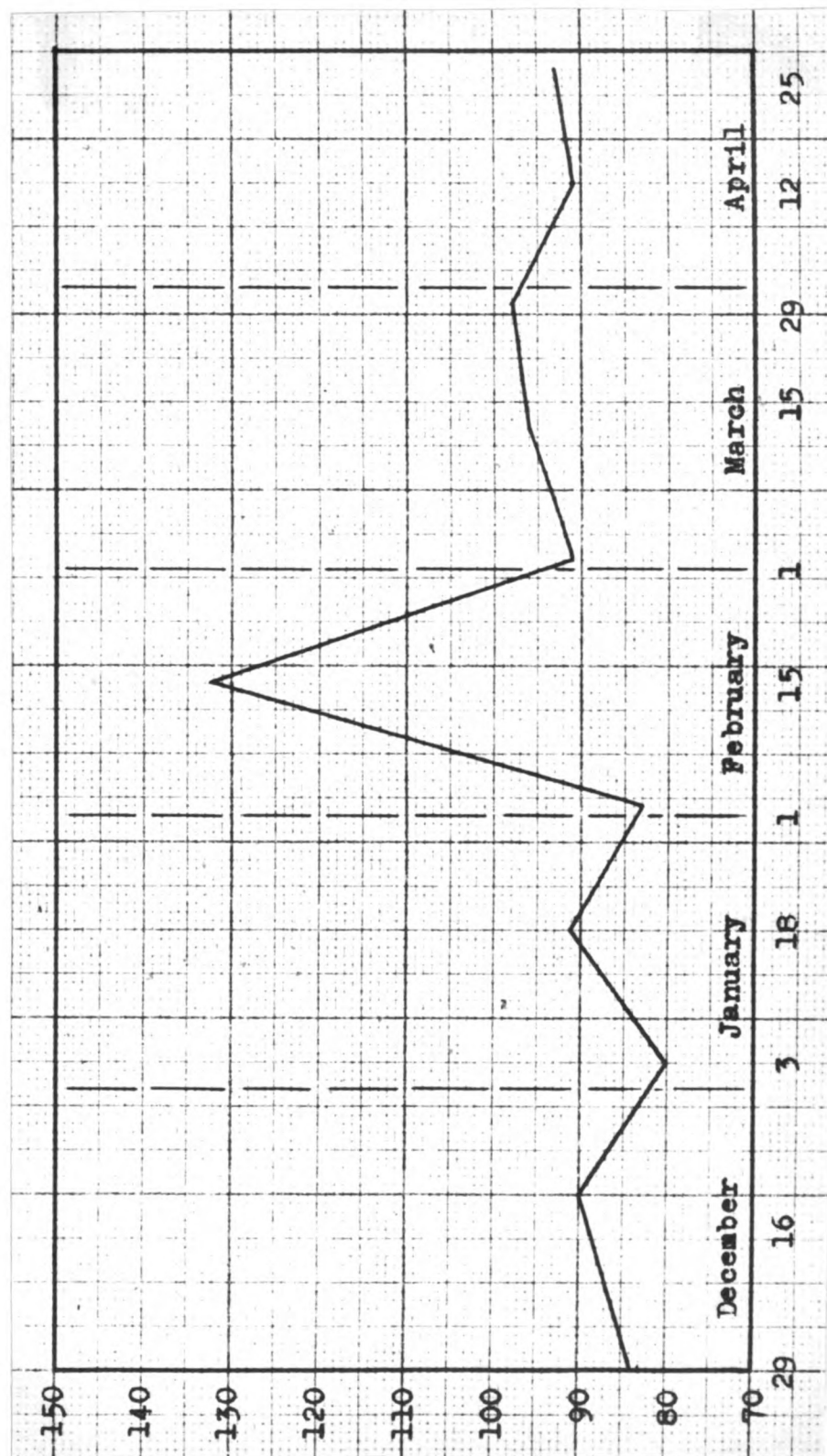


Fig. VI. WEIGHTS OF EWES NOT RECEIVING PHENOTHIAZINE

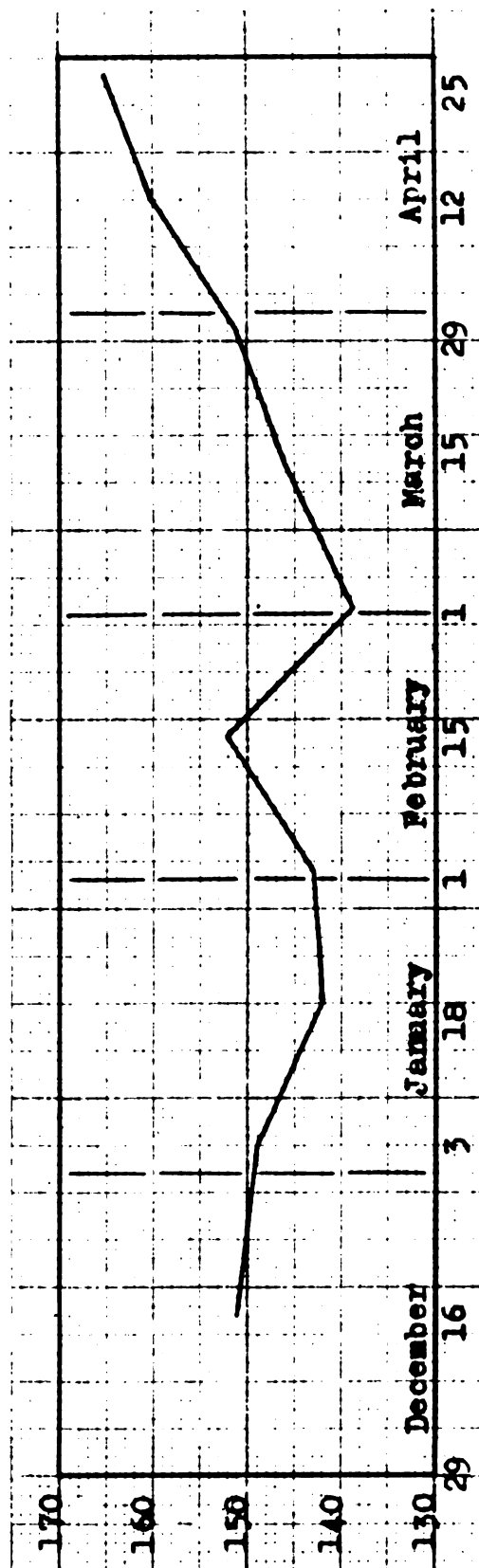
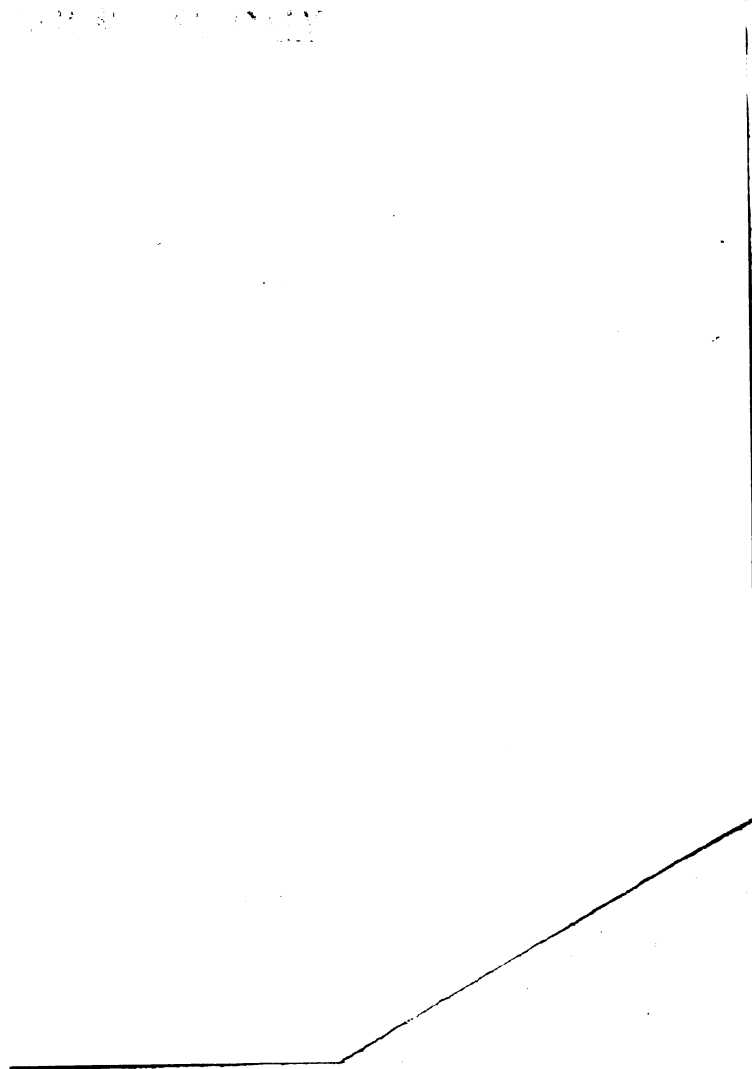


Fig. VII. WEIGHTS OF EWES RECEIVING PHENOTHIAZINE

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