THE RICE INSTITUTE

A CLINICAL STUDY OF THE EFFECTS OF THE AMERICAN SCREW WORM, CALLITROGA HOMINIVORAX (COQUEREL) ON GUINEA PIGS

by

Jack Houston Esslinger

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I. INTRODUCTION

Farmers and ranchers throughout the Nearctic and Neotropical regions of the world are all too well aware of the destruction caused by the American screw worm. This myiasis-producing fly, although long known by reputation, became a taxonomic entity only in 1933 when Cushing and Patton distinguished it from the necrophagous Cochliomyia macellaria, and recognized it as a true parasite, naming it Cochliomyia americana. This name was used tentatively, since the authors were uncertain whether they were dealing with Lucilia hominivorax Coquerel, known to produce myiasis in man in South America. Recently Sabrosky (1953) established the identity of these flies, so Callitroga hominivorax (Coquerel) becomes the correct name.

Although primarily a scourge of livestock, C. hominivorax is also a menace to wildlife (Lindquist, 1937) and is not infrequently found in humans. Micks and Calma (1952) in viewing the incidence of the screw worm in man, especially in the form of nasal myiasis, stress the need for physicians' being acquainted with the identification of the maggots and the treatment of human infestations.

Many papers concerning the ecology and control of C. hominivorax have been published. Numerous other references dealing with the treatment and gross appearance of screw worm infestations can be found. Yet, in spite of its admit-
ted importance, very little is known of the host-parasite relations of the American screw worm. Furthermore, the same situation seems to exist for the myiasis-producing Diptera in general. Two aspects of these relations are immunity and pathogenesis. In the following paragraphs the more important investigations along these lines will be briefly discussed.

Blacklock and Thompson (1923), in working with the Tumbu fly, *Cordylobia anthropophaga*, observed what seemed to be, at that stage of their investigations, a type of age immunity to the maggots of this fly. This concept was not new to them, for several workers had encountered similar situations with this and other myiasis producers (e.g., *Hypoderma*). After attempts to reinfest various animals (guinea pigs, dogs, monkeys) and man, they concluded that this was actually an acquired immunity. Certain inconsistencies were encountered, nevertheless, which could not be explained. Four years later Blacklock and Gordon (1927) solved the riddle and proved conclusively that an acquired immunity does develop against the maggots. This immunity, however, was a local one. Larvae invading an area of the skin which had been previously infested failed to develop and soon died; whereas those exposed to another area developed normally. These workers also showed that the immune region spread until areas which had never been the site of invasion would ultimately become immune. Blacklock *et al.* (1930) in continuing this work, found that the excretions of the larvae possessed antigenic
qualities and whose use in injections produced an immunity without an actual previous infestation. These investigators were among the first in pioneering not only a knowledge of immunity to myiasis-producing flies, but of metazoan immunity in general.

Roubaud and Pérard (1924) echoed the hopes of the times when they expressed the possibility that artificial immunity against such parasites as Hypoderma might be effected by the injections of larval tissues or extracts of these or related flies. Koegel (1933), upon finding specific precipitins in a calf that had been suckled by a cow infected with Hypoderma, suggested that attempts should be made to immunize calves by adding extracts or powdered larvae to their food. According to Tilemann (1940), skin reactions of horses to Gastrophilus sp. antigen showed positive results only after the larvae had left the body, and no antibodies were passed from the mare to the foal. Fures (1939) found that the diagnosis of Gastrophilus nasalis infestation with homologous material was apparently specific, as confirmed in all cases by autopsy. This same author (1942) drew similar conclusions with respect to skin tests for Gastrophilus equi.

An important contribution to knowledge of host-parasite relations of parasitic arthropods was made by Borgstrom (1938). She found that guinea pigs which had been infested with eight larvae (a sublethal dose) of Callitroga hominivorax twenty days previously were able to survive an otherwise lethal infestation of the screw worms in the same area.
of the skin. Furthermore, the exudate from the infested lesions was discovered to have similar immunizing properties. This local immunity, however, did not result in the death or retarded development of the parasites as in the case of *Cordylobia*. It was, rather, manifested by an increased tolerance to the effects of the maggots. Laake and Smith (1939) in confirming Borgstrom's work, found that this tolerance was 50 to 100 percent greater than that of uninfested animals. As in the case of *Cordylobia*, these workers also found an extension of the local immunity. They, as well as Borgstrom, found that other than a slight retardation of death, no tolerance to *C. hominivorax* greater than that in normal guinea pigs resulted from injections of suspended larva tissue.

It is evident, therefore, that both *Cordylobia* and *Callitroga* elicit a local immunity, but the effects of the two are strikingly different. The host reaction to *Cordylobia* apparently leads to the destruction of some essential metabolic product or constituent of the parasite (Chandler, 1953) and consequently the death of the maggot ensues. The immunity to *Callitroga*, on the other hand, appears to be directed against products which, although not essential to the parasite, possess a certain toxicity to the host. That this is the case with screw worms is supported by the conclusion of Laake and Smith (1939) that "no substances deleterious to the larvae were developed by caviies from initial infestations or by as many as three reinfestations."
If a toxic substance, or substances, is to be postulated as an explanation for the immune reaction to *O. hominivorax*, it is obvious that a knowledge of the pathogenesis evoked by the parasite is essential. Although considerable work has been done with the myiasis-producing flies, very few details concerning the clinical picture of infestations with any of these organisms have been disclosed.

The question of pathogenesis in bot and warble infestations is still quite unsettled. By injections of different extracts of *Hypoderma* and *Gastrophilus* larvae, Roubaud and Pérard (1924) studied the effects of the body substances on small laboratory animals. Results showed that these extracts produced a loss in weight, a rise in temperature, diarrhea, and general cachexia. Animals which did not die returned to normal in about eight days. The picture obtained from *Gastrophilus* extracts was more acute than that from *Hypoderma*. It must be noted, however, that nonsterile material was used.

In a series of studies on *Hypoderma* sp., Ono (1932a, 1932b, 1938) and Terada and Ono (1930) found in the midgut of the first and second instar larvae, a substance which they called "hypodermatotoxin." This material was shown to be capable of inducing symptoms resembling anaphylactic shock when injected intravenously into rabbits. It was strongly proteolytic and caused perforation of the skin of guinea pigs at concentrations as low as two percent. Hemorrhagic, coagulating (at low concentrations), and anticoagulating (at higher concentrations) properties were also
demonstrated. Various biochemical aspects of this substance were investigated by Haga (1944), who observed no hemolytic action on the erythrocytes of cattle, horses, sheep, rabbits, and guinea pigs.

Simmons (1939) gives an account of the local reactions of cattle to *H. lineatum* infestations. Lokk (1941) found that extracts of dried larvae of reindeer warble flies (*H. tarandi*) were not particularly toxic to rabbits and mice, but that intravenous doses in cats caused a drop in blood pressure and increased respiration. At low concentrations, heightened coagulation of the blood was noted.

Starkoff (1942), by injecting aqueous extracts of *Gastrophilus* into rabbits, concluded that no hemotoxic substances were present. This worker held that the pathogenic action is caused chiefly by mechanical irritation.

Other references to work on the toxic manifestations of *Hypoderma* and *Gastrophilus* are given by von Brand (1952) who feels that considerable uncertainty exists in both cases.

*Wohlfahrtia vigil* may produce a slight elevation in temperature, irritability, dehydration, and loss of appetite. Large infestations of this primary invader frequently cause death in young mink (James, 1947). Ford (1936) reported a marked cutaneous irritation in children who were exposed to a comparatively slight amount of penetration by the first instar larvae of these flies.

Strike in sheep, caused by *Lucilia cuprina*, *Calliphora* spp., and *Chrysomyia* spp., indicates a certain degree of toxi-
city. Australian workers (Seddon, 1951) have reported that struck lambs show retarded growth and that older sheep lose condition and bear less fleece when badly struck. Deterioration of the whole fleece often results from tenderness of the wool. Rams with infestations on the scrotum may exhibit a degeneration of the semen, and in some cases, temporary sterility. Ewes which are infested often refuse to nurse their lambs; consequently lactation is suppressed and the lambs die. According to Roberts (1940) inflammation of the skin may occur even when the maggots do not penetrate.

Gwatkin and Fallis (1938) found that washings from blow-fly maggots (*Calliphora erythrocephala* and others) possessed certain toxic qualities. The secretions and excretions, when injected intraperitoneally into guinea pigs, caused a rapid fall in temperature and, in many cases, the animals died. *In vitro* tests showed these materials to produce agglutination in slight amounts and hemolysis in higher concentrations. The general symptoms were tenseness of the abdomen and a watery discharge from the eyes. Death usually took place in five to six hours, and autopsy revealed inflammation of the peritoneum and fluid in the abdominal cavity.

Blacklock and his associates (1923, 1927, 1930) reported symptoms resulting from the infestation of man and animals with *Cordylobia anthropophaga*. At first a cutaneous reaction (edema and itching) resulted. Later, pain, enlargement of the lymph nodes, malaise, and fever occurred. Small
animals with infestations of several larvae demonstrated considerable irritation, diminished appetite, and a loss in weight. Necrosis of the infested area was present. Injections of salivary glands, gut, hemocoel fluid, and excreta of the third instar larvae produced marked local and general reactions, and in several cases resulted in death. According to these workers (1930) the excreta were the most toxic of all the substances tested, and an immunity to them could readily be formed. This was demonstrable by precipitin reactions as well as by the reduction of toxic effects produced by subsequent injections. In vitro experiments revealed these metabolic products to have hemolytic properties.

Borgstrom (1938) reported that guinea pigs infested with ten or more larvae of C. hominivorax invariably died. Laake and Smith (1939) established that three larvae per 100 grams body weight was the minimum lethal infestation. Borgstrom found that animals which had a sublethal infestation became inactive, refused food, and lost weight. Daily white blood cell counts on several infested animals showed a drop of about 6500 cells per cubic millimeter by the fifth day. The number returned to normal on the eighth day.

According to Borgstrom, the lesions invariably possessed a nearly pure culture of a gram negative bacillus, Proteus chandleri, for which she postulated a symbiotic relationship to the maggots. It is of interest to note, however, that Emmel (1945) found Streptococcus pyogenes in the healed
screw worm lesions (navel) of calves, and implicated C. hominivorax as a vector of joint ill. Barry (1943) found almost pure cultures of Streptococcus hemolyticus in two cases of nasal myiasis caused by screw worms.

In cases of nasal myiasis (Micks and Calma, 1952) with C. hominivorax, humans exhibited maximum temperatures of 103°F to 104°F, and white blood cell counts above normal.

From the above it is seen that there is very little accurate or detailed knowledge concerning the host-parasite relations of the myiasis-producing Diptera. The purpose of this work, then, is twofold: first, to elucidate the clinical picture presented by screw worm infestations; and second, to provide a basis for future investigations into the apparently antitoxic reactions of hosts previously infested with Callitroga hominivorax.
II. MATERIALS AND METHODS

A. Maintenance of Stock Cultures of Callitroga hominivorax

The original stock of \textit{C. hominivorax} was started from pupae kindly sent by Mr. C. L. Smith, entomologist for the United States Department of Agriculture, Agricultural Research Service, Entomology Research Branch, at Kerrville, Texas. The techniques employed in maintaining the stock cultures were for the most part the same as those practiced at Kerrville (Bushland and Hopkins, 1953). A visit to the laboratories there before the experimental work was begun aided greatly in solving minor problems which had previously been encountered.

The adult flies were kept in screened cages with wooden frames measuring 15 X 15 X 15 inches. Each cage was equipped with a cloth sleeve which permitted manipulation of the contents without the escape of the flies.

During the preoviposition period (about six days after emergence from the pupae) the adults were fed a mixture of ground horse meat and honey. After this time, to avoid the wasting of eggs by oviposition on the food, the meat was removed and a honey and dry oatmeal mixture was substituted. At all times water was provided by a beaker inverted over a pad of filter paper in a petri dish.
By the time the preoviposition period had passed, most of the females had mated and a continual source of eggs was available for two to three weeks. To induce oviposition, the females were isolated in cotton stoppered vials containing a small piece of fresh horse meat. These were placed in an incubator at $37^\circ$ C. and removed after about three hours. The egg masses, which were found adhering to the walls of the vial near the meat, were transferred to moist filter paper in a covered petri dish and allowed to remain overnight at room temperature. Generally, after 12 to 14 hours the first instar larvae had hatched from the eggs.

The artificial medium used to rear the larvae was a modification of that formulated by Melvin and Bushland (1940). Finely ground lean horse meat was substituted for the beef, and citrated human blood from a local blood bank was substituted for the beef blood used by these workers. The following formula represents the medium used throughout these studies.

To 1000 ml. of water add:

- Finely ground lean horse meat..... 1 kg.
- Citrated human blood............... 500 ml.
- Formalin (40% formaldehyde)........ 6 ml.

Previous to inoculation the medium was poured into sterile finger bowls to a depth of about one inch and placed in an incubator at $35^\circ$ C. The newly hatched maggots were then introduced into the warm medium and were kept in the incubator which was provided with a small exhaust pump to assure adequate circulation of air.
After three days the medium became darker and considerable liquefaction had taken place. The maggots were then transferred into fresh medium in a larger enamel pan. During the process of transferring (with a spoon) as little of the old medium as possible was introduced into the fresh. A second transfer was needed in two more days.

About six days after development in the artificial medium the larvae were mature and crawled over the edge of the container into a pan of fine sand. The maggots and sand were removed from the incubator, and the mature larvae pupated at room temperature. The pupae were sifted from the sand, transferred to cylindrical wire-screen containers (capacity about one pint), and placed in a three-gallon crockery jar into which about one inch of water had been added. The pupae were supported on a platform above the water. This arrangement provided the optimum conditions of nearly 100% relative humidity and a temperature of about 80°F. In six to eight days the adults began to emerge and the containers were then removed to the cages, in which a maximum of 150 flies was kept.

B. Experimental Animals

The guinea pigs used throughout this study were obtained locally from a single source. All were females and weighed about 450 grams. Pregnant animals were discarded. During the course of the experiment the guinea pigs were
kept individually in one-half inch wire mesh cages measuring 10 x 14 x 8 inches. Water and food were provided at all times in crockery feeding dishes. The diet was Purina rabbit chow checkers supplemented daily by about 30 grams of fresh lettuce.

C. Method of Infesting

Previous to infestation, an area about two inches in diameter was shaved on the left shoulder of the guinea pig. Animals were anesthetized with ether and were wounded about one centimeter posterior to the left scapula (with leg in sitting position). The wound was made by cutting out a circular piece of skin one centimeter in diameter. The underlying connective tissue and muscle were also cut in several places; care was taken to avoid severing the larger blood vessels.

When the bleeding had stopped, usually within one minute, recently hatched (1-4 hours) first instar larvae were placed into the wound. With a flattened dissecting needle, the larvae were lifted out of the petri dish in which they hatched, counted under a dissecting microscope, and transferred to the tissue just under the edge of the wound. Since preliminary experiments showed exacting sterile precautions to be unnecessary, rubbing the skin with alcohol and flaming the needle immediately prior to infestation proved adequate.
Following infestation the wounds were dressed. The bandages used were two-inch gauze pads bound to the animal by two strips of adhesive tape tied at the ends, one strip around the neck, the other around the thorax. For the first day the bandage was kept moist with normal saline to prevent the wound from drying before the larvae could establish themselves. The bandage was replaced as needed (generally every other day), and by about the fifth day it could be removed without fear of the host's scratching the maggots out of the lesion.

D. Clinical Procedures

In all instances the clinical data were taken daily at the same time, about 10:00 A.M.

Animals were weighed to the nearest gram on the same spring scales throughout the experiment.

Rectal temperature was taken with an oral clinical thermometer, Fahrenheit scale, which was accurate within plus or minus 0.15° F. The bulb was inserted 4 cm. into the anus and was withdrawn after one and one-half minutes. After this time, no increase in the height of the mercury column was ever observed.

Certain general aspects were also considered. These included the general appearance of the animal, whether firm or bony; the activity, mainly on the basis of response to noise or offering of lettuce; and the amount and condition
of the stools. The bandage was untied and turned back to allow observation of the lesion; the size, color, amount of destruction, and the amount and color of the exudate were noted. At this time the condition, size, and number of maggots in the lesion were observed. At intervals of about five days the amount of food consumed was measured, since a known quantity was given at first, and a record of this was kept over the period of clinical observation.

Bacteriological investigations were carried out on nine of the animals (1-9). Daily a direct smear and a streak plate were made of the lesion fluid of each animal. The agar plates were examined after 24 hours' incubation at 37° C. and smears were made of each kind of colony present. The slides were subsequently studied after gram stains had been made.

Blood counts were made with A.O. Spencer Bright-line haemacytometers. Two were used throughout the entire experiment, one consistently for white cells and the other for red cells.

Erythrocytes were diluted 1:200 with Hayem's fluid in standard red cell pipettes. Samples were shaken for two minutes before filling the counting chamber. The cells in the five groups (center and four corners) of 16 small squares of the central square millimeter were counted under 430 X magnification.

Leucocytes were diluted 1:20 with 3% acetic acid (methyl violet added) in standard white cell pipettes. Samples were shaken as for the red cells. Five millimeter squares
(center and four corners) were counted on each side of the haemacytometer (a total of 10 mm$^2$) under 100 X magnification.

The blood was obtained by puncturing a superficial ear vein with a stylet made from the slivered edge of a razor blade. Before puncturing, the ear was rubbed briskly with cotton moistened with alcohol. When the blood welled up and a sufficiently large drop was formed, both samples were pipetted rapidly. After the samples were obtained, the ear was wiped with a tissue, and bleeding usually stopped immediately. Since blood was taken daily, each ear was used only every other day.

E. Grouping of Animals and Experimental Procedure

The experimental animals considered in this study, thirty-five in all, were placed in the following categories. The parentheses show the numbers of the guinea pigs included within the group.

Normal Controls: Animals wounded but not infested (1, 4, 7, 28).

Bacteria Controls: Animals wounded and infested, but which lost all larvae by the third day. Tests showed the typical gram negative bacillus (Borgstrom's Proteus chandleri) to be present (8, 9, 16, 18).

Weight Controls for Food: Animals neither wounded nor infested, but fed a diet reduced to that consumed by the heavy sublethals. With this group weight only was consi-
dered to determine whether loss of appetite alone with its consequent lowered food intake was responsible for the weight loss in the heavily parasitized hosts (32, 33, 34, 35, 36).

Light Sublethal Infestations: Animals surviving an infestation in which 1.5 or fewer larvae per 100 grams body weight matured (2, 5, 10, 14, 20, 22, 23, 24).

Heavy Sublethal Infestations: Animals surviving an infestation in which 1.6 or more larvae per 100 grams body weight matured (3, 6, 11, 12, 13, 15, 17, 29).

Lethal Infestations: Animals which did not survive an infestation (21, 25, 26, 27, 30, 31).

Because guinea pig number 19 was discovered to be pregnant during the course of the experiment, it was discarded.

Since, in this study, a course of infestation as nearly natural as possible was desired, no attempt was made to remove all the larvae when a few started to leave the wound at maturity. Consequently, in some cases a few maggots remained in the host tissues and died when the lesion closed. The number of pupae recovered, therefore, did not represent the actual number of screw worms in the lesion. For this reason the number of larvae infesting the animal, a basis for the above categories, was generally considered to be the number of mature larvae seen within the lesion. As mentioned previously, this was checked each day. It is felt that the number of larvae per 100 grams body weight is a more accurate expression of the degree of infestation than the actual num-
ber parasitizing the animal, and this method is followed here.


Aside from the experimental conditions specified, treatment of all animals, including clinical procedures, was identical. Throughout the entire period the room temperature was about 70° F. Data were collected daily for each animal, starting usually at least three days before wounding, and continuing until three days after the mature larvae had left the lesion. Controls were run for a comparable time.
III. RESULTS

A. General Aspects

The mean fraction of larvae introduced which developed, exclusive of bacteria controls, was 59.2%, a value somewhat lower than that (82%) obtained by Laake and Smith (1939). The figures ranged from 17 to 100%.

The degrees of infestation in the light sublethal group were from 0.7 to 1.4 larvae per 100 grams body weight. The corresponding ranges for the heavy sublethals and lethals were 1.6 to 7.7 and 3.2 to 9.5, respectively. The highest value for the heavy sublethals (animal No. 29) is of interest, since the above workers found that three larvae per 100 grams was nearly always lethal, although they occasionally encountered exceptions.

The duration of the infestation within the groups was quite consistent. In the light sublethal cases the maggots started to emerge from the lesion on the seventh day and most were gone on the eighth, although two animals lost their parasites a day earlier. Larvae in the heavy sublethal group started leaving the wound on the sixth day and were out on the seventh, with one exception in which they dropped out the eighth day.

Most infested animals exhibited marked edema and redness at the site of the wound by the third or fourth day.
No such reaction was noted in either group of controls. The condition of the lesion during the course of the infestation was identical to the description given by Borgström (1938). At first a clear serous fluid was present. Later (by the fourth day) the discharge became dark and extremely malodorous. During the final stages, the lesion became black and dried near the surface. The appearance of the lesion is seen on Plate I. In both sets of controls the lesion had formed a scab by the fifth or sixth day after wounding, and was healed by the eighth or ninth day. In infested animals, except those with very few larvae, the lesion began to increase in diameter by the sixth day and reached a maximum (about 2 cm.) at the time the maggots began emerging. Lesions were closed, in most cases, the fourth day after emergence of the parasites, and the wounds healed in about three more days. In some of the infested animals only, purulence was observed in the lesion after the maggots had left. This was attributed to the presence of dead larvae in the wound.

All controls remained firm and active throughout the study. Light sublethal cases showed little departure from this condition during the first four or five days, but after this time they became more quiet than usual and were somewhat bony. Appetites of these animals were only slightly less than normal.

With heavily infested guinea pigs, the picture was quite different. By the fourth day activity had begun to diminish. Individuals were at first uneasy but later sat
Screw Worms *in situ*

This lesion is typical of the heavy sublethal cases. The mature larvae, seen here with their spiracular plates exposed in the cavity of the lesion, are in their sixth day of development. Note the dark exudate at the bottom of the wound.

*(slightly magnified)*
quietly, occasionally changing their positions with seemingly great effort. Although most animals readily ate the lettuce provided, other food was often neglected. It was found that these animals consumed an average of 27 grams each of chow per day, as compared to 37 grams consumed by the controls. During the last three or four days of the infestation practically no food was eaten. When the maggots left the animals, however, the normal appetite immediately returned. Toward the end of the infestation these hosts were extremely bony and flaccid (see Plate II). Their eyes were partly closed and a watery discharge was usually present. The coat became ruffled and hair in the vicinity of the lesion was easily pulled out.

Lethal cases presented an exaggeration of the conditions found in the foregoing group. Activity was less, food intake lower, and all showed extremely irregular breathing after about the fifth day of infestation. Two animals died on the sixth day while the maggots were still within the wound. Two died on the seventh day as the larvae were emerging, and the remaining two died on the tenth (two days after the emergence of the maggots). Although the most heavily infested (9.5, 6.7) died first, no correlation between the time of death and the degree of infestation could be seen, for the two dying on the seventh day had 3.2 and 6.7 larvae per 100 grams, respectively, and those surviving until the tenth had 5.1 and 5.9.
A Heavy Sublethal Case

This animal (No. 29) illustrates the condition of the host at the time the screw worms begin to emerge from the lesion.
Autopsy of the lethal cases revealed extensive destruction of subcutaneous and muscle tissue near the original lesion. In almost all cases, the muscle had been destroyed to the bone on the left scapula, and a necrotic area about 5 cm. in diameter was seen beneath the skin. In no cases, however, had maggots penetrated the body cavity. Internally, in most animals, no abnormal conditions were observed. One animal (No. 27) had a large amount of clear fluid in the pleural cavity, and another (No. 25) showed slight fibrosis on the surface of one lobe of the liver.

Stools of the lethal and heavy sublethal animals during the last three or four days of the infestation were few, small, and very soft. All other groups of animals produced stools of normal quantity, size, and consistency.

B. Bacteriological Investigations

In the light of Borgstrom's discovering nearly pure cultures of Proteus chandleri in lesions infested with C. hominivorax, and the subsequent findings of other workers (see introduction), it was desired to make at least a cursory bacteriological investigation.

In all infestations, the exudate revealed the presence of a short, gram negative bacillus, in pure culture, from the second day of infestation until the mature larvae emerged. On the basis of tests used by Borgstrom, the organism has been tentatively identified as the same she described. Even
when the lesion became purulent, only this organism was disclosed.

Normal controls, on the other hand, were never found to harbor *P. chandleri*. For the most part, the lesions were sterile, but two animals possessed *Micrococcus pyogenes* var. *albus* and *M. pyogenes* var. *aureus* during the first two days following wounding.

Bacteria controls at first revealed heavy pure cultures of *P. chandleri*, but the organisms began to diminish by the fourth day of infestation, and practically none were present by the time the scab had formed.

C. Weight

Since the individual weights of the different animals at the beginning of the experiment varied somewhat, gains and losses expressed as a percent of the original weight (the day wounded) were found to be more comparable than absolute changes. In nearly all cases, the weight considered for day zero was the mean of the weights of the same animal during the period before wounding (usually three days). These data are graphed on Plate III.

Normal controls exhibited but a slight gain in weight; the operation itself seemingly caused no loss. The mean rise at the end of ten days was 4.2%. Daily fluctuations of individual animals were occasionally as high as this, but the mean changes from day to day were never over 2.2%.
Bacteria controls, on the other hand, showed a marked increase in weight from the time of infestation; no explanation can be found for this difference from the normal controls. At five days the mean gain was 3.8%, and on the tenth day 7.5%. Again, in this group, no effects of the wounding procedure or mechanics of infesting were seen. The bacteria gave no evidence of a deleterious effect manifested by a loss of weight of the host.

Light sublethals showed a slight drop in weight the first day of infestation. For the next six days the weight stayed practically the same as the original. On the eighth day, when nearly all the larvae had dropped out, a rise occurred and continued for the following period. The mean gain by the tenth day was 4.2%. On the whole, the picture was not dissimilar to that of the normal controls.

Heavy sublethal cases began to lose weight consistently from the day of infestation. The main downward trend began on about the third day; the greatest loss occurred between the fifth and sixth days. The lowest point was reached on the eighth day, representing a mean loss of 11.8%. This was two days after the majority of the parasites had begun to leave the wound. From this day on the weight returned slowly, but even by the twelfth day the loss was still 9.5%. As stated previously, these animals ate considerably less food than did the controls. Food controls, fed the same amount of food as the average consumed by the heavy sublethals, lost only 3.4% of the original weight.
WEIGHT

Days Subsequent to Wounding

Mean % Change in Weight from Day Wounded

- Normal Controls
- Heavy Sublethals
- Controls for Food
- Bacteria Controls
- Light Sublethals
- Lethals
over a period of nine days, and the greatest mean loss, occurring on the third day, was only 5.1%.

Lethal cases underwent a precipitous loss in weight from the day infested until death. This is illustrated in the graph. From the seventh day and following, the curve represents increasingly fewer animals. The mean loss in weight at death was 26.3% and the values within the group ranged between 21.3% and 30.5%. As mentioned in the section on general aspects, the animals were in an extremely emaciated condition when they died.

D. Temperatures

In determining the effects on temperatures within each group, values both before and after wounding are considered. The line graphs (see Plate IV) represent the mean daily temperatures. Day zero is the mean of all temperatures (usually three days per animal) before wounding. The bars on each day represent the maximum range of the temperatures, from low to high, recorded for any of the animals; that for day zero shows the range over a three day period before wounding. All temperatures are in degrees Fahrenheit.

Normal controls, previous to wounding, showed a range of lowest temperatures of 101.3° to 101.7° and a high range of 102.6° to 103.3°. Afterwards these were 101.2° - 101.4° and 102.5° - 103.2°, respectively. Since virtually no difference exists, it is evident that the process of wounding by
itself had no effect on the temperature of the animals.

During the period before infestation, bacteria controls showed ranges of 100.5° - 101.8° and 101.4° - 104.0°. After infesting, these were 100.4° - 101.6° and 102.5° - 103.6°. The temperature of 104.0° occurred only once. The next highest value for this same animal was 103.5°, which would express the upper limit of the normal range more accurately. As in the normal controls, the values after wounding fell within or very nearly within the ranges before. Of the four animals, two exhibited the highest temperature on days one and two, respectively, and the others both on the ninth day, about the time the wound was healed. Of the two animals showing the maximum early, only one possessed a temperature exceeding any encountered before wounding. There is no evidence, therefore, that the bacterial infections had any influence on the temperature.

The light sublethal cases before infestation showed a high range of 101.4° - 102.8° and a low of 100.5° - 102.1°. The low range after infestation, 100.7° - 101.7°, was well within the limits of that before. The range of the highest temperatures, however, was 103.2° - 105.0°. These values were all above the corresponding range prior to infestation, with a difference between the means of the highest temperatures before and after wounding and infesting of 2.2°. On the whole, the temperatures began to rise on the first day of infestation and proceeded upward rapidly until the fourth day, upon which the mean temperature was 103.6°. The peak,
a mean of $103.8^\circ$, was reached on the sixth day, after which the values fell rapidly to $102.1^\circ$ on the eighth day. In the period following, the temperatures began to return to within the normal range. Maximum temperatures for individuals, however, did not all fall on the sixth day. One animal showed the peak on the third day of infestation, two on the fourth, three on the fifth, and two on the sixth. Likewise, the return to the normal range was not consistent between animals. Four were back within the normal range on the fifth or sixth day after the peak, one on the third, and one the day after the maximum was reached; two animals retained temperatures slightly above their normal ranges to the end of the period of observation.

Heavy sublethal cases exhibited a similar but somewhat more extreme picture as compared to the above animals. The high range before infestation was $101.7^\circ - 103.1^\circ$. The low for this same period was $101.0^\circ - 102.0^\circ$. After infestation the temperatures were from $103.4^\circ - 105.6^\circ$ in the high range and $99.6^\circ - 102.0^\circ$ in the low range. On a mean daily basis, the temperature began to rise sharply by the second day and reached a maximum on the fourth, representing a jump of $2.4^\circ$ ($101.9^\circ - 104.3^\circ$) from the mean normal temperature. This highest point was during the period of maximum growth of the third instar larvae. The peak remained until the next day, and between the fifth and sixth, one day before the larvae emerged, the temperature dropped precipitously to a value ($102.3^\circ$) within the normal range, near which it remained un-
TEMPERATURE

Days Subsequent to Wounding

Mean Temperature in Degrees Fahrenheit

Normal Controls

Bacteria Controls

Light Sublethals

Heavy Sublethals
til the end of the observations. The mean high temperature was 104.6°, a value 2.3° above the mean normal maximum. One animal reached the peak on the third day, four on the fourth, and three on the fifth. No order was seen in the return to the normal range of temperatures. Some animals dropped irregularly to values below their normal range, but returned to a normal temperature. Some remained slightly higher than the normal range, and others showed a steady decline to normality by the time the maggots had left them. The greatest rise encountered (3.9°) was from a mean normal temperature of 101.7° (animal No. 3 - 2.0 larvae/100 g.) to a maximum, on the fifth day, of 105.6°.

The lethal cases did not lend themselves to generalized treatment as did the preceding groups. Before infestation the low and high ranges for individual animals (100.8° - 101.8° and 101.4° - 102.4°, respectively) were comparable to those in other categories. After infestation the mean high temperature was 104.7°, with a range from 103.7° to 105.6°. The peaks in general fell earlier in these animals than in the other infested groups. Two animals reached a maximum temperature on the second day, one on the third, and three on the fourth. One animal had a peak of 105.6°, representing a rise of 4.0° from its normal mean. In four of the cases, almost immediately after the peak was reached, the temperature dropped to far below the normal range for the animal. Three of these had temperatures below 94.0° on the day of or before death. Two animals, those surviving to
the tenth day, did not experience such a drop.

E. The Blood Picture

(1) Erythrocyte Counts

In this section, for the sake of brevity, a figure such as 5.68 is understood to mean 5.68 million cells per cubic millimeter. In all cases, a comparison was made between the counts during the period before wounding (usually three days) and those on days 7, 8, and 9. These particular days were selected because the lowest counts manifested by the heavy sublethal cases fell principally within this period. Graphs showing the mean daily erythrocyte counts for each group are seen on Plate V. A horizontal line extending from day zero represents the mean count of the days prior to wounding, and therefore depicts the mean normal value of the group.

The normal controls exhibited neither a rise nor a drop in erythrocytes as a result of wounding. The mean count before wounding was 6.23; that after was 6.32, a difference of only 0.09. In all cases daily variations exceeding this occurred during the normal period.

Bacteria controls presented an almost identical picture. The mean before infesting was 5.92, and that during days 7-9 was 5.82. As with the normal controls, larger daily variations than this were invariably encountered within the normal period.
ERYTHROCYTE COUNT

Days Subsequent to Wounding

Mean Erythrocyte Count in Millions/μm$^3$

- Normal Controls
- Bacteria Controls
- Light Sublethals
- Heavy Sublethals
- Lethals
Light sublethals possessed a mean count of 6.00 before infesting. On the fifth day, the mean counts as well as most individual counts began to drop. The mean count on days 7-9 was 4.93, a difference of 1.07 from the normal mean. After this period, for the most part, red cell counts began to return to normal. The low points were reached as follows: two on the sixth day, two on the seventh day, one on the eighth, and one on the tenth. Of the two remaining animals, one showed no counts lower than those it possessed during the period before infestation, and the other only one count (sixth day) which was but slightly lower. In six of the eight animals, the minimum count recorded after infesting was below the lowest count within the normal range. The difference between these two points exceeded the difference between the extremes encountered before infesting.

Heavy sublethal cases, in most instances, showed a more acute drop in the red cell count than did animals in the foregoing group. The mean count before infesting was 5.90, and that during the seventh to the ninth days after infesting was 3.93, thus showing a mean drop of 1.97. The mean drop from the lowest normal value to the lowest count after infestation was 2.03, with values in individual animals from 0.49 to 3.46. In six of the eight animals the drop after infestation was greater, usually considerably so, than the difference between the extremes recorded prior to infestation. In the majority of the animals the most precipitous drop occurred between the fourth and sixth days. By the
eleventh or twelfth days the counts began to rise slightly. The low count was reached for one guinea pig on the sixth day, one on the seventh, two on the eighth, two on the ninth, and two on the eleventh. Low counts were maintained for three or four days, the lowest usually being in the middle of this period. In these heavily infested animals a definite anemia was evident.

In the lethal cases, the erythrocyte count dropped sharply from the third or fourth day on. The mean count prior to infestation was 6.00. The mean of the counts for the two days preceding death was 3.82, a difference of 2.18. Individual counts (the average of the last two days) ranged from 2.37 (a drop of 63.4%) to 4.71 (a drop of 19.0%). In the lethal cases, infestations caused a progressive drop in the erythrocyte count, resulting in a marked anemia at the time of death.

(2) Leucocyte Counts

The picture provided by the white blood cells, in spite of wide normal variations from day to day in normal animals, showed a consistent tendency to a mild leucocytosis for the first two or three days after wounding, followed by a sharp drop to a definite leucopenia in the maggot-infested animals, reaching a low point on the fifth or sixth day. Generally, individual animals conformed reasonably well to the mean counts within the respective experimental groups, and the
results will be expressed for the most part as such means. Graphs indicating the mean daily counts are seen on Plate VI. To reduce daily irregularities, yet still preserve the general contour of the graphed data, values for each two consecutive days, i.e., days 1 and 2, 2 and 3, 3 and 4, etc., have been averaged, and consequently the points fall between the days. The values cited in the text, therefore, are not the same as those plotted on the graphs, but the latter are sufficiently close to give an accurate picture of the trends. As in the preceding group, the counts are abbreviated, and the numbers are to be understood as thousands of cells per cubic millimeter.

The normal controls showed a mean leucocyte count of 14.75 during the period before wounding. A rise was seen on the first or second day to a mean (second day) of 20.11, i.e., an increase of 36.3% occurred in the average count. After this initial rise, some animals dropped back to the vicinity of their normal counts, whereas others remained higher. By the sixth day most counts were close to normal. No evidence of a consistent fall in the leucocyte count below normal was seen.

Quite a similar picture was seen with the bacteria controls. A mean rise the first day from 12.80 to 16.52 (29.1%) was followed in all cases by a return, in a day or two, to within the normal ranges. A normal count was seen throughout the rest of the observational period.
The light sublethals, with a normal mean of 13.51, rose to 17.83 (32.0%) by the first or second day. One animal had a maximum count on the third. After the rise, the counts returned to normal, but about the fifth to seventh days a leucopenia developed; the counts dropped to a mean low of 8.73, representing a 35.4% loss from the normal mean count. Four animals reached a low on the fifth day, three on the sixth, and one on the seventh. A continual rise in the leucocyte count was evident from the day after the low was reached, and by the tenth day values were above the normal range.

Leucocyte counts of the heavy sublethals rose on the first or second day to a mean high of 19.84, a 57.6% increase over the mean normal count of 12.59. This was immediately followed by a very sharp drop in the count, which reached a minimum on the fourth or fifth day. The mean low was 7.53, a 40.2% drop from the normal. As in the light sublethal cases, the count began to rise immediately, and by the sixth or seventh day, most counts were back to normal. Five of the eight animals showed increases above the normal on at least one day after the low was passed; however, these were very irregularly distributed, and occurred either before or after the parasites had left.

Lethal cases, for the most part, showed no initial rise in the leucocyte count. A steady drop was observed in nearly all animals. The lowest counts were found distributed as follows: one on the second day, one on the third, two on
the fourth, and two on the fifth. The mean of these lowest counts, irrespective of the days upon which they occurred, was 6.72, representing a drop of 42.6% from the mean normal value (11.70). Since these low counts are spread over four days (2-5), the leucopenia is not clearly evident on the graph showing the daily means. All animals demonstrated a steady rise after the low count was reached. Of three animals, two surviving to the tenth day and one to the seventh, the increase in the leucocyte count exceeded 100%. Two others reached the normal range before death, and one died on the day (fifth) of the lowest count. No correlation could be seen between the leucocyte picture and the time of death.
LEUCOCYTE COUNT

Days Subsequent to Wounding

Mean Leucocyte Count in Thousands/mm³
IV DISCUSSION

In the preceding section, data have been given illustrating changes in the weight, the temperature, and the blood picture of guinea pigs infested in different degrees with screw worms. As they were presented, little attempt was made to correlate the results, either with each other or with the course of larval development in the host; an integration of these different aspects, as seen in the sublethal infestations, into a generalized picture is presented here. The courses taken by the lethal cases were somewhat different from those of the sublethals, and will be discussed separately.

The most pronounced change over the first two days following infesting was the development of a mild leucocytosis. Controls manifested this same phenomenon, although to a lesser extent. The leucocytic response of guinea pigs to excitement, etc., is quite rapid, changes being noted within an hour after stimulation (Schweizer, 1953). It is felt that for the most part this initial rise in the leucocyte count may be attributed to such factors as anesthetization, wounding, and the consequent post-operative discomfort. However, the extent of leucocytosis was greater in the heavy sublethal cases than in the other groups, and possibly here there was present an additional factor of irritation by the penetration of numerous larvae into the tissues and the secretion of certain products by the invading maggots.
Over this same two-day period a slight rise in the temperature of the host animals was observed. Little effect on the weight, except a slight drop in the heavy sublethal cases, was noted. Erythrocyte counts showed no change. Data collected by Laake et al. (1936) indicate that the maggots undergo ecdysis after about twenty-four hours in the host, becoming second instar larvae. These first two instars, present during the initial two days of infestation, therefore showed very slight if any pathogenic tendencies.

On the third day of infestation, nearly all hosts bore predominantly newly-molted third instar larvae which were easily recognizable by their darkly pigmented tracheal trunks. At this time the infested animals began to exhibit uneasiness. The lesion showed signs of edema and inflammation. Heavily infested animals began to lose weight rapidly, and temperatures continued to rise. Still no effect on the erythrocyte count was seen. The leucocyte count in most cases dropped back to normal, and in some individuals a slightly subnormal value was recorded.

From the fourth to sixth day after infesting, the larvae underwent extremely rapid development, attaining nearly maximum size at the end of this period. Up to this point the mechanical damage had been slight and only a small amount of exudate was seen. Now the maggots began to feed extensively; the exudate became darker and more abundant. It was during these three days that the most pronounced symptomatic characteristics were observed. A precipitous
drop in weight was recorded in the heavily infested guinea pigs. Activity diminished, food consumption fell off, and the animals exhibited a very poor general condition. Defecation fell off and diarrhea appeared. Some of the animals showed signs of dyspnea toward the end of this period. Temperatures reached a peak. A sharp drop in the erythrocyte count, leading in many cases to a definite anemia, occurred during these days. About the fifth day a marked leucopenia was recorded.

On the seventh day, the larvae were mature and most had begun to emerge. Little additional growth of these organisms was evident. The acute general symptoms subsided. Temperatures dropped to normal. Heavily infested animals continued to lose weight, but only slightly; light sublethal cases still showed no gain. Leucocyte counts came back to within the normal range. The anemic condition, however, became more severe.

By the eighth day, nearly all hosts were free of screw worms. Appetites returned to normal. No diarrhea was observed. The animals became more active. The weight loss leveled off and many individuals began to gain. Temperatures continued in the normal range. On this and the ninth day the maximum degree of anemia occurred.

From this point on, the hosts, no longer parasitized, presented a normal picture in most respects. The erythrocyte counts began slowly to return to the pre-infestation level, but even five days after the screw worms dropped out,
the heavily infested animals still maintained a low count.

The development of the parasites in the lethal cases took the same course. The clinical response of the host, in all respects, appeared a day or two earlier than in the sublethal cases. An immediate drop in weight from the first day was evident. Anemia occurred as early as the fourth day, and nearly always by the fifth day, and was very pronounced at death. Peaks in temperature comparable to those of the heavy sublethals fell about a day earlier (the third or fourth). Leucopenia reached a maximum between the third and fifth days. The initial leucocytosis, however, was absent; no explanation for this can be found. One very marked difference in the blood picture of the lethal cases was recorded. Within a day or two after the lowest white blood cell count was reached, a leucocytosis developed, which in some individuals reached as much as 200-500% of the normal value. As the animals approached death, most showed a subnormal temperature. That this drop can be attributed specifically to the influence of the parasites is doubtful, for in all probability the animals were moribund at the time the temperatures were taken. Dyspnea, accompanied by coughing in several instances, was most clearly shown in these lethal cases. In spite of the irregularities encountered, the period of most evident pathogenesis was during the time the third instar larvae were undergoing maximum growth.

The clinical picture seen in screw worm infested guinea pigs is not altogether different from that reported for hosts
infested with other myiasis-producing Diptera.

In some individuals, especially lethal cases, the picture resembled anaphylaxis. Dyspnea, a drop in temperature, and leucopenia would suggest this (Raffel, 1953). However, since no previous exposure to the screw worms existed, and the onset of the symptoms was very gradual, taking several days, it is felt that anaphylactic shock must be eliminated as a possible explanation for the pathogenesis.

Since previously infested animals do not die from otherwise lethal infestations, Borgstrom (1938) discarded mechanical damage as the cause of death. However, in view of the extensive destruction caused by the feeding parasites, this factor as an explanation for some of the symptoms cannot be totally dismissed. As pointed out by Borgstrom, and as also observed in these studies, the exudate contained erythrocytes, but since no readily escaping blood from the wound was observed it seems highly improbable that the anemia encountered in these cases was the result of a simple hemorrhagic loss of blood, especially in view of the entire syndrome.

The picture revealed in these cases, an initial irritation, a rise in temperature, emaciation, diarrhea, leucopenia, and anemia, is very suggestive of some toxic action on the host. It is evident that the body substance of the screw worms is not the toxic agent, for the symptoms appear when no dead organisms are present. Also in support of this is the failure of injected suspensions of larval tissue to
elicit immunity (Borgstrom, 1938, and Laake and Smith, 1939).

A possible explanation, which probably partially accounts for the clinical picture, is the liberation of decomposition products of the host's tissues, since considerable necrosis is present in heavy infestations. Harwood et al. (1937) found that the toxic symptoms produced by the migration of Trichinella through the host tissues were predominantly the result of an auto-intoxication; in this case guanidine retention was the principal factor. However the most extreme symptoms occurred during the period of most rapid growth of the third instar larvae, and before the tissue destruction and decomposition was at its height. At this time secretory and excretory processes of the maggots would be at a maximum. It seems very probable, in considering the toxic and antigenic properties of the excretory materials from other maggots (Blacklock et al., 1930, and Gwatkin and Fallis, 1938), that the metabolic products liberated during the maximum growth phase of the screw worm are the main toxic agents responsible for the clinical picture and consequent death of the host, probably, as noted above, in combination with decomposition products of the host's necrotic tissues. In any case, it seems likely that a reaction in the immune animal serves to neutralize the toxic materials in the region of their production (the vicinity of the lesion) by locally-produced antibodies, thereby preventing the general circulation of lethal amounts of these substances.
During the course of this work other aspects which should be investigated have come to mind. As yet, no detailed information concerning the clinical picture of previously infested and more or less resistant animals is known. Since this and the subsequent death of the host are the principal criteria for the existence of an immunity, such a study is necessary. In most parasitic infestations, an eosinophilia develops. In view of the leucopenia occurring in screw worm infested animals, a consideration of the differential leucocyte picture would be most informative. The nature of the anemia also needs to be determined. The cause may be mechanical, as mentioned, or the anemia may be brought about by hemolysis or by injury to the erythropoietic centers. Reticulocyte counts would elucidate this problem to some extent. Blood sugar determinations and other physiological aspects of the host from the biochemical standpoint should be carefully investigated. The role of Proteus chandleri is still virtually unknown. Attempts to produce infestations in the absence of the bacillus would throw considerable light on the postulated symbiotic function of this organism. To test the hypothesis set forth here that the metabolic products of the third instar screw worm larvae are the principal toxic agents to the host, these substances must be obtained and their effects on the host determined.

The present study, therefore, provides some of the background information needed for these further investigations into the host-parasite relations of Callitroga hominivorax.
V. SUMMARY

A study has been made on the effects of screw worms, Callitroga hominivorax (Coquerel), on guinea pigs. The weight, rectal temperature, erythrocyte count and leucocyte count, as well as the general aspects, were considered for animals subjected to light sublethal, heavy sublethal, and lethal infestations with this parasite. Controls determined the effects of wounding and of infection with the gram negative bacillus, Proteus chandleri (Borgstrom), alone on guinea pigs.

The clinical picture which occurred in all infested animals, to a greater or lesser extent, depending on the degree of infestation, included a loss of weight, emaciation, diarrhea, a rise in temperature, leucopenia, and anemia, all of which reached maximum proportions during the third or fourth to the sixth or seventh days after infesting, with the exception of the erythrocyte count which exhibited its greatest drop within these days. During this same period the third instar larvae underwent their phase of maximum growth. It is postulated that the metabolic products of these third instar screw worm larvae, liberated to the greatest extent during this period, are the principal toxic agents which produce the above clinical picture.

It is also hypothesized that the local immunity acquired to the lethal effects of screw worms, as found by previous
workers, can be attributed to the neutralization of these toxic substances at the site of their production by locally produced antibodies, thereby preventing the general circulation of the toxic agents in lethal amounts.

*Proteus chandleri,* the bacillus almost invariably found in pure culture in the lesions of screw worms, was discovered to possess no pathogenic tendencies in the numbers acquired through infestation by screw worms, and in the absence of the maggots the infection with this organism diminished. The process of wounding caused little more than a slight leucocytosis for the first two days following the operation.

Suggestions for further investigations into the host-parasite relations of *Callitroga hominivorax* are made.
VI. REFERENCES


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