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A PRELIMINARY STUDY OF TICK AND HOST
IN RELATION TO
WESTERN CANADIAN TICK-BORNE DISEASES

JOHN DOUGLAS GREGSON

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A PRELIMINARY STUDY OF TICK AND HOST
IN RELATION TO WESTERN CANADIAN TICK-BORNE DISEASES.

by

John Douglas Gregson, B.A. (British Columbia)

A Thesis Submitted in Candidacy for the Degree

of

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INTRODUCTION.

The Tick in Relation to Disease:

The tick has long been a menace to animal life, acting as a carrier of dangerous diseases, but it is only with the recent advance of civilization into existing tick areas, that this menace has been recognized as a problem of vital economic importance. Not only have severe losses of live-stock occurred in all parts of the world, such losses having involved several hundred animals in certain areas, but human life is itself threatened. Mention need only be made of the deadly Rocky Mountain Spotted Fever in this respect, the death rate of which may be as high as 90% of those affected.

Western Canada, one of whose primary industries is stock raising, is faced with a series of tick problems which threaten this phase of the country's resources. In spite of this, studies of the tick in relation to disease are practically untouched in this area. The investigation of this relationship presents some of the most interesting biological problems to the student, and promises greater security from disease to the animal life of the country.

Nature of Studies followed in this Thesis:

The writer, although realizing the many problems presented by tick-borne diseases in Canada, has, in this present thesis endea-

voured only to prepare a way for their future study. The elementary work that has been followed has been divided into two phases, each of which has been little studied hitherto, and of which a thorough knowledge is necessary before it is advisable to proceed to the more advanced lines of investigation.

The first of these phases deals entirely with the tick as a vector of animal diseases. A preliminary description of the internal anatomy of the tick is followed by histological and cultural studies of pathogenic and non-infectious organisms, particular attention being paid to symbionts. A large amount of data on the technique of tick-flora studies has been collected from other workers, and with the writer's comments, has been listed at the end of this paper.

The second phase is devoted to observations and experiments in connection with the feeding rates of ticks - a factor that plays an important part in the production of tick paralysis. The obscurity of this problem, and the lack of any previous work on it, has necessarily made these studies of a very general nature. The writer, however, considers them well justified, even if they serve only to expose clues that will provide a basis for further investigation. All infestation experiments are hampered by the difficulty of caging ticks safely on animals, and satisfactory results are gained only at the expense of a great deal of time and attention. This fact seriously limits the number of animals under observation. Since hosts and ticks probably show a great physiological and anatomical variation, it is obvious that the conclusions drawn from the ensuing experiments

can only be of an indicative nature.

Extent of Studies:

Little mention has been made of tick-borne diseases that are not found in Canada, and references to studies that have been made in other countries are cited only when their nature has a direct bearing upon the type of work dealt with herein.

The taxonomy of ticks, their life histories, and ecology are all subjects with direct bearing on disease studies. Mention of these however, has, for the greater part, been omitted, as they constitute entirely separate lines of study, reference to which may be obtained from various entomological libraries and research stations.

Sources of Material for Study:

The ticks used for the following investigation were of a large collection of various stages of Dermacentor albipictus and D. andersoni, together with occasional specimens of Haemaphysalis leporus palustris and Ixodes ricinus. The majority of these specimens were gathered by the writer at Kamloops, B.C., during the Christmas of 1935. Through the kindness of the Dominion Entomological Branch at Kamloops, samples of ticks were frequently received from different parts of the province. These were of particular interest for disease study and the writer is most grateful for the cooperation that has been shown while he was away from his duties at that laboratory.

Part I.

AN INTRODUCTION TO THE STUDY OF
WESTERN CANADIAN TICK-BORNE DISEASES.

The order Acarina is divided into two groups, the mites and the ticks. The latter are subdivided into the families Argasidae (Soft ticks), and Ixodidae (Hard ticks). Both of the two genera of the Argasidae are represented in British Columbia. The Ixodidae, a larger family, is comprised of some eight genera, three of which are native to Canada. These genera, Dermacentor Koch, Haemaphysalis Koch, and Ixodes Latreille, include fifteen of the eighteen known species of Canadian ticks.

The ticks studied in this thesis are confined entirely to the Ixodidae of Western Canada. This group of parasites has been extensively investigated during recent years at the Dominion Entomological Laboratory, Kamloops, B.C. In spite of these efforts, however, a knowledge of the taxonomy and life histories of the rarer species is still incomplete, and therefore the following introduction to the ticks will be limited to the most important species. These will be discussed briefly in order of economic standing, their life cycles being given to acquaint the reader with their host relationship.

1. Outline of the Ecology of the Ticks Studied in this Thesis.

Dermacentor andersoni Stiles. This tick is common throughout the dry belts of British Columbia and Alberta, extending north as far as Quesnel, and is most prevalent in the Okanagon and Kootenay districts. In the United States it is recognized as an extremely dangerous pest, being the vector of Rocky Mountain spotted fever. This disease has, fortunately, only appeared in Canada once or twice, although its presence has been indicated in Kamloops ticks. In Canada this tick is responsible for deaths among animals and humans by paralysis. It is also capable of transmitting tularaemia.

D. andersoni, in all of its stages, feeds upon the blood of various warm blooded animals, both domestic and wild. Its hosts include dogs, cattle, horses, sheep, deer, mountain goats, squirrels, rabbits, weasels, mice, groundhogs, and grouse. It also readily engorges upon men.

Mating of the adult ticks takes place on the host while they are engorging. When replete, the female drops to the ground and in about two weeks egg laying commences. During the following ten days or so, as many as eight thousand eggs may be deposited, after which the tick shrivels and dies.

Under favourable conditions, the eggs develop in a month into larval, or "seed" ticks, which have only three pairs of legs. This stage usually appears about midsummer. Its acquisition of a host depends upon the chance passing of a rodent, commonly a groundhog.

Attachment having been accomplished, the larva commences to feed. At the end of five or six days it becomes replete, drops off, and after a dormant period of two to eight weeks, moults into the eight-legged nymph.

The nymph repeats the process, again awaiting a suitable host, which may be a rodent or bird in this instar. It engorges, drops to the ground when replete, and then undergoes a quiescent period of varying duration, to emerge from its nymphal case, a fully formed adult.

The host for this final stage is a larger animal, such as a dog, deer, cow, or man. Attachment takes place a few hours after the chance arrival of the tick upon the animal, and engorging lasts, under optimum conditions, about seven days.

During this period the female tick increases tremendously in size and may reach a length of half an inch. The male does not enlarge to any appreciable extent. Mating occurs, and the life cycle starts anew.

It is thus seen that the interval elapsing between the larval and adult form may be very long, dependent upon the fortune of the various stages to meet with a host. The shortest time in which a life cycle can be completed is one year. Adult ticks have been kept for three years without a host, and even after this period, have fed normally and oviposited. Although the first two instars appear to feed readily at any time of the year, providing the weather conditions are suitable, the adults exhibit a strange inability to engorge after spring has passed.

Dermacentor albipictus Packard. This tick is very similar to the preceding species in general appearance. Its distribution is somewhat wider. Besides inhabiting the dry belt, the species, or variations of the species, are found in the foot-hills as far north as Grande Prairie, Alberta, and along the coast of British Columbia. It is of considerable economic importance to ranchers as large numbers of horses are often lost by heavy infestations. As a vector of at least one disease of moose, it undoubtedly plays an important part in large game epidemics throughout the Northern States and Canada.

Its life cycle differs from that of D. andersoni in that all the three instars are passed on one host. Contrary to the usual season of tick activity, this parasite feeds during the winter, dropping from its host early in spring.

Haemaphysalis leporus palustris Packard, known as the rabbit tick, has a very wide distribution. It is a common parasite of wild rabbits in British Columbia and Alberta, and on account of its ability to transmit tularaemia, is thought to be responsible for the periodic epidemics among these animals.

The genus is an important one from a disease aspect, being responsible, in other parts of the world, for the spreading of piroplasms, and rickettsia bodies, and possibly for a fatal case of human paralysis in Australia.

The life cycle is passed over three hosts in which may be included rabbits and grouse.

Ixodes ricinus californicus (Banks). Known in British Columbia as the Coast tick. This species has in recent years been the cause of considerable attention about the residential outskirts of Victoria and Vancouver, as it is a common parasite of humans, dogs, and deer. Although not yet known to be the vector of any diseases, as is the European variety, this tick is capable of producing serious slow healing ulcers at the site of attachment.

The early life cycle was first observed by Dr. Jellison (15). Further studies of its hosts have been made by the writer (12) and other members of the Dominion Entomological Station at Kamloops.

The larvae and nymphs commonly infest the Coast lizard (Gerrhonotus multicarinatus Blainville). From this reptile they pass to mammalian or avian hosts. Their period of adult activity appears to last through the year, but is at its height during the early warm spring days.

The life histories of these four ticks, alone, illustrate the importance of these parasites to animal life in general. Their assortment of hosts, together with their wide distribution and continuity of activity, makes them extremely powerful agents for the dissemination of disease among human, domestic, and wild life.

2. General Description of Mouth Parts and Alimentary Tract of the Tick (*Dermacentor andersoni* Stiles).

A primary requisite for a study of tick-borne diseases is a thorough knowledge of the alimentary tract of the tick, together with an understanding of the digestive changes that occur within it. To this end, the following description has been compiled by the writer.

The alimentary tract (Fig. 1) may be divided into distinct regions consisting of the buccal cavity, the salivary glands and ducts, pharynx, oesophagus, stomach and caecal appendages, rectal tube, rectal sac, Malpighian tubes, anal canal and anus. The anatomy of these regions will be considered in their proper sequence.

The mouth parts, being the structures which are mechanically responsible for the access to blood will be discussed first. These structures are collectively referred to as the capitulum.

The Capitulum:

This chitinous structure (Figs. 2 and 3), known as the "false head", is composed of the basis capituli, the hypostome, the chelicerae, and the palps, the last structures constituting the first two pairs of body appendages.

The basis capituli is a dense basal part of the capitulum. It articulates with the anterior part of the cephalothorax, of which it forms a part. The integument at the point of articulation is thin and permits a certain degree of extension or depression of the capitulum. Dorsal and lateral movements, however, are prevented by two backwardly

projecting ridges on the latero-dorsal margin of the basis capituli, the cornuae, and two dorsal projections from the cephalothorax on each side of the capitulum, the scapulae. The base of the basis capituli communicates directly with the body cavity by the capitular foremen through which extends the pharynx, salivary ducts, and basal portions of the chelicerae.

The hypostome is a prolongation of the median and ventral lobes, incompletely separated by a vertical suture which dorsally forms a deep longitudinal groove, the hypostomal gutter. Posteriorly this gutter connects with the buccal cavity; anteriorly it merges into the flattened dorsal surface of the hypostome. The distal extremity of the hypostome, called the corona, is broad and blunt, and is beset with an irregular mass of small teeth. Behind the corona these denticles are replaced by definite rows of strong recurved teeth that extend towards the base of the hypostome.

The chelicerae are enveloped by two cheliceral sheaths that lie above the hypostome. These sheaths fuse at the base, and there are continuous with the dorsal surface of the basis capituli. Distally they are armed on the upper and lateral surfaces by fine chitinous denticles. The chelicerae are long and hollow, and are attached to the sheaths near the distal end by a loose flexible membrane, thus allowing a retraction or protraction of the appendages. At the posterior end they are dilated to receive the attachment of extensor and reflexor muscles. At the anterior extremity each chelicera articulates with a pair of dentate processes known as the digits. In each pair the

longer inner digit is bidentate, while the shorter external one bears three teeth. The digits are moved by muscles which extend within the bases of the chelicerae. According to Zebrowski (36), these digits contain relatively large ducts which open to the exterior by pores, the use of these ducts being unknown. The chelicerae and hypostome together constitute the piercing organs of the tick.

The palps are paired appendages that lie closely to each side of the hypostome and chelicerae. Each palp consists of four segments. The proximal articulates with the anterior margin of the basis capituli and is distally fused with the second segment. The fourth or terminal segment is inserted in a cavity on the antero-ventral portion of the third segment, and may be withdrawn or extruded at will. It is the smallest and bears at its tip a tuft of supposedly tactile hairs. Both palps possess sparse irregularly placed hairs on the dorsal and ventral surfaces. Nine long hollow spines extend in a median anterior direction from the inner ventral margins of the first and second segments of each side. The purpose of these spines is not clear. It may be noted that while the tick is feeding, the palps are forced out in a lateral direction, thus permitting the piercing organs to be thrust to their full extent into the tissue. Such a position of the palps would tend to force the mentioned spines into the tissue, and from this fact, they may serve to excrete some substance into the skin of the host.

The Buccal Cavity:

This food channel is bounded dorsally by the cheliceral sheaths and ventrally by the hypostomal gutter. Posteriorly it becomes a

closed tube , surrounded entirely by the fusion of the cheliceral sheaths with the edges of the hypostome. From here it extends back a short distance, ending in a sac which is termed the buccal cavity. A salivary duct enters at each postero-lateral corner of this sac.

The Salivary Glands and Ducts (Figs. 4 and 5):

The large salivary glands lie on each side of the buccal cavity, extending from the capitular foramen back to the stigmal plates. Throughout this region they are in close contact with the caecal lobes of the stomach, portions of the Malpighian tubes, and a ramifying mass of tracheal tubes. Each gland is composed of a number of lobes of loosely connected alveoli, which are of two kinds (Fig. 6). By far the greater number of alveoli are of the first type (Figs. 6, 4). Each of these alveoli is made up of some fifteen large nucleated cells grouped about a central lumen or collecting chamber, and are bordered at their bases by a thin basement membrane which forms the boundary of the structure.

Of these cells there are two types:-

The first type is situated at the opening of the alveolus, and in cross section usually appears as a pair of large cells, one on each side of the duct. The nuclei are small and central, and stain black with haemotoxylin-eosin, red with Mallory's and blue with methylene blue-eosin. The cell contents which are coarsely granular, stain red with these stains.

The other type of cell makes up the greater part of the globular alveolus. Their nuclei are larger, stain a lighter blue with any of the stains mentioned above, and, as a rule, are more distally

placed. The cell cytoplasm is less granular and stains only a blue or purple.

Of counts taken through a hundred random alveolar sections in the gland of one tick, 36% of the cells were of the granular form. (This complies closely with several reconstructed alveoli in which it was usually found that 5 eosin staining cells surrounded the opening of the lumen, and that these were surmounted by a ring of 5 and a distal roof of 5 of the second type of cell.)

The lumen of the alveolus opens into a short efferent duct which in turn joins a lobular duct (Fig. 6, 3). Each lobular duct then empties directly into the central salivary duct. Although this main duct is usually single, the writer upon several occasions has noticed a bifurcation within the gland to form two large glandular lobes. In either case, the main duct extends throughout the whole length of the gland. Anteriorly each duct leaves the gland, continues forward for a short distance, and then turns inwards to open into the buccal cavity.

The second type of alveolus (Fig. 6, 1) is slightly larger than the first, and is found adjacent to the central salivary duct (Fig. 6, 2). These alveoli are most numerous in the anterior half of the gland, often extending in front of the other gland cells. They usually appear on the dorsal and median sides of the duct, but in some cases have been noticed to surround it entirely. The secretory cells of these structures are very resistant to all stains, and the individual cells are distinguishable only by their large poorly staining nuclei.

The lumina open directly into the main duct by short vessels.

The Salivary Gland During Active Secretion:

As the tick commences to engorge, the salivary gland undergoes a morphological and physiological change. The entire gland of a replete female tick is over twice the size of that of an unfed adult. This change in size is due to enlargement of the individual alveoli, which in turn is due to an increase of cell and even nuclear matter. Measurements give the secreting alveolus a diameter of 120 microns, against that of 40 microns for the dormant stage. In many of these alveoli, the two types of cells show a uniform swelling. In others the granular cells remain enlarged but the distal methylene blue staining cells exhibit a marked degeneration and flatten into a thin layer. Cross sections of the latter type have a characteristic signet ring appearance (Fig. 7, 4). The secretion is finely granular and stains with eosin.

The medial non-staining alveoli show little change. The secretion is non staining.

The Pharynx: (Fig. 1)

This is a fusiform tube opening anteriorly into the floor of the buccal cavity and extending posteriorly through the capitular foramen to join with the oesophagus. The entire tube is highly muscular and is supplied with a system of chitinous plates which, with a series of radial constrictor and dilator muscles, forms a powerful pumping organ.

The Oesophagus: (Fig. 1)

The oesophagus is a short delicate tube connecting the pharynx

to the stomach. From the pharynx, it runs posteriorly with a ventral inclination for a short distance, then turns upwards to pass through the brain. It emerges from the posterior border of the latter, and after a short upward course, enters the ventral median surface of the mid intestine or stomach. At the point of entrance there appears a valvular structure, the proventricular fold, which undoubtedly serves to prevent a regurgitation of blood from the stomach.

The Stomach: (Fig. 1)

The mid-intestine, with its caecal appendages, forms the greater part of the digestive tract. Indeed, when engorged, these tubes occupy practically the entire body cavity. The mid-intestine is, itself, a relatively short stout tube, extending anteriorly to the brain, and posteriorly to a point about midway between the brain and anal aperture. At its anterior and posterior extremities it branches to form a number of blind sacs, the diverticula or caecae. These extensions of the gut are of irregular diameter, are usually very long, and are all capable of great dilation. Anteriorly there are two antero-lateral diverticula, each of which continues out at right angles to the mid-gut for a short distance, then gives rise to a short branch which runs forward to the margin of the capitular foramen. Immediately beyond this branch is a second offshoot. This extends out to the edge of the body cavity, follows it forwards, and terminates close to the end of the first branch. The main trunk of the antero-lateral caecum now passes back in a postero-lateral direction. Near the outer margin of the third coxa it sends off a third branch which turns back on itself

and follows the ventral surface of the salivary gland up to the anterior margin of the second coxa. A fourth branch arises a short distance behind the third and extends along the inner margin of the salivary gland, to which it is closely applied. The remaining portion of the diverticulum continues posteriorly till close to the stigmal plate, dips ventrally, and then passes forwards on the floor of the body cavity. It ends near the posterior extremities of the chelicerae, median to and in the same plane as the tips of branches one, four, and two. The postero-lateral diverticula are four in number and present a less complicated system than the anterior appendages. Each of these four caeca extends posteriorly and laterally until close to the margin of the body cavity, then dips ventrally and retraces its course towards the rectal sac near which it terminates blindly.

The Rectal Tube: (Fig. 1)

The waste food from the mid-intestine passes into a rectal sac by a short slender rectal tube. This passage commences as a funnel shaped tube, draining the median ventral portion of the mid-intestine. It extends in a caudo-ventral direction to the rectal sac. The assumption that the rectal tube, in most ticks, is a functionless cord that does not permit the passage of faecal matter is clearly untenable in this case. While engorging, Dermacentor andersoni passes a very large amount of excreta, consisting of products of blood, which can be seen in the sectioned rectal sac where it closely resembles the contents of the diverticula.

The Rectal Sac: (Fig. 1)

The rectal sac lies slightly behind and ventral to the posterior end of the mid-gut. It is a swollen H-shaped structure, with the two posterior extensions larger and more divergent than the anterior lobes. At the antero-ventral surface are three openings, a median one for the entrance of the rectal tube, and two lateral ones for the Malpighian tubules. The anal canal opens from the postero-ventral surface of the rectal sac, immediately anterior to the bifurcation of the posterior lobes.

Anal Canal: (Fig. 1)

This is a short, thin walled tube, passing directly from the rectal sac to the anus. The latter is a single aperture, located on the ventral integument of the tick in a median line and slightly posterior to the stigmal plates.

Malpighian Tubes:

These excretory tubules are two in number and arise blindly near the posterior extremity of each chelicera. Each tube continues backwards in a long tortuous course beside the salivary gland to the stigmal plate region, there turning up and forward to the base of the antero-lateral diverticulum of the mid-intestine. Turning back again, it extends to the posterior region of the body cavity, where its course is again altered to a ventral and forward direction reaching the bases of the posterior diverticula where it finally doubles back and enters the rectal sac. Each tube is unbranched, and varies considerably in diameter according to the engorged state of the tick. The secretion of these organs is a chalky white material and so gives the structures a characteristic whitish colour.

ALIMENTARY TRACT of D. ANDERSONI

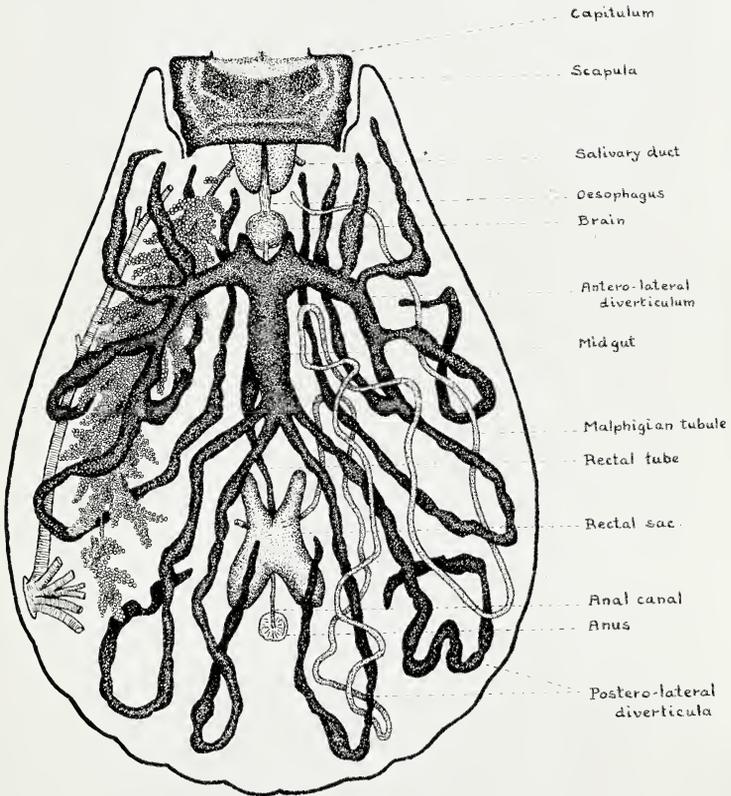


Figure 1.

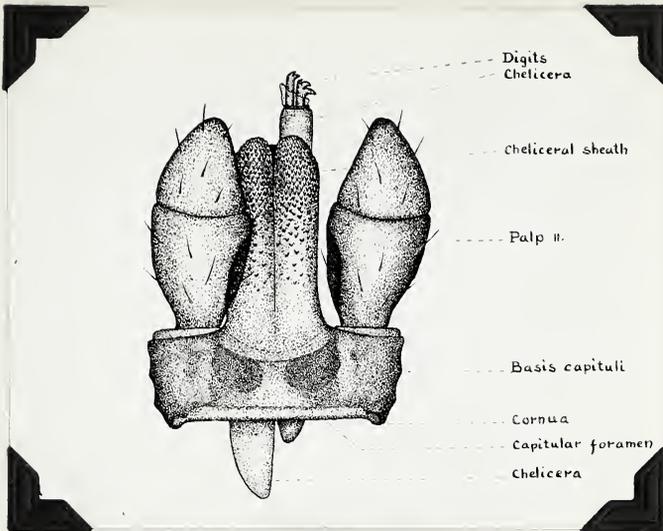


Figure 2.

Dorsal view of Capitulum of *Dermacentor andersoni*.

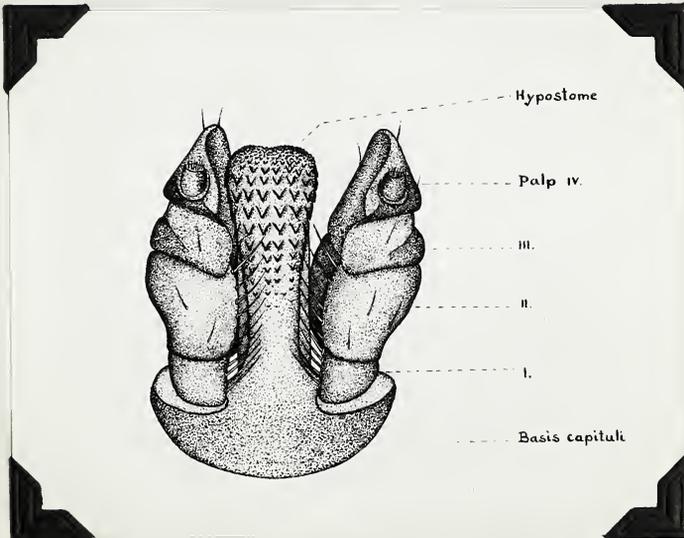


Figure 3.

Ventral view of Capitulum of *Dermacentor andersoni*.



Figure 4.

Salivary gland of an unengorged adult
Dermacentor andersoni tick (x 12).



Figure 5.

Salivary gland of a replete
adult Dermacentor andersoni tick (x 12).

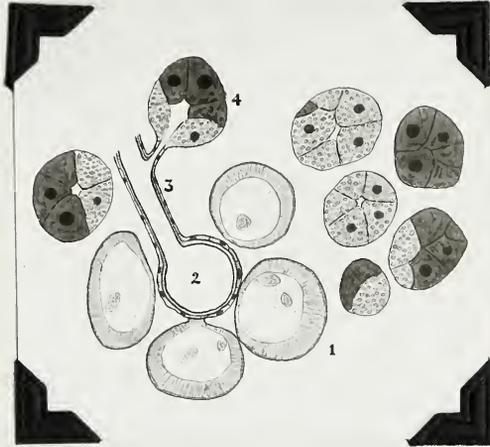


Figure 6.

Section through the salivary gland of an unengorged adult Dermacentor andersoni tick (x250).

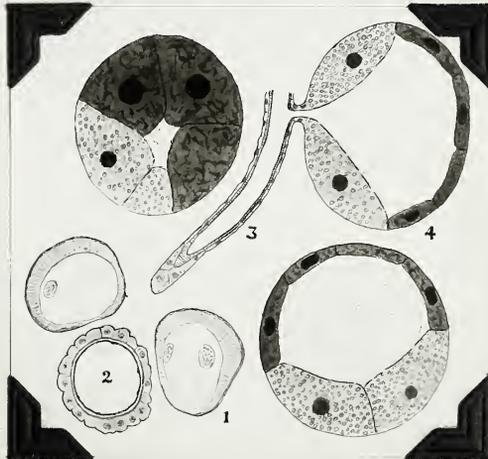


Figure 7.

Section through the salivary gland of a replete adult Dermacentor andersoni tick (x250).

3. The Histology of the Stomach and Process of Digestion.

It was found during early studies that smears of the intestinal contents and excreta of ticks presented a variety of confusing microscopic bodies. These particles bear such a resemblance to bacteria and protozoa that an acquaintance with their nature is essential for detailed symbiotic and parasitic observations of tick-borne microorganisms. Accordingly, an extensive study of the assimilative processes within the tick-gut was made by the writer.

These studies, for convenience, are divided into four phases; the pre-absorptive, absorptive, post-absorptive, and disintegration stages (Figure 8). It must be remembered, however, that the process of assimilation is a continuous one, and in a cross section of an engorging female tick, at least two of the phases are usually present.

Pre-absorptive Stage:

This is the condition of the gut wall of the unfed adult tick, and so presents a picture of its normal histology.

The outer wall of the intestine and diverticula is composed of a very thin network of muscle fibres. Their presence is indicated by occasional spindle-shaped nuclei about the periphery of the gut. Mallory's triple stain, Giemsa's, and methylene blue all bring out these fibres in a dark blue colouration.

Within the layer of muscular fibres is a lining of irregularly shaped epithelial cells, all resting on a delicate basement membrane. These cells of the inactive gut are columnar in general appearance, but vary considerably in size and form and are somewhat compressed. They

usually present two stages of progression. The majority are fairly short and cuboidal in shape. Their nuclei are round and may be basal or central in position. The non-staining cytoplasm of all these cells is evenly filled with numerous black, round granules. (1) The cells of the second type (2) are believed to be similar to those of the first, but in a more advanced condition. They are fewer in number, and are wedged by a narrow stalk between the other cells, the enlarged free ends projecting far out into the lumen of the gut. In these, the granules are of a pale sienna colour - indicative perhaps of some physiological change from their former state. The nuclei are noticeably larger and more distal.

Absorptive Stage:

This stage is present in the gut from the first day of feeding until the tick is semi-replete. The parasite, during this time, is actively feeding from its host, and the digestive tract is markedly dilated with blood. A strong peristalsis is present in the mid-intestine and diverticula, the course of which is usually from the terminus to the origin. By this movement, the blood is carried towards the mid-gut along the periphery of the caecae, and is sucked out distally through a central channel.

Cross sections of portions of diverticula and mid-gut when semi-engorged with fresh haemolysed blood, present a picture of active assimilation. The enclosed fluid within the lumina appears uniform in appearance, is unpigmented, and stains a deep red with Mallory's triple stain (3). Bordering the lumen, and resting on the basement membrane, is an irregular layer of small cuboidal cells. The cell walls of these

are indistinct; the cytoplasm and their small nuclei stain blue with Giemsa's stain or methylene blue. Projecting from between these cells, or appearing to form an inner layer upon them, are large assimilative cells. Whether they are also secretory in nature is not yet known. These cells, formed originally from the primary epithelial cells (described in the preceding phase) are now tremendously enlarged. The cytoplasm of the basal region appears flocculent and stains blue with Giemsa's. In some cells the cytoplasm includes a dense mass of brown granules which may entirely mask any other cell structures (4). This pigment is similar in size and structure to that described in the large central cells of phase I. When present, it appears most dense in the neck of the cell, thinning out at the distal periphery. It was noted that only sections of certain caecae showed these pigmented cells. Other caecae, though similar in appearance morphologically, and even forming the bi-lateral counterpart, contained assimilative cells that were entirely devoid of pigmented granules. In all other respects, the two forms of cells appeared to be identical in nature and function. It is believed by the writer that these granules represent some stage of a symbiont organism. They will be fully discussed from this aspect in a later portion of this paper. The distally placed nuclei of the cells are large and contain distinct chromatin granules and nucleoli. They stain a deep blue with methylene blue, Giemsa's and Mallory's - the nucleoli appearing red with the latter stain - and a purple colour with haemotoxylin-eosin. When stained with Mallory's triple stain, these cells are distinctly seen to be filled with many bright red spherical bodies (5). These droplets are thought to be either globules of the surrounding blood fluid, which

in some manner has been engulfed by the cells, or else to be some form of symbiotic life. As assimilation continues, they increase in size and finally occupy the whole of the intracellular space.

Post-absorptive Stage:

At this stage the tick is replete and leaves its host. The epithelial cells have taken up all the blood that they are able, and continued feeding is no longer of advantage to the tick.

The first stages of degeneration are seen in the elements lining the edge of the gut. Here cell structure has become indistinct and the protoplasm and nuclei are seen only as deeply staining masses between and at the bases of the assimilating cells. The latter now consist of a cell membrane, filled completely by the large, clear, fat-like globules which stain in a manner similar to that of the surrounding fluid (7). Cytoplasm and granular particles, alike, have been compressed to a minimum area between these intracellular drops of fluid. The nucleus alone remains as an undisturbed element of the former cell structure, and retains its affinity for basic stains. As absorption nears completion, these swollen cells project more and more into the lumen of the intestine, many of them becoming detached to form large spherical masses (Text Figure 8, and Plate IV, Fig. 1). The ultimate fate of these cells is complete disintegration. This is preceded by a breaking up of the nucleus into numerous round fragments. Giemsa's stain portrays this process clearly in certain ticks, and particles of the blue nuclear material may be clearly seen within the clear globules by which it has been engulfed (Plate IV, Fig. 2). As many as six fragments have been seen in one droplet. A final breaking of the cell membrane occurs and

the contents diffuse and become lost in the surrounding fluid (9). The blood within the gut, at this stage, has become a thick, dark red, viscid fluid. Microscopically it appears to be full of fine dark granules, varying in size from 1.5 to 2.2 microns (10). These particles which have been extruded from the cell bodies during assimilation, due to similarity in structure, are believed to be related in some way to the previously mentioned yeast-like granules. They are evenly distributed throughout the fluid, except in a narrow region directly surrounding the cells, where the clear fluid forms a distinct halo-like area (Plate IV, Fig. 1). The writer suggests that this "halo" is caused by some chemotactic repulsion. Sections of the intestine gave negative results at all stages of engorgement when submitted to Perle's test for haemosideron.

Physiologically, the digested blood appears to be in two layers at this stage. A heavy staining area surrounds the cells and fills the outer margin of the lumen. Towards the centre is a distinct undulating boundary, within which is a uniform lighter staining area.

Degenerative period:

If the tick is not killed until several weeks after leaving its host, then one of two slightly different pictures will be seen from sections of the gut. In any case there will be a complete disintegration of the epithelial layer of cells, resulting in the subsequent freeing of numbers of the enclosed globules and granules. The globules are now seen as large round bodies that stain red, and sometimes blue, with Mallory's (Plate V, Fig. 2). The edges are usually distinct and the contents of a granular nature. The yeast-like granules (15) are seen as small oval bodies, either brown or colourless, the latter state

Key to Figure 8.

Pre-absorptive stage (eosin-haematoxylin stain)

1. Gut epithelial cell containing pigmented granules.
2. Large gut epithelial cell containing brown granules.

Absorptive stage (Mallory's triple stain)

3. Haemolysed blood within lumen of gut.
4. Gut epithelial cell containing masses of brown, yeast-like granules.
5. Gut epithelial cell inclosing numerous round globules.
6. Cell cytoplasm being compressed by round globules.

Post-absorptive stage (Giemsa's stain)

7. Gut cell containing a large mass of closely packed globules.
8. Floating epithelial body containing nuclear fragments.
9. Disintegrating epithelial body.
10. Blood fluid and granules within lumen of gut.

Disintegration stage (Mallory's triple stain)

11. Malphigian concretion from excretory tubule.
12. Phagocyte from body fluid.
13. Nucleus from disintegrated epithelial cell.
14. Symbiont-like globules from epithelial cells.
15. Yeast-like granules from epithelial cells.
16. Possible fragment of disintegrated nucleus.

SECTIONS THROUGH GUT OF *D. ANDERSONI*
AT VARIOUS STAGES OF ENGORGEMENT

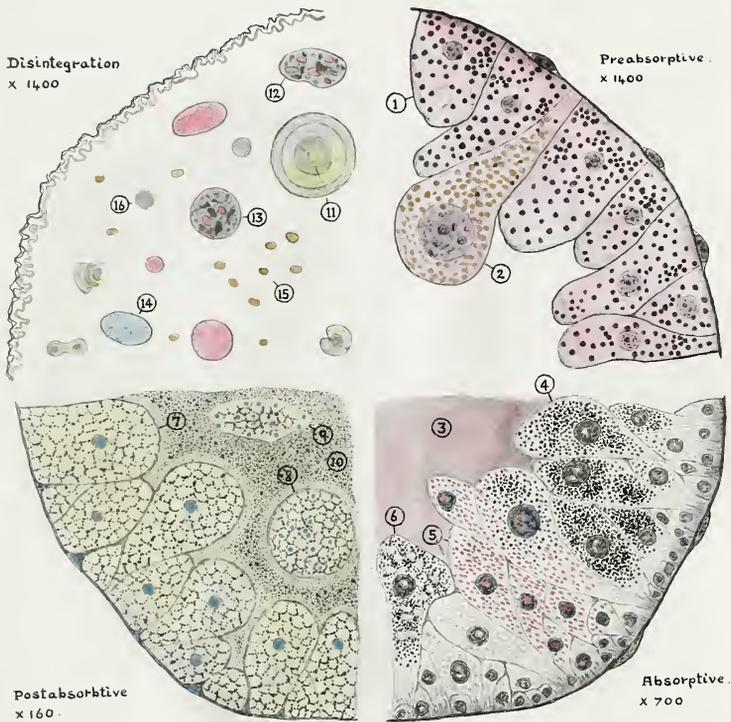


Figure 8.

probably having been brought about by a bleaching action through oxidative reactions.

If the tick is removed before becoming replete, there may also be seen a few nuclei within the lumen of the gut (13). These bodies apparently retain their shape for several weeks after leaving the cells, still possessing the single or paired nucleoli so readily seen with Mallory's stain.

From observations of the nuclei in the replete gut (page 25) it is supposed that all of these cell elements rapidly break down into small masses of nuclear material and are subsequently absorbed by the intestinal fluids. Thus, sections of ticks that have dropped from the host of their own accord would not be expected to contain any nuclear bodies within the intestine. This has not yet been verified.

A further disintegration of the tick tissue may permit excretory particles to flow into the gut, thus adding numerous Malpighian concretions (11), possible tick-blood phagocytes (12) to intestinal inclusions.

4. The Microscopic Picture of Normal Tick Excreta:

Rapidly feeding female adults of Dermacentor andersoni produce a very large amount of excreta - approximately half a cc. per tick. This digestive excretion accompanies feeding throughout the engorging period, and ceases as soon as the replete tick drops from its host. It is extruded from the anus as small droplets at intervals of about three minutes. Each viscid black drop dries almost immediately into a shiny

smooth pellet, which remains adhering to the anal aperture till it is pushed away by the succeeding excrement. If undisturbed for several days, the excreta of the engorging tick is seen in the form of long masses of shiny round globules. It is very brittle and readily breaks into black crystalline particles of varying sizes which readily dissolve in water. It emits a strong acrid odour which is apparent even during the first day that the tick is feeding.

After leaving the host, the tick may continue to excrete one or more small pellets of white matter, identified as guanine.* This Malpighian excrement is also liberated as the tick engorges, but is then masked by the copious digestive waste.

Smears of tick excreta, examined microscopically, show the presence of the following non-staining bodies:

1. Granules of pigment. These appear as uniform round or slightly elongated bodies, about 2 microns in diameter. Their colour is of a greenish brown. These bodies appear in similar form in smears made from the gut-content and represent the extruded yeast-like granules that are mentioned in the preceding studies.
2. Irregularly shaped transparent colourless particles, varying in size from 1 to 5 microns. Mention of these is made in the following paragraph.
3. Large pale green opalescent globular bodies, from 5 to 15 microns in diameter. These particles are fairly uniformly

* Through the kindness of Professor G. Hunter.

distributed throughout the excreta, and represent the greater product of the Malphigian tubules. Examination of the white Malphigian excrement shows an abundance of these bodies, together with a few smaller glassy amorphous particles which are probably of the same material.

Cross sections through the active Malphigian tubules of ticks show the lumina to be closely packed with these spherical particles, although when seen under these conditions they nearly all have a lamellated onion-like appearance (Plate V, Fig. 1).

The main body of the tick excreta is a reddish brown semi-transparent amorphous material. It is readily soluble in water, giving a similar coloured solution, but is insoluble in absolute alcohol. Finely crushed particles of excreta appear microscopically as brown flakes and are seen to contain all of the elements mentioned above. Water soluble particles, which would disappear in a smear, were not noticed in these flakes, but it is probable that such are present since water smears of Malphigian excreta, when evaporated, left a distinct pattern of crystals.

Perle's iron ferricyanide test was negative when applied to all smears of tick excreta.

5. The Tick in Relation to Disease:

The importance of ticks as disseminators of disease cannot be better exemplified than by a summary of the recognized tick-borne diseases from various parts of the world. In no other arthropod group is there such a variety of organisms transmitted by a single family of parasites, and next to the mosquito the tick is probably the most dangerous disease carrier known to man.

The more important tick-borne diseases:

Piroplasms (transmitted exclusively by ticks).

- (1) Texas fever, a haemoglobinuric fever of cattle, occurring in North and South America, Africa and Australia. Caused by Babesia bigemina, transmitted by Boophilus annulatus.
- (2) European Cattle fever. Caused by Babesia bovis, and is transmitted by Ixodes ricinus.
- (3) Malignant Jaundice of Dogs. Present in Europe, Africa, Asia, and Central America. Produced by the piroplasm Babesia canis.
- (4) East Coast Fever of Cattle. An African and Indian disease following the bite of Rhipicephalus ticks carrying Theileria parva organisms.
- (5) Haemoglobinuric anaemia of Sheep from infection by Babesia bovis.
- (6) Biliary fever, caused by the piroplasm Nuttalia equi, transmitted in Southern Europe and America, Africa, and Asia, by the tick Rhipicephalus evertsi.

Bacillus diseases.

- (7) Tularaemia. A European and American disease of about 5% human fatality. Transmitted largely by Dermacentor andersoni.
- (8) Oroya fever, a Peruvian disease claiming many human deaths. Caused by the cocco-bacillus Bartonella bacilliformis,

and transmitted by Dermacentor andersoni.

- (9) United States Moose Disease. A paralysis producing disease of game animals. Organism, *Klebsiella paralytica*; vector, Dermacentor albipictus.

Rickettsia diseases.

- (10) Rocky Mountain Spotted Fever. A severe human disease limited to the mountainous regions of United States. Produced by infection by Dermacentroxenus rickettsi through the agency of Dermacentor andersoni and Haemaphysalis leporus palustris.
- (11) Heart-water, a febrile disease affecting sheep, goats, and cattle in South Africa. Due to a filterable virus in association with Rickettsia ruminantium. Vector, Amblyomma hebraeum.

Spirochaete diseases.

- (12) Relapsing fever, of man and animals. Common in Europe, India and Africa. Vector, usually one of the Ornithodoros ticks.
- (13) Fowl Spirochaetosis. A cosmopolitan disease transmitted by Argas persicus.

Virus or Toxin diseases.

- (14) Tick Paralysis. Various forms of paralysis distributed over America, Australia and Africa.

6. The Status of Tick-borne Diseases in Canada.

The tick-produced diseases of this country at this date present a series of problems that have barely been touched. A knowledge of their etiology is scant and confused and even of the more studied ones is still too scant for a clear understanding of their nature. Because of this lack of data, and since a direct study of any of these diseases

is out of the field of this thesis the following summary is restricted to a brief outline of each type.

1. Tick paralysis. An acute ascending motor paralysis produced by the engorging females of Dermacentor andersoni. Occurs in children, sheep, dogs, and cattle. The paralysis appears about six days after the tick has commenced engorging, and passes rapidly from the hind quarters to the thorax and respiration centres. Death usually follows unless the tick is removed, in which case there is a correspondingly rapid recovery. Cause likely due to a toxin, organism, or virus. (Personal studies.)

(a) A swelling of glands of host noticed on several occasions when Dermacentor andersoni first engorges on humans. May be a mild form of tick paralysis. Unstudied. (Personal observations.)

(Similar diseases occurring elsewhere which may be affiliated are:

Australian tick paralysis, produced in lambs, dogs, and children by Ixodes ricinus and Ixodes holocyclus. Apparently due to a potent salivary toxin which is exhibited in all ticks. Glands of $2\frac{1}{2}$ ticks fatal per mouse. (Ross, (27)).

South African tick paralysis of sheep. Caused by Ixodes pilosus. Paralysis and prolonged weakness for several months. (S. van Rensburg, South Africa Department of Agriculture, Reprint #18, 1928.)

A paralysis of Californian cattle, caused by Dermacentor occidentalis infected with Bacterium tularense. Little studied. (U.S. Pub. Health Rep. Vol. 44, #22, pp 1299-1300, 1929.)

2. Tularaemia. Transmitted by ticks, blood-sucking flies, and lice. A long lasting disease affecting man, rats, squirrels, rabbits, etc. Tularaemia complex in nature is still very little understood. (Parker and Spencer, (24)).

3. Tick Poverty. An ascending weakness of horses caused by heavy infestation by Dermacentor albipictus. Disease unstudied. (Personal observations.)

4. Persistent sores (with one probable case of paralysis) following the bite of the Coast tick, Ixodes ricinus Californicus. (Personal observations.)

5. Canadian Moose Disease. Unstudied. May be a new disease, or of the Illinois or Minnesota varieties mentioned below. (Personal studies. Annual Rept. 1935 Dom. Ent. Lab. Kamloops.)

(Similar diseases occurring elsewhere which may be affiliated are:

Illinois Moose disease. Probably caused by an organism, Klebsiella paralytica, the vector of which is Dermacentor albipictus. Paralysis and weakness.

(Wallace, Cahn and Thomas, (3).)

Minnesota moose disease. Emaciation and weakening of moose without paralysis. All bacteriological, necropsy, parasitological and pathological findings negative.

(Fenstermacher and Jellison, (8).)

6. Rocky Mountain spotted fever. Has been shown to exist in British Columbia rabbit tick in a form of very low virulence. Human cases from Alberta and United States-British Columbia borderline. (Parker, (25).)

7. A Discussion of the Less Known Micro-organisms Invading Tick Tissues.

An outline of the most important tick-borne diseases has already been given. Many of these have been successfully linked up with a causative organism and have had their etiology fairly well established. Since it is the writer's intention to prepare a field for the studying of the lesser known diseases, no further mention will be made of these recognized organisms. A full account of their nature may be found in any bacteriology textbook.

The value and need of a detailed study of tick content flora, whether pathogenic or otherwise, is adequately expressed in the following extract: (Glaser, R.W. (11).)

"The bacteria-like so-called intracellular 'symbionts' or 'rickettsiae' found in some arthropods have been proven to be transmitted and to be associated with the production of fatal disease in man and

domestic animals. To the presumably pathogenic forms belong those associated with exanthemic fever, trench fever, Rocky Mountain spotted fever, and heartwater. Dengue fever and some other arthropod-transmitted diseases, such as the Japanese flood fever, are also suspected to be associated with the 'rickettsiae'. One 'rickettsiae' (*R. melophagi*) is transmitted by its vector (*M. ovinus*) to sheep in which it apparently survives as a harmless parasite. The majority, however, are at present not transmitted to animals, but usually exist within certain specialized cells of the invertebrates and are passed from one generation to the other through their eggs.

Very little is known about either the pathogenic or the non-pathogenic intracellular parasites under discussion because it has been so far impossible to adapt most of them to artificial media. Some claim that the parasites are bacteria, others that they should be placed among the so-called 'rickettsiae', a group erected by da Rocha-Lima in 1916, for certain small microorganisms found in the human body louse infected with the virus of typhus fever. A third class of investigators has concluded that the intracellular inclusions are not microorganisms at all, but products of cellular metabolism. Some of the structures observed might represent bacteria; others a totally new type of microorganism; and lastly, still others might, conceivably, represent products of cellular metabolism.

Many arthropods apparently harbour more than one species of 'symbiont'. In some cases a form pathogenic to vertebrates exists together with a non-pathogenic form. Frequently the two forms resemble one another so closely, morphologically, that cultures become imperative in order to differentiate them, as Noguchi showed in 1926. The cultural possibility also opens up a number of interesting biological problems among which the much discussed question of symbiosis has prominence."

Thus it is only through a systematic and cultural study of all the microbic flora of the parasitic arthropods that definite conclusions as to the nature of the pathogenic group can be obtained. Such studies tend to increase the knowledge of the insect-invading organisms as a whole, and also serve to separate specifically the various groups of economic interest.

The following are the forms of life which have been observed within the ticks and are but partially understood:

1. The Rickettsiae.

The rickettsia organisms have been studied very extensively during the last decade; but there is yet little clarity as to the nature of these microorganisms. Hertig and Walbach (13) define them as being Gram negative, intracellular, minute, coccoid or diplococcoid organisms, 0.3 - 0.5 microns, staining readily with Giemsa, but poorly with other aniline stains, without well defined contour, and difficult to cultivate in vitro. Cowdry (4) suggests that the criterion for distinguishing the rickettsiae from other insect organisms is their minute size.

Nicholson (21) observes differences between rickettsiae bodies and mitochondria in that the former do not establish any definite relation with the nucleus or other cellular components. Dibacillary forms are most abundant in the early stages of infection, later becoming bacillary. He states that after fixation in Regaud's fluid, it is possible to colour the rickettsiae and the mitochondria successively in the same cells and thus demonstrate their independence. The microorganisms are first brought out by staining by Giemsa's method. The tissues are then decolourized and stained with aniline fuschin and methyl green (Bensley's method, Mallory and Wright's Pathological Technique, 7th Ed.). Products of phagocytosis or nuclear degeneration in the same cells may be further demonstrated by decolourizing again and staining with iron haematoxylin.

It was found that the rickettsiae may be stained by both the iron haematoxylin and the aniline fuschin and methyl green technique, the two methods which are primarily intended for mitochondria, and that when appropriately mordanted, the mitochondria may sometimes be coloured by Giemsa's. In both cases, however, the colour reactions were so

atypical that they could not be taken to indicate the existence of a close degree of similarity.

The following are references to some of the recognized forms of rickettsiae; in most cases their systematic position is still very indefinite:

Cowdry (5) observed rickettsia organisms in a variety of Hexapods, among which were included the Araneida, Acarina, Thysanura, Hemiptera, Neuroptera, Diptera, Siphonoptera, and Hymenoptera. It thus appears from these observations that blood is not a definite requisite for the presence of rickettsiae, though they undoubtedly abound more in the blood feeding arthropods.

Kuczynski (18) has obtained cultures of R. prowazeki and Sikora (30) of R. pediculi.

Wolback and Schlesinger (35) obtained virulent cultures of R. prowazeki and Dermacentroxenus rickettsi from tissues of Rocky Mountain spotted fever infected animals.

In 1925 Cowdry (7) observed a Gram -, intracellular, pleomorphic, coccus-like microorganisms in the tissues of goats, sheep, and cattle that were suffering from heartwater. The organism resembled the rickettsia group, and though uncultured at the time was designated as R. ruminantium.

Kligler and Aschner (17) succeeded in isolating and culturing an organism from blood sucking pupipara that closely corresponds to Moeller's (1923) description of R. melophagi.

Noguchi (22) showed that "from the tissues of a spotted fever infected tick, non-pathogenic microorganisms closely resembling D.rickettsi

may be cultivated on special media". Although resembling the spotted fever forms, they bore no relation to infectivity and showed no similarity immunologically. The most frequently isolated species were Bacillus rickettsiformis, B. pseudoxerosis, and B. equidistans. All exhibited pleomorphism.

2. Bacteria.

Wallace, Cahn, and Thomas (33) have isolated an organism, Klebsiella paralytica, which is supposed to be the causative organism of Moose Disease. The strain has been cultured from ticks (Dermacentor albipictus) and has been re-inoculated into guinea pigs with resulting symptoms resembling those of the diseased moose. The bacterium is a capsulated, rod-cocoid organism, producing a sediment in broth, and Beta haemolysis on blood agar. It is a vigorous fermentor, gives a rapid mucoid growth on agar, and produces a powerful toxin.

Dr. Fenstermacher (9), in his studies on moose disease in Minnesota, states that, contrary to reports by Cahn and his associates, paralysis has never been noted as a symptom in the moose he has observed, and he was unable to isolate any pathogenic organism from ticks from diseased moose by bacterial examination.

During the summer of 1935 (An. Rept. Dom. Ent. Lab. Kamloops) the writer isolated a motile, non-capsulated, Gram -, bacterium from Dermacentor albipictus ticks taken from a British Columbia moose. It fermented lactose and grew on common media in white shiny round colonies. Rabbits inoculated with this organism died after 4 to 16 days, exhibiting white lesions on the surface of the liver. Partial paralysis prior to death was noted in two instances. Whether this organism is responsible

for the disease of Canadian moose is not yet known.

It is possible that there may be several types of the disease, that it may be inter-related with tick-paralysis, or, as Fenstermacher states, that an entirely new and hitherto undiscovered pathogen is the cause of the disease among moose and that the conditions studied are brought about through several forces cooperating to reduce the vitality of moose and producing the symptoms observed (8).

3. Other Organisms that have been seen in Ticks.

Through personal correspondence, the writer has been informed that the Rocky Mountain spotted fever laboratory, Hamilton, U.S.A., have observed several species of staphylococci, and recently a spiral-like organism with a single long flagellum, but in very few cases have any of these organisms been successfully cultured.

8. Observations to Determine the Extent of Invasion into Tick Tissues by Microorganisms:

Technique for microscopic examination of ticks for microorganisms:

The following outline of technical procedure is further exemplified at the end of this thesis. Reference, where necessary, will be made to the corresponding page numbers.

Sectioning: This method proved to be both convenient and satisfactory for the demonstration of microorganisms within tick tissues. The ticks are partly opened, then embedded according to the procedure described on page 77. Sections for bacteriological studies were made at 4 microns and stained by Giemsa's and Brown and Brenn's methods (Page 78).

Smears: Smears of tick contents were made on clean slides and spread out with a drop of saline solution. They were studied under dark stage and direct illumination conditions. Dried smears were fixed for a minute in 95% alcohol and then stained for bacteria by Giemsa's and Gram's stains; for spores by Moeller's spore stain; and for spirochetes and Gram negative organisms by Tunnicliff's stain (page 79).

A Discussion of Plant-like Bodies Normally Appearing within the Tick (*Dermacentor andersoni*):

A histological study of the tick for microorganisms reveals the presence of various cell components and digestive products, many of which can easily be mistaken for forms of flora. It is essential that a clear picture of these bodies is gained before passing on to the bacterial studies. To this end a brief description of the appearance and staining properties of each form is given.

Extracellular bodies: These are seen chiefly as digestive wastes, and have been discussed under the section pertaining to assimilative processes of the tick (page 26). They may be tabulated as follows:

- (1) Malpighian concretions (Plate V, Fig. 1). Large round lamellated bodies, approximately 10 microns in size, transparent or slightly green, non-staining, and insoluble in water, alcohol and ether. Normally within the Malpighian tubules, but scattered widely in disintegrating ticks.
- (2) Fragments of the above, exhibiting the same properties.

Intracellular bodies: Any of these may appear outside the cell if disintegration has commenced. Of these bodies, three of the following

forms have not yet been satisfactorily defined. They will be considered under the next section.

- (1) Pigmented bodies.
- (2) Yeast-like granules.
- (3) Intracellular globules.
- (4) Nuclei. These are recognized by the single or paired nucleoli which stain red with Mallory's triple stain.
- (5) Mitochondria. (Plate VI, Fig. 2) A great deal of work has been done in an attempt to define the nature of mitochondria. Many advances have been made suggesting that they are of symbiotic nature. Other investigators deny this theory, stating that they are not capable of independent growth apart from the cells. For the morphologist it will suffice to give the differences that they exhibit from bacteria, as shown by Cowdry (4).
 - (a) They are in general much less resistant than bacteria. They are invariably destroyed by fixatives such as 95% alcohol, Bouin's fluid, or acetic acid. Fixation by Regaud's is necessary for perfect preservation.
 - (b) They show a particular resistance to common histological stains, with the exception of iron haematoxylin. Giemsa's stain, which is best adapted for the demonstration of bacteria, colours mitochondria little, if at all.
 - (c) While some bacteria exhibit a high degree of pleomorphism, it may be said that the mitochondria have been shown to possess a degree of modifiability far greater than that possessed by any known organisms.
 - (d) Mitochondria never appear in the intestinal lumen or apart from cells, as bacteria generally do.

Morphological Characteristics of Organisms Seen in Ticks:

The failure to observe a larger number of invading organisms

in tick tissues must not be taken as an indication that the Western Canadian species of Ixodidae are relatively free from infection. The bacterial studies have merely been curtailed by the wide departure that has been made from the intended plans. As a result, the actual number of specimens examined histologically for bacteria is very small, including not more than fifty individuals, these being comprised of only a very few species largely from one district.

The author, however, feels confident that this extra time has been well spent, and trusts that the previous and following observations of the histology of the tick will fully portray the need for these preparatory investigations.

Bacteria observed in ticks:

A large number of sections were made from adult Dermacentor albipictus ticks that had been collected from a moose at Hulatt, B.C., in the spring of 1935.* Since these specimens had been fixed and preserved in alcohol, smears were not made. All of the sections showed the presence of vast numbers of Gram positive coccoid bacteria (Plate I, Fig. 1). These organisms were seen best when Brown and Brenn's stain was used, staining a dark purple against the yellow tissues. They showed fairly well with Giemsa's, but were harder to differentiate from the surrounding tissue elements with this stain, all of the structures appearing in various shades of blue.

The organisms were scattered throughout the degenerating

* See inoculation experiments with moose ticks, page 39.

tissues, but were most dense along the peritoneal lining of the body cavity. None were observed within the lumen of the gut.

Morphologically the bacteria were all of similar size, being about .6 microns in diameter. About fifty percent were paired, the remainder single. No spores or capsules were noted.

To date no further observations of bacteria have been made.

(April, 1936)

Cell inclusions of doubtful origin:

During the description of assimilative processes within the tick gut (page 23), mention was made of three different intracellular bodies that were observed in the digestive tracts of all adult ticks that were studied. Lack of material and time has prohibited the author from gaining a complete understanding of the nature of these inclusions. Their qualities indeed are still so abstruse that it is with hesitation that they have been discussed among the organisms. They have, however, shown several characteristics that are suggestive of life, and they also bear a certain similarity to some of the recently studied insect symbionts. Until they are cultured, it is unlikely that they will be proven to be true organisms; in the meantime they will be openly considered as either symbionts or inanimate products of cell metabolism.

(1) Pigmented bodies: (Plate I, Fig. 2) These inclusions were observed in all normal cells of the gut epithelium. They appear in unstained sections as numerous minute, round, single, bluish-black particles, distributed throughout the protoplasm, though usually in larger numbers towards the base of the cell. They are constant in size, approximately .2 microns in diameter. Owing to their pigmented nature, it is impossible to determine their staining qualities. They are not decolorized by any of the fixatives used and are not bleached by either ether or acetone. The origin and fate of these coccoid-like particles has not been studied. Since they have not been observed during the later assimilative changes, it is possible that they may develop into one of the following bodies.

(2) Yeast-like bodies: (Plate II, Figs. 1 & 2; Plate VI, Fig.1)
These particles never appeared in the gut cells until assimilation of blood had commenced. During this absorptive stage, the cells of certain diverticula were crowded with these bodies while in other portions of the intestine they were entirely absent. Even in the cross section of a single tube there appeared the same unevenness of distribution. When they were present, they were usually so numerous as to obscure all other cell structures. The newly formed particles are very small, rarely exceeding .4 microns, are round in outline, and possess a characteristic sienna brown colour. They remain visible after passing through the usual fixative fluids, but are decolorized by acetone and ether. They show an affinity for gentian violet and Giemsa dyes, but are not coloured by safranin, aqueous fuschin, or eosin.

As the tick nears repletion, these particles increase in size to 2 microns. They are then readily visible with the oil immersion lens, under which they are seen to be oval in shape. There is an indication of a thick cell wall but no sign of a nucleus. Iodine produces no change in the appearance. No change in size or numbers has occurred by culturing these bodies on agar, though in smears from this medium there is sometimes a suggestion of budding.

(3) Intracellular globules: (Plates III & VII; Plate VI, Fig.1)
At present this is the most specific name that can be given to these bodies that appear in all the epithelial cells of the gut as soon as the lumen becomes filled with blood. At first they

are seen as very small droplets, varying in diameter from 1 to 5 microns, and lying scattered in the protoplasm towards the distal portion of the cell. As assimilation continues they increase in size, until finally, when the tick is replete, they appear as large closely packed globules up to 15 microns in diameter. They stain bright red with aqueous fuschin, blue with Giemsa, are Gram positive, and are coloured brown by iodine.

All of the early forms, and the majority of the final stages, are spherical in shape. A few are oval or of irregular outline. The contents of the bodies are finely granular and homogenous. None of the mentioned stains brings out any indication of a nucleus, although when iron haematoxylin is used there is, in some, a series of concentric areas of different gradation of colour.

Because of their similarity to droplets of blood when stained by fuschin, these globules were first thought to be particles of partly assimilated fluid; this assumption, however, was modified when numbers of the globules were isolated in a smear on agar. Their similarity to organisms then became apparent, and they were definitely seen to be enclosed by a thin membrane. Attempts to culture these bodies on blood and tick excrement agar have so far proven unsuccessful. Certain of the globules, nevertheless, show an astounding similarity to cells in the process of dividing. One body in this state was studied for a week while on agar in a hanging drop, but it failed to undergo any change (see Plate VII).

Discussion: The failure, at this date, to culture success-

fully any of these intracellular bodies has necessarily left their taxonomic position in a very obscure state. Their natures, however, are considered to be sufficiently suggestive of life to permit a comparison to be made with certain of the insect symbionts.

The first mentioned pigment bodies may be excluded from the mitochondria by their characteristic resistance to acetic acid and the more harsh fixatives. Neither mitochondria nor bacteria are normally pigmented, a fact that further denies them a place among the micrococci. Their resemblance to excretory granules has been considered, but here again their pigmented nature excludes them from these bodies. There appear to be only two places left for them. Either they are waste products from previous cell metabolism - and this seems hardly likely, since they give no tests for blood pigments and are of uniform shape and size - or they present some undescribed phase of a spore forming organism. The latter theory seems the more probable, particularly since they are replaced by the following symbiont-like cell inclusions.

Of the three bodies under question, the yeast-like granules present the greatest similarity to living symbiotic units. They are similar in size and shape to members of the yeast group, exhibit apparent changes in growth, possess a thick cell wall, are very resistant to stains, and are occasionally suggestive of budding. Their sudden appearance in only certain of the intestinal cells of semi-replete ticks is suggestive of growth. If they were waste particles of haematogenous origin, they would most likely have appeared in all cells of similar size and position, whether in individual gut sections or in bilateral counterparts. Yeast plants are very commonly found as

symbionts in sap-sucking insects, but according to Buchner (2) there have so far been no records of such symbionts having been seen in any blood-sucking insects.

The intracellular globules, whether living bodies, or inanimate vacuoles of matter, are elements of extreme interest. They have already been shown to play an important part in the assimilative processes, being directly responsible for the greater part of the morphological changes that occur in the epithelial cells as the tick passes towards a stage of repletion. The exact nature of their benefit to the tick, however, is not known, and at present is as little understood as the identity of the bodies themselves.

Bases for assuming them to be merely globules of engulfed protein matter are plentiful: they appear in all epithelial cells as small droplets as soon as the cells are in contact with haemolysed blood; they enlarge, as the time for assimilation increases; they exhibit the same staining reactions as does blood, and even react to the Benzidene test; and they have never been seen in any form of division while in the cell membrane nor yielded any indication of a nucleus. On the other hand, they exhibit equally outstanding qualities that are suggestive of life. They grow from small spheres to larger ones, and since having only blood fluids to assimilate, naturally give a positive Benzidene test for haemoglobin. They possess a resistant membrane about them, will not disintegrate in water, alcohol or fat solvents, and will maintain oval or irregular shapes for many days. They present a general granular appearance and even a concentric structure when treated with special stains; occasionally they stain

blue with Mallory's, instead of red, as if showing signs of age (Plate V, Fig. 2). Full sized globules in replete ticks, when stained with Giemsa's, frequently are seen to have engulfed one or more blue-staining granules, believed to be fragments of epithelial cell nuclei (Plate IV, Fig. 2). They apparently possess an affinity for this material, yet on no occasion have they been seen to take in the surrounding yeast-like bodies.

Whether these cells have been seen in a process of division is not certain. Such a state would definitely prove them to be organisms. Forms have been seen in which there is a budding or cleavage effect, including a distinct membrane between the two adjacent bodies. This appearance could be brought about by either two inanimate globules coming together, the adjacent membranes refusing to break, or by an animate body forming a cell wall preparatory to dividing. From the microscopic picture the latter seems the more probable.

Before departing from the morphological picture of these globules, mention must be made of one other element of the tick's anatomy. In both replete and unfed adults there have been noticed groups of oval cells, 4 microns in size, that invariably stain an even red with Mallory's. These objects appear to infest the sheath of the coelomic muscle fibres, clustering along them like parasites (Plate V, Fig. 1). Although it is probable that they are a form of phagocyte - these cells in insects often attaching themselves to free surfaces of tissues - it is not impossible that they may bear some relationship to the globular masses under discussion.

If the intracellular globules are organisms, they are un-

doubtedly some form of a symbiont. Their type of cell obviously does not fall under the headings of any of the recognized forms of microorganisms, such as the bacteria, protozoa, or yeasts. Nevertheless, the possibility of there being such an organism in existence is not remote, and if present, would resemble very closely the recently studied symbionts of aphid.

Before proceeding further, it will be necessary to digress briefly to the studies of Buchner (2) and Paillot (23).

In most Homoptera there is, in the abdomen, a mass of cell tissue - the pseudovitellus or mycetome. According to Buchner (Arch. Protistenk. 1912) this organ contains great numbers of symbiotic microorganisms which are harboured in cells known as mycetocytes. The association is claimed to result in a mutual satisfaction, the plants living at the expense of the host, and at the same time relieving it of excess waste products or food materials.

Paillot, in dealing with the mycetomes of aphid, describes the symbionts as being elements of rounded form, the general aspect of which varies hardly from one species to another. These bodies, he states, have been temporarily placed with the yeasts. The basis for such a position, however, is quite unsound, since the existence of a differentiated nucleus cannot be demonstrated, and they have nothing in common with the characteristic budding of the yeasts. The symbionts multiply entirely by fission, as in the ordinary micrococci.

When coloured by iron haematoxylin, the organisms present themselves as rounded masses of fairly homogenous structure, the central part appearing more coloured than the peripheral region. There was

nothing that reminded one of the form and structure of a nucleus.

He further adds that sometimes with smears coloured by Giemsa, there appeared chromatophile or basophile granules dispersed through the mass of the cell, or sometimes the central mass of the symbiont was coloured purple while the perimeter remained a pale blue. These organisms often reached a size of six microns.

The classification of these symbiotic globules in the aphid awaits a thorough knowledge of their nature and is dependent on a greater understanding of other symbiotic microorganisms of insects.

These cells of Paillot's thus resemble the tick globules by possessing only the indication of a nucleus, and by being of similar size and shape. In each case have basophile granules been noted under certain conditions when stained with Giemsa's fluid.

Whether the similarities of these bodies from widely differing arthropods is any indication of their both being organisms remains to be seen. Buchner (2) states that to date none of the Ixodoidea have been known to possess mycetomes. In any case, were the epithelial cells of the tick acting in the form of mycetocytes, the gut of the creature could hardly be termed as a mycetome. Further studies will undoubtedly shed more light on the nature of the intracellular globules of the tick, and whatever the fate of their placing, they will invariably be of considerable interest in pathological and physiological studies of the tick in relation to disease.

Part II.

STUDIES ON THE RATE OF TICK FEEDING
IN RELATION TO DISEASE

1. General Discussion of Tick Paralysis.

The ensuing studies have been made in an attempt to throw more light on the problem of tick paralysis. This disease is produced by a variety of ticks and its occurrence appears to be world-wide. Its mysterious and often fatal nature, and the elusiveness of a causative factor have enticed numerous workers to spend more than a passing thought in connection with its etiology, but although several conflicting theories have been advanced, no definite conclusions can yet be drawn that satisfactorily explain its cause or causes.

The disease is prevalent in Southern Alberta and British Columbia, and throughout the North-western Rocky Mountain United States. In these localities it is caused by the female Spotted Fever tick, Dermacentor andersoni. In British Columbia, tick paralysis has attracted considerable attention, particularly among the large cattle ranches. Eight human deaths from the disease in this province were reported recently by Dr. Menzies of the British Columbia Health Service, and every year there are minor cases among children. Sheep, dogs, and cattle are particularly susceptible to the tick, recent paralysis outbreaks in individual cattle ranches having involved, on two occasions, a hundred and two hundred animals with a respective mortality of sixty and thirteen percent.

Tick paralysis is prevalent in Australia and South Africa, proving a 'veritable menace' to sheep farmers in many parts. The causative ticks in these countries are species of Ixodes.

Etiology: Tick paralysis may be produced in an animal by single tick. The symptoms appear only when the tick has been engorging for about seven days and is nearing repletion. The onset is sudden and is characterized by unsteady gait, and an acute ascending motor paralysis of the flaccid type. Pulse and breathing are rapid, there is slight glandular disturbance, and pain is absent. Death usually occurs in twenty-four to sixty hours from paralysis of the thoracic organs. If the causative tick is removed during paralysis, there is a rapid and complete recovery of the patient, normality usually being attained in four to twenty-four hours depending upon the seriousness of the disease. Exceptions to these observations are instanced in the case of a British Columbia man whose recovery required a period of over three months, and in certain South African sheep which remain in a weakened condition for many weeks. There is considerable controversy as to whether an animal can be immunized against the disease. The writer has noted many instances where it has been impossible to induce paralysis even in young lambs with as many as a dozen rapidly feeding ticks.

The facts of rapid recovery, negative necropsy findings, and negative inoculation tests point to the theory that a tick-secreted toxin is responsible for the paralytic symptoms. This supposition is strengthened by experiments that have been performed in Australia in which it was found that an injection of crushed salivary glands

from two and a half ticks proved to be a M.L.D. for the mouse. Opposing this theory are the facts that the latter result has not been obtained in British Columbia by glands from as many as seven ticks, dissected in vivo or while frozen, and injected intravenously, subcutaneously, and intraspinally, into mice, dogs and sheep; that many ticks may feed upon an animal with no ill effect on the part of the host - or one tick may cause a fatal paralysis; and that a bacterial disease, similar in symptoms to paralysis, is produced in the moose by a closely allied tick.

Such is the present state of data on tick paralysis. It must be borne in mind, however, that owing to the wide distribution of this disease it is quite possible that the variations appearing in different countries, and even in same provinces or states, are due to more than one type of disease. If this is true, then much of the confusing and conflicting data may be accounted for.

Variations in the Rate of Feeding of Adult Ticks and Relation of Such to Paralysis:

Tick paralysis is generally thought to be due to a rapid injection of a salivary toxin by the tick into its host. This condition would logically accompany any rapid feeding on the part of the tick. Paralysis in British Columbia is produced only by fast feeding ticks that drop at the end of seven to eight days.

Attacking the problem from this end, the writer has endeavoured to discover the conditions that are conducive to rapid feeding of the tick.

It must first be noted that variations in the rate of tick feeding may be divided into two groups; an individual variation, present among certain ticks, and a general seasonal variation in which all ticks are affected. In the first case, a series of ticks of common origin, feeding under apparently identical conditions, will often show a variation in feeding period from seven to eighteen days. In the second, it is noted that all ticks will feed readily in the spring months over an average period of about nine days, but towards the fall and during the winter months, although apparently willing to engorge, they exhibit a striking inability to do so.

It remains to be seen if there is any relationship between these two types of feeding phenomena.

2. Study of Host and Tick Tissues in Relation to Varying Feeding Rates of Individual Ticks.

On June 11th, 1935, a four month old lamb was infested with twenty-eight pairs of adult Dermacentor endersoni ticks. These ticks were placed in groups of eight over the following areas of grease free and closely clipped wool and were covered by gauze infesting cages.

- A. Directly behind the base of left ear.
- B. Over left shoulder.
- C. To right of spine in lumbar region.
- D. To left of spine in pelvic region.
- E. On right flank.
- F. On right side of belly.
- G. On scrotum.

By June 17th, the following engorging female ticks were in the indicated stages of repletion. (Ticks that had failed to attach during the first day were desiccated by the sun.)

A.	1 tick	$\frac{1}{2}$ -	replete.	
B.	3 ticks	$\frac{1}{2}$	replete;	1 $\frac{1}{2}$ replete.
C.	3 "	"	" ;	1 dropped replete.
D.	3 "	"	" ;	
E.	3 "	"	" .	
F.	3 "	$\frac{1}{2}$	replete;	1 $\frac{1}{2}$ - replete.
G.	3 "	"	" ;	1 dropped replete.

These ticks were all of the same stock - a series of adults that were collected two months previously by dragging in a single area near Kamloops. They all commenced feeding on the same host at the same date and were consequently under common weather conditions. Each infested piece of skin was approximately 1 square inch in area, thus allowing the ticks of each group as little variation in skin structure as possible. Yet in spite of these conditions, similar for each tick, there appeared a great difference in their feeding rates, as shown above (Figures 9 and 10).

Immediately after repletion of two of the ticks (groups C. & G.), the host was chloroformed. Pieces of skin $\frac{1}{4}$ inch deep and $\frac{1}{2}$ inch square were incised around each tick, and, with the latter still attached, were placed in Bouin's. To effect a rapid penetration of the fixative, the dorsal integument of each tick was removed. The entire operation was completed as rapidly as possible, after which the animal was killed. Except for the two replete ticks that had previously detached themselves, not one of the parasites released its hold during the whole of the somewhat drastic procedure.

All specimens were left in Bouin's for eighteen hours; removed to fifty percent alcohol for one half hour; to sixty-five percent for six hours, and stored indefinitely in seventy-five percent.

Comparison of Host Tissues Below Fast and Slow Feeding Ticks (Figure 10):

A series of sections was made from the previously mentioned tissues. (For technique, see page 77). Where possible, these sections were taken through the site of attachment of the tick. Efforts to preserve the mouth parts within the tissue were unsuccessful, due to their chitinous nature, but the depth of penetration could clearly be seen on certain slides, and was marked by a surrounding scab of dried blood. Very little mechanical disturbance appeared to result from the tick's feeding, the mouth parts rarely penetrating beyond the epidermal layer. Opposed to this fact is the common impression that the tick buries itself in the tissues of its host. This, of course, is no more true than the supposition that the tick must be 'unscrewed' to be removed from the skin, though it is probable that the mere twisting may serve to loosen the mouth parts.

(a) The Host Tissue Below Fast Feeding Ticks.

Sections dealt with here were made from the skin below two ticks that were replete at the end of seven days (groups C and G), and from tissue below a two-thirds replete tick (group E). All three series, being pathologically similar, will be described collectively.

The site of attachment of the parasite is surrounded by an area of dried haemolysed blood. Beneath this scab, in two or the three series, a small pocket of pus was present, representing probably a secondary infection. The whole of the dermis presented a picture of an acute inflammation. The blood vessels and capillaries were tremendously dilated, and in two tissues, had ruptured to form haemorrh-

hagic areas of haemolysed blood. An edema was present throughout the tissue, filling lymph spaces with a thick transudate. Surrounding every blood vessel, and extending into the corium towards the tick, there appeared a vast collection of leucocytes. These foci consisted mainly of polymorphonuclears and fibroblasts, but contained a large percentage of eosinophiles, lymphocytes, and histocytes. In no instance could red blood cells be discovered, other than within certain of the unruptured capillaries. It is believed by the writer that this whole area must be saturated with haemolysed blood, but no positive test for haemoglobin (Benzidene reaction) could be obtained outside of the haemorrhage to substantiate this probability.

(b) The Host Tissue Below Slow Feeding Ticks.

These three tissues were taken from areas within $\frac{1}{2}$ inch from the above mentioned ones. In each case, the tick had only reached a semi-replete stage at the time the tissue was fixed. Histological examinations of sections through these areas showed that little change had taken place as a result of the tick. Edema and inflammatory processes were practically absent, and the capillaries were only very slightly dilated.

Discussion:

It has already been shown that an irregular rate of feeding may occur among a common stock of ticks while feeding under identical external conditions and on the same host. This variation was first thought to be due to the fact that certain of the ticks had tapped a blood vessel, and so gained an extra supply of nutritive fluid. This

theory fitted very well with the fact that rapidly feeding ticks usually cause paralysis - any toxic secretion would be readily disseminated by such a position.

Studies from the previous experiment contribute the following data: Rapidly feeding ticks produce an acute inflammation in the underlying dermal tissues. Intense leucocytosis occurs around the blood vessels. The capillaries are very dilated, and a haemolytic area of haemolysed blood is usually present. Slow feeding ticks produce little or no change within the tissue. Capillary counts in both cases were similar.

These facts lead to an assumption that the inability for certain ticks to engorge rapidly during their feeding season is not through an insufficiency of tissue blood. It seems, rather, that the slow feeding ticks lack the power to produce sufficient disturbances within the tissue to cause a liberation of blood from the vessels. Since the mechanical disturbance is very slight, and since the tick does not penetrate deeply enough to obtain blood from the capillary bed, it is entirely dependant upon the production of an edema and haemorrhage for its access to the blood fluid. It probably produces these injuries by the elaboration of a powerful toxin, the evidence of which is suggested by the pathology of the tissues below the fast feeding ticks.



$\frac{1}{2}$ replete
replete

Figure 9.

Tick group G. showing irregular feeding.
(See page 57.)



$\frac{1}{2}$ replete
replete

Figure 10.

Tick group C. showing irregular feeding.
(See page 57.)

COMPARISON of HOST TISSUE BELOW
FAST-FEEDING TICKS and SLOW-FEEDING TICKS

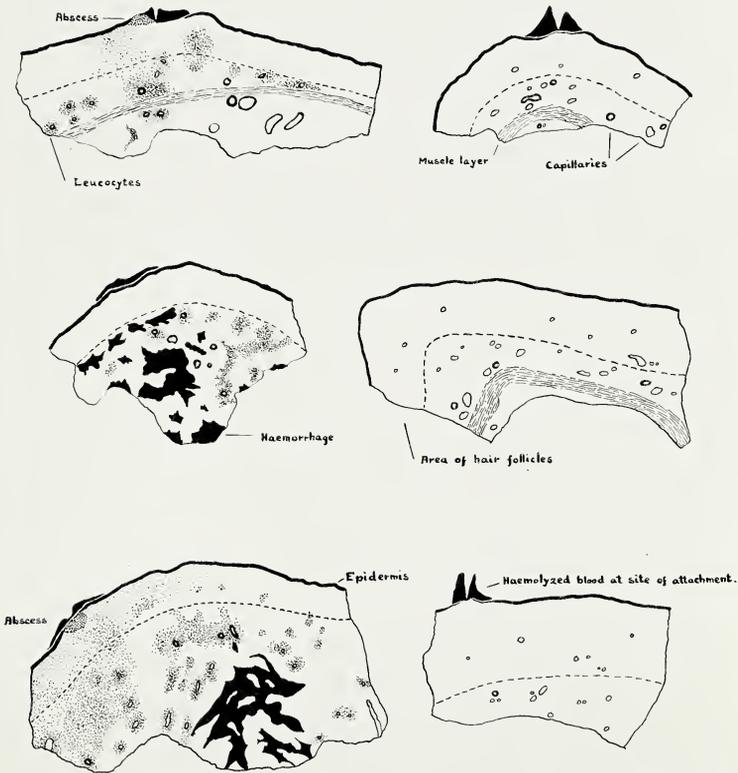


Figure 11.

3. A Study of Host and Tick in Relation to Seasonal Variations in the Rate of Tick Feeding.

In the following series of experiments, mention is made of several investigations that were carried out during the summer of 1935. These were made by the writer while studying other phases of tick problems at the Dominion Entomological Laboratory, Kamloops. He is particularly indebted to the Dominion Entomological Department for the privilege of modifying certain infestation experiments to meet his requirements and for access to the results, the latter which have enabled him to establish the conclusions discussed herein. These summer studies have proven valuable in providing a basis for the ensuing winter experiments, and have also served to lend data for comparison with winter observations. To these ends they are quoted in detail together with the more recent studies.

Studies on the Effect of Age upon the Feeding of Dermacentor andersoni:

The peak of adult Dermacentor andersoni activity, during the year of 1935, was seen to occur in May. Throughout the whole of this month large numbers of these ticks were gathered from tick areas in the vicinity of Kamloops, B.C. All of these specimens taken from the field showed normal activity and, when placed on animals, engorged over an average period of eight days. Conditions were ideal for their feeding.

On the twenty-second of the same month a series of twelve pairs of Dermacentor andersoni adults were placed on a year old ram. These had all emerged from their nymphal state during February, 1935.

Being of known age, an attempt was made to see if the irregular feeding of individual ticks (mentioned in the preceeding pages and noted often during general tick infestation experiments) was due to a difference in the ages of the ticks, or to some condition in the host. Since it is known that adult ticks may wait several years for a host, it is quite within reason to expect that the older ticks would show a weakened ability to engorge and thus take a longer period to feed.

On May twenty-eighth, instead of being two-thirds replete, as expected under the optimum conditions, these ticks were still unengorged. They were still active, and had attached since the date of infestation, so indicating some unfavourable condition was present in either the tick or the host.

In continuance with the above infestation experiment, cross infestations were made to ascertain whether the inability for the ticks to engorge was due to conditions in the tick, or in the host. The following material was used:

- (A) 1 year old ram, upon which ticks 'C' refused to feed during the period from May 22 to May 28. (See above experiment.)
- (B) 1 year old ewe, upon which all ticks 'D' will feed readily.
- (C) Flat adult D. andersoni ticks, the life history of which is as follows: Eggs laid in early June 1934; eggs hatched in late June; larvae kept over damp sand and fed between September and November; flat nymphs fed at end of December; adults emerged in February 1935.
- (D) Flat adult D. andersoni ticks collected in the middle of May 1935 at Rayleigh Bluff, Kamloops. Individual life histories unknown.

The following infestations were made on May 30th:

6	pairs	of	ticks	'C'	were	placed	on	sheep	'A',
12	"	"	"	'C'	"	"	"	"	'B',
12	"	"	"	'D'	"	"	"	"	'A',
12	"	"	"	'D'	"	"	"	"	'B'.

All infestations were made under capsule cages placed over grease free and closely clipped wool. At the end of one week the following observations were recorded:

Nineteen of the twenty-four female ticks 'D' were in various stages of engorgement on both sheep 'A' and 'B'. In no case had any of the eighteen female ticks of group 'C' shown any signs of engorgement. The majority of these latter ticks were still alive and active, about twenty-five percent had attached but still were apparently unable to engorge, and a similar number had dried up from exposure.

Conclusions: This experiment illustrated, with remarkable clearness, that the ticks of 'C' stock were unable to engorge while in their condition at the time of infesting. Their inability to do so was shown to be entirely due to a condition in themselves, and not to one present in the hosts 'A' and 'B'.

Studies on the Effect of Light on
the Feeding of Dermacentor andersoni
Ticks:

During the winter of 1934-35, experimental studies pertaining to the feeding habits of Dermacentor andersoni were made at the University of Alberta. A small number of ticks which had refused to feed on rabbits during the fall were induced to do so when the host was 'modified' by previous gradually increased illumination. Details of this experiment appeared in 'Nature' #3417, Vol. 135, under the title "Winter Feeding of the Tick D. andersoni Styles", Professor Wm. Rowan and J.D. Gregson.

The object of the above experiment, as of the following, was to attempt to show whether the inability of the tick to engorge during the fall and winter months lay in some unfavourable condition in the host, or in the tick, this condition being produced possibly by the shortening fall days.

The following animals were accordingly placed under observation:

- (A) 1 sheep. This was left outside in the open during the entire experiment, and was consequently subjected to increasing days of light until June 21st.
- (B) 2 sheep (ram and ewe, each 1 year old) were subjected to shortening days by enclosing them during the night in an absolutely light proof shed. At the beginning of the experiment their day was from 5 a.m. to 7.30 p.m. On each successive day they were released from their shed five minutes later in the morning and shut up five minutes earlier in the evening .
- (C) A series of eighty pairs of ticks collected by dragging at Rayleigh, Kamloops, during early May, 1935, were placed in a shaded pyrex cylinder over damp earth immediately outside of the sheep shed door. These ticks received increasing days of light until June 21st.
- (D) A series of sixty pairs of ticks of the above stock were placed in a pyrex cylinder over damp earth immediately inside the sheep shed. These ticks consequently received the same decrease of light as the sheep, the door of the shed being left open during the day.

The experiment commenced on May 9th. The sheep were infested on June 16th. The controlled animals at this date were receiving eight and a half hours of light each day, namely from 8 a.m. to 4.30 p.m. The shortening of days was continued to the end of the experiment on June 24th.

Sheep B. (Ram) Eight pairs of ticks 'C' were placed under each of two cages along the back.

Sheep B. (Ewe) Eight pairs of ticks 'C' were placed under each of three cages, one above the shoulder and two over the hips.

Sheep A. Eight pairs of ticks 'C' were placed under each of two cages, one over the left shoulder and one at the centre of the back.

Eight pairs of ticks 'D' were placed under each of three cages, these being placed over the right shoulder, right hip, and centre of back.

The following periods of feeding were noted for the female ticks that attached on June 16th:

Sheep A.	Tick group 'C'	2	dropped in 6 days		
		4	" " 7 "		
		4	" " 8 "		Average engorging
		5	" " 9 "		period for 15 ticks
					was 7.8 days.

	Tick group 'D'	2	dropped in 7 days		
		2	" " 8 "		
		3	" " 9 "		Average period of
		6	" " 12 "		engorging for 13
			(2 remained unengorged)		ticks was 9.9 days.

Sheep B.	Tick group 'C'	4	dropped in 6 days		
(ram)		4	" " 8 "		
		7	" " 9 "		Average engorging
					period for 15 ticks
					was 7.9 days.

Sheep B.	Tick group 'C'	1	dropped in 6 days		
(ewe)		1	" " 7 "		
		7	" " 8 "		Average engorging
		1	" " 10 "		period for 10 ticks
					was 7.9 days.

The results of this experiment have suggested that although light may have some effect on the physiological state of the host, such a change in its condition does not appear to affect the rate of feeding of the tick. This assumption, it will be noted, contradicts the points suggested in the previous experiments, but the latter observations were carried out under slightly different conditions and with different hosts, and do not necessarily refute the former suggestion that the winter feeding of ticks is dependent on the condition of the host.

It would seem, from the average of time taken for the light controlled and uncontrolled ticks to engorge under similar host and weather conditions, that the feeding rates of ticks are impaired by the previous treatment of decreasing light days. Unfortunately, due to the high rate of mortality of ticks between infestation and attaching periods, the number of females under observation was very small. The

results of this portion of the experiment, however, have been of sufficient interest to warrant repetition and further experiments with regard to the effect of light on ticks.

During the fall of 1935 a further experiment was conducted to determine whether light had any effect upon the rate of tick feeding. Since the supply of live ticks at this time was limited, and the infesting of rabbits, even when taking the utmost precaution, involves the risk of losing the parasites, the writer, in this experiment, used himself as the host.

Five adult female Dermacentor andersoni ticks were used, all being specimens that were collected at Kamloops in May, 1935. These ticks were marked and subjected to the following conditions. Their individual behaviour is recorded in the same table. (See page 69.)

Histological Examination of Ticks: All the ticks were sectioned and stained with eosin-haematoxylin and Mallory's stains. Examination of the stained sections gave similar and normal pictures for all ticks. In the intestines of each specimen was seen a certain amount of blood (most in tick # 1). The gut epithelial tissues were all commencing the absorptive stage. Black pigment bodies were abundant in all the cells, and numerous small round globules were present, but in no cases were there present any of the brown yeast-like granules.

The fact that at the areas of attachment an intense itching was produced further suggests that a powerful toxin is injected into the host tissues during the feeding of the tick. The lack of blood from below the four specimens that showed no signs of engorging strengthens the theory that ticks will feed fast only when there is sufficient free blood surrounding their mouth parts.

1935.	Tick #1.	#2.	#3.	#4.	#5.
Nov.3	Irradiated continually for one week under a 60 W. quartz Mazda CX lamp. The ticks were enclosed in a petri dish covered by a damp cloth thus permitting penetration of ultra-violet rays. The source of light was one foot from the ticks, a distance giving an irradiation approx. equivalent to sunlight. (Pers. corres. Gen. Elec.)		No irradiation. These ticks were left in the dark, but were under similar humidity and temperature conditions.		

10th	Placed on right arm under capsule.	Placed on left arm.	Placed on left arm.	Placed on left arm.	Placed on right arm.
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(All ticks were active when placed on the skin and within six hours each had definitely attached itself and was ready to engorge.)

11th	Released its hold during the night, and was found firmly attached to the chest in the morning.	Had all attached near the same site on arm.	Firmly attached.
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(All ticks very active reacting immediately to wafts of breath. No excreta observed. Slight inflammation and intense itching of skin surrounding ticks.)

12th	All ticks still at same sites. Excreta practically negligible. Edematous boil-like elevations filled with clear serum appeared in the skin surrounding the ticks.		
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13th. a.m.	Portion of chest and tick submitted to mercury arc rays for two mins. (The tick at this date still showed no signs of engorging.)
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13th. p.m.	Tick began to engorge very rapidly. Much excreta, with its characteristic odor. Edematous raised area of skin 2 cm. in diameter at site of attachment. Inflamm. region surrounding wound for a distance of two inches.	All other ticks showed no signs of engorgement, although they occasionally liberated small particles of black excrement. Inflammatory areas one inch in diameter. Intense itching.
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All ticks were removed and placed in Bouin's for microscopical examination. Wounds in the skin healed slowly, and itched as long as four weeks after.

Bleeding followed removal of mouth parts.

No blood was evident upon removal.

The interesting results of the previous experiment and the later arrival of more ticks of the same stock led the writer to conduct one more experiment along the same lines. A rabbit was used for the host this time, and instead of the apparently ineffective irradiation of ticks by quartz lamps, the mercury arc method was employed.

The observations commenced on January 8th, when two flat female ticks were placed under gauze capsules on each side of the rabbit (see technique page 73).

The ticks, as usual, attached to their host shortly after being placed on the skin. They were observed carefully for five days, during which time they exhibited practically no tendency to engorge. (Under spring conditions they would have been two-thirds replete by this time.)

On January 13th the infesting capsule was temporarily removed from the right side of the rabbit and the ticks and surrounding skin were exposed to a mercury arc light for ten minutes. This procedure was repeated for three days, a similar dose of ultra violet being given each time. At the end of the third day it was found that all the ticks had commenced to engorge. Furthermore, the two ticks that had been irradiated were noticeably larger than those that had received no treatment. The average size of the ticks from each of the two groups was respectively three eighths and one fourth of the replete stage at this time.

Unfortunately at this date the rabbit succeeded in tearing off the cages and eating all the ticks but one. This remaining specimen, one which had never been irradiated, was observed for a week. At the

end of that time it was still only about two thirds replete.

Discussion: In the above observations it is indicated that irradiation of ticks by quartz (Mazda CX) lamps has little or no effect upon the metabolism of the tick in relation to its feeding. An increase in the engorging rate, however, was noticed on two occasions when feeding ticks and adjacent host tissues were submitted to mercury arc treatment.

Assuming from the previously mentioned experiments (pages 65-68) that light produced no noticeable physiological change in the tick or host in relation to tick feeding rate, and considering the theory that slow feeding ticks are merely suffering from a lack of accessible tissue blood (page 60), it is suggested that the ultra violet treatment of host and tick is effective chiefly through a local pathological condition that is produced in the host. This condition, seen as a hyperaemia, would naturally tend to bring more blood to the subcutaneous tissues surrounding the tick, thus creating a factor conducive to increased assimilation processes.

The tick feeding studies have to date led to the suggestion that the varying rates of tick engorgement are directly due to the varying abilities of ticks to produce a hyperaemia within the host tissues surrounding the site of attachment. This condition is believed to be brought about by some powerful salivary irritant that is injected into the wound.

The reason for the seasonal and individual variations in the potency of this toxin is still unknown. It was at this point in these studies that the various intracellular symbiont-like bodies were noticed

in the digestive epithelium of the tick. It is possible that light and other external physical conditions may play a part in the metabolism of these symbionts, they in turn affecting the physiology of the tick. The possibility of such intricate relationships has led the writer to temporarily discontinue the above experiments until more is known about the normal tick content flora.

APPENDIX

TECHNIQUE FOR THE STUDY OF TICK FLORA

The microscopic studies of tick tissues and their flora are beset with innumerable difficulties, a fact that has been realized by all who have carried out investigations along this line. The following methods used in attacking tick problems have been found to be most satisfactory by the writer.

1. Methods Employed for Infestation Studies:

The writer will make no mention here of large scale infestation procedures, such as are recognized in experimental laboratories where numerous ticks are reared for life history and parasite studies. The following methods only apply to feeding and host reaction studies of individual or small groups of ticks where daily observations are required. All experiments of this nature are rendered doubly difficult by the fact that the student has an animal continually opposing his efforts. The mere attachment of a tick container to a rabbit will usually cause the animal to scratch and bite the object until it is torn off. Cages placed on the backs of sheep are readily rubbed off, or squashed if placed on their heads. The best infesting cages that the writer has tried require at least daily attention.

Sheep are undoubtedly the most suitable animals for the feeding of Dermaceptor andersoni adults. Besides being one of their normal hosts, they provide remarkable facilities for the attachment of infesting cages. The latter are in the form of dome shaped capsules, and are easily made from squares of brass gauze.

The mesh of this screen should be sufficiently close to keep the ticks enclosed, and the wire must be stout enough to give the cage rigidity. The bottom of the capsule is trimmed evenly and covered with adhesive tape to prevent injury to the host's skin. It is important, whenever adhesives are used, that precautions are taken to ensure that there are no sticky portions within reach of the ticks.

The infesting area on the host is clipped and washed free from grease. The cage is then attached by hitching six lengths of stout linen thread to opposite tufts of the surrounding wool, and passing them through meshes near the apex of the container. If the opposite threads are tied in bows, then the cage may readily be removed for inspection of ticks. The writer has found this an exceedingly satisfactory method, providing the threads are renewed whenever they begin to rot or wear.

When the experiment demands a number of hosts, sheep are obviously out of the question. Rabbits have been resorted to in these instances, but have proved to be far from satisfactory. The gauze capsule method was found to be the most efficient for infestations. Each container was fastened by fine cross wires to a stout wire ring of larger diameter than that of the capsule, this ring being held to the animal by a broad band of adhesive tape that entirely encircled the body.

Personal infestations were employed when there was too great a risk in losing the ticks. In these instances the ticks were enclosed in a cork ring that was held on the arm by a cloth bandage. In such experiments, of course, the possibilities of infection must be considered.

2. Technique for Gross Anatomical Studies:

Gross dissections are best carried out under physiological saline solution. The tick is held in position by first spreading it out on a sheet of adhesive tape, embedding the legs into the adhesive by drawing a hot needle over them. The tape is pinned or melted onto a wax bed and the whole tray filled with saline. The chitinous integument of the tick is circumcised with the point of a razor scalpel, and with little difficulty can be detached from the underlying mass of viscera.

Fully engorged female ticks are so distended with blood that an accidental rupturing of the diverticula will cause the contents to flow out and obscure the field. This danger may be avoided by immersing the specimen momentarily in boiling water before placing it on the dissecting tray (due to coagulation of gut contents).

Anatomical studies of the capitulum and other chitinous portions are revealed by careful clearing in 3% NaOH solution and subsequent mounting in Canada balsam or Euparel.

3. Histological Technique for Tick Sectioning:

(1) Killing of specimens.

Ticks are extremely resistant to poisonous fumes and liquids. Adult specimens often crawl about after having been half an hour under 95% alcohol and mercuric chloride. Temporary immersion in hot 70% alcohol will kill and distend larvae and nymphs, and this method is generally used when making whole mounts. For histological studies,

direct killing in the fixative is advised. To ensure an even and rapid penetration of the fixing fluid, part of the integument must be removed. This is done most conveniently by the method previously described. For bacteriological studies, a slice off each side of the tick is sufficient to effect penetration.

(2) Fixing.

Various fixatives have been used, all with fairly satisfactory results. Among these may be mentioned:

Zenker's Bichromate of potash 2%
Sulphate of soda 1%
Mercuric bichloride 5% Dissolved in 100 cc. water.
Acetic Acid (added after) 5%

Bouin's Picric acid sat. aqueous sol. 75 parts,
Formol 25 " ,
Acetic acid 5 " .

Carnoy's Glacial acetic acid 1 part,
Absolute alcohol 3 parts.

Cowdry recommends Regaud's fixative as being particularly good for preservation of mitochondria and bacteria. The solution consists of 4 parts 3% potassium bichromate and 1 part of commercial formalin. The solution must be used immediately after being made and should be changed several times for one or two days. The tissues are washed in running water for 24 hours and then changed to alcohol. The high concentration of formalin in this fixative aids penetration between the plates of chitin, and the bichromate acts as a mordant for Giemsa's stain.

(3) Infiltrating.

The fixed tissues, having been washed in water or alcohol, were passed successively through the following solutions of ethyl and N-butyl alcohols:

18 cc. 50%	ethyl	and	2 cc. N-butyl,
16 cc. 60%	"	"	4 cc. " ,
13 cc. 75%	"	"	7 cc. " ,
9 cc. 90%	"	"	11 cc. " ,
5 cc. absolute	"	"	15 cc. " .

They were allowed to remain in each of the five grades for six hours. These solutions were followed by two changes of pure butyl alcohol, each of 6 to 10 hours duration. The butyl alcohol has the advantage of not hardening the chitin as much as xylol does.

Cedar oil was used with fair results, but the longer period taken for replacement by paraffin tends to harden the tissues to an unnecessary extent.

(4) Embedding and Sectioning.

The butyl alcohol was replaced by a low melting point paraffin (48-52 degrees), five one hour changes being sufficient to complete the removal of alcohol. A temperature of 10 C. was found to be most suitable for the cutting of sections 5 to 10 microns in thickness. The ribbons were flattened over egg albumen in the usual procedure.

(5) Staining.

Sections of engorged female ticks contain a large amount of blood, and because of this show a great tendency to curl off the slide if they are 15 microns or over in thickness. Loss of such sections during staining may be prevented by passing the slice from absolute into a thin solution of celloidin. In the ascending series of alcohols, the absolute is substituted by a carbolxylol solution (25% phenol).

Apart from this deviation, which was followed only in the case of thick sections, the normal staining routine was used.

A variety of stains were tried for histological studies.

Haematoxylin-eosin was found to be the most satisfactory for general purposes. (Delafield's)

Mallory's triple stain was particularly useful for studying assimilative changes within the epithelial cells, the intracellular droplets appearing a bright red, in contrast to the blue cell elements.

Giemsa's stain was of value in studying nuclear changes.

Heidenhain's Iron Haematoxylin was used in several instances to bring out mitochondria and nuclear structures.

Methylene blue-eosin gave a clear picture of connective tissue elements.

4. Bacteriological Technique for the Study of Tick-borne Microorganisms:

Morphological Examination for Microorganisms.

(1) Staining.

All sections that were examined for bacteria and other tick invading microorganisms were cut at 4 microns and stained one or both of the following stains. In either case, the paraffin sections were passed through xylol and brought down to distilled water in the usual manner.

Giemsa. Tissues were stained half an hour, washed with distilled water, and passed immediately through 95% alcohol to absolute.

The following method was found to be very satisfactory for the demonstrating of bacteria in tick tissues:

Brown and Breen's Differential Method for Bacteria in Sections.

1. Stain in freshly filtered alum-haematoxylin (Harris) 4 min.
2. Wash in acid alcohol (3% HCl in 95% alc.) until pink.
3. Wash in ammonia water (1 cc. NH₄OH in 100 cc. water) till blue.

4. Wash in water.
5. In a small vial mix 5 drops of 5% aqueous sol. sodium bicarbonate with 0.75 cc. of 1% aqueous sol. of gentian violet. Pour mixture on slide for 2 minutes.
6. Wash quickly with water.
7. Cover with Lugol's iodine sol. for 1 minute.
8. Wash with water. Blot.
9. Decolourize in one part ether plus 3 parts acetone.
10. Blot.
11. Stain with basic fuschin (0.005 gm. in 100 cc. water) for 5 minutes. (A sat. sol. of fuschin was found to be more satisfactory.)
12. Wash in water, blot, but do not allow section to dry.
13. Pass through acetone.
14. Decolourize carefully in a sol. of 0.1 gm. picric acid in 100 cc. acetone.
15. Pass through acetone, equal parts of acetone and xylol, and xylol.
16. Mount in balsam.

Smears of tick gut contents were fixed for a minute in 95% alcohol and stained for bacteria by Gram's stain.

The following modification of Tunnicliff's stain was used to demonstrate the presence of spirochetes:

1. Cover smear with carbol crystal violet for 30 seconds. (Sat. alc. sol. crystal violet, 10 cc.; 5% aq. phenol sol. 90 cc.)
2. Wash with water.
3. Cover with Lugol's iodine sol. for 30 seconds.
4. Wash with water.
5. Cover with safranin, 30 seconds.
6. Wash with water.

Moeller's Method for Spores.

A method by which bacterial spores are stained red in contrast to the blue staining bodies.

- (1) Wash fixed smear in chloroform for two minutes.
- (2) Wash in water.
- (3) Cover with 5% chromic acid for two minutes.
- (4) Wash in water, and steam in carbolfuschin solution for three to five minutes.
- (5) Decolorize in 5% sulphuric acid for 5 to 10 minutes.
- (6) Wash in water, and stain in aqueous methylene blue for one minute.

(2) Dark Field Illumination.

Examination of fresh smears in saline by dark field illumination is of value, since by this method possible forms of microorganisms may be seen that would otherwise be destroyed beyond recognition by drying and fixing.

5. Artificial Media for the Culturing of Tick Invading Microorganisms.

The preparation of media suitable for the growth of highly adapted forms of microorganisms has long been a problem to the bacteriologist.

Here, in the form of various tick invading bacteria and possible intracellular symbionts, the student is presented with organisms possessing extremely delicate cultural reactions. To date, many of the microorganisms seen within tick tissues remain uncultured on artificial media. Others have been grown only after years of searching for a suitable medium.

The writer lists here media that might normally be used in attempting to isolate strains of tick invading organisms. Whereas ordinary bacteriological media are usually adjusted to a pH between 7 and 7.6, the writer advises the experimenting of media with pH's as low as 6, since it was observed from the reactions of six engorged nymphal ticks that the pH of the body contents was between 6 and 6.2.

Meat Extract Broth. (Zinsser - 37)

To 1 litre of distilled water add:

Difco Meat extract	5 grams;
Peptone	10 " ;
NaCl.	5 " .

Mix, dissolve, cool, and adjust pH.

Boil for 30 minutes, re-adjust, filter, and tube.

Plain Agar. (Zinsser Bact.)

Agar	20 grams;
Meat extract	3 " ;
Peptone	20 " ;
Sodium chloride	5 " ;
Distilled water	1000 cc.

Boil till agar is dissolved, add water to restore volume, adjust pH, filter and tube.

Cooked Meat Medium. (Prac. Bac. Mackie & McCart.)

For cultivation of anaerobes.

Mince 500 gr. fresh heart, place in 500 cc. boiling N/20 caustic soda, simmer for 20 minutes. Neutralization of lactic acid occurs, leaving pH about 7.5. Drain liquid and partially dry meat. Place 5 cc. of meat in each tube and add 1% infusion broth (of adjusted pH) to cover meat by 1 cc. Boil in bath for half an hour to drive off oxygen. Surface may be covered with $\frac{1}{2}$ inch sterile paraffin.

Hottinger Broth. (Per kindness of Dr. Naismith, Kamloops.)

Take about 750 gr. of meat; free it from fascia, cut into finger length pieces and boil in 1500 cc. distilled water. After 15 minutes take out meat and put through grinder. Cool the water to 37 C. and add sodium carbonate, 3 grams. Put 550 grams of the meat into a two litre flask, add the water and fill the remaining space with distilled water. Add pancreatin 3 gr., chloroform 10 cc., and toluene 10 cc. Incubate or leave at room temperature for one to two weeks, shaking two or three times daily. Acidify with HCl. (Brom. thymol blue indicator). Decant the liquid through cheese cloth and add rinsings from the meat. Bottle till ready for use. Dilute to one third for culture broth and autoclave to drive off chloroform.

(Glaser, (11).)

10 cc. of standard nutrient agar is poured into petri dishes into which previously 3 cc. of sterile defibrinated horse blood and 2 cc. of 10% sterile dextrose solution has been added. The final pH should be adjusted to a range from 7 to 7.4. Test for sterility. Spot loops of material over plate.

Peptone-gelatine-blood medium. (Kligner and Aschner, (17).)

Stock solution consists of 10 grams of peptone, 10 grams of gelatine and 100 cc. of water. One cc. of this solution is diluted in 10 cc. Locke solution. Adjust pH, autoclave, and divide into neutral clean test tubes, 2 cc. per tube.

A day or two before use, 0.25 cc. of defibrinated sheep blood is added to each tube.

The writer also used the above medium with a solution of tick excreta, one gram of dry excrement dissolved in 50 cc. water. This medium appeared to favour the growth of organisms, but it was found to be extremely difficult to free the excreta thoroughly from contaminating organisms. Double filtration through Berkefeld filters was only efficient when the medium was made up in agar plates so that the contaminating organisms could be isolated.

Noguchi (22), in his Rocky Mountain spotted fever studies, mentions the following special media:

Leptospira medium.

0.9% saline solution	800 cc.
Fresh rabbit serum	100 cc.
2.0% nutrient agar, pH 7.2	100 cc.
Rabbit haemoglobin (made by taking 1 part of defib. blood with 3 parts of distilled water)	10 to 20 cc.

Novy and McNeal.

Mix equal parts of melted nutrient agar and defibrinated rabbit's blood. When the agar has cooled to about 50°C, an equal quantity of rabbit's blood is added, mixed, and allowed to cool.

Noguchi.

To 9 parts Hiss serum water containing various carbohydrates in series is added 1 part of 2% nutrient agar. N/10 HCl is added to give a pH ranging from 4.5 to 7.4.

6. Methods for the Artificial Cultivation of Tick Invading Microorganisms.

Freeing Tissue from Contaminants:

One of the first difficulties to present itself in cultural studies of tissue invading microorganisms is that of freeing the tissues of contaminating bacteria. This naturally must be accomplished efficiently, and yet too drastic methods must not be employed or the invading organisms themselves will be destroyed.

In the course of the preceding studies a variety of methods for sterilizing the outsides of tissues were tested. Some of these were formulated by the author, others were procedures that had been used by previous workers. The following was found to be the most satisfactory.

Glaser's Method for freeing contaminants from roach tissues. (Glaser, (11).)

"The roach is etherized until immobile. It is then submerged for 15 to 20 minutes in a solution consisting of equal parts of 95% alcohol and 1/1000 mercuric chloride, after which the insect is washed in 70% alcohol. The extremities are amputated at their bases with sterile scissors and the insect is fastened with sterile pins ventral side up in a tray of paraffin recently melted and hardened. Prior to using the paraffin, the tray is flooded with 70% alcohol for a few minutes. After pinning, the ventral side of the abdomen is again washed off with 70% alcohol, and by means of a fine pipette alcohol is forced in between the external abdominal segments. The abdomen is lastly singed with a red hot spatula, and the ventral chitin removed with sterile instruments. The desired tissues are removed, macerated in sterile saline, and transferred to appropriate media."

(Kligner and Aschner (17) state that by dipping the tick into 5% tincture of iodine for 5 to 10 seconds, and then placing it in 95% alcohol for the same time, contaminating bacteria are sufficiently destroyed to permit aseptic dissections of gut and other inner tissues. The writer, in no instance, found this method to be satisfactory.)

The following methods have been used to sterilize the outsides of fly eggs for medicinal purposes. Since the eggs are not destroyed for hatching purposes, it follows that these procedures should be highly

satisfactory for cultural studies of egg inhabiting organisms. For such studies, the treated eggs should be tested for remaining contaminants by incubating in broth for three days. They are then transferred to a suitable medium and crushed with the aid of a sterile rod.

Fine, A. and Alexander, H. Jour. of Bone and Joint Surg., 1934, 16, 572.
Separate clumps of eggs in .85% NaCl with aid of cotton tipped rod.
Wash in 10% formalin for 8 minutes.
Rinse in sterile saline.

Baer, W.S. Jour. of Bone and Joint Surg., 1931, 13, 438.
Wash eggs for 30 minutes in 1 part 1/1000 HgCl₂
1 part 50% alcohol
 $\frac{1}{2}$ 1% HCl.
Rinse in sterile saline.

McLellan, N.W. Can. Med. Ass. Jour., 1932, 27, 256.
Wash eggs for one hour in 50 parts 1/1000 HgCl₂
50 parts 50% alcohol
 $\frac{1}{2}$ part HCl (C.P.)
Rinse in sterile saline. (Few spores killed.)

Child and Roberts, N.Y. State of J. Medicine, 1931, 31, 937.
Wash in sodium hypochlorite to remove albuminous coating, and sterilize by continuous washing with 4% formaldehyde for 3 minutes. (No spores killed.)

7. Elementary Technique for In Vitro and In Vivo Studies of Tick Tissue.

It has been the desire of the writer on several occasions to induce ticks to feed from an artificially constructed tissue. This would essentially consist of a piece of skin placed over a container filled with a suitable medium. The chief difficulty in such an experiment is in keeping the enclosed medium sterile, for whatever would be of best nutrient value to the tick, would also be a medium for bacteria, and it is practically impossible to avoid contamination when the tick penetrates the enclosing skin.

Such studies would undoubtedly throw much light on the nature of tick feeding, their preference for certain hosts, and their relation

to host diseases, and should even open up a new field for the study of tick repellents. That such an experiment is probably not impossible is suggested by the fact that the author has on several occasions been able to induce ticks to attach and feed from a bath of warm blood covered by skin from a rabbit's ear. In each case infection occurred before the 'parasite' had been able to engorge to any appreciable extent.

One further experiment was performed in which portions of the gut of an unfed adult tick were incised under aseptic conditions and were placed in hanging drops of serum, haemolysed, and defibrinated blood. After being cultured for three days at a temperature of 30°C. they were fixed and sectioned.

Microscopic examinations showed the digestive epithelium to be still preserved, though no apparent assimilative changes had taken place. It is probable that assimilation of blood by the gut cells is stimulated by other factors than the mere presence of blood - possibly distension or digestive enzymes play a part in the process.

It is hoped that further experiments in this line will be planned in which sterile extracts of the body fluid will be added to the blood medium. To this end, the following methods of Frew (10) are quoted.

Method of Obtaining Bacteria-free Tissues:

"Sterile tissues may be obtained from larvae reared in the ordinary way by the tedious but quite effective method of repeated washing of the fragment of tissue in sterile physiological saline. Twelve changes of saline have been found effective. It is left for about two minutes in each change with constant agitation with a needle. Sterile needles are used for each transference to a fresh washing. After the final washing it was either put up in culture whole, or cut into fragments depending on the type of growth required; well over 90% of such cultures were free from bacterial infection."

It is noted by Frew that the above method is only successful for portions of ganglia, muscle and fat bodies. Fragments of gut and salivary glands invariably showed bacterial infection after several days.

Method of Obtaining Bacteria-free Body Fluid:

"It is an easy matter to obtain sterile body fluid by filtration through a Berkfeldt candle, but it has proved an extremely difficult matter, and one not successfully overcome, to ensure that the fluid so filtered is unchanged in any way except in being freed from bacterial contamination.

"After autoclaving, the Berkfeldt candle must be dried, and it is essential that the drying should be done at a low temperature (30°C.) as otherwise it has a very marked effect on the pH of the filtrate. The nozzle of the candle projects into a small waxed glass tube, which is introduced with aseptic precautions into the container after the whole assembled filter has been dried at the temperature mentioned above. Paraffin oil is now poured into the reservoir and sucked through the candle, gradually filling the small glass tube. The candle itself must be kept completely covered with oil and the suction is continued until air bubbles, at first numerous, practically cease to emerge from the nozzle of the candle. The fluid to be filtered is then pipetted into the reservoir below the oil which covers the candle. On passing through the candle the fluid is delivered below the surface of the oil in the small glass tube, to the bottom of which it sinks. In this way the body fluid is collected, filtered, and stored without once coming in contact with the air. In the absence of this special paraffin oil filtration there is quite enough air in the candle to cause pronounced oxidative changes in the fluid. The use of the oil has the further and minor advantage, in dealing with small quantities of fluid, that almost the very last traces of the fluid can be forced through the filter."

SUMMARY

This preliminary study of the tick and host in relation to western Canadian tick-borne diseases has been divided into two parts. The first deals with the tick as a reservoir of microorganisms, pathogenic or otherwise.

A brief discussion of the systematic position of the ticks studied and the life histories of the most important Canadian species introduces the topic.

After a morphological description of the alimentary tract of the tick Dermacentor andersoni Stiles, the histology of the stomach and process of digestion is fully described. The assimilative changes of the gut are divided into four phases: the preabsorptive, being the condition of the gut before entry of blood; the absorptive, during which time the epithelial cells are actively assimilating blood; the post-absorptive, when the epithelial cells become distended and break loose from the gut lining; and the degenerative period, involving a gradual disintegration of the gut elements.

The tick-borne diseases of the world are tabulated and followed by a summary of the status of tick-borne diseases in Canada. As an introduction to the study of the latter, the writer briefly reviews the studies that have to date been made with regard to the less known micro-organisms, these including the rickettsia bodies and certain forms of bacteria. A preparatory study of the tick as a reservoir for micro-organisms brings to light several symbiont-like bodies that possibly play a very important part in the life history of the tick.

Part II includes a series of studies of the physiology of tick and host in relation to varying rates of tick feeding. In connection with this, the problem and etiology of tick paralysis are discussed. Studies of host tissues below fast and slow feeding ticks, the effect of light on host and tick, and observations from the feeding of ticks on the writer all lead to the suggestion that the varying rates of tick feeding are directly due to the varying abilities of ticks to produce a hyperaemia and haemorrhage within the host tissues surrounding the site of attachment. This condition is believed to be brought on by some powerful salivary irritant that is injected into the wound. The reason for the seasonal and individual variations in the potency of this toxin is unknown, but may possibly be indirectly due to the effect of external physical conditions on certain intracellular symbionts of ticks.

The thesis is concluded by a discussion of technique in attacking problems pertaining to the study of tick-borne diseases.

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PLATE I.

Figure 1.

Gram + cocci as seen in the tissue of
a Dermacentor albipictus tick stained by
Brown and Brenn's method.

x 1700.

Figure 2.

Pigmented bodies in gut epithelial cells
of Dermacentor andersoni, as seen in un-
stained tissues.

x 1750.

PLATE I.



Figure 1.



Figure 2.

PLATE II.

Figure 1.

Portions of diverticula of an engorging
Dermacentor andersoni tick showing certain
cells filled with yeast-like granules.

x 150.

Figure 2.

One cell of the above showing the dense
mass of pigmented granules.

x 1050.

PLATE II.



Figure 1.

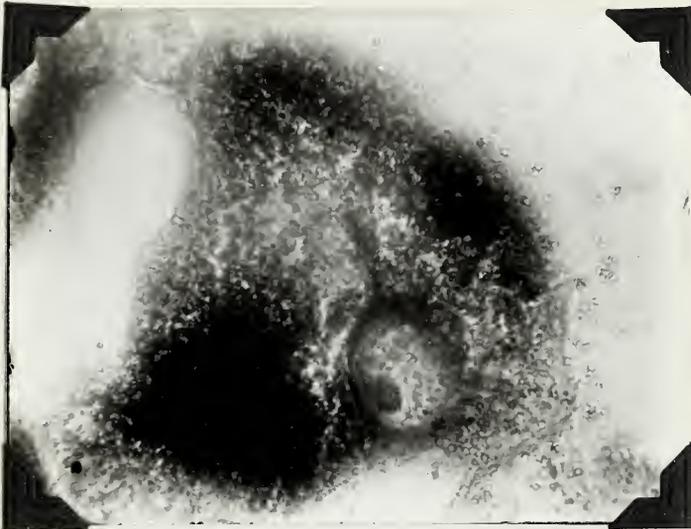


Figure 2.

PLATE III.

Figure 1.

Cross section of a diverticulum of an engorging adult Dermacentor andersoni tick showing proliferation and expansion of epithelial cells.

x 170.

Figure 2.

Portion of above section showing the fuschin staining intracellular globules.

x 500.

PLATE III.



Figure 1.

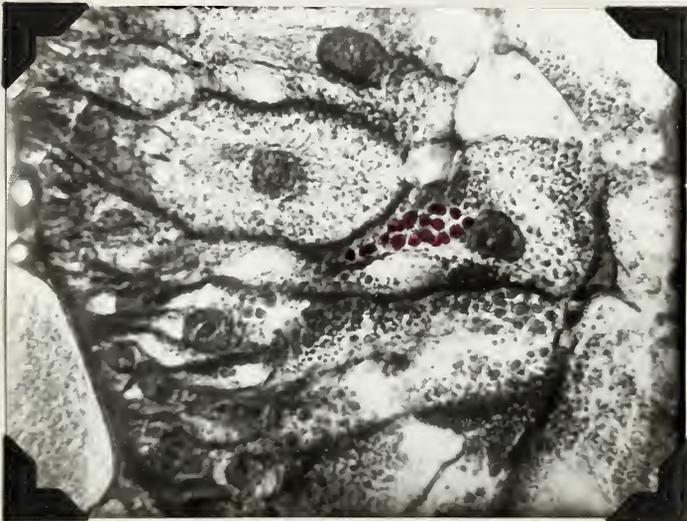


Figure 2.

PLATE IV.

Figure 1.

Portion of diverticulum (and integument) of a replete adult Dermacentor andersoni tick stained with Giemsa's stain. This picture shows several 'floating' epithelial cells, one with the nucleus still intact, two others with only nuclear fragments. Note the pigmented particles in the gut fluid and the halo-like area surrounding the epithelial cells.

x 160.

Figure 2.

Epithelial cells of above section filled with large clear globules. Two whole nuclei are shown in cells at the bottom. In a neighboring cell the nucleus has disintegrated and is being engulfed by the globules.

x 500.

PLATE IV.

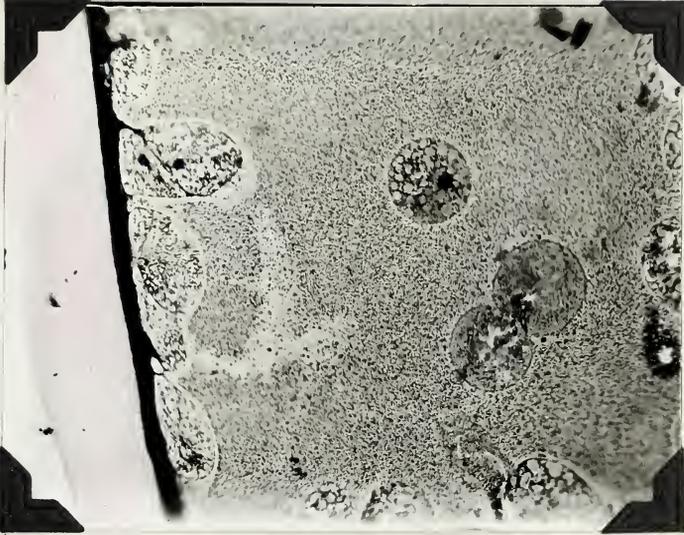


Figure 1.

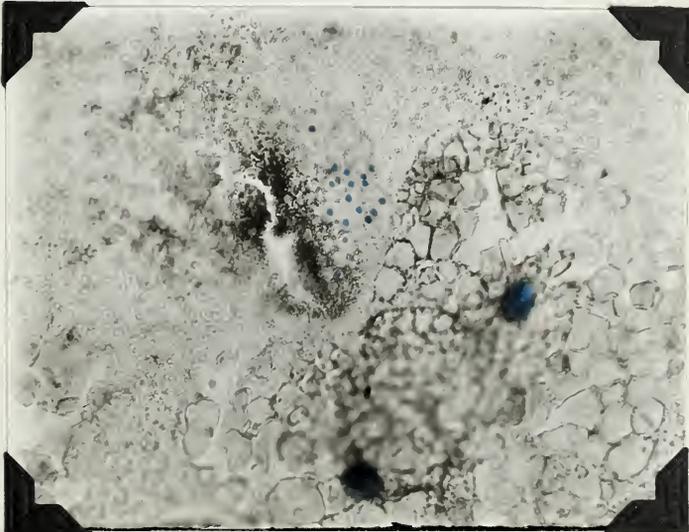


Figure 2.

PLATE V.

Figure 1.

Portion of body contents of a disintegrating Dermacentor albipictus tick stained by Mallory's. At one side are seen members of Malphigian concretions. In neighboring tissues and within the muscle sheath are numerous symbiont-like bodies which stain red with fuschin.

x 375.

Figure 2.

Another portion of the above section showing red and blue staining symbiont-like bodies within the gut membrane. These globules are believed to be the same as those shown in Plates III and IV. Note the complete absence of epithelial cells. The clouds of clear particles are likely decolorized yeast-like granules (Plate II).

x 375.

PLATE V.

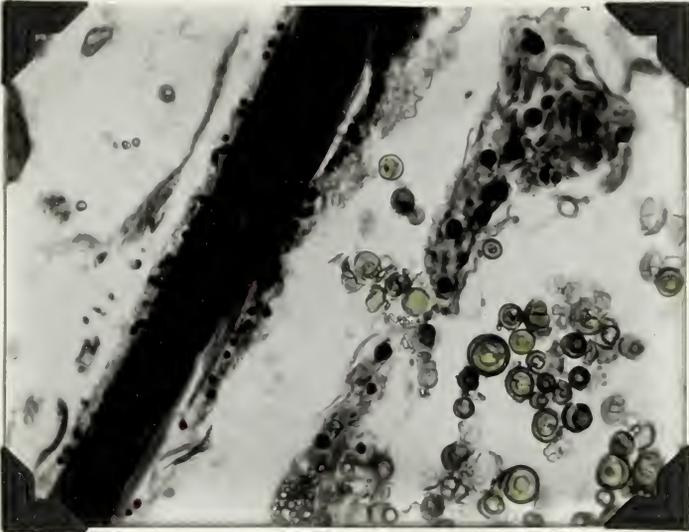


Figure 1.

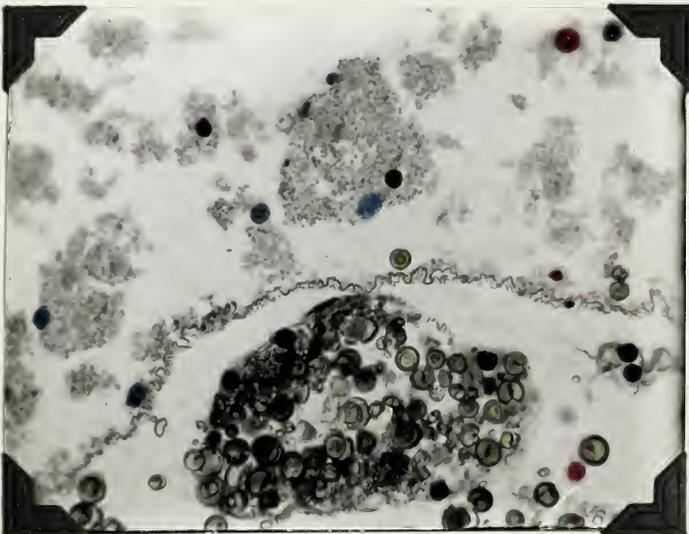


Figure 2.

PLATE VI.

Figure 1.

A smear from the gut of a replete Dermacentor albipictus nymph, stained with Mallory's.

Note the oval non-staining yeast-like particles and the large blue and red intracellular globules.

x 1050.

Figure 2.

Portion of the egg of a tick stained by iron haematoxylin to show the mitochondria.

x 1050.

PLATE VI.



Figure 1.

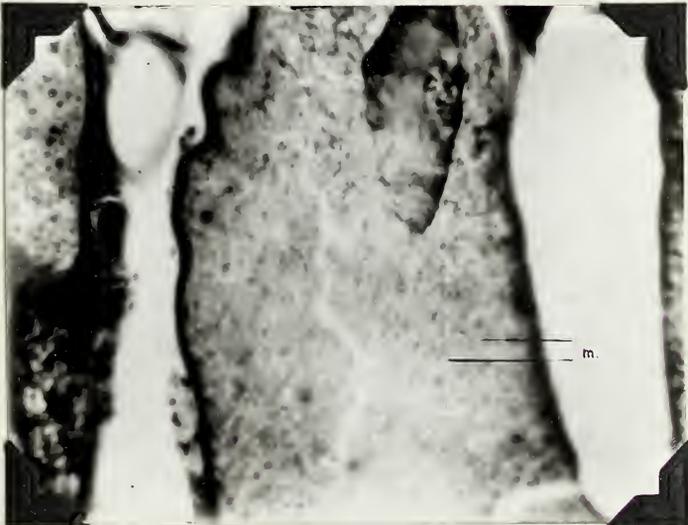


Figure 2.

PLATE VII.

Figures 1 and 2.

Photomicrograph of a culture of tick gut content on agar. Note the pigmented yeast-like bodies and the appearance of division in the large clear globule.

PLATE VII.

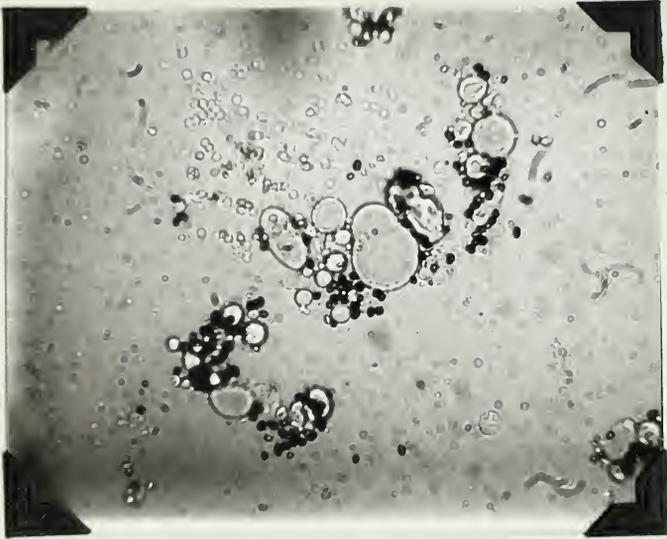


Figure 1.

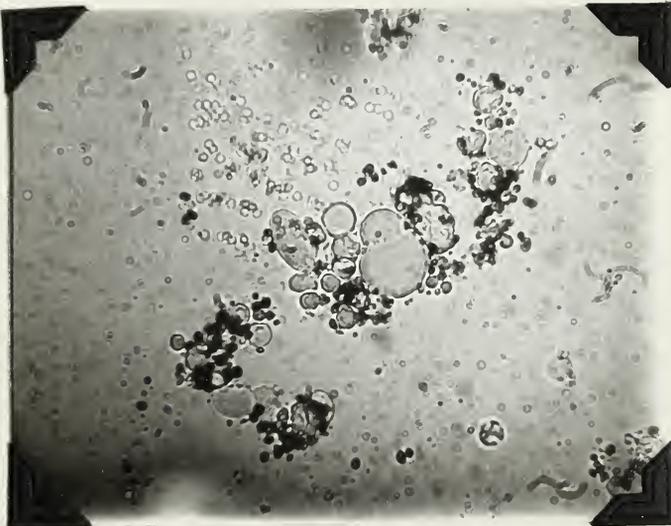


Figure 2.

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