A Group of Micro-organisms Transmitted hereditarily in Ticks and apparently unassociated with Disease (1).

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While studying the relation of *Rickettsia ruminantium* (2) to heart-water, it was found that a micro-organism previously reported in *Amblyomma hebraeum* (3) was present not only in the eggs of adult females, but also in infected larvae hatched therefrom and in nymphae. Profiting by the unusual opportunities afforded for the investigation of all stages in the life-history of ticks in Sir Arnold Theiler's laboratory, an attempt was made to ascertain whether this micro-organism in *Amblyomma hebraeum* was an isolated example of hereditary transmission or whether it was one of a group of parasites thus transmitted.

A review of the literature indicated that the micro-organism in question might possess points of affinity to the so-called symbionts briefly discussed by Buchner (4), and to a micro-organism observed by Godoy and Pinto (5) to have a predilection for the ovaries in certain species of Brazilian Ixodidae, and presumed by them to be not a parasite but a symbiont. These authors named it "Ixodisymbionte," and noted that it was first discovered by R. Koch in *Rhipicephalus*. The micro-organism in *Amblyomma hebraeum*, as seen in the Malpighian tubules, also resembled in morphology, in arrangement, and in its feeble affinity for dyes some bodies reported by Hindle (6) in the Malpighian tubules of *Argas persicus* (see his fig. 5), and believed by him to be stages in the life-cycle of *Spirochaeta gallinarum*. And, furthermore, as observed in egg-cells, the micro-organism presented somewhat similar morphology and staining reactions as some intracellular protozoa described by Hertig and Wolbach (7) in the ovaries of *Dermacentor variabilis* and *Dermacentor venustus*, and considered by these investigators to resemble superficially certain phases of *Theileria*.

Attention was first directed toward the study of unfed larvae, because the probability seemed to be a strong one that any micro-organisms found in them must have been inherited through the eggs, since the larvae had hatched out from eggs deposited by the female in sterile test tubes plugged with cotton, which were carefully kept from contamination.

Soon somewhat similar micro-organisms, characterized by relatively large size as compared with most Rickettsia, great pleomorphism, Gram-negative properties, and intracellular habitat, were observed in both smears and sections of the unfed larvae of *Boophilus decoloratus*, *Haemophysalis leachi*, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, and *Rhipicephalus simus*, in addition to *Amblyomma hebraeum*. Fortunately, these six species of ticks were being reared in the laboratory for other purposes, so that material was at hand in which to trace the history of the micro-organisms through some of the subsequent stages of development. Photographs

of the micro-organisms, as seen in smears, are given in Plate 1; camera lucida drawings, illustrating their degree of pleomorphism, are reproduced in Plate 2; and their appearance in sections is shown by the drawings in Plate 3.

The survey was extended by the inclusion of certain phases in the life-history of other ticks available in the laboratory and by a re-examination of specimens previously described (2, 3). In this way it was found that micro-organisms of this general type, and probably also transmitted hereditarily, were present in at least fifteen species represented by the plus (+) signs in Table 1, which gives full information regarding the material examined. Unless stated to the contrary, it is to be understood that the ticks were obtained in Sir Arnold Theiler's laboratory. To these the laboratory numbers are appended for reference. The methods employed in handling the larvae, nymphae, and adults, and the technique applied were the same as those used in an earlier study on *Rickettsia ruminantium* in ticks (2).

TABLE 1.

Material Examined.

Species.	Unfed Larvae.	Nymphae.	Adults.
Amblyomma americana +	—	—	Sections of 2 from Columbia (S.C.), 5 from Washington (N.C.), and 10 from Baton Rouge (La.) fixed in Regaud's fluid (3).
Amblyomma hebraeum +	Smears of 25 and sections of 25 fixed in Regaud's fluid (517); dark field examination; also 112 engorged larvae prepared by a variety of methods (2)	300 prepared by many methods, some infected with heartwater (2)	Sections of 20 fixed in formalin and Regaud's fluid (3); also sections of 6 infected with heartwater (2).
Amblyomma maculatum —	—	—	Sections of 6 from Van Cleave (Miss.) fixed in formalin and Regaud's fluid (3).
Amblyomma tuberculatum —	—	—	Sections of 1 from Perkinston (Miss.) fixed in formalin and Regaud's fluid (3).
Argas mineatus —	—	—	Sections of 2 from Southerland (Fla.) fixed in Zenker's and Regaud's fluids (3).
Argas persicus +	Smears of 25 and sections of 25 fixed in Regaud's fluid; also dark field examination	—	—
Boophilus decoloratus +	Smears of 50 sections of 50 and dark field examination of several, some infected with Redwater (517, 570, 608, 622)	—	Sections of 10 fixed in formalin and Regaud's fluid (3).
Dermacentor albipictus —	—	—	Sections of 2 from Regina (Canada) fixed in formalin and Regaud's fluid (3).
Dermacentor variabilis +	—	—	Sections of 5 from Urbana (Ill.) and 8 from College Park (Md.) fixed in formalin and Reguad's fluid (3).

Species.	Unfed Larvae.	Nymphae.	Adults.
Dermacentor venustus +	—	—	Sections of 9 from Hamilton (Montana) and 2 from Wawawai (Wash.) fixed in Regaud's fluid and some infected with Rocky Mountain spotted fever.
Haemophysalis leachi +	Smears of 25 and sections of 25 fixed in Regaud's fluid; also dark field examination (629)	—	—
Haemophysalis leporis palustris —	—	—	Sections of 19 from Raleigh (N.C.) fixed in formalin and Regaud's fluid (3).
Hyalomma aegyptium +	Sections of 25 fixed in alcohol	—	Sections of 5 fixed in Regaud's fluid; also dark field examination.
Margaropus annulatus +	—	—	Sections of 8 from Columbia (S.C), 10 from Washington (N.C.), and 2 from Baton Rouge (La.) fixed in Regaud's fluid (3).
Margaropus annulatus australis +	—	—	Sections of 6 from Jamaica fixed in formalin and in Regaud's fluid. Sections of 3 from Trinidad fixed in the same way (3).
Margaropus winthemi —	Sections of 25 fixed in alcohol	—	—
Ornithodorus megnini +	Sections of 25 fixed in Regaud's fluid; also dark field examination	—	—
Ornithodorus moubata —	Sections of 25 fixed in alcohol	—	—
Ornithodorus turicuta +	—	—	Sections of 1 from Arizona fixed in Regaud's fluid (3).
Rhipicephalus appendiculatus +	Smears of 25, sections of 25 fixed in Regaud's fluid; also dark field examination (621)	—	Smears of 1 and sections of 5 fixed in Regaud's fluid. Some infected (?) with anaplasmosis; others exhibited many bacteria in tracheal system (602, 612).
Rhipicephalus capensis —	Sections of 25 fixed in alcohol	—	—
Rhipicephalus evertsi +	Smears of 10, also dark field examination; sections of 25 fixed in alcohol and of 25 fixed in Zenker's fluid (633)	Smears of 2 and sections of 11 fixed in Regaud's fluid and infected with East Coast fever (644, 645, 646, 650)	Sections of 20 fixed in formalin (3).
Rhipicephalus pulchellus +	—	Sections of 2 fixed in Regaud's fluid and infected with East Coast fever (618)	Smears of 1 infected with East Coast fever (639).
Rhipicephalus sanguineus +	—	—	Sections of 14 from Hawaiian Islands, fixed in formalin and Regaud's fluid (3); also smears of 3 (555).
Rhipicephalus simus +	Smears of 25 (624), sections of 25 fixed in alcohol, and of 25 fixed in Regaud's fluid: also dark field examination	Smears of 2 and sections of 2 fixed in Regaud's fluid and infected with anaplasmosis (611)	Smears of 7 and sections of 6 fixed in Regaud's fluid and infected with anaplasmosis (561, 564).

Group Characteristics of the Micro-organisms in Unfed Larvae.

The micro-organisms were not detected in the six species mentioned with sufficient facility and constancy by direct illumination of living cells, with or without the addition of vital dyes, to delimit their properties.

Dark field examination (8) of living cells revealed only a few of the morphological forms in the case of each species of tick. In some cases the entire micro-organisms appeared brightly luminous, while in others the luminous material was confined to a thin marginal layer. In no instance was definite motility observed.

In air-dried smears, after fixation for about fifteen minutes in absolute alcohol, the micro-organisms stained light red or pink with Giemsa's stain, that is to say, much more faintly than most bacteria; neither did they possess the sharp contours often seen in the case of bacteria. They were very pleomorphic, varying from spherules to straight and curved rods and filaments. They were found to be larger than most Rickettsia, particularly in their diameter, which varied from about 0.3μ to 1.5μ. Filaments were frequently met with, sometimes as much as 5μ in length, or longer in the case of *Boophilus decoloratus*, in which branching was seen (fig. 2). Their ends were generally uniformly and evenly rounded; but occasionally they were sharply pointed. This slight affinity for stains and pleomorphism served as a basis of differentiation between the micro-organisms and certain refractile coccoid, rod-like, and dumb-bell-shaped concretions contained in the Malpighian tubules, which otherwise might have constituted a source of error.

After fixation in Regaud's fluid, the micro-organisms in sections were coloured dark red or purple by Giemsa's stain. They were uniformly Gram-negative in both smears and sections. They stained feebly by Unna's alkaline methylene blue and other basic aniline dyes. They were coloured faintly red by Pappenheim's pyronin-methyl green method. By all methods of staining, when they were embedded in cytoplasm, some were observed to exhibit distinct halos. It was also in sections that their intracellular habitat was most clearly distinguished. Their location was very characteristic, being restricted to the cytoplasm of the epithelial cells of the Malpighian tubules of larvae, nymphae, and adults. Occasionally they were seen in the cells lining the rectal sac, which is a continuation of the Malpighian tubules. They were found also in the eggs of adults of ten species (10). In no instance were they found to invade other tissues.

Differential Properties of the Micro-organisms in the Different Species of Unfed Larvae.

While resembling each other by: (1) their relatively large size; (2) the restriction of their intracellular habitat to the Malpighian tubules and to the egg cells of the females; and (3) certain of their microchemical reactions, it was found possible to clearly distinguish the micro-organisms present in each species of tick by characteristic differences in their morphology.

Thus, in *Amblyomma hebraeum* (figs. 1, 7, and 15) the micro-organisms were noticeably plumper and of larger diameter than in the case of any of the other ticks. They were, moreover, often grouped together in a unique fashion, suggestive of multiplication by some kind of budding.

In *Boophilus decoloratus* the micro-organisms differed from those in the other ticks by showing marked red and blue staining materials (fig. 8), by the fact that some of the morphologic types were coloured rather intensely by Giemsa's stain (fig. 8), and by their branching (fig. 2). They were never grouped together in the same fashion as the micro-organisms in *Amblyomma hebraeum*.

Haemophysalis leachi, *Rhipicephalus appendiculatus* and *Rhipicephalus evertsi*.

The micro-organisms in *Haemophysalis leachi* were easily distinguished from all the rest by a peculiar tendency of the filaments to be grouped side by side, by the presence of unusually marked chromophobe areas within the filaments, and by the occurrence of forms indicative of the possibility of the occurrence of multiplication by longitudinal splitting (figs. 3, 9, and 18).

In *Rhipicephalus appendiculatus* the micro-organisms were of particularly small size, and were grouped distinctively (figs. 4, 10, and 14). In *R. evertsi*, still another type of grouping was manifest (figs. 5, 11, and 16) which called to mind certain forms of *Rickettsia lectalaria*, as described by Hertig and Wolbach (7), and represented in their figs. 25 and 26; while *R. simus* differed sharply from the two above-mentioned species of the same genus by the fact that it contained micro-organisms were not grouped in any fashion other than in diplo-formation (figs. 6, 12, and 17). These micro-organisms in *R. simus* more closely resembled those in *Boophilus decoloratus* than those in any of the other five species to which reference has already been made; but they differed from the micro-organisms in *Boophilus decoloratus* by their failure to exhibit red and blue staining materials and in the absence of branching forms.

On the basis of these observations, it would be quite possible to identify unfed larvae of any of these species by an examination of the contained micro-organisms.

In addition, micro-organisms having somewhat similar general properties were observed in unfed larvae of *Argas persicus* and *Ornithodorus megnini*, but differing slightly as between their respective hosts. The failure to detect like micro-organisms in alcohol-fixed specimens of *Hyalomma aegyptium*, *Margaropus winthemi*, *Ornithodorus moubata*, and *Rhipicephalus capensis*, as noted in Table 1, may have been due to imperfect preservation, and, consequently, cannot be interpreted as conclusive evidence of the absence of micro-organisms of this general type in the unfed larvae of these species.

Evidence of the Hereditary Transmission of the Micro-organisms.

That the finding of the micro-organisms in the unfed larvae of seven species (9) in the eggs of ten species (10) and in smears of the adults of four others (11) is indicative of hereditary transmission on a fairly large scale is supported by tracing them through the entire life-cycle of two species.

Thus, in *Amblyomma hebraeum* and in *Rhipicephalus evertsi* they were observed not only in the Malpighian tubules of unfed larvae, carefully protected from bacterial contamination, but also in the Malpighian tubules of nymphae and in the Malpighian tubules and eggs of adult females.

A series of preparations of *Amblyomma hebraeum* made at close intervals for the study of *Rickettsia ruminantium* was particularly valuable. It was observed that throughout the periods of engorgement and moulting the micro-organisms exhibited with constancy the morphological and microchemical properties already alluded to, so that the likelihood of error in their identification was almost negligible.

TABLE 2.

Geographic Distribution of the Micro-organisms.

Similar Micro-organisms Present in	Collected at
Amblyomma americana	1. Columbia, South Corolina. 2. Washington, North Carolina. 3. Baton Rouge, Louisiana.
Dermacentor variabilis	1. Urbana, Illinois. 2. College Park, Maryland.
Dermacentor venustus	1. Hamilton, Montana. 2. Wawawai, Washington.
Margaropus annulatus	1. Columbia, South Carolina. 2. Washington, North Carolina. 3. Baton Rouge, Louisiana.
Margaropus annulatus australis	1. Jamaica, British West Indies. 2. Trinidad, Leeward Islands.
Rhipicephalus sanguineus	1. Honolulu, Hawaiian Islands. 2. Onderstepoort, Union of South Africa.

FREQUENCY OF OCCURRENCE OF THE MICRO-ORGANISMS.

Although technical difficulties prevented the detection of the micro-organisms in all the individual ticks examined, favourable preparations always revealed their presence in 100 per cent. of the ticks when careful and prolonged search was made, and it is believed that they were invariably present in the specimens of the following

species which were studied: *Amblyomma americana*, *Argas persicus*, *Boophilus decoloratus*, *Dermacentor variabilis*, *Dermacentor venustus*, *Haemophysalis leachi*, *Hyalomma aegyptium*, *Margaropus annulatus*, *Margaropus annulatus australis*, *Ornithodorus megnini*, *Ornithodorus turicata*, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, *Rhipicephalus sanguineus*, and *Rhipicephalus simus*.

The failure to observe them, as indicated by the negative sign (–) in Table 1, in *Amblyomma maculatum*, *Amblyomma tuberculatum*, *Argas mineatus*, *Dermacentor albipictus*, *Haemophysalis leporis palustris*, *Margaropus winthemi*, *Ornithodorus moubata*, and *Rhipicephalus capensis*, is open to two interpretations: either that they are absent or that further search in better preparations would bring them to light. In favour of the latter hypothesis may be mentioned the fact that the material available for their study was much less satisfactory than it was in the case of the species in which the micro-organisms were actually discovered, and, further, the consideration that these ticks all belong to genera, other species of which were found to contain the micro-organisms.

There is no indication that the season of the year has any influence upon the association of the micro-organisms with their arachnid hosts. The evidence at hand hardly permits a statement of the geographical distribution of the micro-organisms. In other words, it is possible that South African ticks are especially prone to harbour micro-organisms of this kind by reason of favourable climatic conditions and other unknown factors, and that the same species elsewhere might not possess them. Against this interpretation, however, was the observation of apparently identical micro-organisms in individuals of a species collected in widely separated localities. For convenience, the available information on this point is summarized in Table 2.

It will be noted that the majority of the ticks examined came from tropical or temperate regions, but this does not necessarily mean that temperature is in any sense a controlling factor in their distribution, since they also occur in specimens of *Dermacentor venustus* originating at Hamilton, Montana, where the winter temperature falls far below zero.

From the geographic point of view, Honolulu and Onderstepoort are very widely separated. Not only is their location almost antipodal, but Honolulu is hot and humid and situated at sea-level, while Onderstepoort is at an altitude of about 4,000 feet, somewhat cooler, and very dry in winter, when the specimens were collected. Yet examples of *Rhipicephalus sanguineus* collected at these two places harbour micro-organisms which are indistinguishable in their morphology and tinctorial properties, in their location in the tissues, and in the probability of their hereditary transmission.

If Godoy and Pinto (5) are correct in assuming that they found the same micro-organism in Brazilian ticks that R. Koch observed in *Rhipicephalus* in West Africa, and supposing, further, that this micro-organism belongs to the same general type as those under discussion, this discovery would likewise constitute a case of wide geographic distribution.

In being closely bound to their hosts hereditarily and geographically, these micro-organisms in ticks closely approximate to *Rickettsia lectularia* and certain other kinds of Rickettsia on the one hand and to the bacteroids of the *Blattidae* and many other insect symbionts on the other.

Discussion.

The exact systematic position of these hereditarily transmitted micro-organisms is not easily established, but they may profitably be compared with Rickettsia and with the symbionts of insects.

Their Gram-negative properties, intracellular habitat, bacterium-like shape, and their presence in arthropods would justify their provisional inclusion under the general heading of Rickettsia as was done in an earlier paper on Rickettsia (8). This general definition of Rickettisa, originally proposed by me, was used as a working basis by Hertig and Wolbach (7) in their studies on Rickettsia-like micro-organisms in insects. These investigators very properly remark that: "This definition is patently inadequate, in that it takes no account of certain points more or less characteristic of the group, namely, the very small diameter, usually less than 0.5μ, the presence at some stage of a minute, coccoid, or diplococcoid form staining densly with Giemsa, but poorly with ordinary bacterial dyes, the lack of clean-cut outline as compared with familiar bacteria, and the difficulty of cultivation in vitro. On the other hand, as to these characteristics, the Rickettsia so intergrade and overlap with each other and with bacteria that sharp lines cannot be drawn, and the pleomorphism is so great that a definition which would include all known phases of all known Rickettsia would be so broad and vague as to be valueless."

The micro-organisms reported in this paper also fulfil some of these additional criteria for the identification of Rickettsia, although they differ sharply from them in possessing a diameter usually in excess of 0.5μ, and often reaching as much as 1 to 1.5μ. At some stage they exhibit minute, coccoid, or diplococcoid forms, staining densely with Giesma, but poorly with ordinary bacterial dyes, and their outlines are frequently less clear cut than those of most bacteria. It is true that we may assume that they are difficult to cultivate, since they occur in ticks in company with spirochaetes and pathogenic protozoa, which have been repeatedly studied by cultural methods without bringing the micro-organisms in question to light, but this resistance to artificial cultivation is even more a characteristic of certain types of symbionts, particularly those in the *Pediculidae* (12), which will be mentioned subsequently, than it is of the Rickettsia.

It remains, however, to mention three other properties suggestive of some Rickettisa, namely, (1) the occasional observation in the multiplicative phases of two kinds of material, one staining red and the other blue by Giemsa's method (the micro-organism in *Boophilus decoloratus*); (2) the fact that none of the micro-organisms seem to cause injury to the cells containing them; and (3) that they all seem to be transmitted hereditarily.

The position assumed by Hertig and Wolbach in attempting to restrict the use of the term "Rickettsia" is progressive and well taken, and in view of the above-mentioned considerations, it seems possible to recognize, almost within the ranks of the Rickettsia, this group of micro-organisms, the members of which differ from Rickettsia in being of considerably larger size and in the fact that none of them is known to be pathogenic.

We are faced by a similar state of affairs when we compare the micro-organisms with the symbionts of insects. Points both of similarity and of dissimilarity come to light. The symbionts, themselves, like the Rickettsia, are hard to define, since their properties

vary not only in different groups, but also in different species of the same genus. Even the word "symbiont," itself, meaning literally "the act of living together," is sometimes employed in this sense, and, at others, with the added implication of the existence of benefit, either mutual or one-sided, between the micro-organisms and their arthropod hosts. Seldom is its use backed by any physiologic experimentation to determine the exact degree of association. Often the condition is more properly one of commensalism (or of eating from the same table), particularly when the micro-organisms in question are found in the alimentary tract and the element of advantage to one or both of them is greater. When there is injury, or the slightest harmful influence is exerted upon either the micro-organisms or their hosts, the condition verges on parasitism; but the line of division is not easily established. Moreover, the *Arthropoda* comprise so many forms in such diverse habitats that the problem becomes very complicated.

Excluding from the discussion the "biophytes" of aphids (13), certain types of bacteria said to be associated with luminescence, the bacteria of the mid-intestinal caeca of hemiptera (14), the bacteroids of the *Blattidae*, and many questionable forms, and confining ourselves to the "symbionts" of the *Pediculidae* and of the tsetse flies, which, latter, were first reported by R. Koch, some definite points of similarity with the micro-organisms in ticks come to light. Mention may, for example, be made of the relatively large size and of the staining reactions of the symbionts. Perhaps the branching in the case of the micro-organism in *Boophilus decoloratus* is significant, but these micro-organisms do not form definite structures annexed to the alimentary tract at all comparable to the so-called "mycetomes" of the *insecta*. Another point of distinction from symbionts, as usually described, is the restriction of the micro-organisms in ticks to the Malpighian tubules in addition to the egg-cells.

The observations herein reported indicate, therefore, that certain bacterium-like, Gram-negative intra-cellular micro-organisms, which are transmitted hereditarily and are apparently unassociated with disease, are of common occurrence in the *Argasidae* and the *Ixodidae*. If it is found that the "symbionts" mentioned in mites by Reichenow (16) belong in the same category, then we must also include the family *Thrombidiidae*.

Ectoparasites belonging to these three families, that is to say, to the order Acarina, play an important rôle in the transmission of disease to man. It will suffice to mention Rocky Mountain spotted fever, Tsutsugamuchi disease, relapsing fever, and perhaps pseudo typhus fever (17). They are, moreover, most harmful to domestic animals, and constitute the vectors of Theileriosis. In consequence of their great pleomorphism there is a chance that some of the forms which they assume may be mistaken for micro-organisms which are pathogenic. Attention has already been called to their similarity to Rickettsia and to certain phases of the intracellular life-cycle of spirochaetes. The latter is noteworthy in the case of the micro-organisms found in adult specimens of *Rhipicephalus simus*, which are often of somewhat spiral shape with pointed ends. Unfortunately knowledge of the wide distribution of these micro-organisms does not afford a clue to the etiology of any specific disease, but it may supply a useful background in the study of diseases the sources of which are well-known, but of which the nature is doubtful, and of other diseases which it is

possible that future investigation will show to be associated with ectoparasites of this general type.

And, furthermore, if Roubaud (15) is justified, as he appears to be, in claiming that the symbionts of tsetse flies play an active part in the process of assimilation in these insects, then the possibility is opened that these observations may also reveal a hitherto almost unsuspected factor in the normal physiology of the *Acarina*.

Evidently recognition of this infestation, if we may logically use this term, with bacterium-like micro-organisms might readily have been suspected in advance for the reason that so many lice and flies, which are likewise adapted to a diet exclusively of fresh blood, exhibit similar, though not identical, flora. The fact that these micro-organisms have attracted so little attention probably finds explanation by a consideration of the natural lines of progress in the investigation of arachnid-borne diseases. The ease with which some of the most destructive may be controlled, once the identity of the arachnid concerned has been ascertained, at an early date removed the urgency of the demand for information regarding micro-organisms, both pathogenic and benign, which might occur within the vectors. Moreover, immediately that harmless micro-organisms were excluded from casual relationship to disease, their presence would become a matter merely of scientific interest.

However this may be, detailed studies of the various groups of insects and arachnids, particularly those which are blood-feeding, or insectivorous, like the spiders (18), may be expected ultimately to supply a perspective view of the chief types of symbiosis, commensalism, and parasitism exhibited, and lay a basis upon which a rational classification of the micro-organisms occurring in the arthropods may be based, including even those the nature of which remains at present very obscure.

Results.

1. Pleomorphic, bacterium-like, Gram-negative, intracellular micro-organisms, which stained much less intensely with ordinary dyes than most bacteria, were found in fifteen ticks comprising examples of both the *Argasidae* and the *Ixodidae* and belonging to the following species:—*Amblyomma americana*, *Argas persicus*, *Boophilus decoloratus*, *Dermacentor variabilis*, *Dermacentor venustus*, *Haemophysalis leachi*, *Hyalomma aegyptium*, *Margaropus annulatus*, *Margaropus annulatus australis*, *Ornithodorus megnini*, *Ornithodorus turicata*, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, *Rhipicephalus sanguineus*, and *Rhipicephalus simus*.

2. No morphological or tinctorial evidence could be adduced of injury to the tissues of their arachnid hosts other than physical distension of the cells to accommodate them in large numbers. Since microscopic examination of favourable preparations of the species which contained them revealed an incidence of 100 per cent. not only in ticks collected in South Africa, but also in others from Jamaica, Trinidad, Honolulu, and several parts of the United States, it is probable that the micro-organisms were in no sense harmful to their hosts.

3. In consideration of the detection of the micro-organisms in the egg of ten species, in the unfed larvae of seven species, and at

very close stages throughout the life-cycle of two others, the conclusion was reached that they were transmitted hereditarily.

4. The micro-organisms in several respects resembled Rickettsia, but differed from them in being of larger size. They also resembled the symbionts of certain lice and blood-feeding flies, but never gave rise to definite organ-like structures comparable with the mycetomes, and were restricted in their distribution to the Malpighian tubules and the egg-cells, as contrasted with the digestive tract to which the symbionts of these insects are confined.

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(8) Using the precautions previously described (2).

(9) *Amblyomma hebraeum*, *Argas persicus*, *Boophilus decoloratus*, *Haemophysalis leachi*, *Ornithodorus megnini*, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, and *Rhipicephalus simus*.

(10) *Amblyomma americana*, *Amblyomma hebraeum*, *Boophilus decoloratus*, *Dermacentor variabilis*, *Dermacentor venustus*, *Boophilus annulatus*, *Boophilus annulatus australis*, *Ornithodorus turicata*, *Rhipicephalus evertsi*, and *Rhipicephalus sanguineus*.

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(18) A collection of South African spiders was made, and it is hoped subsequently to report the micro-organisms contained in them. A Rickettsia-like micro-organism in *Salticus scenicus* was described in an earlier paper (3).

PLATE I.

Photomicrographs, made with apochromatic objective 3 mm., 1.40 aperture, and compensating ocular 8, giving a magnification of 1,400 diameters, of air-dried smears of unfed larva, fixed in absolute alcohol and coloured by Giemsa's stain.

Fig. 1.—*Amblyomma hebraeum.*
Fig. 2.—*Boophilus decoloratus.*
Fig. 3.—*Haemophysalis leachi.*
Fig. 4.—*Rhipicephalus appendiculatus.*
Fig. 5.—*Rhipicephalus evertsi.*
Fig. 6.—*Rhipicephalus simus.*

PLATE II.

Drawings of selected micro-organisms from smears of unfed larval ticks to show extent of pleomorphism. All the drawings were made from air-dried smears, fixed in alcohol, and coloured by Griemsa's stain, with apochromatic objective 1.5 mm., compensating ocular 8, and camera lucida.

Fig. 7.—*Amblyomma hebraeum.*
Fig. 8.—*Boophilus decoloratus.*
Fig. 9.—*Haemophysalis leachi.*
Fig. 10.—*Rhipicephalus appendiculatus.*
Fig. 11.—*Rhipicephalus evertsi.*
Fig. 12.—*Rhipicephalus simus.*

PLATE III.

All the figures are drawings of portions of the Malpighian tubules of unfed larval ticks, made with apochromatic objective 1.5 mm., compensating ocular 8, and camera lucida.

Fig. 13.—*Boophilus decoloratus.*
Fig. 14.—*Rhipicephalus appendiculatus.*
Fig. 15.—*Amblyomma hebraeum.*
Fig. 16.—*Rhipicephalus evertsi.*
Fig. 17.—*Rhipicephalus simus.*
Fig. 18.—*Haemophysalis leachi.*

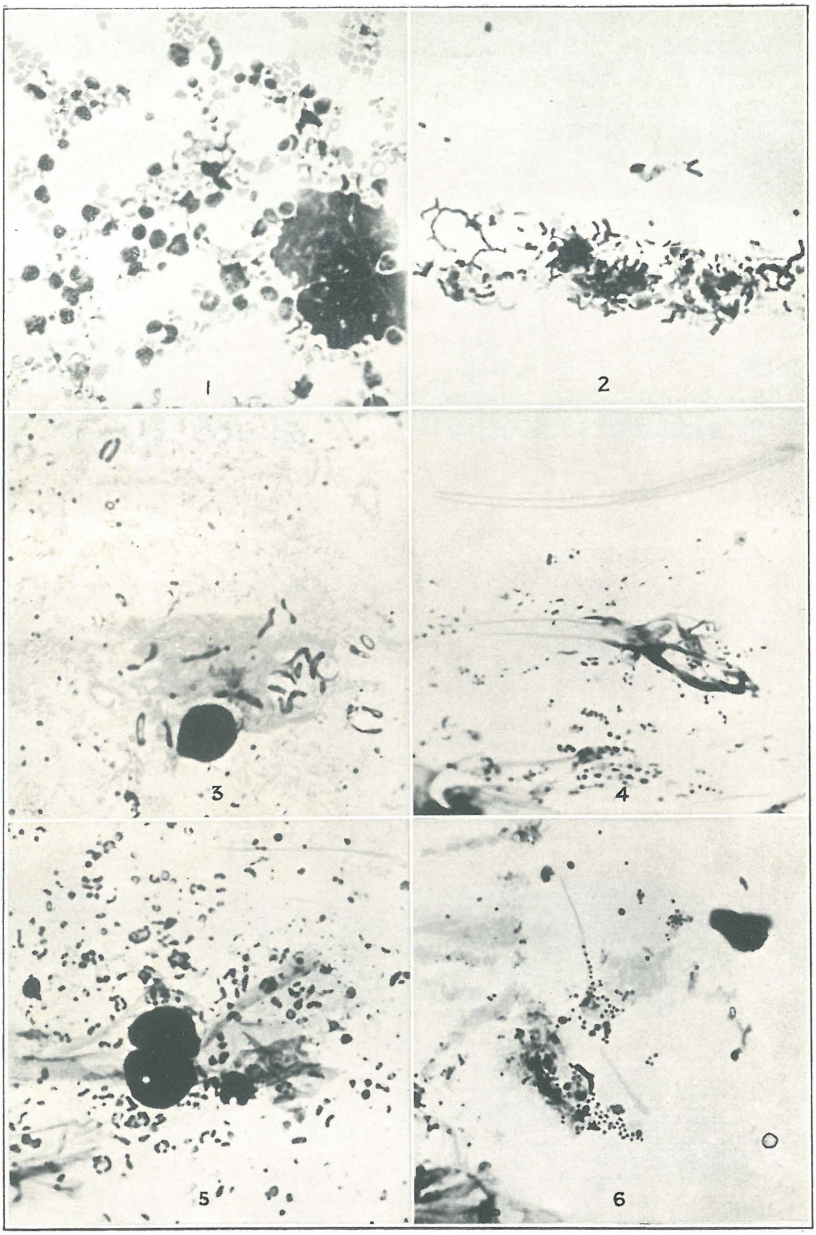

Plate I.] [*E. V. Cowdry.*

MICRO-ORGANISMS IN TICKS.

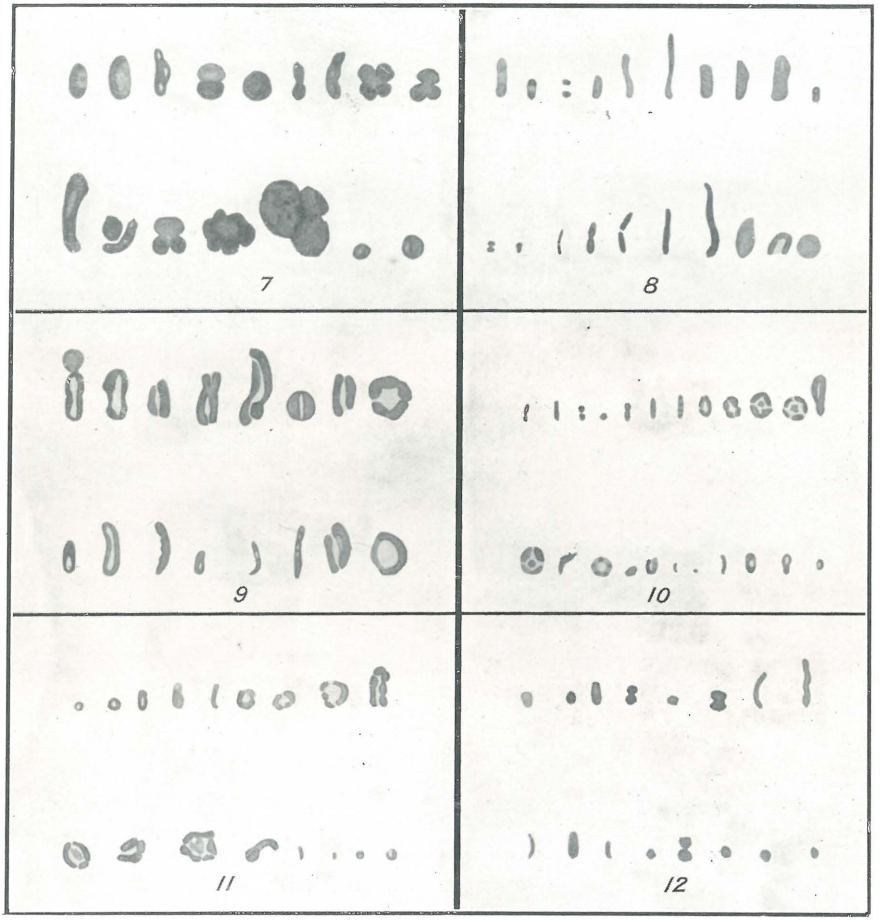

Plate II.

Micro-organisms Transmitted in Ticks.] [*E. V. Cowdry.*

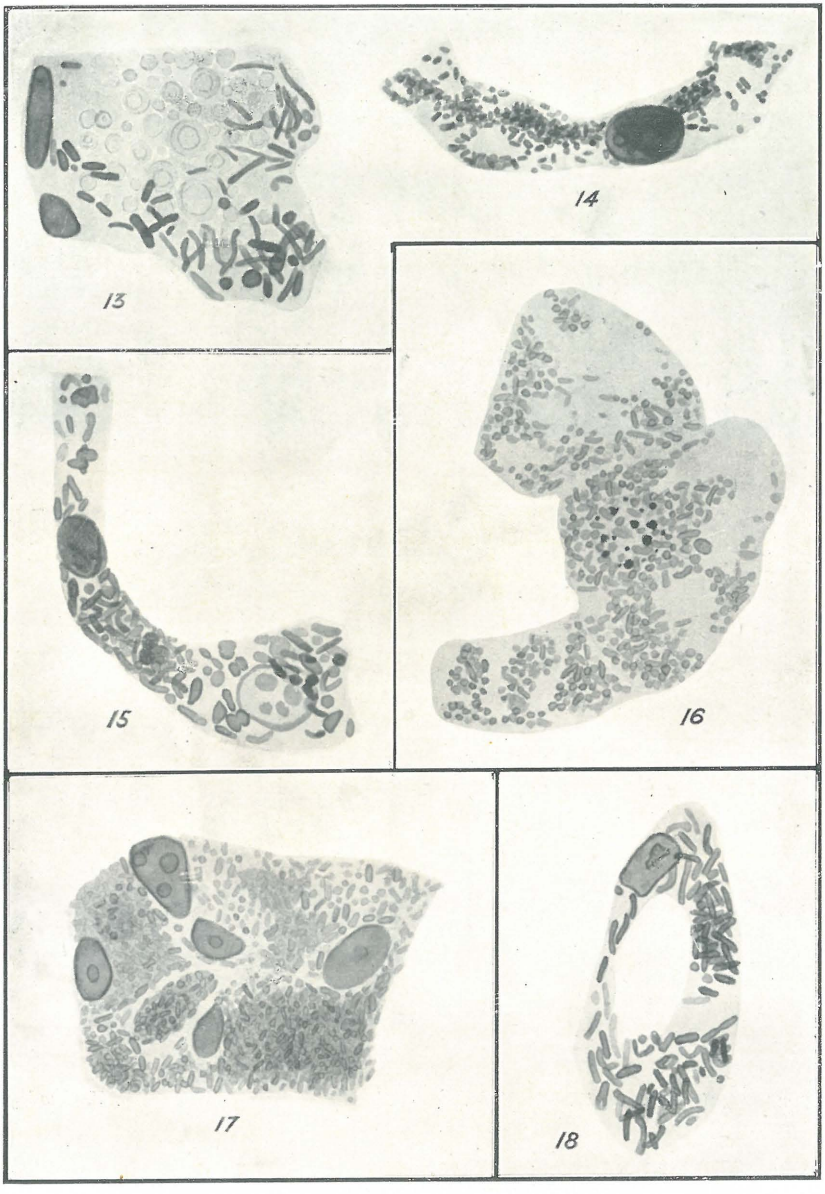

Plate III.

Micro-organisms Transmitted in Ticks.] [*E. V. Cowdry.*