STUDIES ON RICKETTSIA-LIKE MICRO-ORGANISMS IN INSECTS *

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SYNOPSIS

INTRODUCTION

A survey of the rapidly growing list of micro-organisms described under the name "Rickettsia," including not only pathogenic forms transmitted by blood-sucking insects, but harmless parasites and symbionts of non-blood-sucking insects as well, indicates that this type of organism is one more or less generally distributed throughout the whole group of arthropods. Further, micro-organisms described as Rickettsia,

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while possessing certain characteristics in common, may exhibit very great differences in pathogenicity for vertebrates, in range of morphology, staining reactions, relation to host, and cultivability. As a result, this group without doubt will more and more include wholly unrelated forms. The present study was undertaken with two objects in view: first, to determine the incidence of Rickettsia-like organisms in insects; and second, to arrive at a basis for defining the limits of *Rickettsia* as a generic term.

Historical Review of the Known Rickettsiae

Since 1916, when Rocha-Lima described *Rickettsia prowazeki* as the cause of typhus, the Rickettsia-like organisms reported in the literature have increased to over forty. A summary of the then known Rickettsiae, some sixteen in number, was given by Wolbach, Todd and Palfrey in their work on typhus (1922, pp. 116–131). This list has been more than doubled in the last two years. Rosenberger (1922) has published a detailed report of *Rickettsia rocha-limae*, establishing it as distinct from *Rickettsia prowazeki*. Reichenow (1922) has reported a rickettsia from a *Dermanyssus* of doves, these organisms being found in but a single individual out of four examined. Guimarais (1922) found organisms of this type in the public louse, *Phthirius pubis*. Sikora (1922) has considered certain organisms found in the intestines of several Mallophaga to be rickettsiae. Arkwright (1923) has reported a rickettsia from the intestine of the goat-louse *Trichodectes climax*. Cowdry (1923) in his report of an extended survey of the arthropods has added seventeen rickettsiae to the total. In addition, we have found a number of hitherto undescribed rickettsiae in the course of the present study, which are discussed in another section.

Aside from the question of the probable identity of *Rickettsia pediculi*, *Rickettsia quintana*, and *Rickettsia wolhynica*, there are perhaps two subtractions to be made from the list of rickettsiae given by Wolbach, Todd, and Palfrey, namely those of the Japanese harvest-mite and of the mouse-flea. Sikora (1920) reported the former on the basis of an oral communication to her, which has not received confirmation in any published re-

port known to us. In the case of the mouse-flea, Sikora (1918) suggested that certain granules in the Malpighian tubes might be rickettsiae. These bodies were not mentioned by Sikora in a later paper (1920) in her enumeration of the known rickettsiae, and no record of their further investigation has been found.

The data concerning the various rickettsiae are so scattered throughout the literature and so incomplete, with the exception of the pathogenic forms, that it has been thought advisable to present in tabular form a summary of the information available. In addition, certain of the rickettsiae are considered in some detail in the discussion. The information summarized in the appended table includes the arthropod hosts and certain characteristics of the organisms themselves, as well as reference to the authors who have described or discussed them. This tabular summary is necessarily inadequate in view of the mass of information on the rickettsiae of typhus, trench fever and Rocky Mountain spotted fever, but will permit a ready comparison of these with other rickettsia-like organisms, including those described by us.

Materials and Technique

The insects examined were for the most part collected in Boston or in nearby towns. After being chloroformed or decapitated, they were dissected in sterile 0.8 per cent sodium chloride solution or in Ringer's solution. Smears were made by teasing the organs, separately for the most part, in drops of salt solution on the slide. This was done under the binocular dissecting microscope with sharp needles or knives made from needles. The object was to obtain as many isolated cells as possible, rather than to crush the tissues indiscriminately. The drop was then spread out so that it dried quickly; great care was taken not to remoisten or disturb any part of a film thus made after it had dried. Smears were fixed fifteen minutes in absolute alcohol (a cotton-plugged tube of anhydrous copper sulphate being kept in the fixing jar).

Specimens for sectioning were dissected free of the chitinous exoskeleton except in the case of very minute insects. Zenker's

fluid without the addition of acetic acid was used chiefly, though Carnoy's acetic acid-alcohol-chloroform fixative and sublimate-alcohol were also employed. Paraffin sections were cut as thin as possible, usually about 3μ in thickness. The technique for staining smears and sections with Giemsa, which was used as a routine, was that described by Wolbach (1919) and Wolbach, Todd, and Palfrey (1922). Goodpasture's (1919) stain was occasionally employed for sections. It has proved simple and reliable for the demonstration of rickettsiae in insects and is a valuable adjunct to the more difficult Giemsa technique. As the method is comparatively new, we have copied the following from Goodpasture's and Burnett's paper.

"1. Tissues fixed thoroughly in Zenker's fluid.
2. Steam thin paraffin sections for five minutes in a few drops of the following poured over the section fixed to the slide:

Basic fuchsin	0.5 gram
Carbolic acid (crystallized)	1.0 c.c.
Anilin oil	0.5 c.c.
Alcohol, 30 per cent	100.0 c.c.

Dissolve fuchsin in the dilute alcohol and add the other reagents.
3. Wash off excess of stain rapidly in tap water.
4. Differentiate and decolorize with 40 per cent formalin, poured over section a few drops at a time until no more color is discharged.
5. Rinse in water and counterstain for one minute in a saturated aqueous solution of picric acid.
6. Dehydrate quickly in 95 per cent and absolute alcohol; xylol; balsam."

Cowdry (1923) has employed sections as a routine technique in his search for rickettsia-like organisms, supplemented to a certain extent by Giemsa smears. For mere survey purposes we have found Giemsa smears much superior to sections. The saving of time and labor is obvious. Further, we believe that small numbers of minute organisms are more likely to be detected in smears, if properly made, than in sections. However, the chief disadvantage of the section technique for rickettsia is that some of these organisms by our present methods stain with difficulty in sections. It has been the experience of Buchner (1923) and of ourselves in connection with the bedbug rickettsia that the minute cocci and rods stain very poorly in

sections. Even where there are large masses of organisms demonstrable in control smears, the corresponding groups in sections frequently appear as poorly stained, evenly granular areas which are difficult or impossible to resolve into individual organisms. This is strikingly true of the mycetome sections where rarely are any forms but the filaments to be made out clearly. Our sections of mosquitoes, drug-store beetles and book-lice have proved similarly disappointing. It is perhaps this difficulty which accounts for Cowdry's negative findings in certain of his bedbug strains, and for the failure of earlier investigators of the drug-store beetle symbionts to find the rickettsiae present in all individuals of our two American strains of this insect. It goes without saying that good sections as well as film preparations are indispensable in any study beyond the survey stage. Such further study of the rickettsiae is urgently needed. The difficulties of staining these organisms are probably due to imperfect fixation of the insect tissues, due either to the attempt to secure fixation without removing the integument or to the action of the liquids in which the insect is immersed during dissection.

Criteria for distinguishing rickettsiae from cell granules and artefacts. It is well known that in Giemsa smears of insect tissues there commonly occur granules, precipitates of the dye and other artefacts which would tend to mask the presence of any minute organisms accompanying them. The following considerations aid in distinguishing rickettsia-like organisms from such other bodies:

1. The bacterium-like morphology. Rickettsiae in smears differ from most bacteria chiefly in being typically smaller, less deeply stained, and less sharply outlined. Even when the rickettsiae are most nearly cocciform, and are as a result most easily confused with artefacts, they are nearly always accompanied by diplococcoid forms and usually also by short rods.

2. Numbers and grouping — the "rickettsia picture." Since individual artefacts often do simulate coccoid and even diplococcoid and bacillary rickettsiae, isolated or widely scattered forms cannot be judged with certainty. Rickettsiae usually

occur in groups or masses within or upon cells. In well made smears, i.e., where many cells are isolated but nearly intact, such masses furnish a characteristic "rickettsia picture," particularly where a cell has been ruptured and the rickettsiae have been spread out around it. In searching for rickettsiae we have considered only those occurring in numbers and associated preferably with tissues or isolated cells. The outline of the individual rickettsia is not as sharp as that of most bacteria. A rickettsia group rarely presents the uniformity of a field of ordinary bacteria, due to minor departures from a regular spherical or oval form, such as slight variations of diameter and length, and the presence of short rods mingled with coccoid forms. The rickettsiae characterized by filaments, long rods, and curved forms, are at once obvious.

3. Giemsa artefacts. Precipitates of the stain are usually identified without difficulty. Areas with appreciable amounts of precipitate are of course to be discarded at once. Some Giemsa smears appear covered in whole or in part with fine, coccus-like dots, the individual dots simulating rickettsiae. This condition is quickly recognized after a little experience, but in any event, the even scattering of such dots and the fading out of the dots at the edge of such areas show them to be artefacts. Where artefacts are particularly abundant and deceptive, as sometimes occurs when portions of a smear already dried are rubbed up again with the liquid, duplicate smears or smears of other specimens serve as controls.

4. Insect granules. Most insect cells in the living condition are seen to be filled with a great number of granules of various sorts (Hertig, 1923). The staining of some of these resembles that of rickettsiae and other organisms. Such stained granules are usually larger than rickettsiae, are very uniformly spherical and occur in small numbers evenly scattered. Other granules, which are found especially in the Malpighian tubes and appear blue-black or green-black and which are sometimes refractive, are totally unlike rickettsiae. Granules, precipitate and other artefacts are particularly common in smears from ova containing much yolk, though here the chief difficulty so far as rickettsiae are concerned is in their becoming surrounded with

material which interfered with their staining or obscures them altogether. Many insect granules visible in fresh tissues do not stain at all but appear as vacuoles. Particles or strands of chromatin from injured cell nuclei occur frequently but are rarely confusing. They may be identified by their characteristic redness on comparing with neighboring uninjured nuclei.

It is of course desirable to support the finding of rickettsiae in any insect by examination of a number of specimens and to demonstrate them in sections if possible.

Observations on Various Rickettsiae

Rickettsia melophagi in the Sheep-Ked, *Melophagus ovinus*. This rickettsia was discovered and named by Nöller (1917 *a*) in the course of his work on the flagellates of the sheep-ked (sheep-"louse" or "tick"), *Melophagus ovinus*, and has since been studied by Jungmann (1918), Sikora (1918, 1920), Hindle (1921) and Arkwright and Bacot (1921). These investigators have established the occurrence of the organism in keds in practically one hundred per cent, except in the case of Hindle who found them in a "large proportion" of sheep-keds from the Continent and England. We have found every one of our stock of keds, taken from near Boston, infected.

The organisms occur characteristically in large numbers upon and in the cuticular layer covering the epithelium of the mid-intestine. In section they appear in closely packed rows perpendicular to the epithelial surface (Fig. 1). This arrangement, according to Sikora (1918) is due to the fact that they occupy corresponding vertical cavities in the cuticular border. Sikora found the rickettsia layer occupying the two middle quarters of the mid-intestine. According to Jungmann it extends as a continuous layer clear to the anus. That this layer is not always an unbroken one is shown in our sections, where in some preparations only tiny isolated patches are found. Clumps and individuals may also be found in the intestinal lumen and in feces. Jungmann found the mouth-parts and fore-intestine to be always free of rickettsiae. The organisms vary greatly in number in different specimens. Sikora found the infection to be rather light in young individuals and heavy in older ones,

there being a few rickettsiae in the intestine of newly emerged, unfed keds. Jungmann, on the other hand, found in those freshly emerged from "eggs" (pupae) which had been kept in the incubator, an infection as great as that of the older specimens. Both of these investigators concluded that the parasites were transmitted hereditarily on the basis of finding rickettsiae in the pupae. Jungmann found none in the young eggs in the ovary, but showed them to be constantly present in the newly formed intestine of the embryo. In the larva the intestine was filled with rickettsiae. Hindle found a few rickettsia-like bodies in developing eggs but not in sufficient numbers to be certain of their identity. We have found considerable numbers of rickettsiae in developing eggs. These facts, together with the apparently universal infection, seem to establish the hereditary transmission of the organism.

Nöller at first (1917 *a*) considered that the rickettsiae occasionally penetrated cells, but later (1917 *b*) stated that this was an error due to the great masses of organisms found in smears which simulated an intracellular position. In his sections he found only extracellular forms. Jungmann, however, in six out of thirty individual sections, stated that he found unquestionable ("einwandfrei") intracellular rickettsiae in the anterior portion of the mid-intestine. Such cells appeared more deeply stained, even with low magnification. Jungmann found occasionally infected cells in other parts of the mid-intestine. It may be noted that in the anterior portion of the mid-intestine of the sheep-ked the epithelium of a certain region is greatly developed, forming a definite, whitish collar visible under the dissecting microscope. These cells Sikora (1918) found to be filled with parasites resembling those found in similar cells of the tsetse fly, *Glossina*. It is difficult to conceive, however, that these rather large, yeastlike bodies could have been confused by Jungmann with rickettsiae. Nevertheless, Arkwright and Bacot (1921) have also considered these yeasts found in their smears along with the coccoid rickettsiae, to be stages of the latter. They and Jungmann were apparently unaware of the existence of similar yeastlike "symbionts" in other pupiparous Diptera (Roubaud, 1919). No intracellular forms, except the

yeastlike organisms, have been found by us in sections of seven keds, although in tangentially cut sections the masses of rickettsiae in the cuticular border may simulate intracellular masses.

Rickettsia melophagi is not pathogenic to sheep, rabbits, guinea-pigs or mice when inoculated into the body (Jungmann), nor are any ill effects noted when the sheep-keds are allowed to feed naturally on various mammals, including man (Sikora, 1918). Indeed, Jungmann believed that the rickettsiae do not get into the blood of the host at all, but Nöller and Kuchling (1923) have just reported the cultivation of a rickettsia from the sheep's blood. We are unable to infect guinea-pigs by intraperitoneal injections of suspensions of the intestinal tracts of sheep-keds. We also experienced no untoward results from allowing the keds to feed upon our persons.

The organisms found in the intestine correspond closely in morphology with the coccoid forms of *Rickettsia pediculi*. They are quite uniform in size and occur characteristically in pairs. The individuals measure 0.4μ to 0.6μ in diameter, in the form of spheres, or about 0.3μ to 0.4μ $\times$ 0.5μ to 0.6μ in the form of rods. The staining with Giemsa is a deep purple. The organisms are Gram-negative, stain fairly well with carbol-fuchsin and gentian violet, but very faintly with safranin and methylene blue. In the egg the organisms stain bluish with Giemsa and rather less intensely than those in the intestine. There is also a tendency to form rods as well as paired and single cocci. In some cases the rods are somewhat swollen, curved and more faintly stained (Fig. 2).

Following Nöller (1917 *a*) we have succeeded in cultivating *Rickettsia melophagi*. We substituted beef bouillon for horse meat bouillon, and rabbits' blood for hare blood in the following medium recommended by him for use in test tubes:

Agar	25.0
Glucose	20.0
Weakly alkaline horse meat bouillon	1000.0

The agar is prepared in the usual way and tubed. Sterile, defibrinated horse blood in equal volume to double volume is added to the melted medium immediately after removal from a steam bath or boiling water

and therefore approximately at 100° C. The completed medium is slanted and the tubes sealed with rubber caps to prevent evaporation.

The following technique was used to obtain material for the cultures: The sheep-ked was clasped across the anterior end of the abdomen with small sterile dissecting forceps, and transected in front of the forceps with a delicate thermo cautery. (This cautery was improvised from flattened platinum wire and operated by a single dry cell.) If the operation is performed at sufficient speed, using a red hot cautery with a cutting edge, an aseptic approach is achieved without heating the abdominal contents to a temperature destructive to the micro-organisms in the intestines. The intestinal tract was removed with delicate sterile forceps and passed through ten or more changes of sterile salt solution as an additional precaution against contaminations. This washing was done in drops of salt solution distributed in a large-sized sterile Petri dish. Before inoculating the medium a fresh preparation of the intestinal contents was examined in order to prove, by the presence of motile flagellates, that the material had not been overheated. Growth of the flagellates was inhibited by incubating the tubes at 30° to 32° C. Colonies in the form of minute colorless elevations became visible in three to five days. In heavily seeded tubes, a fine ground glass appearance was produced. We did not obtain the continuous growth of the colonies during long periods as described by Nöller, who reported colonies as large as 0.4 to 0.6 mm. after 35–40 days growth. In our cultures the colonies remained at a barely visible size. We did not maintain our cultures beyond second transfers, by which time we decided that the behavior of the organism corresponded to that of a bacterium and that the morphological range was not great. The organism in cultures is slightly longer, more definitely rodlike than in the sheep-ked. Paired forms are common. It decolorizes by Gram's method, and it stains readily with ordinary stains as compared with *Rickettsia prowazeki*, and *Dermacentroxenus rickettsi*. In typical growth the individuals measure quite uniformly 0.3μ to 0.35μ in width, in length from 0.4μ to 1.0μ, the average length being about 0.7μ (Fig. 3). Elongated forms, straight or curved, appeared in a few cultures with poor growth but did not appear in actively growing cultures. After five to six weeks, the micro-organisms disintegrate rapidly, passing through stages similar to many bacteria when conditions become unfavorable for growth (B. influenzae, gonococcus, meningococcus), i.e., becoming swollen to faintly staining globular forms containing deeply staining granules and reduction to amorphous granular material. In every respect *Rickettsia melophagi* in cultures behaves like a bacterium.

Woodcock (1923) in looking over his smears made in connection with a study of the flagellate *Crithidia melophagia*, which is found in the intestine of a large proportion of sheep-keds, came to the conclusion that the rickettsiae are derived from the degenerating bodies of the flagellates. He believed certain granules in the cytoplasm of the flagellates to be, or to

give rise to, the rickettsiae. We have observed such granules, but they do not occur in all preparations showing flagellates, while the rickettsiae are present in large numbers without exception. While we are not able to pass on the nature of such bodies within the flagellates, a number of facts establish quite clearly that the large masses of rickettsiae are living organisms and are independent in origin of the flagellates, whatever the crithidia-granules may be. A small proportion of keds are entirely free of flagellates, but these never lack the masses of rickettsiae. Further, the rickettsiae are present constantly in the intestine of the embryo, larva, and pupa and in freshly emerged, unfed keds, and they persist throughout life — an unbroken morphological sequence finding a parallel in many other cases of insect "symbiosis." These facts, together with their characteristic coccoid or diplo-bacillary morphology, seem to obviate any possibility of identifying the rickettsiae with degenerating flagellates. Woodcock disposed of these facts, however, by claiming the embryonic, larval and pupal rickettsiae to be the result of "lysis" of the insect's cells, while in later life (with no apparent hiatus in their morphological continuity) they are derived from flagellates. Their relatively easy artificial cultivation by Nöller (1917 *a*), Jungmann (1918) and by ourselves, establishes beyond any further question their organism nature. Woodcock attempts to set this evidence aside on the ground that Nöller's rickettsia colonies occurred only after two or three weeks in cultures also containing *Crithidiae*, and are hence degenerating flagellates. One of Nöller's flagellate cultures which showed no rickettsiae, i.e., a pure culture, Woodcock regarded as "a most interesting physiological case," comparable to a mutation. In Jungmann's cultivation studies, which Woodcock has ignored, he obtained pure cultures of rickettsiae, the colonies appearing after eight to ten days. His cultures were maintained for several months by successive transfers. All our own cultures, with appreciable growth after eight or nine days of rickettsiae in pure culture, were entirely free of flagellates at all times. Thus both organisms, *Rickettsia melophagi* and *Crithidia melophagia*, have been grown separately and in pure culture.

It may be noted in passing that Woodcock has further, with the same sort of argument, but without the redeeming feature of any first-hand observation whatsoever, attempted also to designate *all other* rickettsiae as cell granules, debris and the like. The soundness of the "conclusions" to which this writer is led by his prejudice against the organism nature of rickettsiae in favor of his own theory of "hematophagy," is illustrated in the case of the bedbug rickettsia, "As indicating end products of different modes of hemataboly." The pleomorphism of this organism, including the filamentous forms, "does not trouble" him "at all." Even were there not abundant other evidence, its organism nature is at once proclaimed by the active motility of the filaments.

Rickettsia in the Mosquito, Culex pipiens. In a postscript to a paper by Sikora (1920) Nöller reported the finding of a rickettsia in the "sucking stomach" or oesophageal diverticula of this mosquito. This rickettsia was associated with the yeasts described by Schaudinn (1904) and formed a layer lining the wall of this organ, with masses here and there. Nöller informs us in a personal communication that these were seen incidental to other studies in only a few specimens, and that further data are not available. They resembled the sheep-ked rickettsia but were perhaps somewhat larger. The yeasts mentioned were found by Schaudinn to be constant inhabitants of the oesophageal diverticula of *Culex pipiens* and certain other mosquitoes. He considered these organisms to be the producers of the gas bubbles found in these diverticula, and on the basis of certain experiments, believed them to be the cause of the itching wheals resulting from mosquito bites. He believed this fungus, which was found in all his specimens, to be a stage in the life-cycle of one of the higher, mycelium-forming fungi, possibly one related to the *Entomophthoraceae.* These organisms he found in the stomach, where they multiply, at first yeastlike in form, later forming a mycelium, and then producing a tiny fruiting form (Fruchtform) which Schaudinn also found in the eggs of *Culex.* The yeast form he also observed in the intestine of the larva and in pupae. The organism was considered to be a commensal

transmitted hereditarily. The description of the yeasts is limited to the statement that they are very small, oval or spherical bodies; the tiny fruiting forms were not described at all.

During the summer of 1922 we examined thirteen females of *Culex pipiens* taken near Boston. The oesophageal diverticula of six of these were examined in smears. Nöller's organism was not found, with one possible exception, in which groups of cocci measuring quite uniformly 0.6μ to 0.7μ in diameter, occurred in numbers together with other larger cocci (Fig. 4). While in several cases large numbers of different bacteria have been found in the oesophageal diverticula as well as in smears of the intestine, no organism has been found which corresponds to the meagre description of Schaudinn's yeast. In all these thirteen specimens, however, as well as in nine females and three males from Boston and Minneapolis, dissected in 1923, there have been found in smears of the ovaries (Fig. 5) or testes (Figs. 6 and 6 *a*) a tiny rodlike or coccoid, Gram-negative organism. We have no basis on which to judge whether this is or is not Schaudinn's "Fruchtform." Careful search of separate smears of other organs, including the oesophageal diverticula, mid- and hind-intestine, Malpighian tubes, fat-body, heart and pericardial cells, salivary glands and accessory reproductive organs, as well as smears including all the remaining portions of the body, have so far failed to reveal the constant presence of any organism comparable to the form always present in the gonads.

In smears of the adult ovaries the organisms are characterized by lack of uniformity of shape, size and staining. There is a large proportion of small curved and bent rods, usually constricted at the center. They measure 0.2μ to 0.3μ in width by 0.4μ to 0.6μ in length. Slender, wavy rods up to 3.0μ in length occur in varying proportions. There are always to be found at least a few swollen forms. In one case such swollen granules, 0.6μ to 0.8μ in diameter, were the dominant form in one ovary while in the other ovary of the same female the organisms were chiefly rods. In its variety of forms, this rickettsia exhibits many striking parallelisms with the organisms of the bedbug

mycetome (Figs. 7 and 8). With dark field illumination the organisms appear as luminous points or rods, and short, twisted filaments. They lack the non-refractive interior noted in the rods and filaments of the bedbug rickettsia.

Sections of the viscera have failed to reveal these organisms, and even in the ovaries the granular masses could not be resolved into individuals with certainty. The same difficulty, it may be noted, is met with in sections of the bedbug mycetome and ovary.

An attempt was made to follow the development of these organisms in larvae reared in the laboratory from egg-masses laid by two females, one of which was shown later by ovarian smears to be infected, while the other was not examined. The head, thorax, and abdomen of the very young larvae were smeared separately. With older larvae it was possible to smear some of the abdominal organs separately. The nearly mature larvae were cut down the mid-dorsal line, the digestive tract removed and its parts smeared individually. The parts forming the body-wall were scraped off the chitin for still other smears. In such preparations, particularly of the abdomen, there were usually to be found a few scattered individuals of rods similar to those of the adult, their number increasing with the age of the larvae. In only one case out of the twenty examined, were their numbers or association with cells such as to indicate appreciable development of the organism in the larva. In this one instance, a young second-instar larva, there were associated with one or two crushed cells, a group of organisms indistinguishable from those found in such large numbers in the pupae. If they occur in only such small numbers in the larvae, the failure to find them except as occasional individuals, is readily explained. However, in all cases, after the larvae had become pupae, at which stage the gonads first become recognizable under the dissecting microscope, smears of these organs showed enormous numbers of small rods filling the cytoplasm of certain cells. Some of these are so densely packed that they can be distinguished at low magnification. The organisms in the young pupa are typically very uniform in morphology, mostly rods in pairs or chains, the individuals measur-

ing 0.25μ to 0.3μ in width by 0.5μ to 1.2μ in length. The staining is rather faint. Filaments were not found (Fig. 9). In some of the very heavily infected cells there occur densely staining, somewhat irregular granules, and curved and bent rods, with a striking similarity to the forms found in the bedbug.

Examination of limited numbers of common mosquitoes, including among others *Culex stimulans*, *Culex sollicitans*, *Culex impiger*, *Culex pulchriventer* (?), *Uranotaenia* sp.?, and *Anopheles maculipennis*, revealed no organisms associated with the ovaries, or any rickettsia-like organisms associated with the other organs which were examined.

In one *Culex pipiens* female, caught in Brookline, Mass., the Malpighian tubes were observed during dissection to be greatly swollen. Smears showed the cause of the swelling to be due to masses of a large spirochaete, having the general characteristics of the spirochaetes of relapsing fever.

Summary of *Rickettsia* of *Culex pipiens*. All of twenty-five males and females from Boston and Minneapolis, taken during two seasons, contained numbers of a somewhat pleomorphic, rodlike, Gram-negative, intracellular organism. These apparently infect only the ovaries and testes. The organisms are found in the eggs. A tentative cycle of development is suggested as follows. All the progeny become infected via the egg. The infection in larvae is apparently very scanty, and the cells or organs concerned are not known. However, at about the time of pupation, the organisms associated with the gonads increase rapidly in number, keeping pace with the rapid development of the gonads themselves. The pupal gonads always contain many heavily infected cells. The organisms during this rapid growth are quite uniformly tiny rods. Pleomorphism is apparent after cells have become crowded and becomes increasingly more marked from this time on through adult life. Only the gonads of this one species of *Culex* are known to be infected. Further details of development within the mosquito can be determined only after a careful study with the aid of sections has been made.

Rickettsia in the Sand-Fly, Culicoides sanguisuga. Two lots of this sand-fly, obtained from Ipswich, Mass., during two summers, were studied by teasing out the whole body on slides, separating the organs where possible. In nine out of twenty-seven were found varying numbers of tiny, rickettsia-like cocci, 0.3μ to 0.4μ in diameter, diplococci and very short rods, measuring 0.3μ $\times$ 0.4μ to 0.5μ. These organisms are rather clear-cut and stain reddish purple (Fig. 10). For the most part they occurred free in smears of the abdomen, though in one or two cases lobes of the fat-body were seen to contain them in great numbers (Fig. 11). They apparently do not occur in the intestine or Malpighian tubes. While the morphology and staining are for the most part quite uniform, in two cases where the ovary was recognizable, the surrounding organisms were notably larger, more irregular, and less deeply stained. Marked irregularity of ovarian forms has been noted also in the rickettsiae of the bedbug, mosquito and drug-store beetle. In four additional sand-flies stained with Gram no organisms were found. It is not known whether these sand-flies were infected or not, although a third of others of the same batch were infected. Unfortunately further material for Gram and other stains and for sections was not available.

Rickettsia-like micro-organisms in Tabanidae. Chrysops. In smears of about fifteen deer-flies, consisting of three species of *Chrysops*, the fat-body in nearly all cases showed quantities of tiny, very uniform coccus bodies, 0.2μ to 0.3μ in diameter (Fig. 12). Frequently these stained deep red or purple. They occurred singly and in pairs. In some of these cases, however, what were apparently the same bodies took the stain so faintly as to be scarcely discernible. Sections of three deer-flies failed to reveal any granular bodies at all in the fat-body. Other organs in smears and sections lacked such granules. Many of the other tabanids mentioned below also contained in the fat-body similar very uniform coccus bodies, the staining of which varied from very faint to a deep red. Sections of such tabanids also failed to reveal granules in the fat-body. These bodies at times simulate, in numbers and grouping, rickettsia-like organ-

isms, but in the extreme regularity of their spherical shape, their near-failure to stain at times with Giemsa, their entire absence in sections and their regular occurrence in the fat-body alone of various tabanids, they are comparable to normal cell granules of other insects. From our present information they appear to be merely cytoplasmic inclusions characteristic of tabanid fat-cells.

Tabanus pumilis. But a single individual of this species, from Attleboro, Mass., was dissected in 1922. It appears not to be abundant near Boston as no other specimens were obtained that year in spite of special search. In the Malpighian tubes and in the pericardial cells were large numbers of delicate rods and filaments (Fig. 13). The filaments were not numerous in the Malpighian tubes and in morphology resemble the rickettsia of the bedbug. These are curved, and beaded forms and others resembling chains of minute bacilli. The individual rod forms are curved, of uniform thickness and varying length (Fig. 14). They measure 0.25μ to 0.3μ thick by 1.0μ to 3.0μ long. Many have tapering ends.

In 1923 nine specimens of this species, obtained in Sudbury, Mass., were dissected. All were entirely negative for rickettsiae. The fat-bodies of many contained granules similar to those seen in the fat-body of chrysops.

Tabanus costalis. Thirteen specimens collected in the Lynn (Mass.) marshes were examined. In the pericardial cells of one were found clusters of typical rickettsia-like micro-organisms (Fig. 15). These are short rods single and paired. Many show polar granules, and bear a considerable resemblance to the micro-organisms of Rocky Mountain spotted fever. They measure 0.2μ to 0.25μ in width by 0.4μ to 0.5μ in length. In this same specimen were found, apparently in cells of the rectal glands, a number of large cocco-bacilli and throughout the whole intestinal tract considerable numbers of a micro-sporidian parasite. The cocco-bacillus was also found in the rectal glands of several other specimens. In nine out of thirteen of the species, the fat-body preparations showed numerous uniform, red spherical granules resembling somewhat those of

Chrysops except that they stained a brighter and less intense red. They were not seen in any number in unbroken cells and could not be identified with certainty in sections.

Tabanus trispilis. One specimen from Mansfield, Mass., was examined. It showed large numbers of densely staining diplococci in the intestine, apparently within epithelial cells. Rickettsia-like micro-organisms were not found.

Tabanus lineola. Six specimens examined were negative.

Tabanus abdominalis. One specimen examined was negative.

Rickettsia lectularia in the Bedbug, Cimex lectularius. This organism, universally present in the bedbug, *Cimex lectularius*, and described by Arkwright, Atkin, and Bacot in 1921, is an especially interesting one on account of its pleomorphism, and hereditary transmission. It multiplies intracellularly in various organs, exhibiting coccoid, rodlike, and filamentous forms. A complication arises from the fact that simultaneously with the report of this rickettsia there appeared an account by Buchner (1921) of a "new symbiotic organ" of the bedbug, containing bacteria. This author, however, did not mention the infection of other organs with the rickettsia of Arkwright, Atkin, and Bacot, nor did the latter authors describe the special "organ" containing the symbiotic bacteria. Buchner has since (1923) published an extended account of the mycetome,* its development in the embryo and nymphs, and of the "bacteria" contained therein, but still has made no mention of the British investigators' work. Both were certainly concerned with the same organism in part, and from our own work we believe that the organisms found in the mycetome and the rickettsia of Arkwright, Atkin, and Bacot are the same.

Arkwright, Atkin, and Bacot found *Rickettsia lectularia* in practically every specimen of three English strains, and in all bugs of two Polish strains. We have found this organism in all bugs examined from one Porto Rican and six American sources. Cowdry (1923) found them in all bugs of lots from New York,

* A term proposed by Buchner (1912) for an "organ" or group of cells given over exclusively to symbiotic fungi or bacteria.

Honolulu, Antigua, and Peking. Bugs from a second New York source, Trinidad, Jamaica, and Dutch Guiana he found to be entirely or mostly negative. As Cowdry indicated, poor fixation probably accounted for these negative results. However, he had unfortunately overlooked Buchner's earlier report of the mycetome. We believe that this "organ" with its rickettsiae is invariably present, though infection in other tissues is frequently very light.

Not only does the mycetome seem to be invariably present in the common bedbug, but it apparently occurs in other species of cimex as well. Patton and Cragg (1913) have figured as an "accessory lobe" of the testis of *Cimex rotundatus* a structure identical in general appearance and position with the mycetome of *Cimex lectularius*. Arkwright, Atkin, and Bacot found in *Cimex hirundinis* an infection somewhat similar to that caused by *Rickettsia lectularia*, and further study may reveal a mycetome for this species as well. Cowdry refers to "*Rickettsia hirundinis*" but we are unaware of the authority for this name.

Arkwright, Atkin, and Bacot considered as the typical rickettsia-like form of the parasite, minute coccoid and diplococcoid bodies, staining deep purple with Giemsa. These forms they found in the ovaries along with patches of ill-defined granules, in developing eggs and in various organs, including the alimentary canal, testes, Berlese's organ and Malpighian tubes. Accompanying these coccoid forms and very often predominating in numbers, these authors found minute bacillary, lanceolate and "thread" forms, which stain red rather than purple with Giemsa. The threads attain their greatest development in the cells of the Malpighian tubes which are greatly swollen as a result (Figs. 16 and 17). Many forms intermediate between the bacillary or lanceolate bodies and the threads were found, some being curved. The rods and threads when stained with Giemsa appeared to possess an "outer sheath which took the eosin rather lightly, while in the interior were granules or groups of granules which stained more deeply and of a purplish hue." With dark field illumination these authors observed the threads to evacuate granules which they consid-

ered similar to the granules seen in smears within the threads. Their attempts at artificial cultivation failed. A tentative developmental cycle of the parasite was outlined, in which the typical rickettsia forms in the ovary developed through a bacillary stage into the threads, the latter in turn releasing granules which were assumed to be the rickettsia forms.

Buchner's description of the "bacteria" found in the mycetome and developing eggs agrees closely with that of the English investigators for the rickettsiae found in the ovaries and eggs. Both inclined to consider the ill-defined granules as forms of the parasite. Buchner also found filaments or rods in the mycetome, ovary fat-cells and Berlese's organ, but did not mention an infection of the Malpighian tubes. In addition, his specimens were often heavily parasitized in various organs with a large bacterium, but here again the Malpighian tubes were not mentioned. This latter organism, a large densely staining, oval bacterium, 3.0μ long, is apparently peculiar to Buchner's specimens, since it certainly does not correspond to any forms described by Arkwright, Atkin, and Bacot, or to any organism we have seen in our specimens. The entire absence of organisms in the Malpighian tubes of Buchner's specimens we find difficult to explain, since infection here is usually strikingly present.

The mycetomes have been described in detail by Buchner (1923). They are paired structures occurring without exception, so far as known, in both sexes. They are sharply outlined, oval or pear-shaped in form, and slightly opaque. We have observed that the size and shape are by no means constant and that the mycetomes may exhibit in whole or in part a glassy transparency. They lie on either side in about the third abdominal segment, near the gonads. In the male each mycetome is connected with the base of the corresponding testis by a delicate strand which gives it the appearance of being a part of this organ. The mycetomes are easily recognized after one or two dissections, although they lie among lobes of the fat-body which they superficially resemble. A tracheal branch from the fourth abdominal spiracle supplies the mycetome in each sex and aids in identifying this structure.

We have been able to confirm in general the morphological findings of both Buchner and Arkwright, Atkin, and Bacot. In our bedbugs during the summer of 1922, the tiny coccoid forms were very much in the minority, short rods and filaments being overwhelmingly dominant (Figs. 18 and 19). In the winter and spring of 1923, and winter of 1923–24, however, in the same strains the coccoid forms (Fig. 20) had largely replaced the others though rods and filaments were always to be found. The rods and filaments seem always associated, together with all gradations between the very short and the very long forms, though any particular cell or cell group may contain exclusively one or the other. The short rods measure 0.2μ to 0.3μ in width by 0.4μ to 0.5μ in length. The filaments, which may be considered those curved or wavy forms longer than three or four microns, exhibit a great range of morphology. They are nearly always irregularly curved. They may be very slender, with sharp outlines, staining homogeneously, measuring 0.25μ to 0.3μ by 3.0μ to 8.0μ (Fig. 21). In other specimens thicker filaments may exhibit irregularly alternating light and dark portions measuring up to 0.5μ $\times$ 8.0μ. In the latter case the ends are nearly always unstained, appearing to fade out. The diameter of these filaments, which are the granular threads of Arkwright, Atkin, and Bacot, usually exceeds that of the normal ones and may be two or three times their diameter (Figs. 22 and 23). Arkwright, Atkin, and Bacot considered that these granules, or deeply stained portions of the filaments, may represent the coccoid forms, but on this point our preparations are not convincing. Filaments, together with the short rods, have been found chiefly in the cells of Malpighian tubes and of Berlese's organ, though occasionally cells of the mid-intestine are found swollen with twisted masses of filaments. In the mycetomes, ovaries and eggs, the short rods and the irregularly bent and curved forms predominate, though a few filaments are nearly always to be found.

While no motility at all was observed by Arkwright, Atkin, and Bacot, the filaments in the mycetome were found by Buchner to be motile. A certain proportion of the filaments in nearly all our preparations have been found to be motile, in

some cases very actively motile. The filaments are quite flexible, apparently possess a terminal flagellum, and when at rest undergo constant, almost undulating, movements, due possibly to molecular movement. In translatory motion, one end describes a spiral. The line of progress is usually straight. It often occurs that only two or three of many filaments are motile, but in other cases, notably when large numbers pour out of ruptured Malpighian tubes, the whole field is a mass of whipping, darting filaments. The short rods seem to possess no motility. Motile filaments occur chiefly in the Malpighian tubes, but we have also observed them in the unruptured mycetome and in preparations of ovaries.

With dark field illumination the appearance of the rods and filaments is characteristic. We have studied particularly the organisms from the Malpighian tubes. These forms are usually of the same diameter and are uniform throughout their length. The ends are rounded, or perhaps slightly tapering, making the very short rods oval or lanceolate. Their contour is evidenced by a definite, unbroken, luminous line, with typically a clear, non-refractive interior. The filaments may contain a greater or less number of tiny, spherical, refractive granules as described by Arkwright, Atkin, and Bacot. In certain cases there are short rods which also contain refractive material. We have seen no extrusion of granular material from rods or threads in preparations watched for several hours. The tiny coccoid forms were not plentiful in the specimens examined with the dark field, and as a result we could not distinguish them with certainty. The many tiny bodies which appear as luminous points may be the coccoid forms or merely some of the many granules normally found in most insect cells. The curved and bent organisms of the mycetome differ in appearance from those of the Malpighian tubes only in their size and shape, the refractivity being the same. The irregular granules of the mycetome, which appear often to be disk-shaped are also of the same refractivity as the other forms.

The organisms of the mycetome (Figs. 18, 19, and 24) in smears present a characteristic picture. Most commonly there is great lack of uniformity of size and shape of the organisms,

together with a large proportion of curved and bent forms. There are sharp differences in density of staining between individuals or groups of both curved and straight rods. Occasional filaments and varying proportions of coccoid forms are noted. In addition there are the bodies mentioned by Buchner, which appear as unevenly stained granules. In the above, which may be termed the typical mycetome picture, there is really a mixture of all forms found throughout the body of the bedbug, with a particularly large proportion of curved forms and with the granules in addition. In other mycetome smears any one of the above types may predominate, almost to the exclusion of the others, or two or more forms may occupy different portions of the mycetome, as indicated in smears by homogeneous groups. Whole fields or groups of sharply bent rods, C-shaped and ring-formed organisms are especially common (Fig. 25). The mycetomes of the same specimen may present strikingly different pictures in smears. This is correlated perhaps with differences in their gross appearance, e.g., the degree of opacity, as between portions of the same mycetome, or the two mycetomes of the same bug. Ovarian smears (Fig. 28) resemble somewhat those of the mycetome, but the rods and filaments usually predominate in the former.

The somewhat irregular granular forms ("Bläschen" of Buchner) are among the most puzzling features of the mycetome. Buchner was at first inclined to consider them forms of the organism, but in his last paper was wholly uncertain as to their nature. We are inclined to believe they are forms of the parasite. Throughout our preparations, and usually in the same smear, there are to be found in large numbers all morphological intermediates between (1) the circular or oval granules, (2) the same with dense periphery and light center, (3) ring-shaped or C-shaped organisms, and (4) the bent or curved rods. In many of the ring forms no break is obvious, but in others there is clearly the picture of a rod twisted into a circle, 0.75μ to 1.0μ in diameter (Fig. 25), with the two ends overlapping or bent inward, but otherwise indistinguishable as to size and staining from surrounding straight or curved rods. Forms with two nearly complete turns are occasionally seen.

We are not able to state what the exact structure of these granule-ring forms is, but there is the unescapable impression on studying slide after slide that either the rods are in process of curling themselves up, or else in their growth from some smaller form they have been restrained by some force which has made them curl on themselves, later becoming free and straightening out. This force would seem not to be the pressure of neighboring organisms, since they are never entangled with each other, but rather something enveloping each organism. That the granules bear some relationship to the organisms finds support in fresh preparations. In some of our dark field mounts, curved or straight rods were quite rare, the whole mycetome appearing to be filled with round or oval granules, which were in many cases flattened. The surface and edge frequently presented rounded irregularities. The contents of these spheroid or disk-shaped granules were not homogeneous, though no definite internal structure could be made out. In other preparations these granules seemed to have given way to corresponding numbers of unmistakable organisms. Ring-shaped forms have not been recognized as such in dark field mounts in spite of their constant presence in smears, but the disk-shaped granules correspond exactly in diameter to the ring forms, with a refractivity which prevents resolution of internal structure. It is difficult to believe that the mycetomes exhibiting almost exclusively discoid granules should be practically uninfected, but it seems rather that these structures represent merely one of the many forms that characterize this rickettsia. We are inclined to believe that the flattened disks represent the ring forms characteristic in whole or in part of many mycetome smears.

Buchner has also mentioned larger, strongly refractive bodies of variable size. We have found these in usually small numbers in the mycetome. They are more refractive than the smaller disks, but like them are rather irregular as to surface contour and are not homogeneous as to contents. We have been unable to resolve any definite inner structure either with ordinary or dark field illumination. However, in groups of these bodies we have seen others of about the same size, less

refractive, and in which portions of a filament could be resolved. Accompanying such groups were unquestionable filaments irregularly, and more or less tightly, coiled or "crumpled" into a ball of about the same size as the granules. Here again we are unable to affirm anything as to the structure of the refractive spheroids, but there are in fresh preparations morphological intermediates between them and unquestionable organisms, the filament-balls. In stained slides the refractive spheroids are perhaps represented by large, usually densely but unevenly stained bodies, 2 to 3 microns in diameter, which accompany groups of filaments (Figs. 26 and 27). As with the smaller curved and ring forms, something seems to have prevented these long filaments from becoming entangled with their immediately adjacent neighbors. While large numbers of the filament-balls are not common, in several cases associated with starved or dying bugs,* the organisms of the mycetome, and in one case of the ovary, were mostly swollen filaments, half of them crumpled more or less tightly into balls (Fig. 26). The free filaments had a very much crumpled appearance as if they had just been released. What relationship any of these forms may have to a life-cycle of the organism, if such there be, we have as yet no basis for judging.

Summary of Rickettsia lectularia. A Gram-negative, intracellular, pleomorphic organism, which has not as yet been cultivated, causes a general infection of many organs of the bedbug, *Cimex lectularius*, the world over. The degree of infection varies

* The literature indicates quite generally that bedbugs can be raised successfully when fed on a variety of mammals. However, in our one experience, using a male guinea-pig, the blood formed a solid mass in the mid-intestine which broke up readily into crystals which almost penetrated the intestinal wall. Of our Porto Rican stock, two-thirds of the adults and eight-ninths of the young nymphs died with such crystals in the intestine. Our numerous "Gloucester" strain fared somewhat better on this diet, but there was considerable mortality and reproduction ceased entirely. Except for the ten-day period when these two strains were fed on one guinea-pig, all our bedbugs were fed on ourselves. The effect of the guinea-pig blood on our bugs was similar to that noted when lice are fed on other than humans. Nevertheless, during the winter of 1922–23 there occurred an abundant infestation of *Cimex lectularius* from some unknown source in one of the animal houses.

greatly but the organism seems never to be pathogenic, though it causes hypertrophy of certain cells. The different forms of this rickettsia include minute coccoid and diplococcoid bodies, minute curved, bent and straight rods, bacillary forms and filaments. There is considerable variability as to size and density of staining of these forms, as well as the proportions in which they are present in any infected organ. The filaments are frequently actively motile. In addition to this general infection there is invariably present a pseudo-organ, or mycetome, the cells of which are filled with varying proportions of the above types, but usually with a large proportion of curved, bent, and ring forms. Certain discoid granules in the mycetome seem to be genetically related to the ring forms. Other larger granules are perhaps related to long filaments which appear crumpled into a ball. The ovaries and eggs are always infected, thus providing for the infection of each embryo, in which the organisms and certain host cells undergo a definite development leading to the formation of the mycetome in nymphs and adults. As far as the mycetome and its organisms are concerned, the phenomenon parallels many other cases of intracellular "symbiosism" in insects.

Rickettsiae in Siphonaptera. Rickettsia ctenocephali Sikora. This organism was found by Sikora (1918) in a varying proportion of different strains of cat-fleas (*Ctenocephalus felis* *). In one hundred fleas from one cat, twenty were scantily and five heavily infected. The organisms were found in the body cavity or on the surface of organs in the body cavity, though in one case Sikora found them in the intestinal lumen. There are both large and small forms and this writer suggested that there are perhaps two species, a larger one resembling *Rickettsia pediculi*, and a much smaller coccus form resembling *Rickettsia melophagi*. The infection is transmitted hereditarily, Sikora believed, though the organism has not been demonstrated in the egg. No strain was entirely free from the rickettsiae. While early attempts to infect *Pediculus* with flea-rickettsiae

* Given as *Ctenocephalus canis* by Hindle (1921). Sikora's reference was merely to the "Katzenfloh."

failed (Sikora, 1918), later it was reported (1920) that they multiplied readily in the louse coelom.

We have found two, or possibly three, infected specimens out of seven cat-fleas examined in smears. The organisms, which stain reddish with Giemsa, are rather irregular in their morphology, varying from tiny cocci, measuring 0.3μ to 0.4μ in diameter, to rather large, swollen, curved rods, 0.3μ $\times$ 1.5μ to 2.0μ. Short, red-stained rods with deeply stained poles are common, the central portion of such rods being unstained or very faintly stained (Fig. 29). Material for Gram stain was not available.

Other Siphonaptera. Cowdry (1923) found in sections of one out of four dog-fleas (*Ctenocephalus canis*) a rickettsia-like organism occupying cells of many organs. In one out of twenty-five human fleas, *Pulex irritans*, the same investigator found filamentous rickettsiae in the body cavity and fat-cells, and very tiny Gram-negative rods in the intestinal lumen. Three specimens of another flea, *Spilopsyllus simplex*, Cowdry found to be negative.

Rickettsiae in Corrodentia. Psocus sp.? Sikora (1918, 1920) has noted the occurrence of a very small rickettsia-like organism which forms a thin layer over the surface of the stomach epithelium of the dust-louse, *Psocus* sp.? All individuals are infected and transmission is apparently via the egg. Attempts to infect *Pediculus* with this organism failed. No further information on this organism is available.

Dorypteryx pallida. In smears of all of eleven nymphs and adults of this book-louse taken in Boston during two seasons, we have found a very tiny rickettsia-like organism in the cells of the Malpighian tubes, and also in the ovaries in those smears in which this organ could be recognized as such. The infection is commonly very light, but occasionally the Malpighian tubes are packed with the organisms. The presence of the rickettsiae was also confirmed in sections of several lots, though the sections were not satisfactory for their study. In the Malpighian tubes the rickettsiae are densely and evenly stained coccoid

and rodlike forms of uniform diameter, measuring 0.25μ to 0.3μ × 0.5μ to 0.6μ (Figs. 30 and 31). They are Gram-negative. In the ovary, as seen in two heavily infected specimens, the organisms are mostly cocci 0.4μ to 0.5μ in diameter, or ovals of 0.4μ to 0.5μ × 0.5μ to 0.8μ, of appreciably greater diameter than the Malpighian tube rickettsiae, while they stain less densely and are distinctly more reddish (Fig. 32). Curved rods were noted very rarely.

In small numbers of three other Corrodentia, namely a winged bark-louse (*Psocidae* genus?), the book-louse *Troctes divinatoria*, and an undetermined wood-louse, we have found no rickettsiae.

Rickettsiae in Mallophaga. Hindle (1921) has described under the name *Rickettsia trichodectae* an organism which he found in seven to eight per cent of his specimens of the horse-louse, *Trichodectes pilosus*. They occur in the alimentary canal and closely resemble *Rickettsia melophagi*. They measure 0.3μ to 0.5μ × 0.5μ to 0.9μ, with occasional longer forms. The organisms are passed out with the feces and Hindle assumed that infection is accomplished via the digestive tract. Arkwright (1923) has reported the presence of a rickettsia-like organism in the intestine of the goat-louse, *Trichodectes climax*. No information was given as to the reaction of these two organisms to Gram stain. If the intracellular criterion for rickettsia is maintained, these two extracellular organisms would have to be excluded from the genus, unless they can be shown to occur in cells as well as in the intestinal lumen.

Sikora (1922) found rickettsia-like organisms in the intestinal lumina of *Lipeurus baculus* from doves, *Trinoton* sp.? from the black martin, and occasionally a few rickettsia-like forms in smears of *Menopon* sp.? from chickens. These organisms were not described.

Menopon pallidum. Cowdry (1923) has found in the intestinal lumen of this hen-louse, Gram-positive diplococci and smaller Gram-negative cocci, about 2μ in diameter. We have found in the crop of each of six hen-lice great numbers of a small

bacterium mixed in with the debris of feathers, and present in apparently pure culture. They are clear-cut, slender, straight rods, measuring 0.25μ × 0.6μ to 1.2μ (Fig. 33). They stain reddish purple with Giemsa in smears. Some of the rods are so short as to be coccoid. That there is a specific relationship of this organism to its host is indicated by the fact that it occurs in great numbers in this species while in other Mallophaga from the same chicken, the crop contains almost no organisms of any sort, though filled with similar feather debris.

In the smear of one adult *Menopon pallidum* near the ovaries were found several large masses of an organism very much resembling *Rickettsia melophagi*. These stained deep bluish purple and exhibited coccoid forms and very short rods.

In two other specimens of *Menopon* from the same chickens, there were found in the mid- and hind-intestine, numbers of very delicate, faint pink filaments curved into various shapes. A few were also found in the smear of the Malpighian tubes (Figs. 34 and 35). Also, in one of these two specimens and in two others as well, there have been found in smears and unassociated with any particular organ, little clumps of a tiny, reddish, lanceolate organism about 0.3μ × 0.4μ, with sharply tapering ends and a central darker portion.

What the interrelationships of the organisms seen by Sikora, Cowdry, and ourselves may be, we are unable to say. From our present data we do not consider the clear-cut rods of the crop to be rickettsiae. As to the rickettsia-like organisms seen in small numbers by Sikora and ourselves, which may be intracellular or intracoelomic, further study is needed.

In two other species of Mallophaga, *Goniocotes hologaster*, and *Lipeurus variabilis*, rickettsia-like organisms have not been found with certainty. In one adult of *Goniocotes* there were found in the intestine and Malpighian tubes the same delicate filaments noted in the same organs in two specimens of *Menopon*. In a smear of *Lipeurus* were noted a number of large bacteria filling the cytoplasm of scattered cells, which may be part of an ovarial mycetome such as that found by Sikora (1922) in *Lipeurus baculus*. Masses of bacteria were also noted within cells of a nymph of *Goniocotes*.

It is thus seen that while a number of very small organisms are found in the intestinal lumina and elsewhere in various Mallophaga, in no case is there sufficient evidence for judging their relation to the rickettsiae.

Rickettsiae in Coleoptera. The Drug-Store Beetle, Sitodrepa panicea. We have found specimens of this insect, the so-called drug-store beetle, obtained from heavily infested dog-biscuit stored near the laboratory, to be infected in all cases with a somewhat pleomorphic rickettsia. Ten adult beetles were examined. All the abdominal organs, and especially the fat-body, Malpighian tubes and ovaries, seem to be infected. One form of the organism is represented by minute cocci and rods occurring singly and paired, measuring 0.2μ × 0.2μ to 0.4μ (Fig. 36). In the ovaries occur swollen coccoid and ring forms comparable to those of the bedbug mycetome. A second form comma-shaped, found chiefly in the Malpighian tubes, is rather larger, measuring 0.5μ to 0.6μ × 0.8μ to 1.4μ (Fig. 37). The latter may be a separate organism. Both types are Gram-negative and stain poorly with stains other than Giemsa. Large numbers of the smaller type were also found in two adult beetles taken from infested spice at Minneapolis.

The ovaries are always infected and transmission is apparently via the egg. The organisms were found in small numbers in the four eggs examined, and each of five larvae was infected.

In this beetle are also found the symbiotic yeasts inhabiting certain hypertrophied cells of the mid-intestine, studied by Karawaiew (1899) and Escherich (1900). Neither of these investigators made any mention of bacterium-like organisms infecting their specimens.

Rickettsia in the Argasid Tick, Ornithodoros moubata. In sections of one of fourteen ticks dissected in 1912 masses of peculiar granules were noted in the salivary gland. A restudy of these slides show in salivary gland acini of both types intracellular clusters of typical rickettsiae in large masses. These are illustrated in Figure 38 and are obviously different in size and distribution from those described by Cowdry (1923) in *Ornithodoros turicata.*

Intracellular Protozoa found in the Ixodid Tick, Dermacentor. In the ovaries of eleven out of twelve females of *Dermacentor variabilis* from Cape Cod, were found large numbers of bodies which we believe are protozoa. The organisms are somewhat irregular, reddish-purple granules surrounded by a pale bluish, irregular and indefinite halo. In certain specimens, however, the purple granules were surrounded by sharply outlined, pale blue material. The granules are frequently elongate and paired. They are of the size and resemble superficially certain phases of *Theileria*. These bodies occurred in the ovarian epithelium rather than in egg-cells and were not found in other organs of the body. They could not be demonstrated in sections stained with Giemsa or haematoxylin.

Half-grown embryos from eggs laid by this lot of ticks were examined in smears. Painstaking and prolonged search of forty or fifty smears revealed in three several cells containing what appeared to be the same organisms.

The ovaries of a specimen of *Dermacentor venustus* received from Montana and apparently not infected with the rickettsia of Rocky Mountain spotted fever, also contained the same organisms.

In a paper not available to us, Godoy and Pinto (1922) are reported to have described as symbionts certain organisms in the ovaries of Ixodid ticks, similar to those previously noted by Koch in Rhipicephalus.

Arthropods examined by us and found to be negative as to rickettsia-like organisms include the following:

Certain of the Tabanidae, Culicidae, Mallophaga, Corrodentia and specimens of Dermacentor, as noted in the test.

Stomoxys calcitrans, stable-fly (four specimens).

Musca domestica, house-fly (one specimen).

Haematobia irritans, cattle horn-fly (one specimen).

Tipulid crane-fly (one specimen).

Diabrotica sp., striped cucumber beetle (one specimen).

Anasa tristis, squash bug (two specimens). Caecal bacteria found in large numbers.

Pedicinus longiceps, monkey-louse (one specimen). Large bacterial? symbionts characteristic of Pediculidae present.

Unidentified Collembolan, spring-tail (about twelve specimens).

Unidentified red mites from mosquito (five specimens).

Sarcoptes mutans, from "scaly-leg" of chickens (about twenty specimens).

Other Bacterial Organisms of Insects. There are several groups of bacterium-like organisms found in insect tissues, some of which may be related to the rickettsiae. Well-known examples of such bacterial forms are the symbionts of cockroaches and carpenter-ants, and the bacterium- or fungus-like organisms of the Pediculidae. In their staining and general characteristics, however, these symbionts are not rickettsia-like.

The photogenic organs of certain insects contain symbiotic bacteria which it is claimed cause the luminescence (Buchner, 1921). These organisms are very minute and may possess other rickettsia-like characteristics.

Krassilstschik (Buchner, 1921) found in many species of aphids masses of bacteria in definite regions of the body-cavity. These micro-organisms, which were termed "biophytes," are in some cases very minute, are transmitted hereditarily and some of them have been cultivated. Their possible relation to the rickettsiae has not been investigated.

Glasgow (1914) has described certain mid-intestinal caeca characteristic of many of the Hemiptera belonging to the Pentatomidae, Lygaeidae, Coreidae, and other families. These caeca always contain bacteria in large numbers, particularly in the lumen but in some cases within cells. The transmission is hereditary. The organisms are of a number of types and some have been cultivated, but our information concerning them is as yet very limited. It was doubtless these organisms which Cowdry found in large numbers in the intestine of two Pentatomids. These caecal bacteria seem to have escaped notice in the literature of insect symbiosis.

Discussion

Relation of Rickettsiae to their Hosts. There are many obvious parallels in their behavior between some rickettsiae and the intracellular symbionts of insects. The term "symbiosis" has been used rather loosely to designate the extremely delicate equilibrium existing between many yeast- and bacterium-like organisms and their arthropod hosts. (See Buchner 1912 and 1921 for literature and summary of this whole field.) These organisms, familiar examples of which are the bacteroids of cockroaches and the yeasts of aphids and other Hemiptera, are found in all individuals of the species concerned, where they inhabit certain cells or groups of cells, forming at times pseudo-organs, the mycetomes of Buchner. They are transmitted without fail to each of the progeny via the egg. Their development in the embryo is as definite and constant for any given species as the development of any organ of the embryo itself. The hosts apparently do not suffer from the presence of these organisms.

Certain rickettsiae are similar to the symbionts in that transmission is via the egg. The most striking instance is that of the bedbug rickettsia. Here, at least so far as the mycetome and its development in the embryo are concerned, this rickettsia is not essentially different from other intracellular symbionts. However, it causes in addition a more or less general infection of the organs of the host, with at times hypertrophy of certain cells. The rickettsia of *Culex pipiens*, in that it is transmitted hereditarily and is found only in connection with cells of the gonads, behaves very much as a symbiont. In other instances where the rickettsiae are found in all individuals of a given species and are transmitted apparently through the egg, as in the sheep-ked, certain Corrodentia (*Psocus* and *Dorypteryx*) the drug-store beetle, and the several ticks described by Cowdry, further study may reveal a relationship approximating symbiosis rather than mere infection. In the case of Rocky Mountain spotted fever, where the tick becomes infected from mammalian blood, the transmission of the parasite via the egg for many generations is not essentially different from such trans-

mission where no external sources of infection are known. A comparable instance of the continuous hereditary transmission in an arthropod of a micro-organism pathogenic for vertebrates is that of the spirochaete of African relapsing fever in the tick *Ornithodorus moubata*. On the other hand, many rickettsiae can be considered only as more or less harmless parasites, well adapted to living in arthropod tissues, although the typhus organism is somewhat pathogenic to the louse. Thus, the relationships of the rickettsiae to their arthropod hosts present all gradations from pathogenic parasitism to a completeness of adaptation comparable with that of many intracellular symbionts, with a tendency on the part of most rickettsiae toward such symbiosis.

The pathogenicity of the three rickettsiae causing disease in man would seem to be either an accidental or acquired characteristic so far as the group is concerned. In this connection, however, it may be noted that while the rickettsiae typically inhabit arthropod tissues, an arthropod reservoir of the parasites is unknown in typhus and trench fever, while in Rocky Mountain spotted fever, although the infection may persist for generations in the tick, it is by no means certain that the parasite can be indefinitely maintained in ticks. The hereditary transmission of the trench-fever organism has not been demonstrated.

To what extent the non-pathogenic rickettsiae of other blood-sucking insects get into the blood of the vertebrate hosts is almost wholly unknown. While Nöller and Kuchling (1923) have recently reported the cultivation of a rickettsia from the blood of the sheep, this is certainly not the primary source of the sheep-ked's infection.

Nature and Relationships of the Rickettsiae. The nature of the rickettsiae has been the subject of debate from the first. It has been suggested that they are mitochondria, normal cell inclusions, granules, cell debris, etc. The control work done by Wolbach (1919) and Wolbach, Todd, and Palfrey (1922) in studies of Rocky Mountain spotted fever and typhus, in their comparative studies of uninfected and infected ticks and lice were sufficient to exclude the possibility of the rickettsiae of

the diseases being mitochondira, for there is no reason to believe or possible explanation that mitochondria should stain in infected arthropods and not in normal ones. Cowdry (1923) has shown quite clearly that by staining methods rickettsiae are "easily distinguishable from mitochondria and all other cytoplasmic and nuclear granulations." Nicholson (1923) has corroborated this for the parasite of Rocky Mountain spotted fever. Rocha-Lima (1916 *b*) considered them different from bacteria, while Töpfer (1916) held them to belong to the bacteria. Jungmann and Sikora have suggested that they are Chlamydozoa. Cowdry finds no basis for the latter view, but affirms in no uncertain terms that they are true micro-organisms. For the purposes of his study Cowdry arbitrarily restricted the term *Rickettsia* to include only Gram-negative, intracellular, bacterium-like organisms. While implying that they are not "merely bacteria" he did not attempt to establish their systematic position.

We feel that it is as yet impossible to designate the systematic relationships of the rickettsiae. Indeed, no entirely satisfactory basis even for defining *Rickettsia* as a genus has been found. We have used as a working basis Cowdry's definition, — namely, Gram-negative, intracellular, bacterium-like organisms found in arthropods, — recognizing as he points out that at least some of such organisms are probably merely bacteria. 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 densely 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 rickettsiae 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 rickettsiae would be so broad and vague as to be valueless. One difficulty lies in the fact that we do not as yet have wholly adequate information about the

morphology or other characteristics of any one of the rickettsiae. Furthermore, the type of the group or possible genus *Rickettsia prowazeki*, is itself a pleomorphic organism. The degree and range of pleomorphism to which other members of the group are to be restricted cannot at present be delimited. In addition, the question of possible life-cycles for at least some of the rickettsiae is yet to be settled.

The results of the present investigation and the work previously done by Cowdry, covering even a larger range, establish the fact that minute micro-organisms morphologically and tinctorially similar to the pathogenic rickettsiae are widely distributed in arthropods, without relation to their feeding habits. Our brief analysis of the morphology and distribution of the rickettsia-like organisms in arthropods, and of their relationship to their hosts, makes it quite evident that adequate premises for classification cannot be obtained from these sources. Bacteria botanically related, for instance the acid-fast bacteria, show comparable extremes in nature of habitat and pathogenicity. Without keeping this in mind, it would be very difficult to reconcile the grouping together of micro-organisms, which exhibit the delicate adjustments of a true symbiont, and therefore invariably present and specific for a given species of insect (*Rickettsia lectularia*), and those which are pathogenic both for vertebrates and their insect hosts (*Rickettsia prowazeki*). Intermediate between these two extremes is the parasite of Rocky Mountain spotted fever, which, as already noted, is non-pathogenic for the arachnid host and is transmitted hereditarily. Its rôle in the tick is probably that of a harmless parasite rather than that of a symbiont. If the indefinite perpetuation of the parasite of Rocky Mountain spotted fever is possible in the tick, it is hard to account for its very restricted distribution in ticks without assuming that it is a parasite in process of adaptation to the tick.

Although it is possible to arrange in a series the rickettsia-like micro-organisms, according to the degree of perfection of adaptation to their arthropod hosts, and while it is satisfying to speculate that these micro-organisms represent various stages in the development of true symbionts, we are also confronted

with the fact that most of the non-pathogenic micro-organisms in insects are wholly unlike rickettsia (the Gram-positive bacteroids of Blattidae and the symbionts of Pediculidae). It is conceivable that further study may make it possible to construct other series, with the intention of showing gradations of other characteristics, and connecting symbionts greatly different in size and morphology. With all these considerations in mind, it seems to us unfortunate that specific names have been given to non-pathogenic rickettsia-like micro-organisms. These considerations also make precarious the conception that *Rickettsia* represents a type of micro-organism widely distributed in arthropods, with occasional representatives which have accidentally or gradually acquired pathogenic properties. *Rickettsia prowazeki* is clearly the type micro-organism, if rickettsia is to have generic status. The name for the present serves a useful purpose, just as the term Pasteurella did for the designation of a group of bacteria.

A grouping of the rickettsiae, which may be justified by further study, can be made as follows: (1) the intracellular, pleomorphic organisms exhibiting coccoid, rod and filamentous forms, e.g., *Rickettsia prowazeki*, *Dermacentroenus rickettsi*, *Rickettsia lectularia*, and the rickettsiae of the mosquito and possibly of the drug-store beetle; (2) the intracellular, uniform coccoid and diplococcoid organisms such as those of the sand-fly, *Culicoides* and the book-louse, *Dorypteryx;* and (3) those in which the dominant phase is an extracellular one in the intestinal lumen of the host, such as *Rickettsia pediculi*, *Rickettsia melophagi*, and the organisms of the dust-louse, *Psocus*, and of various *Mallophaga*. The latter group, of which *Rickettsia melophagi* and *Rickettsia pediculi* (Sikora, 1921) have been cultivated artificially, clearly seems to be closely allied to the bacteria, and may in fact be bacteria, though the difficulty of separating them from other rickettsiae is encountered immediately in the fact that *Rickettsia pediculi* grows readily intracoelomically and at times intracellularly, and that *Rickettsia melophagi* is transmitted via the egg (hence presumably intracellular at some stage) and in cultures exhibits atypical forms comparable to stages of the bedbug rickettsia. The swollen

granules associated with ring and curved forms, as in the bedbug, mosquito, drug-store beetle and possibly several others, may provide a basis for sub-grouping within the rickettsiae.

The importance of a knowledge of the distribution of rickettsia-like micro-organisms in insects is very apparent in the contemplation of researches upon the etiology of insect-borne diseases of both plants and animals, and demands from the outset rigorous control experimentation. We wish to present for consideration the wisdom of restricting the term "Rickettsia" to proved pathogenic micro-organisms having the following characteristics: Small size, pleomorphism, slight affinity for aniline dyes, and intracellular habitat.

In the meantime "Rickettsia" will doubtless continue to be a loose but convenient group name for certain minute micro-organisms associated with arthropods. The name of the arthropod host usually serves the purpose of specific reference.

We wish to acknowledge our indebtedness to Dr. William A. Riley for living bedbugs sent to us from Porto Rico, and to Dr. O. A. Johannsen and Dr. Nathan Banks for identifying some of our specimens.

TABULAR SUMMARY OF RICKETTSIA-LIKE ORGANISMS IN ARTHROPODS

The table includes organisms described as rickettsiae which, doubtless, later will be excluded from the group

ARACHNIDA		Arthropod Hosts · Name of Rickettsia	Occurrence in Body	Morphology	Authors
ARANEIDA	ATTIDAE	*Salticus scenicus*, jumping spider[1] (Organism unnamed)	Intracel. f–b., nerves, egg-cells	Fil.	Cowdry, 1923
ACARINA	TROMBIDAE	*Atomus* sp., mite (Organism unnamed)	Intracel. hypoblast	Diplococ.	Cowdry, 1923
		Lucoppia curviseta, mite (Organism unnamed)	Intracel. int. epi.	Bac.	Cowdry, 1923
	ARGASIDAE	*Ornithodorus turicata*, tick (Organism unnamed)	Intracel. mal. t., eggs	Bac.	Cowdry, 1923
		Ornithodorus moubata, tick (Organism unnamed)	Intracel. sal. gl.	Cocco-bac.	Wolbach and Hertig, 1923
	IXODIDAE	*Amblyomma americana*, "lone-star" tick [1] (Organism unnamed)	Intracel., all tissues, egg-cells	Bac.; pleo.	Cowdry, 1923
		Amblyomma hebraeum, tick [1] (Organism unnamed)	Intracel. sal. gl., mal. t., egg-cells	Bac.; pleo.	Cowdry, 1923
		Boophilus decoloratus, tick [1] (Organism unnamed)	Intracel. egg-cells, mal. t.	Fil.; pleo.	Cowdry, 1923
		Dermacentor venustus, Rocky Mountain spotted fever tick *Dermacentroxenus rickettsi* [2,3,4]	Intracel., all organs	Coc., bac.	Wolbach, 1919
		Dermacentor variabilis, dog-tick (Organism unnamed)	Intracel. mal. t., eggs	Fil.	Cowdry, 1923
		Margaropus annulatus, tick [1] (Organism unnamed)	Intracel. egg-cells, mal. t., rectal sac	Bac.	Cowdry, 1923
		Margaropus annulatus, australis [1] (Organism unnamed)	Intracel. egg-cells, mal. t.	Bac.	Cowdry, 1923
		Rhipicephalus evertsi, tick [1] (Organism unnamed)	Intracel. int. epi., egg-cells, mal. t.; Extracel. int. lum.	Bac., fil.	Cowdry, 1923
		Rhipicephalus sanguineus, tick (Organism unnamed)	Intracel. mal. t., egg-cells	Bac.; pleo.	Cowdry, 1923
	GAMASIDAE	*Dermanyssus* sp., mite from doves [5] (Organism unnamed)	Extracel. int. lum.	Coc.	Reichenow, 1922
		Dermanyssus avium, bird-mite [5] (Organism unnamed)	Intracel.? int.	Cocco-bac.	Nöller, 1920

[1] Transmission via egg probable.
[2] Pathogenic to man.
[3] Cultivated in tissue culture, rickettsiae filamentous and bacillary (Wolbach and Schlesinger, 1923).
[4] Transmission via egg demonstrated.
[5] Not sufficient data from which to judge relationship to rickettsiae.

HEXAPODA		Arthropod Hosts Name of Rickettsia	Occurrence in Body	Morphology	Authors
THYSANURA	CINURA	*Lepisma saccharina*, "silverfish" (Organism unnamed)	Intracel. int. epi., f-b., ganglia	Bac.	Cowdry, 1923
CORRODENTIA	PSOCIDAE	*Psocus* sp., dust-louse [1] (Organism unnamed)	Extracel. int. lum.	Coc.	Sikora, 1918, 1920
	ATROPIDAE	*Dorypteryx pallida*, book-louse [2] (Organism unnamed)	Intracel. mal. t., ov.	Coc., bac., gran.	Wolbach and Hertig
MALLOPHAGA	TRICHODECTIDAE	*Trichodectes pilosus*, horse-louse [3] *Rickettsia trichodectae*	Extracel. int. lum.	Coc., bac.	Hindle, 1921
		Trichodectes climax, goat-louse [3] (Organism unnamed)	Extracel. int. lum.	Coc.	Arkwright, 1923
	PHILOPTERIDAE	*Lipeurus baculus*, from doves [3] (Organism unnamed)	Extracel. int. lum.	Coc.?	Sikora, 1922
	LIOTHEIDAE	*Trinoton* sp., from black-martin [3] (Organism unnamed)	Extracel. int. lum.	Coc.?	Sikora, 1922
		Menopon pallidum, hen-louse [3] (Organisms unnamed)	Intracel.? Coelomic?	Coc.	Sikora, 1922 Wolbach and Hertig
HEMIPTERA	PEDICULIDAE	*Pediculus humanus*, body-louse [4, 5] *Rickettsia prowazeki*	Intracel. mid-int. epi.	Coc., bac., fil.; pleo.	Rocha-Lima et al., 1916
		Pediculus humanus, body-louse [6] *Rickettsia rocha-limae*	Intracel. mid-int. epi.; extracel. int. lum.	Coc., bac., fil.; pleo.	Weigl; see Rosenberger, 1922
		Pediculus humanus, body-louse [2, 6, 7] *Rickettsia pediculi* (? *R. quintana*, *R. wolhynica*)	Extracel. mid-int. lum.	Coc.	Topfer, 1916; Munk and Rocha-Lima, 1917
		Linognathus stenopsis, goat-louse [3] *Rickettsia linognathi*	Extracel. mid-int. lum.	Coc.	Hindle, 1921
		Phthirius pubis, public louse [3] (Organism unnamed)	Extracel. int. lum.	Coc., bac.; pleo.	Guimarais, 1922
	ACANTHIIDAE	*Cimex lectularius*, bedbug [2] *Rickettsia lectularia* (Probably similar organisms in other species of *Cimex*)	Intracel. mycetome, mal. t., ov., f-b., int. epi., etc.	Coc., bac., motile fil., gran.; very pleo.	Arkwright, Atkin, and Bacot, 1921; Buchner, 1921, 1923
NEUROPTERA	CHRYSOPIDAE	*Chrysopa oculata*, lace-winged fly (Organism unnamed)	Intracel. f-b.	Bac.	Cowdry, 1923

[1] Transmission via egg probable.
[2] Transmission via egg demonstrated.
[3] Not sufficient data from which to judge relationship to rickettsiae.
[4] Pathogenic to man.
[5] Virus of typhus fever cultivated in tissue culture (Wolbach and Schlesinger, 1923).
[6] Cultivated on artificial media.
[7] Cultivated in coelom of *Pediculus humanus*, body-louse (Sikora, 1921).

		Arthropod Hosts Name of Rickettsia	Occurrence in Body	Morphology	Authors
DIPTERA	CULICIDAE	*Culex pipiens*, mosquito [1] (Organism unnamed)	Extracel. oes. div.	Coc.	Nöller, Sikora, 1920
		Culex pipiens, mosquito [2] (Organism unnamed)	Intracel. ov., testes	Coc., bac., fil., gran.; pleo.	Wolbach and Hertig
	CHIRONOMIDAE	*Culicoides sanguisuga*, sand-fly [3] (Organism unnamed)	Intracel. f–b., ov. coelomic?	Coc., bac.	Wolbach and Hertig
	TABANIDAE	*Tabanus pumilis*, horse-fly [3] (Organism unnamed)	Intracel., mal. t., pericard. cells	Bac., coc., fil.	Wolbach and Hertig
		Tabanus costalis, greenhead [3] (Organism unnamed)	Intracel. pericard. cells	Coc., bac.	Wolbach and Hertig
	HIPPOBOSCIDAE	*Melophagus ovinus*, sheep-ked [2,4] *Rickettsia melophagi*	Extracel. mid-int. lum.; Intracel.?	Coc.	Nöller, 1917, a–b, Jungmann, 1918
SIPHONAPTERA	PULICIDAE	*Ctenophalus felis*, cat-flea [3,5,6] *Rickettsia ctenocephali*	Coelomic	Coc., bac.	Sikora, 1918, 1920
		Ctenophalus canis, dog-flea (Organism unnamed)	Intracel. sal. gl., f–b., int., mal. t., etc.	Bac.	Cowdry, 1923
		Pulex irritans, human flea (Organism unnamed)	Intracel. f–b., Coelomic; Extracel. int.?	Bac., fil.	Cowdry, 1923
COLEOPTERA	PTINIDAE	*Sitodrepa panicea*, drug-store beetle [2] (Organism unnamed)	Intracel. mal. t., f–b.	Coc., bac., gran., comma-shaped; pleo.	Wolbach and Hertig
HYMENOPTERA	ICHNEUMONIDAE	*Casinaria infesta*, ichneumon-fly (Organism unnamed)	Intracel. int. epi.; coelomic	Fil.	Cowdry, 1923

[1] Not sufficient data from which to judge relationship to rickettsiae.
[2] Transmission via egg demonstrated.
[3] Reaction to Gram-stain not known, but otherwise organism typical rickettsia.
[4] Cultivated on artificial media.
[5] Cultivated in coelom of *Pediculus humanus*, body-louse (Sikora, 1921).
[6] Transmission via egg probable.

ABBREVIATIONS

bac.	bacilliform	*intracel.*	intracellular
coc.	coccoid	*mal. t.*	Malpighian tubes
epi.	epithelium	*mid-int.*	mid-intestine
extracel.	extracellular	*oes. div.*	oesophageal diverticula
f–b.	fat-body	*ov.*	ovary
fil.	filamentous	*pericard.*	pericardial cells
gran.	swollen coccoid granules	*pleo.*	pleomorphic
int. lum.	lumen of intestine	*sal. gl.*	salivary gland

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DESCRIPTION OF PLATES XXVII–XXX

All the illustrations are stained with Giemsa's stain, with the exception of Figures 16 and 17, which have been stained with Goodpasture's stain.

PLATE XXVII

Fig. 1. Rickettsia of sheep-ked. Drawing at about 1400 diameters of cross-section of the gut of a sheep-ked, showing flagellates (*Crithidia melophagia*), and *Rickettsia melophagi*.

Fig. 2. *Rickettsia melophagi* in smear preparation. 2000 diameters.

Fig. 3. *Rickettsia melophagi* in an eleven day old pure culture. 2000 diameters.

Fig. 4. *Culex pipiens*. Micrococcus from oesophageal diverticulum. 2000 diameters.

Fig. 5. Rickettsia of *Culex pipiens*. Smear from ovary. 2000 diameters.

Figs. 6 and 6*a*. Rickettsia of *Culex pipiens*. Smear, testis of pupa raised in laboratory. 2000 diameters.

Fig. 7. Rickettsia of *Culex pipiens*. Smear, ovary. 2000 diameters.

Fig. 8. Rickettsia of *Culex pipiens*. Smear, ovary. 2000 diameters.

PLATE XXVIII

Fig. 9. Rickettsia of *Culex pipiens*. Smear, testis, of pupa. 2000 diameters.

Fig. 10. Rickettsia of *Culicoides sanguisuga*. Smear from fat-body, to show distribution of organisms in cell. 2000 diameters.

Fig. 11. Rickettsia of *Culicoides sanguisuga*. Smear from fat-body, to show distribution of organisms in cell. 2000 diameters.

Fig. 12. Smear of fat-body, Chrysops, showing cytoplasmic inclusions characteristic of tabanid fat cells. These inclusions simulate rickettsia.

Fig. 13. Rickettsia of *Tabanus pumilis*. Pericardial cell. Smear preparation. 1000 diameters.

Fig. 14. Rickettsia of *Tabanus pumilis*. Smear, pericardial cell. 2000 diameters.

Fig. 15. Rickettsia of *Tabanus costalis;* pericardial cell. 2000 diameters.

Fig. 16. *Rickettsia lectularia*. Cross-section of Malpighian tube showing cell distended with filamentous forms. 1000 diameters.

PLATE XXIX

Fig. 17. *Rickettsia lectularia*. Drawing, 1500 diameters. Cell of Malpighian tube filled with filamentous coccoid or rod forms.

Fig. 18. *Rickettsia lectularia*. Smear from mycetome, with variety of forms. 2000 diameters.

Fig. 19. *Rickettsia lectularia*. Smear from mycetome, with variety of forms. 2000 diameters.

Fig. 20. *Rickettsia lectularia*. Smear, Malpighian tube, showing predominance of coccoid forms.

Fig. 21. *Rickettsia lectularia*. Smear from ovary, showing long bacillary or filamentous forms. 2000 diameters.

Fig. 22. *Rickettsia lectularia*. Smear from Malpighian tube. Long bacillary or filamentous forms. 2000 diameters.

Fig. 23. *Rickettsia lectularia*. Smear from Malpighian tube showing extreme size of filamentous forms with irregular staining. Illustrative of Arkwright's and Bacot's "granular threads" which they believe give rise to the coccoid forms. 2000 diameters.

Fig. 24. *Rickettsia lectularia*. Smear from mycetome. Coccoid and granular forms of Buchner. 2000 diameters.

Fig. 25. *Rickettsia lectularia*, of mycetome, showing sharply bent rods, C-shaped and ring-formed organisms. 2000 diameters.

Fig. 26. *Rickettsia lectularia*. Smear from mycetome from a bedbug fed on guinea-pig, shows rolled up filaments or filament balls. 2000 diameters.

PLATE XXX

Fig. 27. *Rickettsia lectularia*. Smear of ovary showing filament balls. 2000 diameters.

Fig. 28. *Rickettsia lectularia*. Smear of egg, showing forms similar to those found in the mycetome. 2000 diameters.

Fig. 29. *Rickettsia ctenocephali*. Smear from coelomic cavity. 2000 diameters.

Fig. 30. Rickettsia from book-louse, *Dorypteryx pallida*. Smear from Malpighian tube. Organisms within cell. 2000 diameters.

Fig. 31. Rickettsia from book-louse, *Dorypteryx pallida*. Smear from Malpighian tube, coccoid forms and small rods. 2000 diameters.

Fig. 32. Rickettsia from book-louse, *Dorypteryx pallida*. Smear from ovary, showing the slightly larger coccoid and ovoid forms. 2000 diameters.

Fig. 33. Hen-louse, *Menopon pallidum*. Smear, bacteria constantly found in the crop. 2000 diameters.

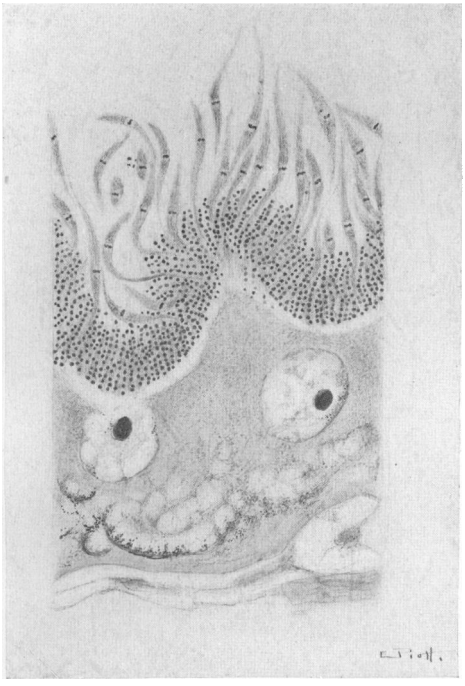
Fig. 34. Rickettsia of hen-louse, *Menopon pallidum*. Smear, Malpighian tube. 2000 diameters.

Fig. 35. Rickettsia of hen-louse, *Menopon pallidum*. Smear, Malpighian tube. Isolated, deeply stained rickettsiae. 2000 diameters.

Fig. 36. Rickettsia of drug-store beetle, *Sitodrepa panicea*. Smear, Malpighian tube, to show organisms within cell. 2000 diameters.

Fig. 37. Rickettsia of drug-store beetle, *Sitodrepa panicea*. Isolated organisms from Malpighian tube, intensely stained. 2000 diameters.

Fig. 38. Rickettsia of African tick, *Ornithodorus moubata*. Section of salivary gland, showing clumps of innumerable minute rickettsiae. 1300 diameters.



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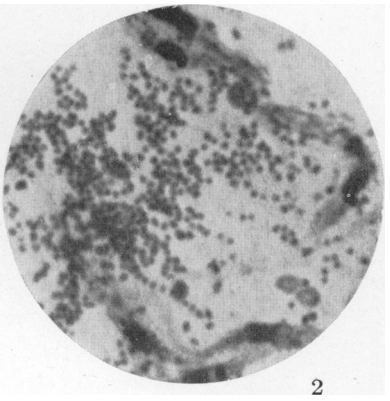

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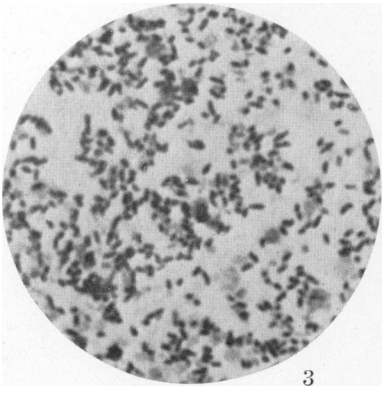

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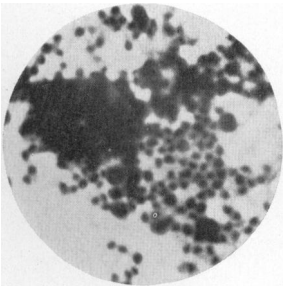

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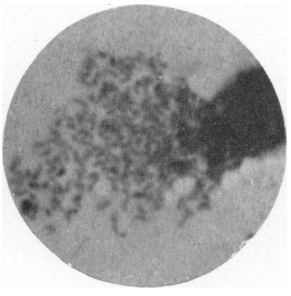

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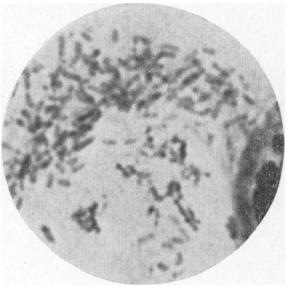

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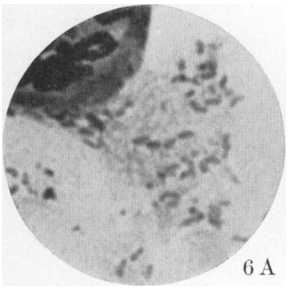

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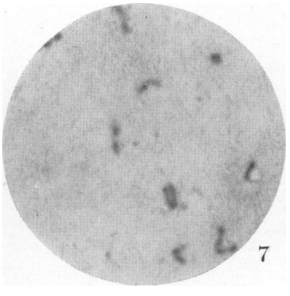

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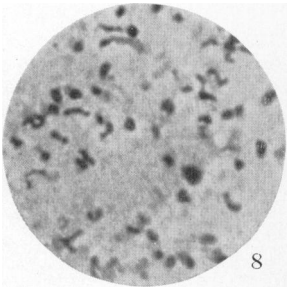

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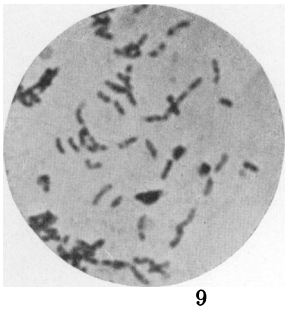

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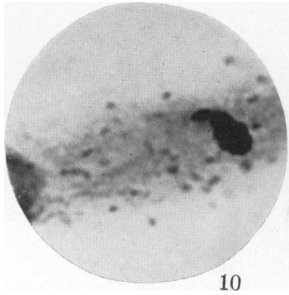

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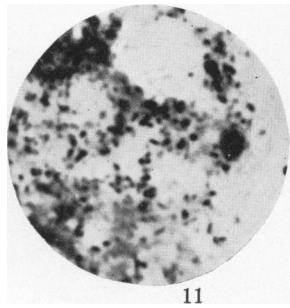

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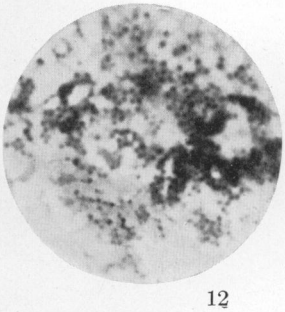

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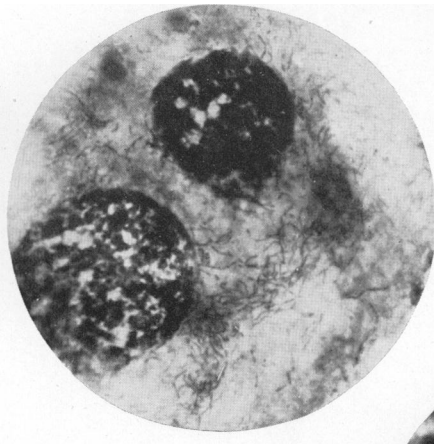

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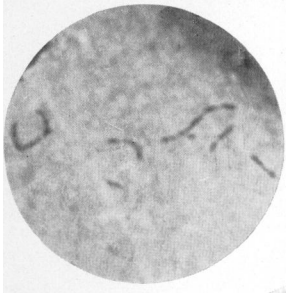

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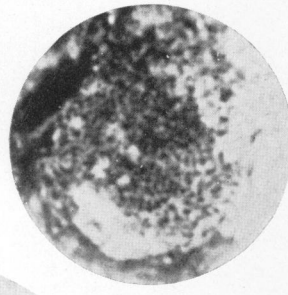

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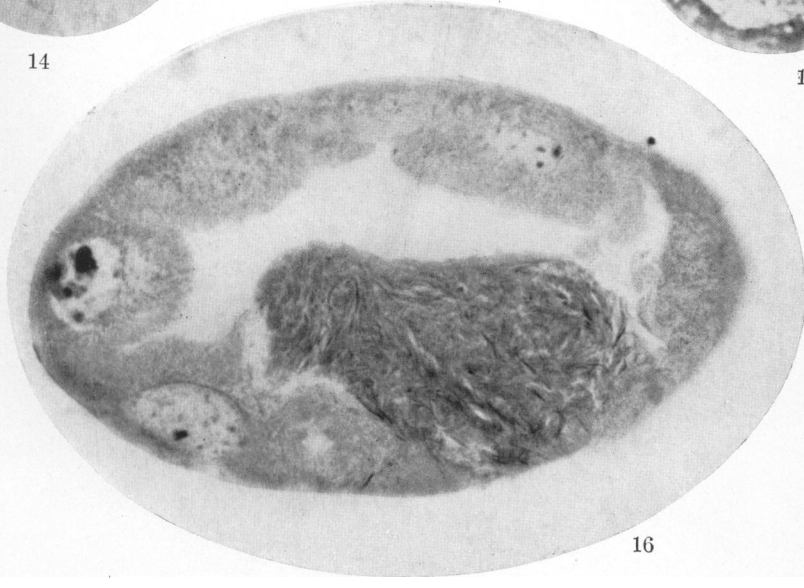

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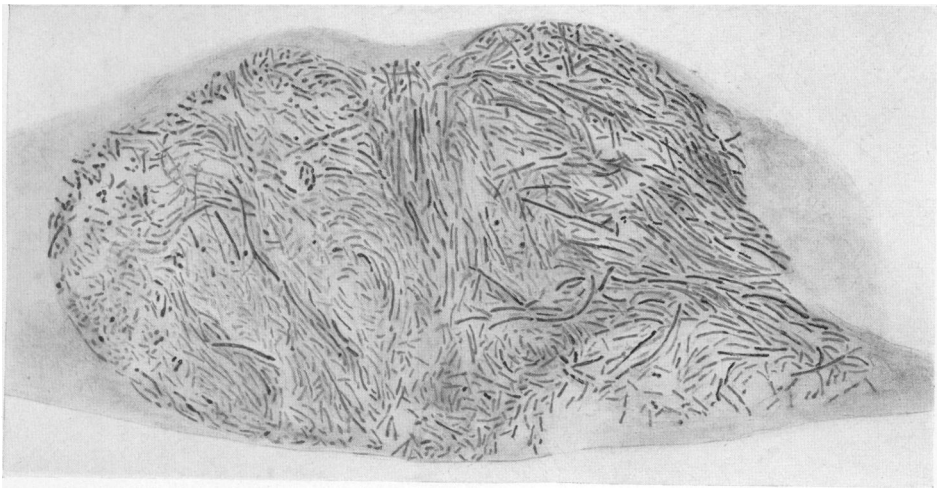

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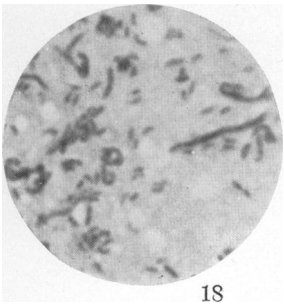

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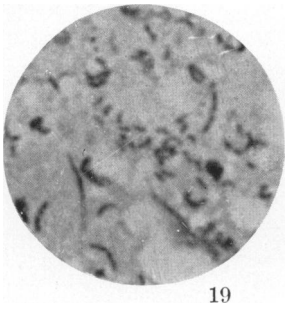

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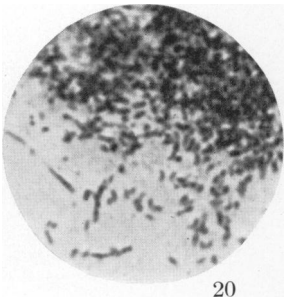

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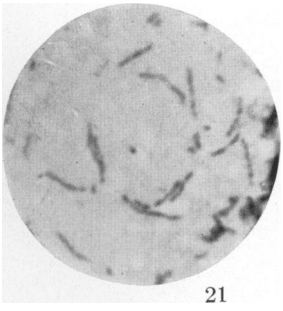

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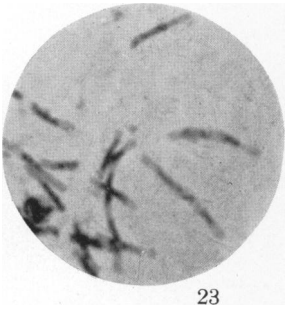

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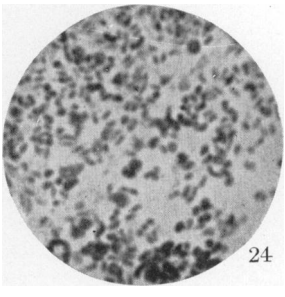

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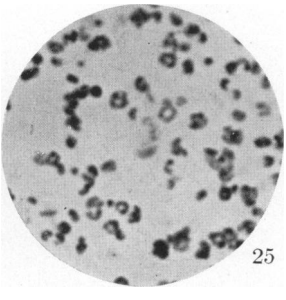

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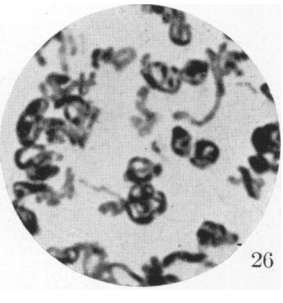

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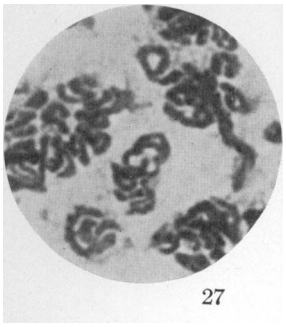
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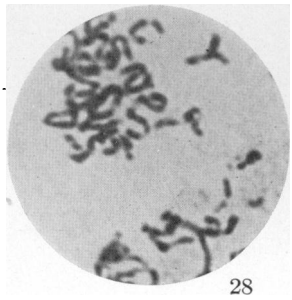
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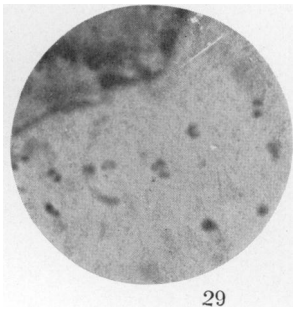
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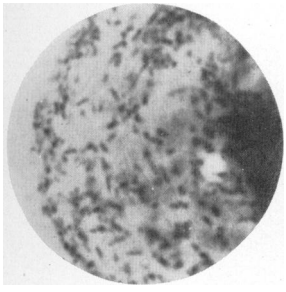
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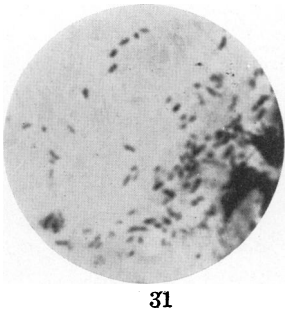
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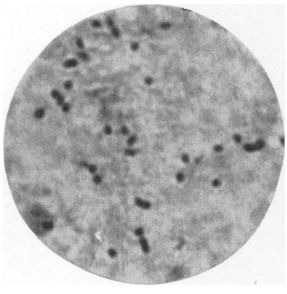
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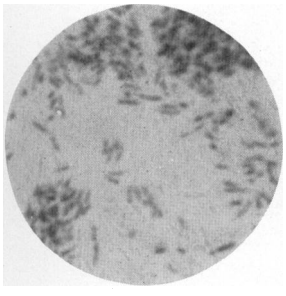
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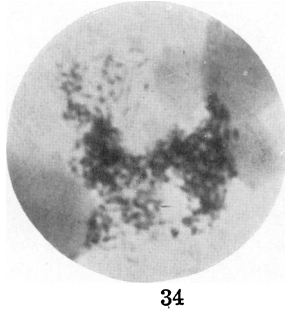
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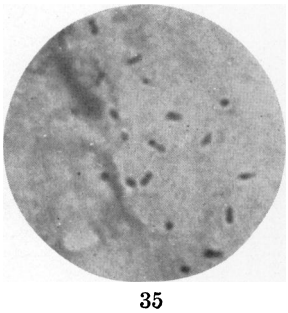
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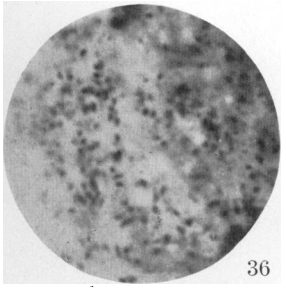
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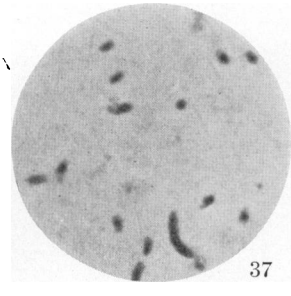
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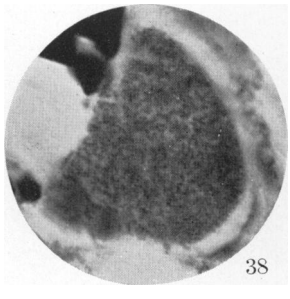
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