HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOLUME 24 APRIL, 1956 NUMBER 17

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EDWARD A. STEINHAUS, MARIECE M. BATEY, and CATHARINE L. BOERKE

UNIVERSITY OF CALIFORNIA · BERKELEY, CALIFORNIA

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EDWARD A. STEINHAUS,[2] MARIECE M. BATEY,[3] and CATHARINE L. BOERKE[3]

INTRODUCTION

SINCE the early years of the nineteenth century it has been known (e.g., see Dufour, 1833)[4] that the posterior end of the midgut in certain groups of Heteroptera is characterized by the presence of saclike appendages opening into it. These evaginations, called "gastric caeca" or simply "caeca," apparently serve a purpose different from that of the so-called gastric caeca of the Acrididae, and they appear almost always to contain specific bacteria. The bacteria from different Heteroptera may vary in their morphology, but they are relatively constant for a given insect species. The caeca themselves also vary a great deal in their morphology according to species, showing simple to complex arrangements, and apparently reflect basic phylogenetic relationships.

Although there have been a number of studies (e.g., those by Forbes, 1892; Glasgow, 1914; Kuskop, 1924; Rosenkranz, 1939; Schneider, 1940) very little has been accomplished toward revealing the nature, kind, or function of the caecal bacteria. Of their function we are most ignorant; certainly it can be said that the role of the bacteria, and their relation to the host insect, are not clearly understood. Glasgow (1914) assumed that the caecal bacteria not only inhibited the development of "foreign" or adventitious bacteria but excluded them altogether. Others (e.g., Kuskop, 1924) have thought they might play a nutritional role.

In size and shape, the bacteria, as they occur in different species of Heteroptera, range from small coccuslike bacilli to large vermiform or spirochetelike forms. Attempts to cultivate the bacteria on ordinary bacteriological media have not met with much success. Glasgow (1914) reported the cultivation of the symbiote from the caeca of the squash bug, *Anasa tristis* (De Geer), and of certain other coreids, in nutrient broth and agar; and Stein-

[1] Contribution from the Laboratory of Insect Pathology, Department of Biological Control, University of California, Berkeley. Part of a report on an investigation generously supported by a grant from the U.S. Public Health Service, Grant No. G4069(C). Submitted for publication September, 1955.

[2] Professor of Insect Pathology and Insect Pathologist in the Experiment Station, Berkeley.

[3] Senior Laboratory Technicians.

[4] See "Literature Cited" for citations referred to in the text by author and date.

haus (1951) found the symbiotes from the cactus bugs *Chelinidea vittiger* Uhler and *Chelinidea tabulata* (Burm.) capable of growing on nutrient agar and glucose nutrient agar. In none of these instances was any attempt made to identify the bacteria.

Recently, the opportunity presented itself again to study the symbiotes of *Chelinidea vittiger,* as well as those of several other Heteroptera, especially the squash bug, *Anasa tristis* (De Geer), the consperse stinkbug, *Euschistus conspersus* Uhler, and a bordered plant bug, *Euryophthalmus cinctus californicus* (Van Duzee). To report some of the findings resulting from this study is the principal objective of this paper.

MATERIALS AND METHODS

With the exception of certain immature stages reared in the laboratory, all specimens of *Chelinidea* used in this investigation were collected from *Opuntia* cacti on or near the campus of the University of California at Riverside, California. After being received in our Berkeley laboratory the bugs were placed in cages containing *Opuntia* plants, and so reared until used.

Specimens of *Anasa* were obtained from various cucurbitaceous plants near Riverside and San Jacinto in southern California. Some of these after being received in the laboratory were reared on banana squash and crook neck squash. The *Euschistus* used in these experiments were reared on green beans in our own laboratory from material originally collected near Berkeley. The *Euryophthalmus* were collected near Pacific Grove, California, and held on lupine in the laboratory.

Dissections were made in the usual manner using iridectomy instruments and minuten nadeln. The internal organs of the insect were exposed by removing the dorsal body wall. Dissections were made under sterile saline or dry. Standard aseptic procedures were followed.

Cultivation of the symbiotes was attained by transferring the caecal region of the insect's midgut to plates of nutrient agar or nutrient agar containing one per cent glucose. A variety of other media was tried but none proved any more suitable for cultivating the symbiote. On the surface of this medium the caeca were broken up and the contents smeared out. The plates were incubated at room temperature (23–25° C). Growth usually began to appear within 24 to 48 hours.

EXPERIMENTATION AND RESULTS

Chelinidea vittiger Uhler (Coreidae)

Anatomical Aspects

The alimentary tract of the adult female *Chelinidea vittiger* usually measures from 23 to 26 mm in length, with that of the caecal portion measuring from 7 to 8 mm. The alimentary tract of the adult male is approximately the same as that of the female, but the caecal region averages slightly less, about 6 to 7 mm.

The numerous outpocketings of the caecal region are arranged in two rows extending along opposite sides of the tubular fourth stomach. This caecal portion of the gut is slightly undulated. (Figures 1, 2, and 3.)

The Symbiotes

Previously successful attempts (Steinhaus, 1951) to cultivate the symbiotes from the caeca of *Chelinidea vittiger* were performed with only a few specimens of the insect, and no effort was made to identify the bacterium isolated. During the present study, the caeca from 80 bugs were removed aseptically and streaked on plates of nutrient agar containing one per cent glucose. Abundant growth was obtained in all instances. However, the colony appearance of the growth obtained from some of the bugs was not the same as that obtained from others. In one case the colony growth was of a thick, slimy, mucoid consistency, in the other it was smooth and glistening but not mucoid in character (figures 8 and 9).

The mucoid strains of the symbiote were isolated somewhat more frequently than the nonmucoid strains, the latter constituting about 37 per cent of the isolates. In only two instances were both the mucoid and the nonmucoid strains isolated in abundance from the same insect. Occasionally, on a plate showing one type of growth, a few colonies of the other type were noticed. Just what factor or factors determine whether an insect harbors the mucoid or the nonmucoid strain has not been determined. It does not appear to be associated with the sex of the insect, nor its stage of development. Nutritional factors were not exhaustively studied, but both types were isolated from different insects feeding on the same host plant in the laboratory. No relation could be detected between the occurrence of the two types and the host plants from which they were collected in nature (see table 1).

Most of the mucoid and nonmucoid strains were rather shortlived when held on artificial media such as nutrient agar. Occasionally a culture would die within a week or 10 days after being streaked. Usually, however, the mucoid strains remained viable for about 10 weeks, and the nonmucoid strains for about 14 weeks.

For the purpose of making a detailed cultural and biochemical study of the two types (mucoid and nonmucoid), two strains of each type were selected. The two mucoid strains carried culture numbers 20-3-1 and 20-45-1. The two nonmucoid strains were numbered 20-2-1 and 20-44-1. Strains 20-2-1 and 20-45-1 did not produce acid in glucose while strains 20-3-1 and 20-44-1 did have this ability. (The continuous and repeated culturing on a glucose substrate of the strains that did not attack glucose did not, in the few attempts made, give rise to strains that did attack this sugar.)

Serology. That the bacteria cultivated were in fact the caecal symbiote was indicated by: (1) morphological and tinctorial similarity between the symbiote in the insect and the cultivated forms; (2) the markedly abundant growth obtained from the caecal region of the gut in which no other types of bacteria could be seen upon microscopic examination, such growth almost invariably was a pure culture; (3) the constant repetition with which the bacteria were isolated from the caeca; and (4) the serological identification of the cultivated forms with the symbiotes of the insect.

In performing the latter tests, immune sera against cultivated strains 20-2-1 and 20-3-1 were prepared in rabbits. With this antisera, microscopic and macroscopic agglutination tests were run using as antigens suspensions

of symbiotes prepared directly from pooled caeca. In general, the symbiotes agglutinated in the antiserum prepared against the cultivated strains in titers as high as 1:1280, whereas in normal rabbit serum the symbiotes partially agglutinated only to a titer of 1:20 (occasionally 1:40). The mucoid and nonmucoid strains agglutinated to slightly higher titers in their respective antisera than they did in each other's antisera. The bacteria grown in cultures agglutinated in the homologous antisera to titers of 1:1280 and slightly in that of 1:2560.

TABLE 1

TYPE OF COLONY GROWTH OBTAINED FORM GROUPS OF *CHELINIDEA VITTIGER* UHLER COLLECTED FROM CACTI IN DIFFERENT LOCATIONS (IN THE SAME GENERAL AREA) AND SEPARATED ACCORDING TO SEX

Lot number	Insect number	Sex	Type of growth
A	1	♀	Mucoid
	2	♀	Nonmucoid
B	1	♀	Mucoid and nonmucoid mixed
C	1	♀	Nonmucoid
	2	♂	Nonmucoid
	3	♂	Nonmucoid
D	1	♂	Mucoid
	2	♂	Nonmucoid
	3	♀	Mucoid
	4	♀	Mucoid
E	1	♂	Nonmucoid
	2	♂	Nonmucoid
	3	♂	Nonmucoid
	4	♀	Nonmucoid
	5	♀	Mucoid
	6	♀	Mucoid and nonmucoid mixed
F	1	♂	Mucoid
	2	♂	Mucoid
	3	♂	Nonmucoid
	4	♀	Nonmucoid
	5	♀	Mucoid
	6	♀	Mucoid

Taxonomic Description

So far as is known, the gastric caeca of a given species of Heteroptera characteristically harbor a single species of bacterium, regardless of where or under what environmental conditions the host insect is found. For this reason, if for no other, the repeated isolation from the cactus bug of two distinct colony types (usually from different insects) suggested that here we may be concerned with two variants of the same bacterial species. Thinking that the two colony types isolated might represent S (smooth) and M (mucoid) forms of the well-known M $\rightleftharpoons$ S $\rightleftharpoons$ R type of dissociation, efforts were made to induce the development of such variants on artificial media. Since with most

bacteria the smooth form shows a tendency to change to the rough form, it was hoped that the latter would be revealed upon repeated subculturing or from the plating out of old broth cultures. No variants were thus revealed although we do not consider our experiments exhaustive in this regard. Nevertheless, it was felt that this evidence of nonmutability, as well as other cultural differences, did not constitute sufficient reason for considering the two types as representing distinct species.

TABLE 2

COMPARISON OF PHYSIOLOGICAL PROPERTIES OF TWO MUCOID AND TWO NONMUCOID STRAINS OF THE *CHELINIDEA* SYMBIOTE

Test or substrate	Mucoid type		Nonmucoid type	
	20-3-1	20-45-1	20-2-1	20-44-1
Gelatin liquefaction	–	–	–	–
Litmus milk	AR	R	Alk	Alk, sl R
Hydrogen sulfide	–	–	–	–
Nitrate reduction	+	+	+	+
Indole	–	–	–	–
Methyl red	–	–	–	–
Voges-Proskauer	–	–	–	–
Citrate	+	+	+	+
Starch hydrolysis	–	–	–	–
Uric acid	–	–	–	–
Glucose	A	–	–	A
Mannose	–	–	–	–
Arabinose	A	–	–	A
Xylose	–	–	–	–
Rhamnose	–	–	–	–
Sucrose	–	–	–	–
Maltose	–	–	–	–
Lactose	–	–	–	–
Trehalose	–	–	–	–
Inulin	–	–	–	–
Raffinose	–	–	–	–
Mannitol	–	–	–	–
Cellulose	–	–	–	–
Growth at 6° C	Abundant	Abundant	Scant	Scant
Growth at 25° C	Abundant	Abundant	Abundant	Abundant
Growth at 37° C	Very scant	Very scant	Moderate	Moderate
Growth at 42° C	Negative	Negative	Negative	Negative
Growth at 50° C	Negative	Negative	Negative	Negative

A = acid; Alk = alkaline; R = reduction.

It is admittedly difficult, for taxonomic purposes, to decide which of the supposed variations should be considered the parent form. It might be assumed that the mucoid type is a simple variant of the smooth type. Some authorities, however, do not believe that the mucoid character is only a more advanced expression of the smooth property of a culture since M and S strains can vary independently of each other.

Accordingly, we have decided tentatively to consider the mucoid and nonmucoid types as variants of, or representing a single bacterial species. The variations we have observed in fermentation reactions within each of the types also, in our opinion, indicate instabilities of a subspecific character.

The fact that the symbiote from *C. vittiger* is a gram-negative, polar-flagellate, straight rod suggested that it probably belongs to the tribe Pseudomonadeae in the family Pseudomonadaceae. Of the six genera in this tribe, as listed in the sixth edition of *Bergey's Manual of Determinative Bacteriology,* all but the genus *Pseudomonas* can be readily eliminated. Two more recently named genera, *Aeromonas* and *Zymonas,* can also be eliminated because their members possess physiological properties not characteristic of the symbiote from the cactus bug.

Pseudomonas is, in many respects, not a very clearly defined genus. Some species produce water-soluble pigments of a greenish hue; others, including the species with which we are concerned, produce no pigment of any kind. The habitat of most species is soil or water; a few are animal pathogens and many are plant pathogens. They frequently ferment glucose but do not ferment lactose. A careful comparison of the morphological, physiological, and cultural characteristics of the cactus-bug symbiote with the published descriptions of other species of *Pseudomonas* revealed no case where a close similarity or identity of species was involved. Accordingly, because the symbiote in numerous respects appears to be distinct from all previously described species, we provisionally assign it to the genus *Pseudomonas* and name it *Pseudomonas excibis* n.sp. (Gr. *ex,* out of, from; *kibisis,* pouch).

PSEUDOMONAS EXCIBIS N.SP.

Rods: 0.6 to 0.8 by 1.5 to 2.5 microns, occurring singly and in pairs. In insect host filamentous forms up to 25 microns in length may be common. Motile with polar flagella, usually monotrichous. In insect, generally nonmotile. Gram-negative.

Gelatin stab: No liquefaction.

Nutrient-agar colonies; mucoid type: Up to 5 mm diam., circular, entire, low convex, opaque center with translucent edge, grayish white, smooth, glistening. No diffusable pigments produced. Nonmucoid type: Up to 7 mm diam., circular, entire, low convex, opaque, grayish white, smooth, glistening. No diffusable pigments produced.

Glucose-agar colonies; mucoid type: Up to 23 mm, circular, entire, raised, opaque, grayish white, smooth, glistening, thick and mucoid in consistency. Nonmucoid type: Up to 10 mm diam., circular, low convex, opaque, center (3 mm) dull white, remainder of colony may be dull cream colored.

Nutrient-agar slant; mucoid type: Moderate, white, glistening, translucent, filiform. Nonmucoid type: Moderate, white, glistening, translucent, filiform.

Dextrose-agar slant; mucoid type: Abundant, white, glistening, opaque, thick, slimy, filiform, running to bottom of tube. Nonmucoid type: Abundant, white, glistening to dull, opaque, dry, filiform.

Nutrient broth; mucoid type: Turbid, thin pellicle, moderate sediment. Nonmucoid type: Turbid, moderate pellicle, slight sediment.

Litmus milk; mucoid type: Slight acid, reduction. Nonmucoid type: Very slightly alkaline.

Indole not formed.

Nitrates are reduced to nitrites.

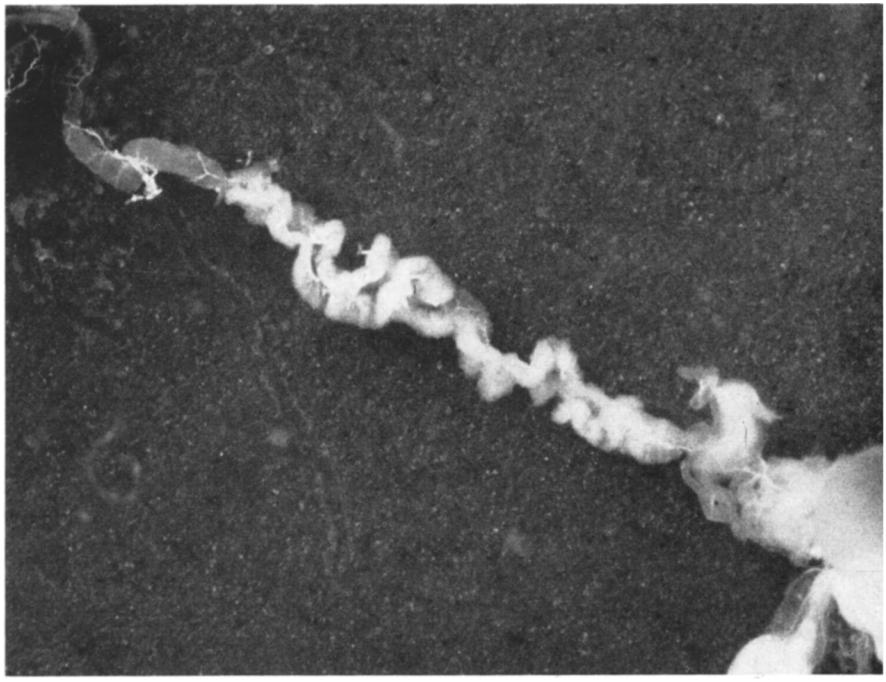

Fig. 1. Caecal region of the gut of *Chelinidea vittiger* Uhler (male).

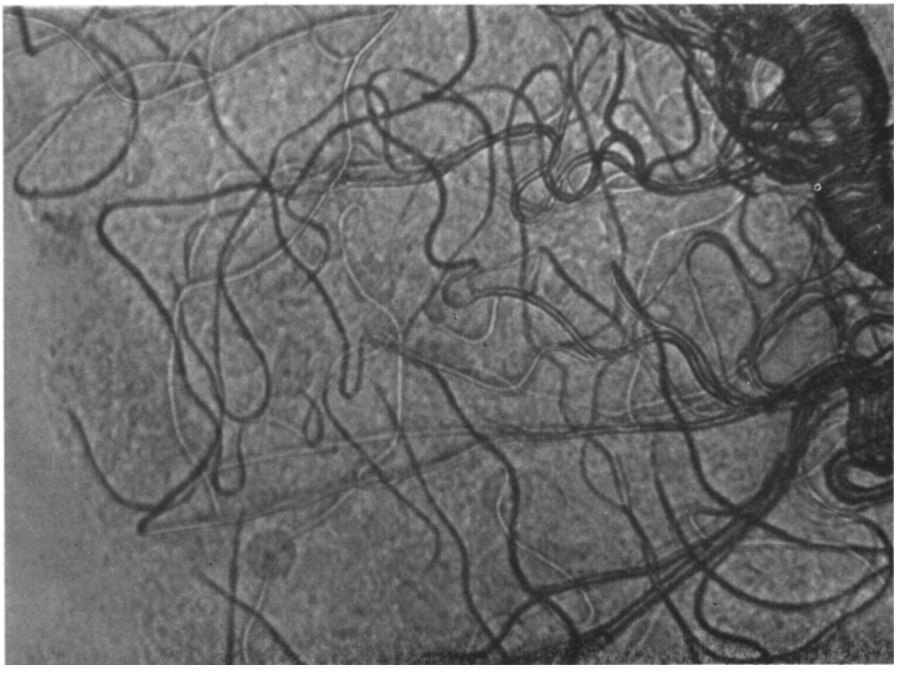

Fig. 2. Wet mount of caeca of *C. vittiger* showing abundant tracheation.

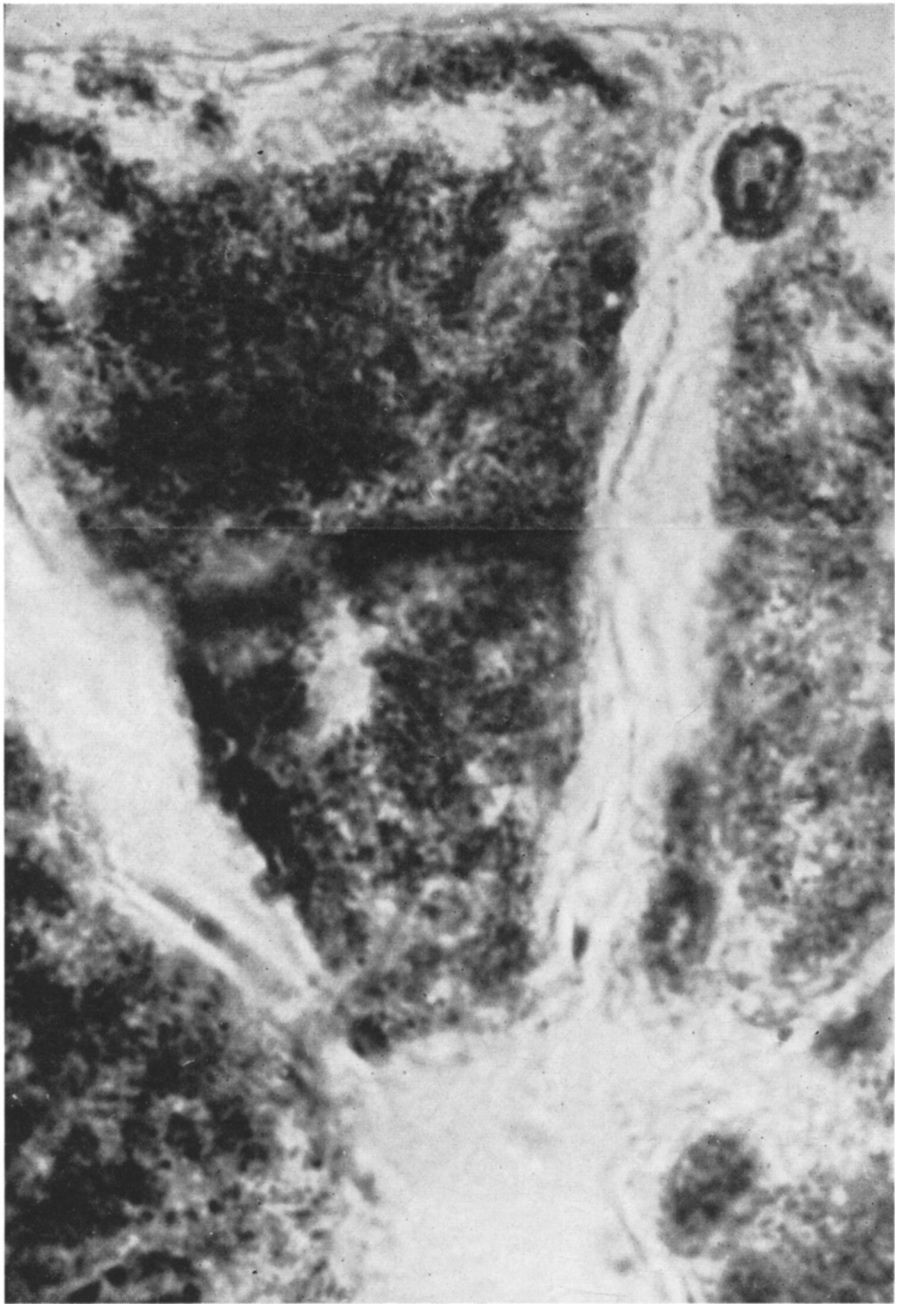

Fig. 3. Cross section of caecal region of *C. vittiger* showing symbiotes in lumen of caeca. (Fixed in Bouin's fluid; stained with Giemsa solution.)

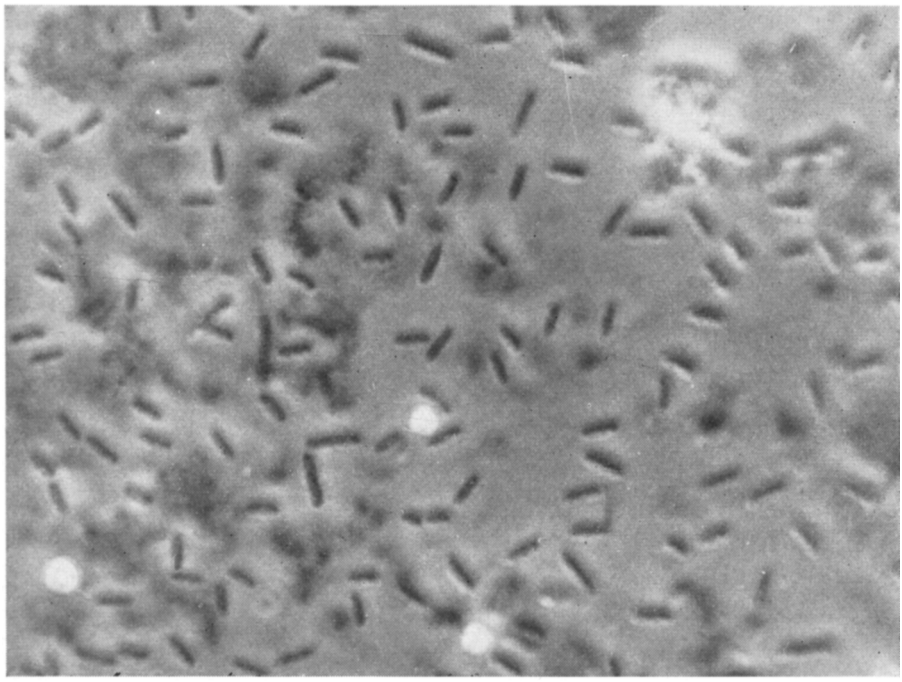

Fig. 4. Wet mount from caeca of *C. vittiger* showing symbiote (*Pseudomonas excibis* n.sp.) as seen by phase microscopy.

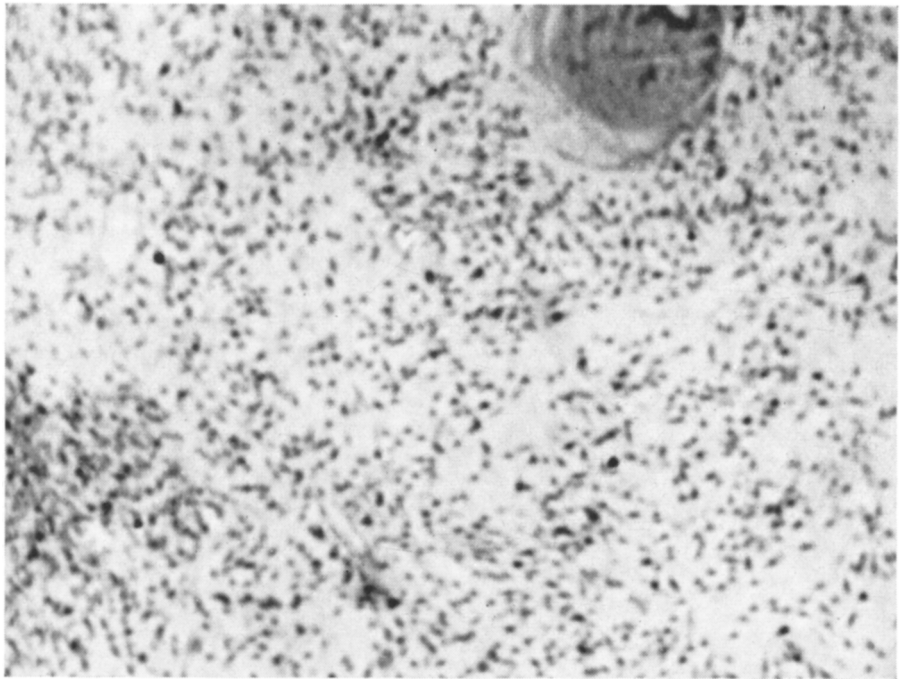

Fig. 5. Giemsa-stained smear of symbiotes in caeca of *C. vittiger*.

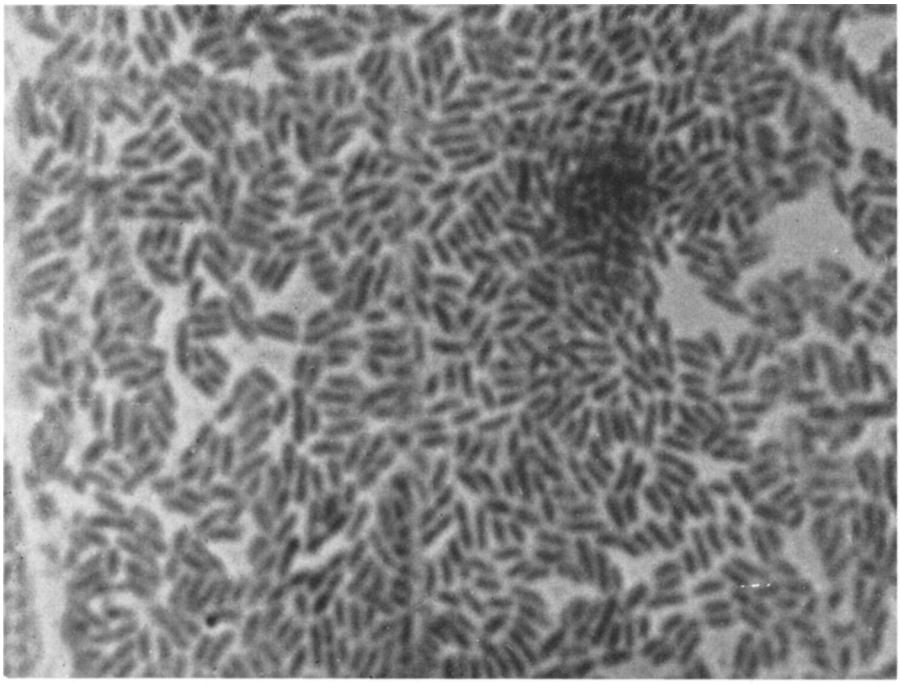

Fig. 6. Gram-stained smear of 24-hour glucose-agar culture of symbiote (mucoid strain) from caeca of *C. vittiger*.

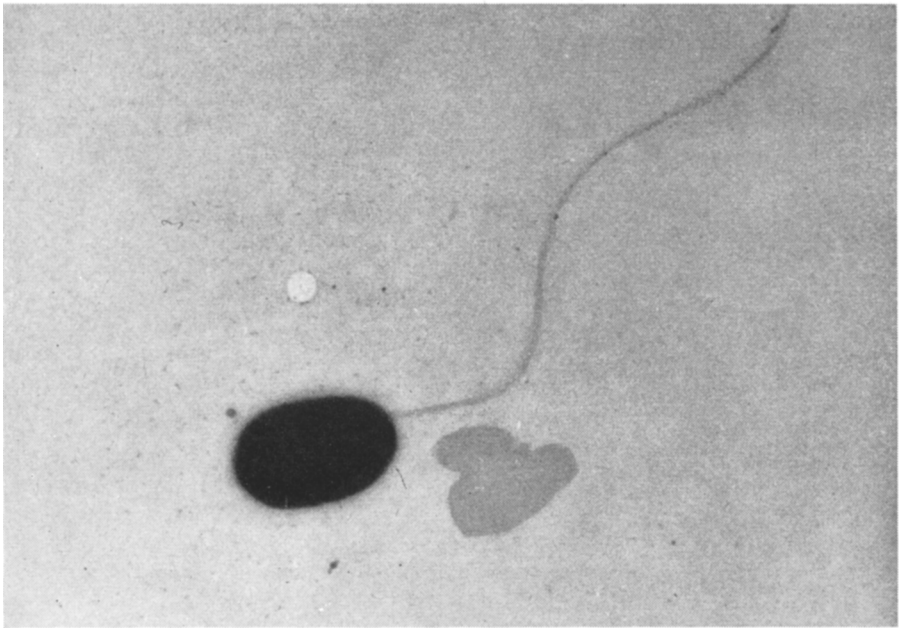

Fig. 7. Electron micrograph of symbiote isolated from caeca of *C. vittiger*, and showing the characteristic single flagellum.

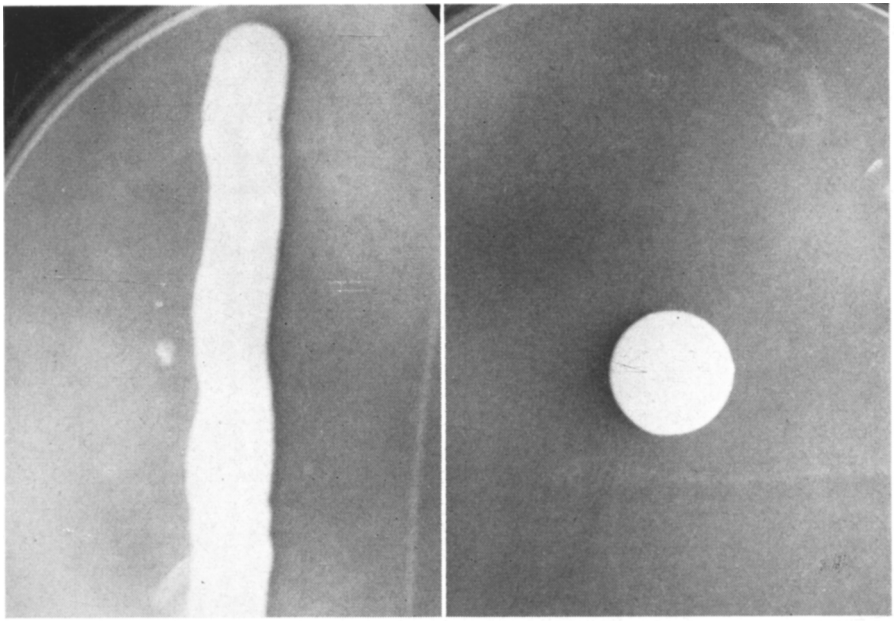

Fig. 8. (Left) Streak of nonmucoid strain of symbiote from *C. vittiger*, growing on glucose agar. (Right) Colony of nonmucoid strain.

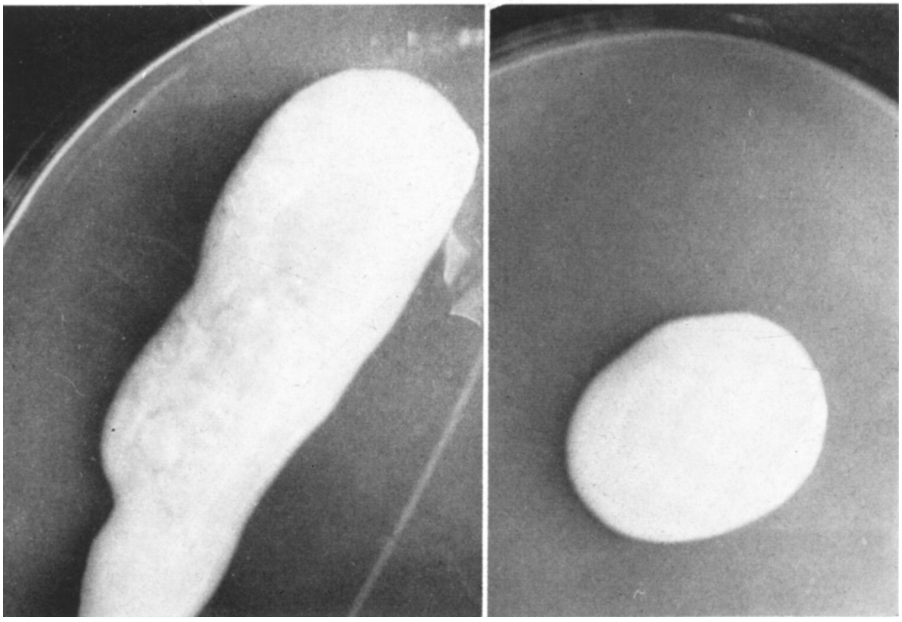

Fig. 9. (Left) Streak of mucoid strain of symbiote from *C. vittiger*, growing on glucose agar. (Right) Colony of mucoid strain. Compare with figure 8.

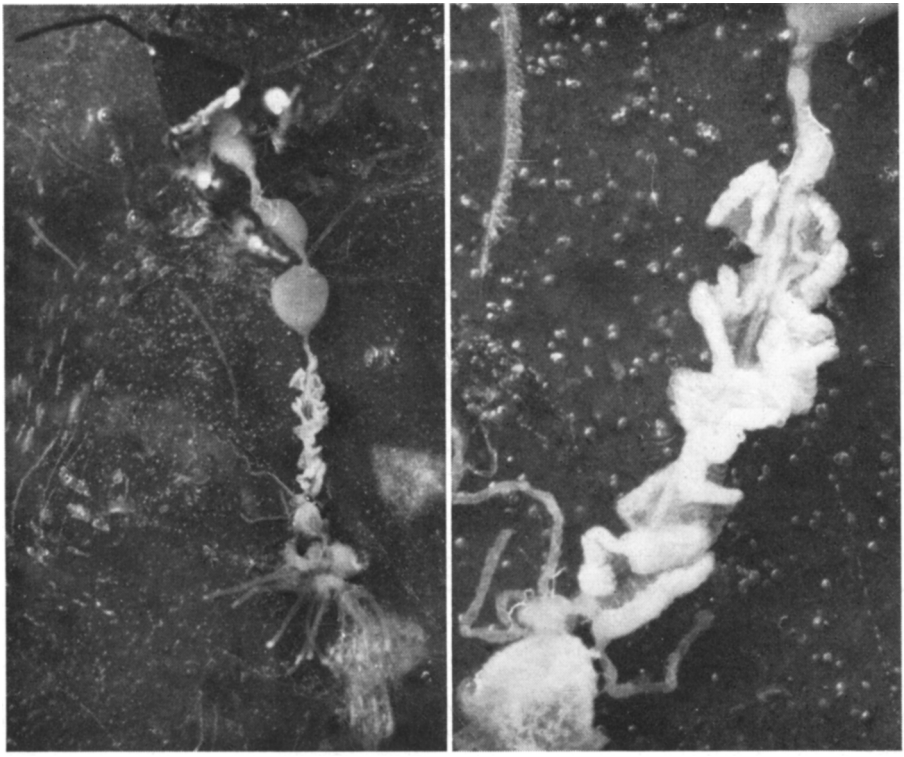

Fig. 10. (Left) View of entire alimentary tract of *Euryophthalmus cinctus californicus* (Van Duzee). (Right) Close-up showing caecal region.

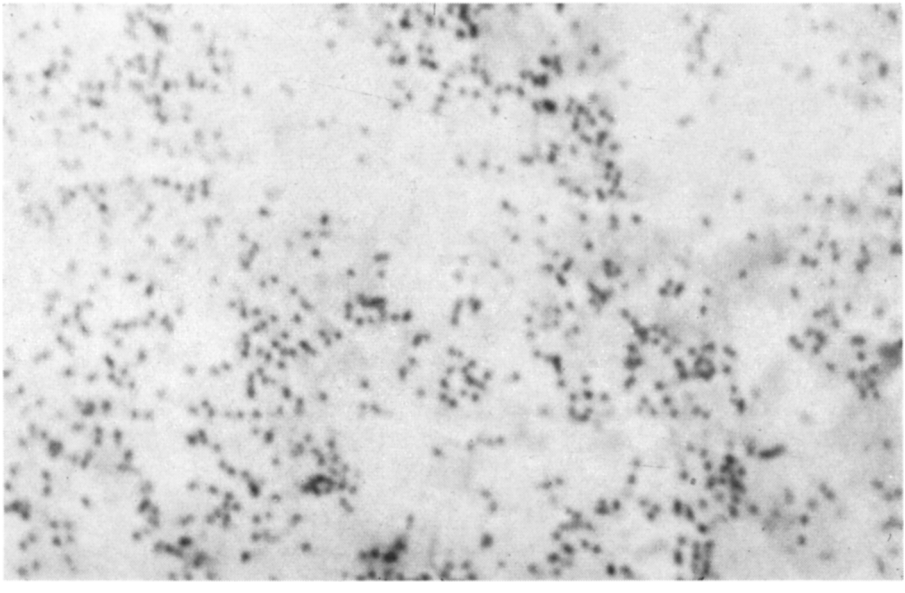

Fig. 11. Giemsa-stained smear of symbiote as it occurs in caeca of *E. cinctus californicus.*

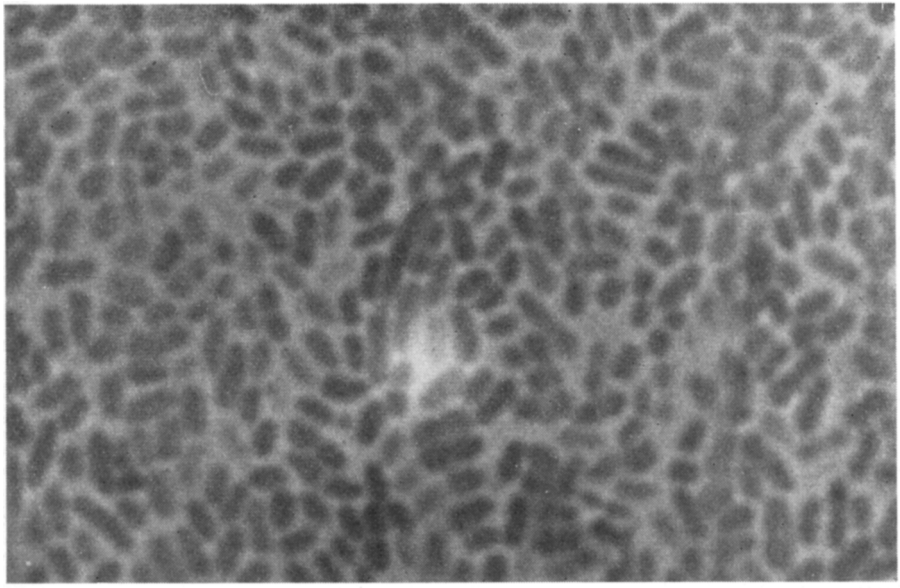

Fig. 12. Gram-stained smear of bacterium (*Pseudomonas nactus* n.sp.) from *E. cinctus californicus* grown on glucose agar for 48 hours (magnification slightly larger than figure 11).

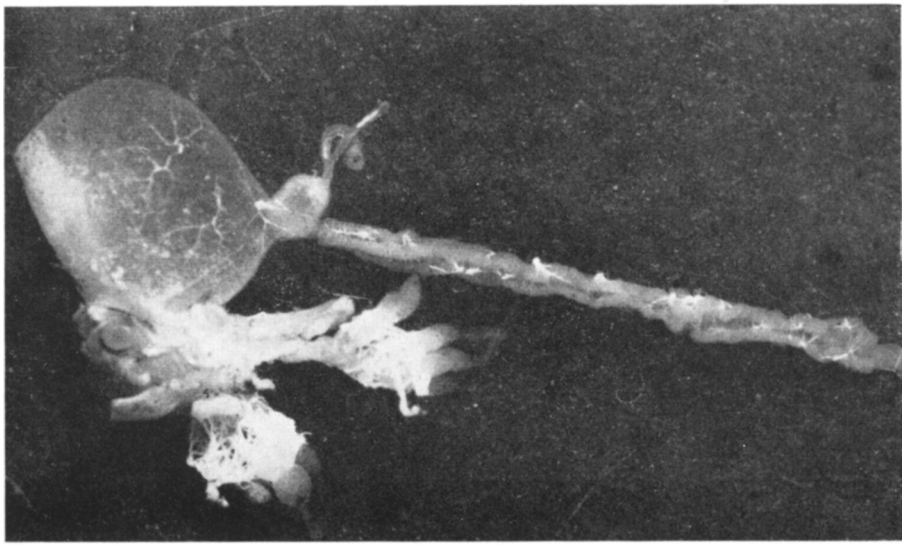

Fig. 13. Caecal region of the gut of *Euschistus conspersus* Uhler (female).

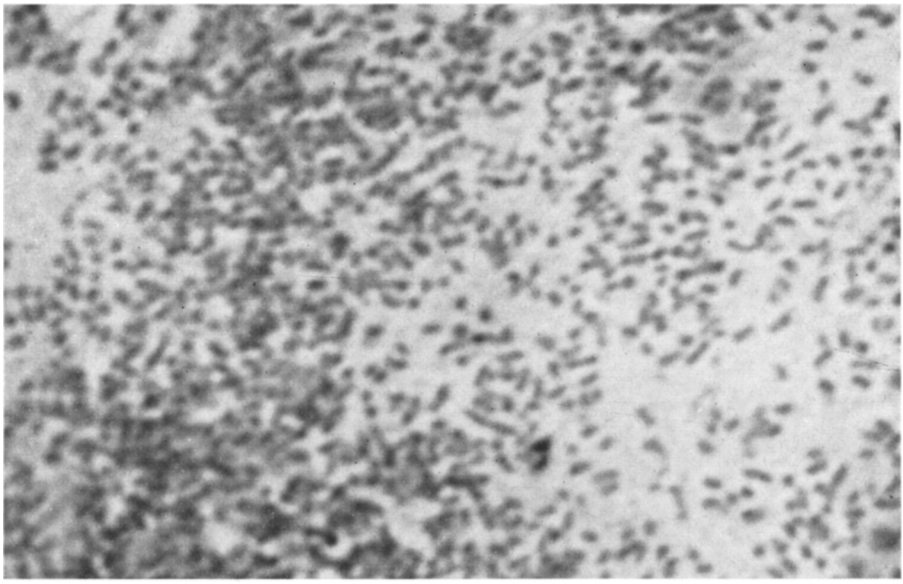

Fig. 14. Gram-stained smear of symbiotes directly from caeca of *E. conspersus.*

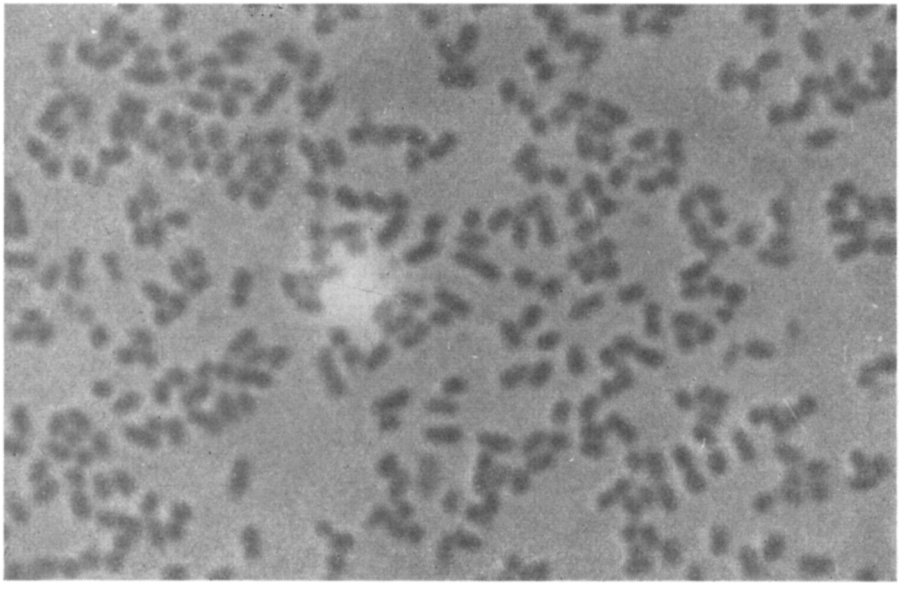

Fig. 15. Gram-stained smear of bacterium (*Pseudomonas* sp.) cultured from caeca of *E. conspersus.*

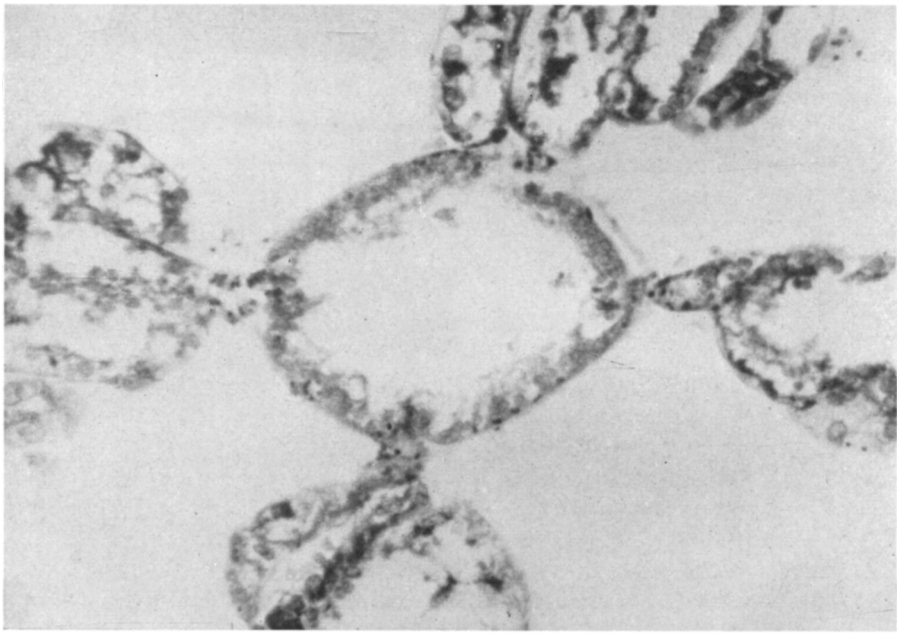

Fig. 16. Cross section of caecal region of the gut of *E. conspersus*. (Fixed in Bouin's fluid; stained with Giemsa solution.)

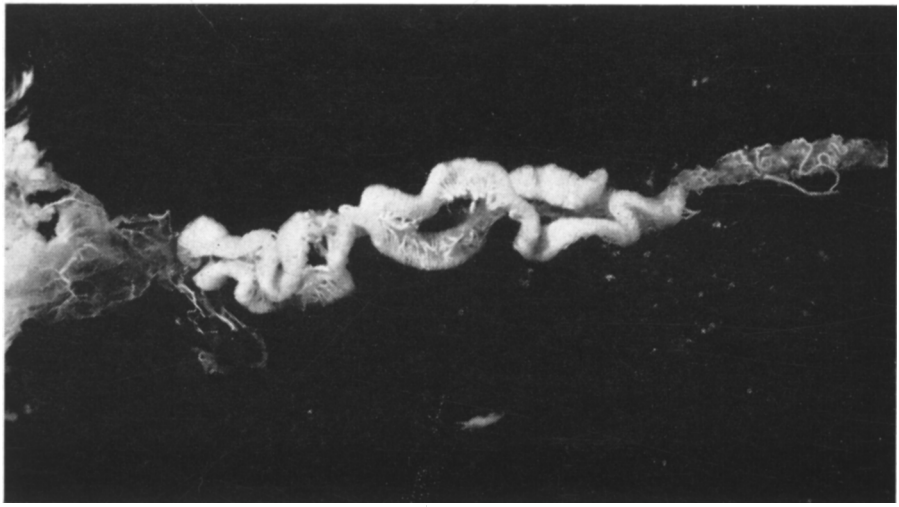

Fig. 17. Caecal region of the gut of *Anasa tristis* (De Geer) (male).

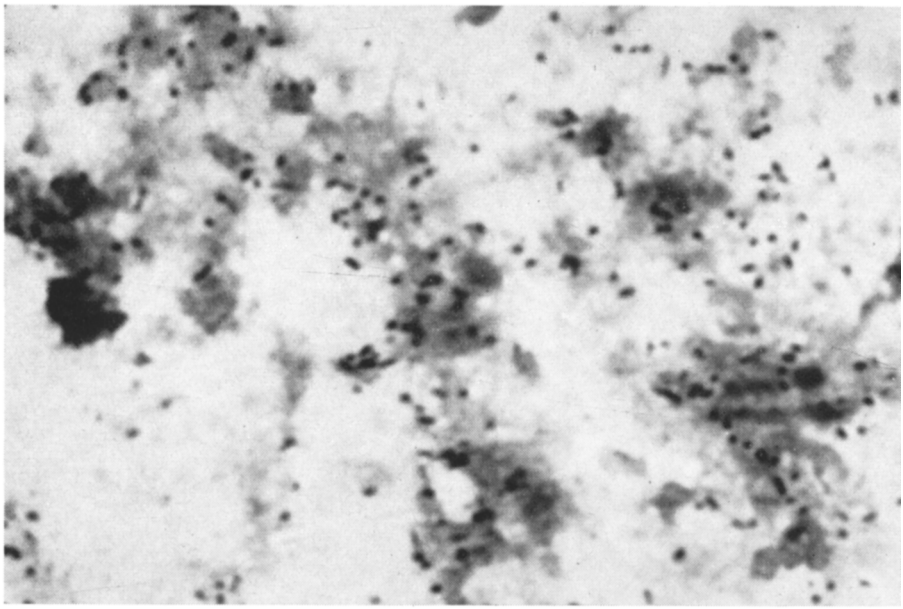

Fig. 18. Giemsa-stained smear of symbiotes directly from caeca of *A. tristis*.

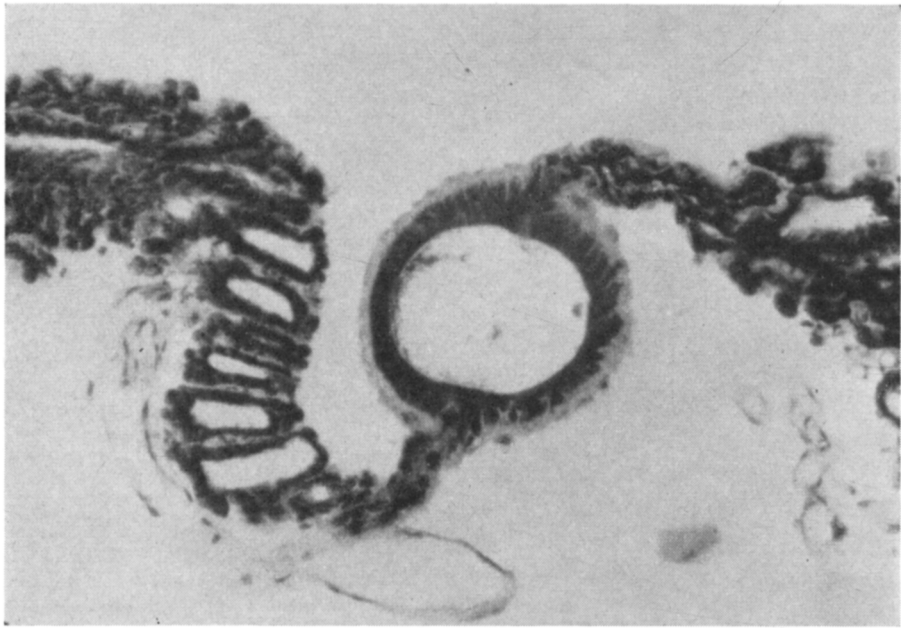

Fig. 19. Cross section of caecal region of the gut of *A. tristis*. (Fixed in Bouin's fluid; stained with Giemsa solution.)

Action in carbohydrates; mucoid type: Glucose and arabinose usually attacked with formation of acid. Mannose, xylose, sucrose, maltose, lactose, trehalose, raffinose, rhamnose, and mannitol not attacked. Nonmucoid type: Glucose and arabinose sometimes but not usually attacked; other carbohydrates not attacked.

Methyl-red test negative. Acetylmethylcarbinol not formed.

Citrates used as sole source of carbon. Hydrogen sulfide not formed.

Starch not hydrolyzed.

Urea not decomposed.

Aerobic, facultative.

Optimum temperature: Approximately 25° C. No growth at 42° and 50° C. Nonmucoid type grows moderately well at 37° C while the mucoid type grows very poorly at this temperature. At 6° C the mucoid strains grow well but the nonmucoid strains only scantily.

Nonpathogenic for the following insects, as tested by injection: *Cirphis unipuncta* (Haw.), *Prodenia praefica* Grote, *Malacosoma fragilis* (Stretch), *Heliothus armigera* (Hüb.), *Bombyx mori* Linn., *Sabulodes caberata* Guenée, *Junonia coenia* Hübner, and *Colias philodice eurytheme* Boisd.

Source and habitat: The gastric caeca of the cactus bug, *Chelinidea vit tiger* Uhler.

Caeca and Symbiotes of Other Heteroptera

Upon the completion of our study of the symbiote of *Chelinidea vittiger,* attempts were made to cultivate the symbiotes from the caeca of other Heteroptera, especially those of *Euryophthalmus cinctus californicus, Euschistus conspersus,* and *Anasa tristis.* Although encouraging and perhaps definitive results were obtained, it became necessary to abandon the project before certain inconsistencies could be worked out. Also, the isolation of the symbiotes did not proceed with the ease and uniformity which attended that of the symbiotes of *Chelinidea.* Therefore, the results about to be recounted are presented with certain qualifications and the bacteria isolated from the insects concerned, while probably new species, are, with one exception, not given names.

Euryophthalmus cinctus californicus (Van Duzee) (Pyrrhocoridae)

The caeca of *E. cinctus californicus* are arranged in two rows extending along opposite sides of the tubular fourth stomach. They appear as numerous outpocketings, the rows of which, apparently because of the heavy tracheation, take on an undulating appearance (fig. 10). The caecal region measures between 6 and 7 mm in the adult female, and slightly less in the male.

Like the symbiote from *Chelinidea vittiger,* that from *E. cinctus californicus* is a gram-negative, polar flagellate, straight rod, suggesting that it, too, belongs to the Pseudomonadeae. It differs from the *Chelinidea* bacterium in that it produces acid and gas in various carbohydrates, while the former produced no gas in any carbohydrate and formed acid in only two of them. Its other general characteristics, however, indicate that the wisest procedure at this time would be to assign the organism provisionally to the genus *Pseudomonas.*[5] Although definitive proof of the cultivated bacterium's

[5] After the manuscript of the present paper was submitted for publication, there appeared a paper by Gaby (1955) in which certain taxonomic problems relating to the

identity with the symbiote is lacking, a comparison with known species of *Pseudomonas* indicates that it is a new species. Accordingly, we hereby name it *Pseudomonas nactus* n.sp. (L. *nactus,* obtained, pp. of *nanciscor,* to obtain or to meet with).

The caeca from 15 of the bugs were removed, crushed, and placed on glucose agar. Moderate to abundant growth occurred on all plates. Except in four instances, all isolates appeared to represent the same bacterial species. (Morphologically the symbiotes, as they occur in the caeca of the insects, appear as very short gram-negative rods [fig. 11] slightly smaller than the bacterium [fig. 12] cultivated on artificial medium.) Three strains were selected as typical, and these were studied in some detail. The following description is based on this study.

PSEUDOMONAS NACTUS N.SP.

Rods: 0.7 to 0.8 by 1.0 to 1.5 microns, occurring singly. Motile with polar flagella. Monotrichous. Gram-negative.

Gelatin stab: Infundibular liquefaction.

Nutrient-agar colonies: 2 to 6 mm diam., circular, entire, slightly convex, smooth, glistening, opaque white; periphery somewhat mottled, especially on larger colonies, central portion smooth and glistening. No diffusable pigment produced.

Nutrient-agar slant: Opaque white, glistening, filiform, edge slightly wrinkled.

Nutrient broth: Turbid, slight sediment.

Litmus milk: Alkaline coagulation, reduction, peptonization.

Indole not formed.

Nitrates are reduced to nitrites.

Acid and gas produced in glucose, mannose, arabinose, xylose, sucrose, maltose, raffinose, and mannitol. Acid and sometimes gas formed in rhamnose and trehalose. Lactose and inulin not attacked.

Methyl-red test negative. Acetylmethylcarbinol is formed.

Citrate used as sole source of carbon.

Hydrogen sulfide not formed.

Starch not hydrolyzed.

Urea not decomposed.

Aerobic, facultative.

Optimum temperature: Approximately 25° C. Grows at 6° C. No growth at 42° or 50° C.

Source: The gastric caeca of a bordered plant bug, *Euryophthalmus cinctus californicus* (Van Duzee).

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identification of species within the genus *Pseudomonas* were discussed. Gaby pointed out the confusion now reigning in the genus, criticized the assignment of species to the genus solely on the basis of flagellation, and suggested certain biochemical characteristics for the genus. In all probability a bacterium such as the one herein described from *Euryophthalmus* would be excluded by Gaby from the genus *Pseudomonas,* although the present (6th) edition of *Bergey's Manual* admits it. Under the circumstances we shall temporarily assign it to this genus until the systematics of the bacteria concerned are elucidated. The distinctiveness of the species appears to be clear regardless of its eventual generic assignment.

Euschistus conspersus Uhler (Pentatomidae)

The caeca of *E. conspersus* consist of small outpocketings arranged in four rows extending along the sides of the posterior end of the midgut; the rows are usually very much undulated (figures 13 and 15). In the adult female the caecal region, when extended, measures between 6.5 and 7.5 mm; in the male, between 5 and 6 mm. The color of the caecal portion of the gut is usually a yellowish white; occasionally the last few posterior segments may be distinctly yellow in color.

As found in the caeca, the symbiote is a gram-negative small rod, approximately 0.7 by 1.5 microns in size, occurring singly and in pairs (fig. 14). Upon removing the caeca and streaking them out on plates of glucose nutrient agar (as well as numerous other media), bacterial growth is occasionally, but not always, obtained. Of a total of 59 attempts to cultivate the symbiote from the excised caeca, cultures were obtained in only 17 instances. Of the 17 strains isolated, 14 appeared to be a single species and probably represented the symbiotes (fig. 16). This was also indicated by the fact that when growth was obtained it was obtained abundantly, such as occurred in culturing the symbiote of *Chelinidea,* and such as is not characteristic of the growth of adventitious forms. Of the 14 virtually identical strains, three were selected for taxonomic study. A description based on this study follows. (Assignment to the genus *Pseudomonas* is provisional.)

PSEUDOMONAS SP.

Rods: 0.8 to 1.0 by 1.0 to 1.7 microns, occurring singly and in pairs. Sometimes exhibit a bipolar appearance. Motile with polar flagella. Monotrichous. Gram-negative.

Gelatin stab: Liquefaction.

Nutrient-agar colonies: 3 to 7 mm diam., circular, entire, low convex, opaque white, smooth, glistening. Some strains show a tendency to be mucoid especially on glucose agar on which the growth is usually thicker and more raised than on nutrient agar.

Nutrient-agar slant: Opaque grayish white, glistening, filiform, edge smooth, sometimes slightly wrinkled.

Nutrient broth: Moderate turbidity; slight sediment.

Litmus milk: Alkaline coagulation, peptonization.

Indole not formed.

Nitrates are reduced to nitrites.

Acid and gas produced in glucose, mannose, and sucrose. Only acid produced in maltose, trehalose, and mannitol (see text). Arabinose, xylose, rhamnose, lactose, inulin and raffinose not attacked.

Methyl-red test negative. Acetylmethylcarbinol is formed.

Citrate used as sole source of carbon.

Hydrogen sulfide not formed.

Starch not hydrolyzed.

Urea not decomposed.

Aerobic, facultative.

Optimum temperature: Approximately 25° C. No growth at 6°, 42°, or 50° C.

Source: The gastric caeca of the consperse stinkbug, *Euschistus conspersus* Uhler.

Of interest is the fact that small amounts of the bacteria cultivated from the caeca of *Euschistus,* when inoculated into the body cavity of normal, healthy bugs of the same species, frequently caused a septicemia that killed the insect. However, when a concentrated suspension of pooled triturated caeca was injected into the body cavity no untoward results occurred. Incidentally, many of the *Euschistus* examined in these experiments were found to harbor flagellates (Trypanosomidae) in their body cavities.

Anasa tristis (De Geer) (Coreidae)

Inasmuch as Glasgow's (1914) attempts to cultivate the caecal bacteria succeeded primarily with the squash bug, *Anasa tristis,* we thought it would be of interest to attempt to repeat the salient features of his work. Accordingly, experimental procedures we had been using were applied to squash bugs collected in southern California.

The caeca of *A. tristis* are arranged in two rows of outpocketings extending along opposite sides of the posterior end of the midgut (figures 17 and 19). The caecal portion of the gut is somewhat curled and undulated. In the adult female it measures between 8 and 10 mm, and somewhat less in the male (6 to 7 mm).

We found the bacteria contained in the caeca to be gram-negative small rods approximately 0.6 to 0.7 by 1.0 to 2.0 microns in size (fig. 18).

Using tubs of nutrient broth as a culture medium, Glasgow isolated from the eggs and caeca of *A. tristis* a bacterium which he did not identify but of which he says "clearly belongs in the large group of fluorescent bacteria that are so common in water and in soil generally." He was able to obtain pure cultures of the bacterium regularly and with ease from the caeca of the insect, and concluded that it was indeed the symbiote. When freshly isolated the bacteria were very short motile rods, averaging 0.7 to 0.9 micron in length, and usually arranged in pairs. After several days in nutrient broth the cultures frequently showed a variety of involution forms.

Our experience in attempting to cultivate the symbiote from *A. tristis* proved to be somewhat different from that of Glasgow's. In the first place, inoculating tubes of nutrient broth with the excised caeca usually did not yield pure cultures or yielded no growth whatever, so no great advantage could be seen in using this technique rather than a solid form of the same and similar media. However, occasionally the inoculated tubes did yield pure cultures of a bacterium that morphologically appeared to be identical to that occurring in the caeca of the insect. Of a total of 58 caeca streaked on plates of nutrient agar and/or glucose nutrient agar, only 32 yielded bacterial growth of any kind. In all but five instances ostensibly pure cultures were obtained; the second form in most of the cases of mixed growth was a coliform bacterium.

Of the 32 primary cultures isolated on solid media, 15 liquefied gelatin and produced acid or acid and gas in glucose, maltose, and sucrose, but not in lactose; six produced acid and gas in lactose as well as in the other carbohydrates and did or did not liquefy gelatin, and 11 attacked glucose slowly

but did not attack any of the other substrates (except gelatin after 3 weeks). However, all were gram-negative motile rods and could have represented the bacteria isolated, but not studied further, by Glasgow. Ten of the strains (Group I in table 3) we isolated were very similar, in fact almost identical to the strains isolated from the caeca of *Euryophthalmus* and which we have already described. This fact, at first, gave us some feeling that possibly these represented the symbiote. Such a supposition, however, would not explain the nature or source of the remaining 22 strains, particularly the group of 11 (Group II in table 3) that showed little physiological activity. These latter were virtually identical in morphology to the bacteria in the caeca, and to the pure cultures found in tubes of nutrient broth, and could very well represent the symbiote. Unfortunately, time did not permit the verification of any of the possibilities.

Thus we have been unable, on the one hand, to confirm Glasgow's results and conclusions, and on the other, to come to any definite conclusions of our own, as far as the caecal symbiote of *A. tristis* is concerned. The reactions and properties of the two groups of strains we considered most likely to represent the symbiote are included in table 3, which is presented in a manner that permits a comparison of the symbiotes or probable symbiotes of all four of the Heteroptera concerned. All are gram-negative, polar flagellate, straight rods, which suggests that they may all belong to the tribe Pseudomonadeae. However, since the taxonomy of this group is still rather vague and confused, we can assign these entomogenous bacteria to it only provisionally.

INCIDENTAL OBSERVATIONS ON OTHER SPECIES

During the course of this investigation we took advantage of the opportunity to examine the caecal arrangement of a few other species of Heteroptera that came our way, all of them collected in California. In each of these instances only from one to three specimens were available at the time. Nevertheless, sufficient information was obtained to justify the following report.

In addition to *Euschistus conspersus* Uhler, with which we have already dealt, six species of Pentatomidae were examined. These were: *Trichopepla californica* Van Duzee, *Cosmopepla conspicillaris* (Dallas), *Thyanta custator* (Fabricius), *Neottiglossa tumidifrons* Downes, *Peribalus abbreviatus* Uhler, and *Murgantia histrionica* Hahn. The caecal region of the alimentary tracts of all six consisted of numerous outpocketings arranged in four rows more or less along opposite sides of the tubular fourth stomach. In some species (*T. californica, N. tumidifrons,* and *P. abbreviatus*) the rows of outpocketings were more straight and parallel than in other species (*T. custator, C. conspicillaris,* and *M. histrionica*) in which the units more or less spiral about the alimentary tract. In all of these insects the symbiotes were gram-negative rods, usually of small dimensions (0.5 to 0.8 by 1.1 to 2.0 microns). In the case of *M. histrionica,* however, they appear as large convoluted forms, as has been described in previous publications (Glasgow, 1914; Steinhaus, 1951).

Incidentally, in the course of some experiments on the effects of antibiotics on the caecal flora, it was observed that streptomycin (Merck) ap-

parently caused marked morphological changes in the symbiotes of *M. histrionica*. Although these bacteria are normally very convoluted and pleomorphic, those in insects that have ingested streptomycin were even more pleomorphic and bizarre, with many vacuolated and apparently disintegrating forms. The streptomycin was administered via the vascular system of the host plant (kale).

TABLE 3

REACTIONS AND PROPERTIES OF THE PRINCIPAL BACTERIUM ISOLATED FROM THE CAECA OF THE FOUR HETEROPTERA CONCERNED

Test, substrate, or property	Bacterium from caeca of				
	Chelinidea	Anasa		Euschistus	Euryophthalmus
		Group I	Group II		
Gram-negative small rods	+	+	+	+	+
Motile (Polar flagella)	+	+	−	+	+
Gelatin liquefaction	−	+	± (slow)	+	+
Litmus milk	AlkR	AlkCP	−	AlkCP	AlkCR
Hydrogen sulfide	−	−	−	−	−
Nitrate reduction	+	+	−	+	+
Indole	−	−	−	−	−
Methyl red	−	−	−	−	−
Voges-Proskauer	−	+	−	+	+
Citrate	+	+	−	+	+
Starch hydrolysis	−	−		−	−
Uric acid	−	−		−	−
Glucose	A ±	AG	A	AG	AG
Mannose	−	AG		AG	AG
Arabinose	A ±	AG	−	−	AG
Xylose	−	AG	−	−	AG
Rhamnose	−	−	−	−	A
Sucrose	−	AG	−	AG	AG
Maltose	−	AG	−	A	AG
Lactose	−	−	−	−	−
Trehalose	−	AG		A	AG
Inulin	−	−	−	−	−
Raffinose	−	AG	−	−	AG
Mannitol	−	AG		A	AG
Growth at 6° C	+	+		−	+
Growth at 25° C	+	+	+	+	+
Growth at 42° C	−	−		−	−
Growth at 50° C	−	−		−	−

A = acid; Alk = alkaline; C = coagulation; G = gas; P = peptonization; R = reduction.

Three species of Scutelleridae were examined with regard to their caeca and symbiotes. Two of these were distinct but new species of *Eurygaster* (to be described by John D. Lattin), and one was *Homaemus parvulus* (Germar). The caecal region in each of these species consisted of four rows of outpocketings extending along the posterior end of the midgut, being considerably spiraled, and similar in appearance to that found in *Murgantia histrionica*. The symbiotes harbored in the caeca of these insects are gram-negative short rods. Our few observations confirmed the generalization made by Rosenkranz (1939) to the effect that all scutellerids and pentatomids

characteristically possess four rows of caeca. Quadruple rows of caecal units were also found in *Podops vanduzeei* Barber and Sailer (Podopidae), the symbiote of which is also a gram-negative small rod.

We were able to examine three species from the family Cydnidae: *Corimelaena extensa* Uhler and *Corimelaena nigra* Dallas, and *Aethus testudinatus* (Uhler). In these insects the caeca were found to consist of two rows of outpocketings occurring on opposite sides of the gut. The symbiotes were gram-negative small rods.

Narnia pallidicornis Stål (Coreidae) was found to possess a double row of caeca much like that of *Chelinidea* in appearance. The caeca were filled with gram-negative small rods. The caecal region from one was streaked on glucose nutrient agar from which abundant growth developed. It is entirely likely that this culture represented the symbiote. In its general characteristics it is very similar but not identical to the nonmucoid type strains isolated from *Chelinidea.*

Other species examined included an unidentified species of *Scolopostethus* (Lygaeidae), the caeca of which consist of four fingerlike tubes, two on each side of the gut. The symbiote is a gram-negative small rod, sometimes having a coccoid appearance.

Two of the Heteroptera examined were found not to possess caeca or outpocketings of any kind on the posterior portion of the midgut. These were *Oncopeltus fasciatus* Dallas (Lygaeidae) and *Leptocoris trivittatus* Say (Corizidae).

SUMMARY

The experiments and results described in this paper represent simply an attempt to cultivate on artificial media the caecal symbiotes of certain Heteroptera, and to learn more concerning the nature of these bacteria. Considerably more extensive studies are required before phylogenetic significance can be attached to the identity of the various species of caecal bacteria. The present study does highlight some of the difficulties that would attend such an effort. This report, therefore, is concerned primarily with certain bacteriological aspects of the subject; other authors have dealt with or touched on the significance of differences in the morphology of the caeca, on their presence or absence in certain species, on the function of the symbiotes, and on methods of transmission from generation to generation.

Most of our cultivation work was accomplished using the cactus bug, *Chelinidea vittiger* Uhler. From the caeca of this insect was regularly isolated a gram-negative bacterium which is described in detail and to which we have given the name *Pseudomonas excibis* n.sp. This symbiote was found to occur in one of two forms: in one the colony growth was of a thick, slimy, mucoid consistency; in the other it was smooth and glistening but not mucoid in character. The mucoid strains were isolated somewhat more frequently than the nonmucoid strains. In only two instances were both the mucoid and the nonmucoid strains isolated in abundance from the same insect. The factor or factors that determine the type of strain harbored by a particular insect were not determined, but several possibilities were eliminated.

Bacteria were isolated from the caeca of four other Heteroptera studied: *Euryophthalmus cinctus californicus* (Van Deuzee), *Euschistus conspersus*

Uhler, *Anasa tristis* (De Geer), and *Narnia pallidicornis* Stål. That from *E. cinctus californicus* was named *Pseudomonas nactus* n.sp. It is probable that the principal isolates obtained from each of these species represent the symbiote. Unfortunately, it became necessary to abandon the project before certain inconsistencies could be worked out.

Incidental observations on the caeca and symbiotes of 13 additional species of Heteroptera were made, and the essential features of these recorded.

ACKNOWLEDGMENTS

We wish to express our sincere thanks and appreciation to several individuals who kindly provided us with specimens of certain of the insects used in this investigation. All the specimens of *Chelinidea* were collected by Irvin M. Hall from cactus in the vicinity of Riverside, California. Dr. Hall also collected for us some of the *Anasa,* others of which were obtained from a colony being reared by Robert van den Bosch and E. J. Dietrick at Riverside. The *Euschistus* were reared in our own laboratory by Robert L. Langston. To John D. Lattin we are greatly indebted for specimens of most of the remaining species studied in this investigation.

LITERATURE CITED

BREED, R. S., E. G. D. MURRAY, and A. P. HITCHENS
1948. Bergey's manual of determinative bacteriology. 6th ed. Williams and Wilkins Company, Baltimore, 1529 pp.

DUFOUR, L.
1833. Recherches anatomiques et physiologiques sur le Hémiptères. Bachelier, Paris, 333 pp.

FORBES, S. A.
1892. Bacteria normal to digestive organs of Hemiptera. Illinois State Lab. Nat. Hist. Bul. **4**:1–7.

GABY, W. L.
1955. Taxonomic problems relating to the identification of species within the genus *Pseudomonas.* International Bulletin of Bacteriological Nomenclature and Taxonomy **5**:153–60.

GLASGOW, H.
1914. The gastric caeca and the caecal bacteria of the Heteroptera. Biol. Bul. **26**:101–70.

KUSKOP, M.
1924. Bakteriensymbiosen bei Wanzen. Arch f. Protistenk. **47**:350–85.

ROSENKRANZ, W.
1939. Die Symbiose der Pentatomiden (Hemiptera Heteroptera). Ztschr. f. Morph. u. Ökol. der Tiere **36**:279–309.

SCHNEIDER, G.
1940. Beitrage zur kenntnis der symbiontischen Einrichtungen der Heteropteren. Ztschr. f. Morph. u Ökol. der Tiere **36**:595–644.

STEINHAUS, E. A.
1951. Report on diagnoses of diseased insects, 1944–1950. Hilgardia **20**(22):629–78.

The journal *Hilgardia* is published at irregular intervals, in volumes of about 600 pages. The number of issues per volume varies.

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