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Symbiotes and the Nutrition of Medically Important Insects *

MARION A. BROOKS [1]

A discussion of this subject must revolve about two quite different subjects, since much of our information on the physiology of symbiotes is derived from insects of non-medical importance. As a generalization, arthropods possess symbiotic micro-organisms only if they feed on nutritionally inadequate (i.e., incomplete) diets during their *entire* life-cycle. Examples of such inadequate foods are wood or stored grains (rich in cellulose and deficient in nitrogen); wool, hair or feathers (rich in keratin, deficient in vitamins); plant juices (deficient in nitrogen); and blood or serum (deficient in B-vitamins).

Many of the blood-sucking insects with complete metamorphosis are scavengers or omnivorous feeders during their larval stages, and evidently at that time store up an adequate amount of vitamins to carry them through the reproductive phases of adult life. Insects capable of this type of feeding are represented by the sandflies, mosquitos, blackflies, horseflies, deerflies, and fleas, the larvae of all of them feeding on detritus, yeast or other micro-organisms, or on animal droppings. No one has found symbiotic bacteria or yeasts in constant internal association with these insects, although recently evidence has been presented that an apparently symbiotic *Rickettsia* is found intracellularly in the midgut of *Culex pipiens fatigans* (Micks et al., 1961).

On the other hand, blood-feeders with incomplete metamorphosis which feed on blood during all their nymphal stages do have symbiotic micro-organisms while, as a rule, their intestines are otherwise sterile. These arthropods are represented by bed-bugs, assassin-bugs, sucking- and chewing-lice, and ticks and mites. In addition, those blood-feeding Diptera which nourish the larval stages internally, the tsetse-flies and the Pupipara, also have symbiotes.

Cockroaches, which are of minor medical importance, are enigmatic since they are omnivorous feeders yet possess prominent and vitally important symbiotes. Finally, insects of non-medical importance which bear symbiotes and which have been well studied are termites, aphids, and stored-products beetles.

Because of the coincidence between possession of symbiotes and the eating of a blood-diet, it was for long assumed that the symbiotes are contributing either to digestion of the blood or to synthesis of vitamins. In tsetse-flies, the *Rickettsia*-like micro-organisms are transmitted *via* the milk-gland ducts of the female to the larva. Certain cells of the larval midgut wall, which had previously enlarged, now become infected with the symbiotes. Wigglesworth (1929) presented histological evidence against the blood-digestion hypothesis in three species of *Glossina*, since blood haematin is released from the erythrocytes in the midgut *posterior* to the zone of cells containing the symbiotes.

Rhodnius prolixus, a blood-feeding hemipteran parasitic on small mammals, is of little consequence to human health, but is closely related to *Triatoma infestans*, the vector of the flagellates of Chagas' disease, and thus is frequently studied. The symbiotic bacteria in *Rhodnius* are *Nocardia rhodnii*, located in crypts between the midgut cells produced by foldings of the epithelium, and thus they are readily expelled into the gut lumen where they become mixed with the ingested blood and are passed out with the faecal material. Since the eggs are laid in the detritus of the host's burrow, the eggs become contaminated with the insect faecal material containing the bacteria. During hatching, the young bugs inadvertently eat some bacteria and thus become infected. By surface-sterilizing eggs and rearing the bugs in aseptic or even simply clean surroundings, it is possible to obtain insects free of symbiotes and thus to study the additional nutri-

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[1] Associate Professor, Department of Entomology, Fisheries and Wildlife, University of Minnesota, St. Paul, Minnesota, USA.

tional needs of the aposymbiotic strain. The symbiotes of *Rhodnius* and *Triatoma* can be cultured on simple glucose agar.

Geigy, Halff & Kocher (1953, 1954) isolated and cultured the bacterial organisms from *Triatoma* and, although remarking on their similarity to *Nocardia rhodnii*, pointed out that they could not be identified with certainty; the authors thought the organism is very near to *Streptococcus viridans*. On a semi-synthetic medium, the cultured symbiote produced minimal amounts of a number of growth factors, but only folic acid was produced in significant excess. By comparing the production of folic acid with the folic acid requirement of the related *S. faecalis*, it was estimated that *S. viridans* (the cultured symbiote?) was producing 2500 times its own requirement. Although the aposymbiotic bugs could be fed on an artificial diet or sterile serum through a membrane, their requirement of folic acid could not be demonstrated; the authors ascribed this to use of an improper concentration of the vitamin.

Bewig & Schwarz (1955, 1956) studied the symbiotes of *Haematopinus suis*, *Glossina submorsitans*, *Rhodnius prolixus*, and *Triatoma infestans*. They concluded that the symbiotes of *Rhodnius* and of *Triatoma* are identical, both fitting closely the description of *Nocardia rhodnii*. Both kinds of bugs suffered similar deficiency symptoms of slow growth, crumpled wings, and failure of reproduction when reared free of their symbiotes. The bacteria from the two species could be reciprocally exchanged and were equally effective in alleviating the deficiency symptoms. However, the authors could not compensate for the bacteria by direct rectal injections of B-vitamins into the aposymbiotic bugs. Microbiological assays for vitamin B_{12}, folic acid, and folinic acid all failed because of the presence of an inhibitory factor in the culture filtrate. (They used *Leuconostoc citrovorum* and *Lactobacillus leichmannii*.) It was possible to replace the symbiotic *Nocardia* with free-living species of *Nocardia* or with *Mycobacterium phlei*. A number of other organisms (contaminants from the outer body surface) were also found in the gut lumen after inoculation, but they were without influence on the deficiency symptoms. The authors concluded that it was impossible to make any definite statement as to whether the symbiotes of *Rhodnius* and *Triatoma* provide their hosts with B-vitamins.

Baines (1956) attempted to elucidate the problem by injecting various concentrations and combinations of B-vitamins into the host's blood-stream immediately before the aposymbiotic bugs fed. He found that the omission, singly, of thiamine, pyridoxine, calcium pantothenate, or B_{12} were all detrimental to growth. This, of course, was only circumstantial evidence in favour of synthesis of these vitamins by the symbiotes. He concluded that niacin, folic acid, and biotin are all present in adequate concentrations in the host's blood to meet the requirements of the aposymbiotic bugs. The difficulty with the experiment lay in injecting an adequate amount of certain vitamins without injuring the mammals.

Harington (1960b) fed aposymbiotic *Rhodnius* through a rubber membrane on small, but known, amounts of oxalated horse-blood fortified with vitamins, and then allowed the bugs to feed to repletion on a live host. With this method he found that the addition of folic acid alone was not beneficial; thiamine alone was of doubtful value; but both vitamins at once supported quite good growth. Unfortunately, he used (or at least reported) results for only two aposymbiotic bugs on each experimental diet, so that one may not place much significance on these values. Microbiological determinations of the amounts of thiamine and of folic acid (using *Lactobacillus fermenti* and *Streptococcus faecalis* respectively) synthesized by *Nocardia rhodnii* in pure culture were more convincing, there being a 2·6-fold increase in the thiamine level and a fourfold increase in the folic acid level in seven days. The difficulty here lay in not knowing the amount of the vitamins required by the organisms for their own growth, so that it was not possible to determine the excess available for the host.

Various species of *Pediculus* have been studied for many years but the evidence from them is also less than definite. Aschner (1934) discovered that the unpaired, medial mycetome—the "stomach disc"—which can be seen glistening through the body wall, can be removed surgically from young lice or the embryonated eggs can be centrifuged early in development to dislocate the stomach disc so that the symbiotes are unable to migrate normally to the ovaries. Nymphs resulting from these eggs without symbiotes feed and develop normally for five or six days and then suddenly die. Adult lice deprived of their mycetome after the symbiotes have migrated out of it show no symptoms. Puchta (1955) found that, while defibrinated, haemolysed blood was inadequate for growth of the lice (either aposymbiotic or normal), he could rear them by rectal injections of large doses of either yeast extract or

B-vitamins. The aposymbiotic males were functional but the aposymbiotic females died without reproducing. Interestingly, overdoses of the vitamins or yeast extract were injurious, particularly to *normal* lice. Puchta concluded that aposymbiotic lice continue to synthesize significant amounts of B-vitamins, and that the function of the symbiotes is to regulate the proper concentration of particular B-vitamins.

Haddon (1956a, 1956b) has designed an artificial membrane and feeding arena which takes into consideration the feeding position and anatomy of the mouth-parts of lice. He has reared several generations of *Pediculus humanus corporis* on this device, using sterile, defibrinated, haemolysed human blood, although Puchta (1955) found this medium unsatisfactory. It now remains for someone to use this feeding technique for determining the additives needed by aposymbiotic lice.

The symbiotes of *Pediculus* are highly pleomorphic, changing with their site in the host, as well as with the latter's age and sex. Their identity is unknown.

The paired, glassy-white mycetomes of *Cimex lectularius* are located against the fat lobes in the third abdominal segment of the female, and along the vasa deferentia at the bases of the testes in the male. The symbiotes are minute, pleomorphic coccoid, diplococcoid, bacillary, lanceolate, or thread forms, designated as *Symbiotes lectularius* (*Rickettsia lectularia*). They also occur in the alimentary tract, Malpighian tubules, and accessory sex organs as well as in the gonads. They infect the nurse cells in the ovary, from where they pass along the nutrient cords to the oocytes. In the embryo they accumulate in the spherical aggregate forming the mycetome of the next generation. Although some work has been done on the efficiency of conversion of blood to body-weight (Johnson, 1960), I know of no one who has interrupted the cycle of transmission to study the nutritional requirements of the aposymbiotic bedbug.

The symbiotes of ticks occur mostly in the Malpighian tubules and ovaries, rather than in mycetomes or in connexion with the gut, as in insects (Roshdy, 1961). The symbiotes are minute rods or granules, arranged in rows or chains vertical to the base of the cell in the Malpighian tubules. They pass to the ovary and infect the developing oocytes. Among the various ticks, there are numerous minor differences in the particular cells which house the symbiotes and in the method of egg infection.

Mites have one or more mycetomes associated with the digestive tract and if there is more than one type of symbiote each is restricted to a separate mycetome. Transmission is *via* the egg.

We can thus summarize what is known of the contribution of symbiotes to the nutrition of medically important insects, ticks and mites by the simple statement that it is very little. We know that the arthropods which feed on blood all their lives possess symbiotes; that removal of the symbiotes causes severe growth impairment and body malformations, lack of reproduction, and premature death; and that supplementing the blood meal with B-vitamins partially alleviates the growth factor deficiencies. But we do not know if the symbiotes actually provide any particular vitamins, or if they are otherwise involved in some aspect of intermediary metabolism.

The reasons for our lack of precise information are numerous. Probably the most important reason is that only the extracellular symbiotes of the reduviids have been cultured, since only in culture is it likely that we can analyse their synthetic capacities. But even so, it does not necessarily follow that the behaviour of the micro-organisms *in vitro* will be identical to that *in vivo*. Confusion of free-living or contaminating forms with the symbiotes is undoubtedly a big obstacle. The intracellular forms which resemble rickettsiae or highly-pleomorphic bacteria, as found in *Glossina*, *Pediculus*, *Cimex*, ticks, and mites have not been cultured (although much work is done on the human pathogenic rickettsiae of the Acarina). Also of prime importance is the fact that few of the arthropod hosts of the intracellular micro-organisms have been rendered aposymbiotic, so that we cannot test their requirements. The transmission of micro-organisms from the mother to the embryo *via* internal routes is difficult to intercept.

Inducing blood-feeding insects to feed through a membrane is an additional problem, although this is certainly not insurmountable (Bar-Zeev & Sternberg, 1962; Haddon, 1956a; Harington, 1960a; Tarshis, 1959). Identification of blood components which induce gorging, such as adenylic acid, is also helpful (Hosoi, 1959).

As to the remainder of the medically important insects, primarily the various Diptera and the fleas, according to our present information they do not have symbiotes. That is not to say that they are not dependent on yeasts and bacteria for many nutritional factors, but the micro-organisms vary from time to time according to the environmental

conditions and thus are not strictly symbiotes. It is not yet possible to rear these insects on a completely synthetic (i.e., chemically-defined) diet that is sterile. Either the diet must include some crude natural product such as casein or yeast, or it must be inoculated with at least one species of bacteria or yeast.

Little by little the nutritional requirements are becoming better defined but, with the best refinements to date, there is still further improvement of growth by the addition of yeast or yeast extract, e.g., *Aedes aegypti* (Akov, 1962); *Phormia regina* (Cheldelin & Newburgh, 1959); *Stomoxys calcitrans* (Gingrich, 1960).

As mentioned at the beginning of this note, most of our information on symbiote physiology is derived from insects which are not medically important; this has been reviewed recently (Brooks, 1963a, 1963b). In brief, a number of stored-products beetles and plant-feeding bugs contain yeast or bacteria in pouches or rows of crypts along the intestine, and these organisms are transmitted by being smeared on the egg-shells. It is a simple matter to surface-sterilize eggs of this type to obtain aposymbiotic offspring. The aposymbiotic insects require the B-vitamins of yeast; the symbiotes can be cultured; and the cultured micro-organisms can be fed to replace the symbiotes (Pant & Fraenkel, 1954). This is the simplest type of symbiotic relationship known, and is like that of the reduviids.

In aphids, the mycetomes are not connected to the intestine, the symbiotes are inherited by the embryo before it is born, and no way has been devised to interrupt the cycle. Mashes of aphid tissue containing symbiotes have been studied with labelled nitrogen and, in contrast to the many claims for it, the fixation of atmospheric nitrogen is not performed by these symbiotes, according to the results of Smith (1948). It is more likely that these symbiotes are involved in turnover of urea or some nitrogenous intermediary. Actually, in spite of the low nitrogen content of plant sap, careful calculations show that such large volumes of phloem pass through the intestinal tract of the aphids as to provide enough combined nitrogen to double their nitrogen content in 58 hours.

The symbiotic flagellates of termite guts were at one time thought to fix atmospheric nitrogen or to convert cellulose to glucose but experimental evidence rules out both of these ideas. Hungate (1943) found that a suspension of flagellates (washed to remove excess bacteria) decomposed cellulose to carbon dioxide, hydrogen, acetic acid, and a small amount of unidentified acids. The termites then oxidize acetic acid as their carbonaceous source of energy.

The intracellular bacteria-like symbiotes of cockroaches can be prevented from passing on to the next generation, *via* the egg, by feeding the mother antibiotics or by withholding manganese and zinc from the diet (Brooks, 1960). The aposymbiotic offspring are weak and pale-coloured, they fail to grow, and they die prematurely unless they are fed much yeast or whole liver. No single nutritional factor has been found which can replace these symbiotes.

The intracellular symbiotes of some scale-insects determine the sex of the embryos by whether or not they enter the eggs (Buchner, 1955).

We can conclude that a great variety of insects possesses symbiotes—indeed, I would venture to predict that all insects might be found to harbour them if we but knew where and how to look for them. The symbiotes undoubtedly are involved in some aspect of nutrition in its broadest sense, but this is complicated by their effect on morphology and reproduction. The relationship is so old and of such an intimate nature that the processes involved in many cases may be more akin to those carried out by cell organelles rather than to those of independent bacteria.

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