STUDIES ON THE SYMBIOTIC YEASTS OF TWO INSECT SPECIES, LASIODERMA SERRICORNE F. AND STEGOBIUM PANICEUM L.[1]

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The organs and tissue cells of many insects harbor living microorganisms which seem to exert no harmful effect on their hosts. In fact, some of them have been found to bear a symbiotic relationship with the insects. It has been shown in many instances, especially in termites, blood-sucking insects and two anobiid beetles, *Lasioderma serricorne* F. and *Stegobium* (*Sitodrepa*) *paniceum* L., that symbionts can play an important part in the nutrition of their host (Fraenkel, 1952; Buchner, 1953).

The mycetom which houses the yeast-like symbionts of *Stegobium* was first described by Karawaiew (1899), and the true nature of the symbionts first recognized by Escherisch (1900). Later Buchner (1912), Heitz (1927), Breitsprecher (1928), Koch (1933), Fraenkel and Blewett (1943), Blewett and Fraenkel (1944) and Pant and Fraenkel (1950) added to our knowledge of the relationship of the symbionts to their insect hosts in *Lasioderma* and *Stegobium*. Koch (1933) recognized that the symbionts of *Stegobium* exerted a function in the nutrition of the host which was similar to that of yeast. Blewett and Fraenkel (1944) showed that the symbionts were sources of many of the vitamins of the B-complex for their hosts. This present study continues and extends the previous work by Blewett and Fraenkel and is also concerned with the culture of the yeasts outside the body and the effect of transplanting them into the foreign host. Some of the results have already been briefly reported (Pant and Fraenkel, 1950).

Materials and Methods

Cultures of *Lasioderma serricorne* and *Stegobium paniceum* were maintained on wholemeal flour and wheat bran with the addition of 5% dried debittered brewers yeast. The cultures had to be covered with tightly fitting wire gauze tops because of the tendency of the adult beetles to cut through muslin covers. In order to obtain a large number of eggs, adult beetles were maintained on a small quantity of white flour plus 5% yeast. Eggs laid in this medium were recovered by sifting through a 60-mesh sieve.

The basic diet was the same as that described previously for *Tenebrio molitor* (Fraenkel *et al.*, 1950) and consisted of 20 parts casein, 80 parts glucose, 1 part cholesterol and 2 parts McCollum's salt mixture no. 185. To this mixture the

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following vitamins of the B-complex were added (expressed as μg. per gram of the dry diet) : thiamin 25, riboflavin 12.5, nicotinic acid 25, pyridoxin 12.5, pantothenic acid 25, choline chloride 500, inositol 250, folic acid 2.5 and biotin 0.1. In some cases the water-insoluble residue of yeast was used in the place of biotin (Fraenkel and Blewett, 1943).

The tests were carried out in 2 × 1 inch shell vials closed by one-holed corks with muslin tops. Ten newly hatched larvae were added to each tube and each test was performed in duplicate. Each tube contained two grams of food. All tests were carried out in a constant-temperature-humidity chamber of 27° C. and 70% relative humidity.

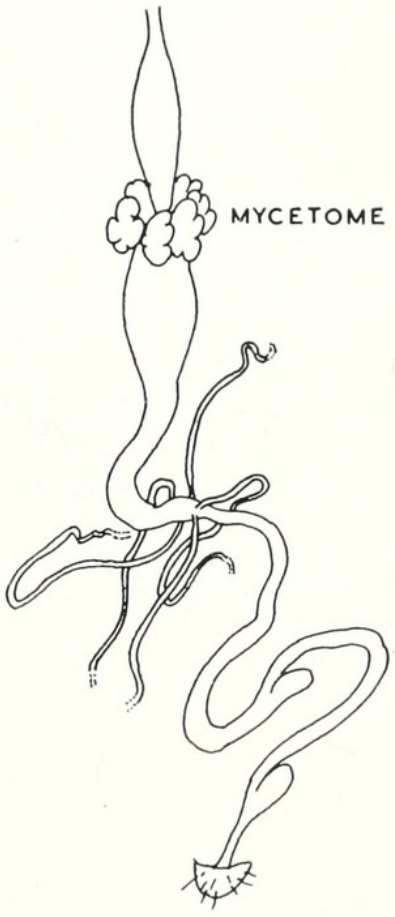


FIGURE 1. Intestine of the adult *Lasioderma serricorne,* showing the mycetoms at the junction of fore- and midgut.

For the study of the symbionts *in situ,* sections were cut and stained with Delafield's hematoxylin and eosin or orange G. Smears of mycetomic tissue were stained with Wright's stain or were smeared on to the slide with a drop of India ink. The latter method proved very suitable for the study of the shape of the cells.

For the graphic representation of results, the total number of adults formed was plotted against time. Both *Lasioderma* and *Stegobium* spin a cocoon previous to pupation. Most of these cocoons are fixed on to the glass wall of the vials leaving open a window to the inside of the cocoon through which the insects can be observed. Thus the time of pupation and emergence of the adults can be accurately determined by frequent inspections.

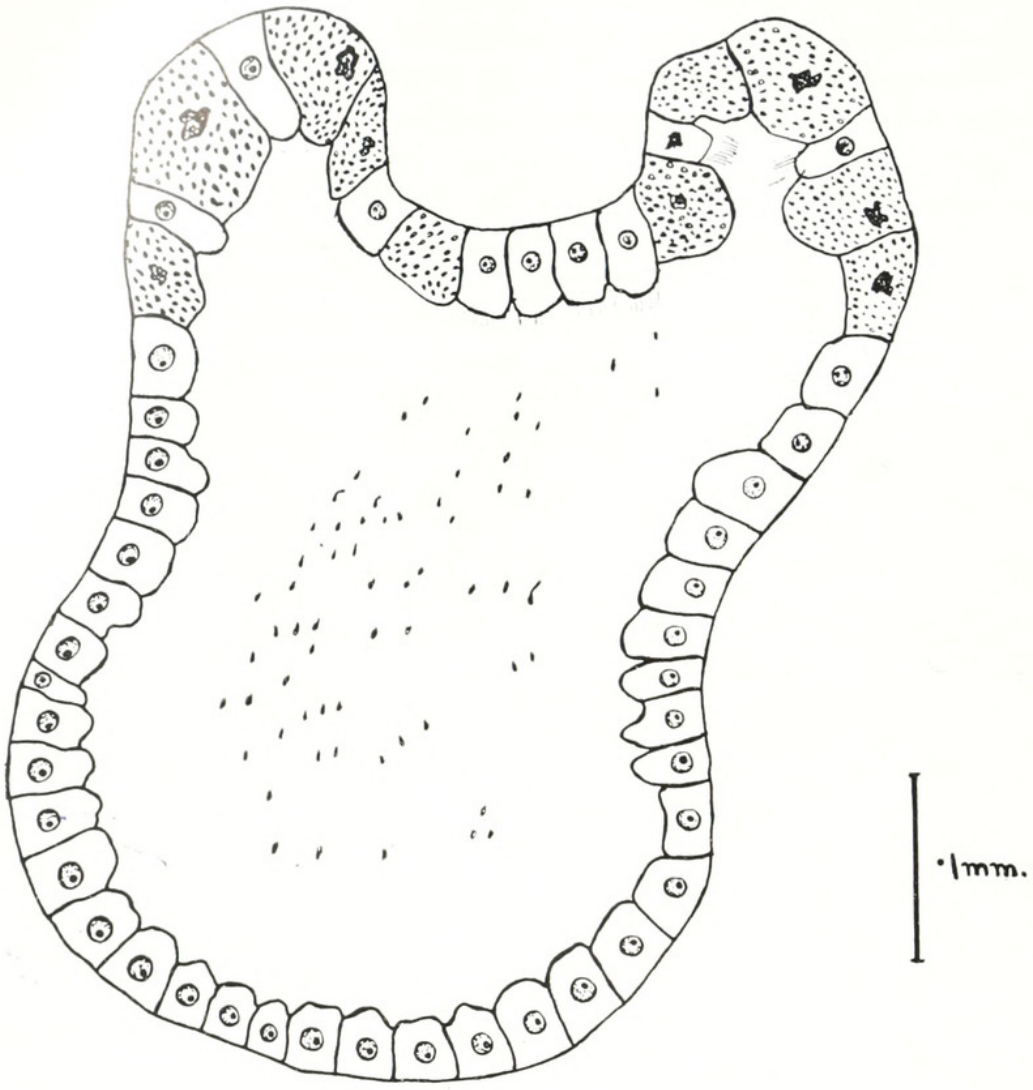


FIGURE 2. Transverse section through two lobes of the mycetom of the *Stegobium paniceum* larva, showing location of symbionts in the mycetom and lumen of the intestine.

OBSERVATIONS AND EXPERIMENTS

1. Mycetoms and symbionts

The mycetoms of *Lasioderma serricorne* are similar to those of *Stegobium paniceum* which have already been described several times (Karawaiew, 1899; Buchner, 1912; Heitz, 1927; Breitsprecher, 1928). However in *Lasioderma* they consist of 6 lobes or protrusions situated at the junction of fore and midgut which are evaginations of the wall of the midgut, and which are in continuation with the lumen of the gut (Fig. 1). As in *Stegobium,* they consist of large cells containing symbionts and irregular shaped nuclei. The other cells of the epithelium are without microorganisms and have round nuclei and fringed borders (Fig. 2).

In newly hatched larvae the mycetoms are not externally differentiated. It is after a period of 5–7 days that the mycetoms acquire their characteristic lobed shape. In the prepupae the mycetomes become reduced in size and are no longer prominent. They are further reduced in the pupae, but are fully developed in the adults, where each lobe is outwardly subdivided into three sub-lobes. In *Stegobium,* which has a four-lobed mycetom, the shape of the organ changes markedly in the adult, by the development of six tubular appendages which arise from each lobe.

The symbionts of *Stegobium* were first identified by Escherisch (1900) as organisms which look like yeasts and multiply by budding. They differ markedly in size and shape in the two species (Figs. 3 and 4). In *Lasioderma* the cells are broadly oval, 2 to 3.5 μ wide and 2 to 4.5 μ long, with only one bud attached to a cell, except for the pupa, where two buds per cell are common. The buds are apical and the point of attachment is broad. No mycelium or spores were ever observed. In the adults the symbionts are slightly smaller and budding is less common. In *Stegobium* the yeasts are elongate, pear-shaped, and pointed at one end to which a single bud may be attached. The point of attachment is very narrow. The size varies from 1.5 to 3.5 μ width and 3 to 6 μ length. These differences in size, shape and budding are so characteristic that the two types can be readily discerned.

2. Cultivation of the symbionts

Escherisch (1900) claimed to have cultivated *Stegobium* but Heitz (1927) and Breitsprecher (1928) failed in subsequent attempts. We have successfully cultivated the yeasts of both species in Hansen's solution (peptone 1.0 gm., glucose 5 gm., potassium dihydrogen phosphate 0.3 gm., magnesium sulfate 0.3 gm., and water 100 ml.). The larvae were sterilized by submerging them in 5% chloramine in 70% alcohol for two minutes. They were then washed with sterilized water, and the mycetom dissected out under sterile conditions and transferred into the culture medium. In the case of *Lasioderma,* inoculated medium was kept at 29° C. for 15 to 20 days after which it turned turbid, with the cells settling down

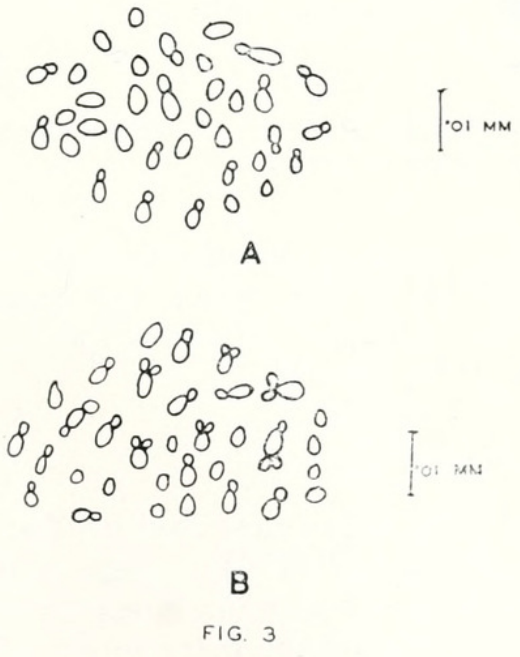


FIG. 3

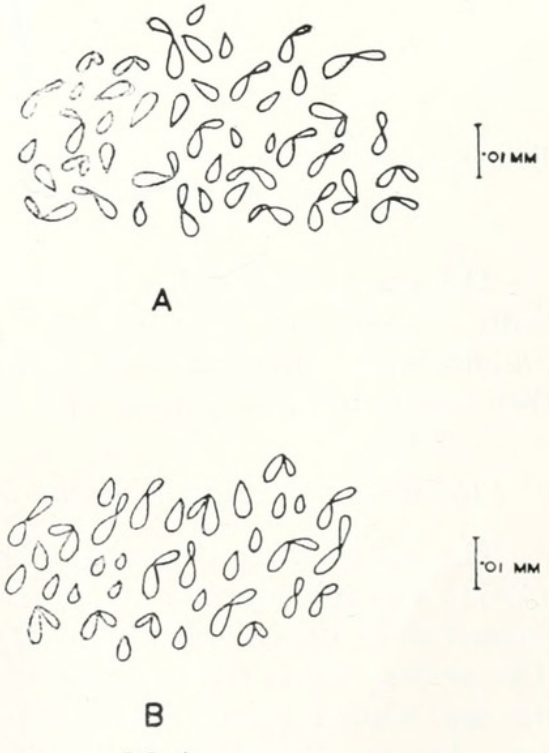


FIG. 4

FIGURE 3. The yeast-like symbionts of *Lasioderma serricorne.* A. In the larva. B. In the pupa.

FIGURE 4. The yeast-like symbionts of *Stegobium paniceum.* A. In the larva. B. Cultivated symbionts.

at the bottom. The suspension was then inoculated on to an agar medium where colonies were formed (Fig. 5). The incubation period for the yeasts from *Stegobium* was longer, up to 35 days, and growth was poor compared with that of *Lasioderma* yeasts. No colonies were obtained on the agar medium.

The cultivated yeasts showed the same morphological characteristics as those *in situ* (Figs. 3 and 4). But in very old cultures chains of yeast sometimes developed. *Lasioderma* symbionts could be grown in such profusion that it was possible to conduct feeding trials with them. For inoculation of media mycetoms of larvae, pupae and adults were used with success. Eggs were also employed but given up, owing to the fact that the outer surface, which contained the yeasts, could not be sterilized.

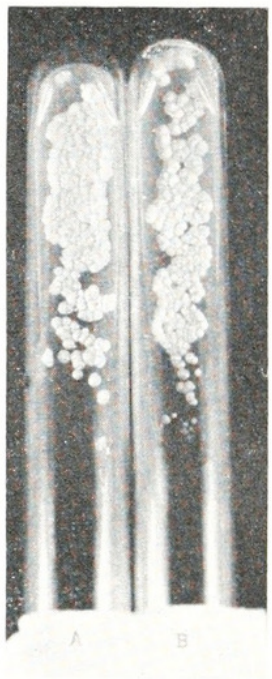


FIGURE 5. A. Cultures of the symbionts of *Lasioderma* on an agar medium. B. Culture of the same symbionts after implantation into *Stegobium* larvae.

The yeasts from either species have never been accurately identified taxonomically. They were provisionally placed by Buchner (1930) under the genus *Saccharomyces* and named *S. anobii*. However they differ from *S. cervicae* in that they cannot ferment glucose with development of carbon dioxide.

3. Elimination of yeasts from the insects, and reimplantation

The insects were deprived of their yeast by a method first described by Koch (1933) and subsequently employed by Blewett and Fraenkel (1944). Eggs were treated with 5% chloramine in 70% alcohol for one minute at room temperature. The yeasts, which are normally transmitted by smearing on the outer surface of the egg, while it passes through the common oviduct, are hereby killed. The eggs are subsequently washed in distilled water. The larvae which hatched from these eggs did not contain symbionts, although the mycetoms developed normally. The offspring from eggs treated in this way will never acquire symbionts, unless reinfected.

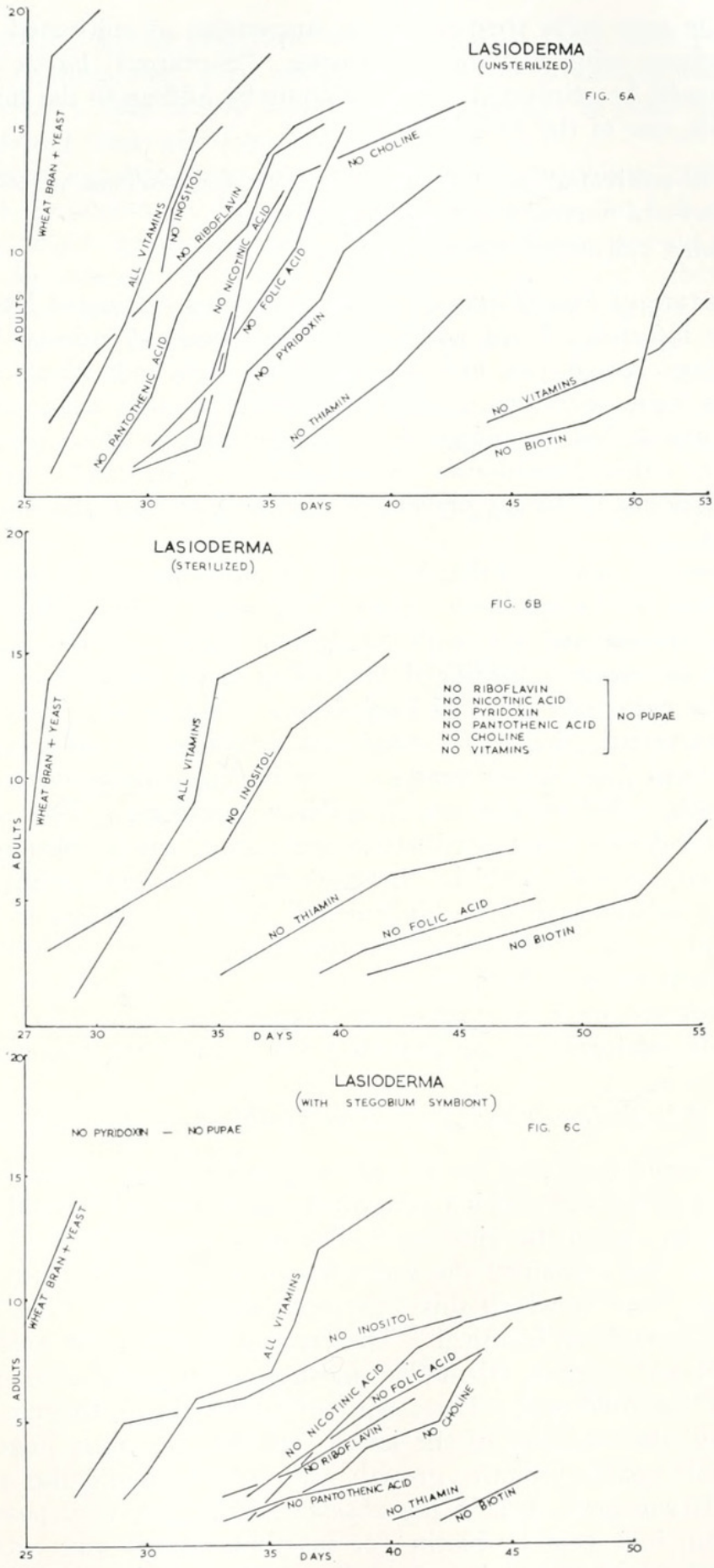


FIGURE 6. Growth of the larvae of *Lasioderma serricorne* on artificial diets in the presence and absence of certain vitamins of the B-complex. A. Normal larvae. B. Larvae without symbionts. C. Larvae which contain the symbionts of *Stegobium*.

When sterile eggs were treated with a suspension of cultivated yeasts the ensuing larvae became reinfected with symbionts. "Sterilized" larvae of *Lasioderma* or *Stegobium* could be reinfected with symbionts by adding to the flour, which was otherwise sterile, one of the following:

1. Culture of cultivated yeast (done only with *Lasioderma* yeasts);
2. 0.1 g. faeces of normal insects in 2 g. of flour;
3. flour from a culture of normal insects.

Sterilized larvae of *Lasioderma* on a diet containing cultivated *Lasioderma* yeast readily became infected. Food which contained faeces of normal larvae did not affect *all* sterilized larvae, but, after two months, *some* individuals of *Lasioderma* and *Stegobium* were found to contain symbionts in their mycetoms. Flour on which normal insects had been feeding previously had no effect on the sterility of the mycetoms of either *Lasioderma* or *Stegobium*. This can be explained by the much smaller amount of faeces present in this sample than the one where faeces had been added.

It was, therefore, concluded that larvae are readily infested by way of mouth, by admixing a culture of the symbiotic yeasts to the food. This method could only be used with *Lasioderma* and not with *Stegobium* whose symbionts did not grow sufficiently well in culture. Sterilized *Stegobium* larvae could, however, be readily infested with the cultivated yeast of *Lasioderma*. The yeasts in the mycetoms retained the characteristic shape of *Lasioderma* symbionts. Cultures were successfully obtained from *Lasioderma* yeasts grown in *Stegobium* mycetoms under circumstances which failed to produce *Stegobium* symbionts. The adults of *Stegobium* with *Lasioderma* yeast produced a generation which contained the typical *Lasioderma* yeast, and this could be observed through several generations.

Stegobium symbionts could be "inoculated" into *Lasioderma* by smearing cultures of them on to sterilized *Lasioderma* eggs. The mycetoms became filled with yeast of the typical shape of *Stegobium* symbionts. The mycetomic tissues of such larvae of *Lasioderma* were inoculated into Hansen's medium and the growth was found to be poor, analogous to the growth of the normal *Stegobium* symbionts.

4. Physiology of Lasioderma and Stegobium symbionts in their normal host

Larvae of *Lasioderma* were grown in the presence and absence of the normal symbionts on artificial diets which contained either the full vitamin complement, or were lacking in one of the vitamins. The tests were performed in two series, one in which the diet contained the water-insoluble fraction of yeast (which contains biotin), and one in which this was replaced by biotin. The results of the two tests were essentially identical. The normal larvae grew relatively well in diets which did not contain riboflavin, inositol, choline chloride, nicotinic acid, pantothenic acid or folic acid. Diets without pyridoxin or thiamin were slightly inferior although the majority of the larvae reached the adult stage. In the absence of insoluble yeast or biotin, growth was very slow and the mortality high. The sterilized larvae grew well in the absence of inositol, very poorly in the absence of thiamin, folic acid or biotin (or insoluble yeast) and entirely failed to grow in the absence of riboflavin, nicotinic acid, pyridoxin, pantothenic acid and choline chloride (Fig. 6).

When sterilized larvae had been reinfested with their own yeast by feeding them on yeast cultures, they grew about as well in the absence of single vitamins as did normal larvae. This clearly indicates that growing the yeast cells in pure culture did not change them physiologically.

A comparison was also made between growth of *Stegobium* in the presence and absence of the symbionts on diets which contained either the full vitamin complement, or had a single vitamin omitted. With the symbionts present the larvae grew very well in the absence of inositol; omission of pyridoxin, riboflavin, nicotinic acid, folic acid or pantothenic acid delayed growth very slightly, but little or no growth took place in the absence of biotin or thiamin respectively. With the symbionts absent, no growth occurred in the absence of thiamin, riboflavin, nicotinic acid, pyridoxin, pantothenic acid, biotin or folic acid. Growth was markedly delayed in the absence of choline chloride and unaffected in the absence of inositol (Fig. 7).

In general, the effect of symbionts in diets lacking in certain vitamins was similar in *Lasioderma* and *Stegobium*. Certain differences which emerged from these

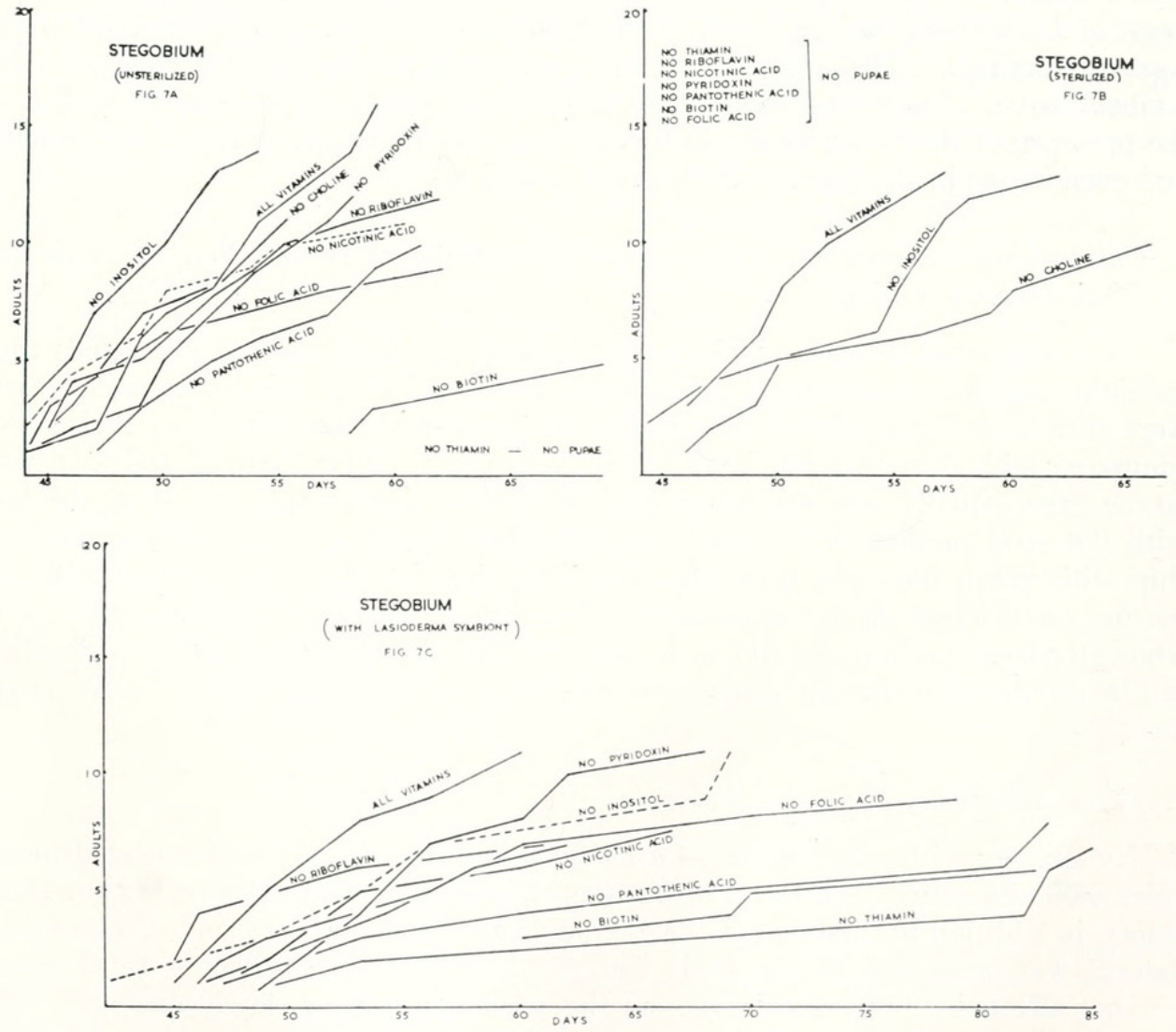


FIGURE 7. Growth of the larvae of *Stegobium paniceum* in the presence and absence of certain vitamins of the B-complex. A. Normal larvae. B. Larvae without symbionts. C. Larvae which contain the symbionts of *Lasioderma*.

tests appear to be connected rather with the requirements of the insects than the properties of symbionts. The normal *Lasioderma* grew slowly in the absence of thiamin, but so also did the larva after removal of the symbionts. Neither the normal nor sterilized *Stegobium* grew in the absence of thiamin. It seems, therefore, that neither *Lasioderma* nor *Stegobium* symbionts supply sufficient quantities of thiamin. The sterilized *Lasioderma* failed to grow in the absence of choline chloride, while choline was not required by the sterilized *Stegobium*.

5. The effect of the symbionts of Lasioderma and Stegobium in the abnormal host

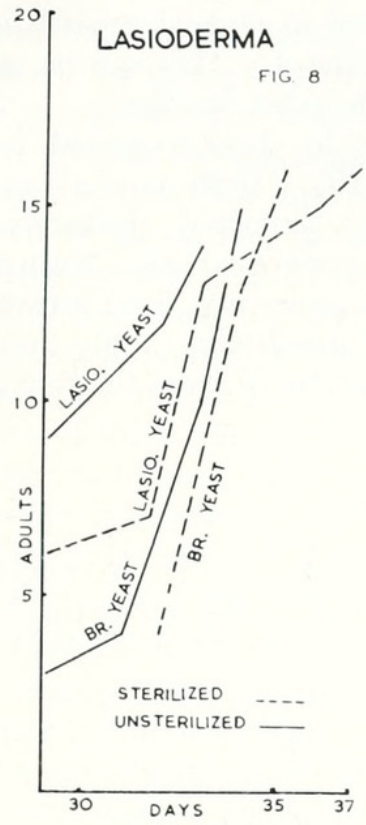
Sterilized *Lasioderma* was infected with the symbionts of *Stegobium* by the method of smearing a culture of *Stegobium* yeasts on the sterilized eggs. The resulting larvae were then submitted to the series of diets, which were lacking in certain factors, as has been described before. Growth of these larvae was better than that of sterilized larvae but inferior to that of normal larvae (Fig. 6). In most instances growth was delayed and the mortality increased. However, in only one case, in the absence of pyridoxin, did the *Lasioderma* larvae with *Stegobium* symbionts entirely fail to develop. In the converse experiment cultivated yeast of *Lasioderma* was mixed with the food on which sterilized *Stegobium* larvae were maintained. The offspring of these larvae were submitted to the tests described above. *Stegobium* larvae with *Lasioderma* yeasts grew about as well as in the presence of their own yeasts in the case of most individual vitamin deficiencies, and even better in the absence of thiamin (Fig. 7).

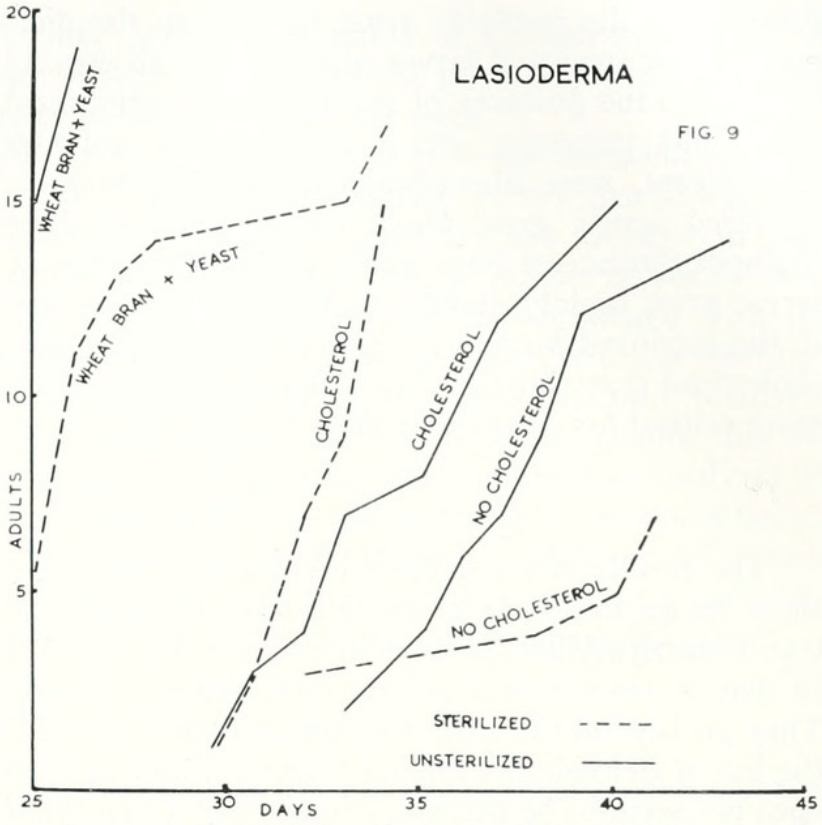
6. Comparison between the effect of dried brewers yeast and that of cultivated Lasioderma symbionts

Lasioderma yeasts were cultivated on an agar medium and the yeast cells then carefully separated from the medium and dried; 2.5% of dried symbiotic yeasts were then added to the basic diet, which did not contain vitamins, and their effect compared with that of 2.5% of dried brewers yeast. Both normal and sterilized larvae grew equally well on either diet (Fig. 8). This result was in agreement with the tests previously reported. *Lasioderma,* owing to its symbiotic relationship with yeast, does not need vitamins like other insects. The reason why the normal *Lasioderma* did not grow optimally in the absence of pyridoxin or thiamin, while sterilized *Lasioderma* did so in the presence of *Lasioderma* yeast in the diet, can be attributed to the far larger amounts of symbiotic yeast present in the latter case.

7. The symbionts as a source of sterols

By analogy with other insects, *Lasioderma* and *Stegobium* were expected to require a dietary source of a sterol and it was of interest to find out whether the symbionts, in addition to vitamins of the B-complex, also supplied sterols. When cholesterol was omitted from the basic diet of *Lasioderma,* growth of normal larvae was not affected. Sterilized larvae, on the other hand, grew slowly and with high mortality in the absence of cholesterol. When cholesterol was added the efficiency of the diet was at once restored (Fig. 9). It is evident that a sterol deficiency does not arise as long as symbionts are present in the insect. In another test the





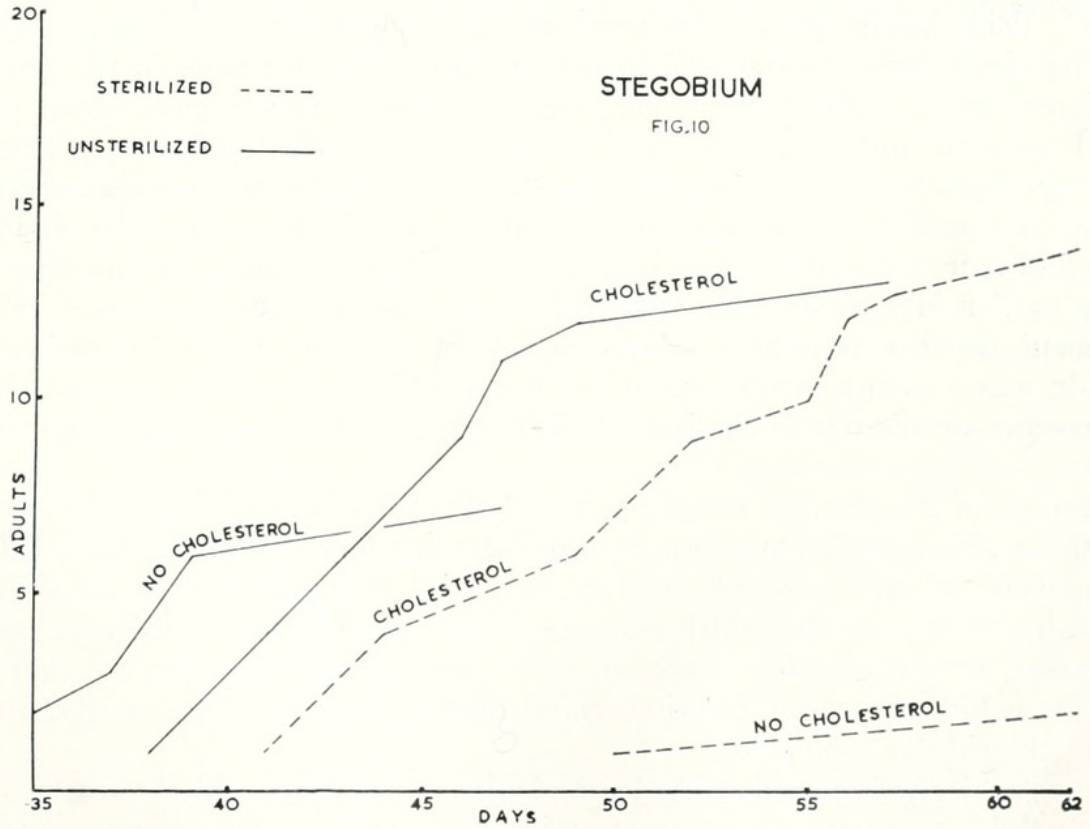


FIGURE 8. Growth of normal and symbiont-free larvae of *Lasioderma* in the presence of dried symbionts or dried yeast as sources of vitamins. L.Y. = Dried *Lasioderma* yeast. Br. Yeast = Dried brewers yeast.

FIGURE 9. Growth of normal and symbiont-free larvae of *Lasioderma* in the presence and absence of cholesterol.

FIGURE 10. Growth of normal and symbiont-free larvae of *Stegobium* in the presence and absence of cholesterol.

presence of the insoluble yeast fraction in the diet resulted in normal growth in normal and sterilized larvae alike, in the absence of cholesterol. This can be attributed to the presence of sterols, largely ergosterol, in the yeast fraction.

Results regarding sterol requirements, corresponding to those reported for *Lasioderma,* were also obtained with *Stegobium* (Fig. 10). Both normal and sterilized larvae grew about equally well in diets which contained cholesterol. Without cholesterol there was a marked difference in both types of larvae. Normal larvae grew rapidly, though there was a high mortality, but there was little growth in the sterilized larvae. It seems that the symbionts of *Lasioderma* supply more cholesterol than those of *Stegobium,* also that the absence of sterol from the diet is more critical for *Stegobium* than *Lasioderma.*

Discussion

The results of the present investigation restate and largely confirm the conclusions drawn in an earlier investigation (Blewett and Fraenkel, 1944), that the intracellular symbionts of *Lasioderma* and *Stegobium* have a nutritional effect similar to that of yeast in a synthetic diet, namely to supply vitamins of the B-complex. They go beyond the earlier results in that folic acid and biotin have been added to the list of individual vitamins tested. Folic acid, like many of the other vitamins, does not seem to be necessary in the diet when the larvae have their ordinary complement of symbionts, but becomes a critical requirement in the absence of the symbionts. With biotin the results were not quite conclusive. Neither *Lasioderma* nor *Stegobium* larvae grow well in the absence of biotin, even in the presence of symbionts, while without symbionts, the *Lasioderma* larvae gave about the same growth pattern and the *Stegobium* larvae entirely failed to grow. There were some minor discrepancies between the present and earlier investigation which might be due to a variety of factors, such as differences in the basic diet (addition of biotin and folic acid), the casein, and the environmental factors, or the insect stock. In the earlier investigation, but not in the present one, choline was a critical requirement for the normal *Lasioderma* larva. On the former occasion choline chloride was a critical requirement for the sterilized *Stegobium* larva, while now the presence or absence of choline had little effect on both the normal and sterilized larva.

Results on the vitamin requirements of the *Stegobium* larva, which agree well with those given earlier by Blewett and Fraenkel (1944) and again in this publication, were recently reported by Lemonde and Bernard (1953), who succeeded in growing them on a diet which contained only two B-vitamins, thiamin and biotin. The larvae grew noticeably slower than on wheat flour; however, no improvement could be achieved by the addition of riboflavin, nicotinic acid, pantothenic acid, choline chloride or folic acid.

In general, the impression has been obtained that the symbiotic yeasts of *Lasioderma* exhibit a greater efficiency of action than those of *Stegobium. Lasioderma* yeasts in the *Stegobium* host have about the same effect as, and with regard to thiamin, an even better effect than, the *Stegobium* yeasts. *Stegobium* yeasts are less efficient in *Lasioderma* than the normal symbiont. This could be, however, attributed to the same unexplained phenomenon that *Lasioderma* yeasts are easier to culture than *Stegobium* yeasts. All these phenomena can be reduced to one

common factor, namely the greater synthetic faculties of *Lasioderma* yeast. It would be interesting to compare vitamin contents of the two species of yeast, and also with those of known varieties of bakers or brewers yeast.

Finally the question remains to be discussed as to how the intracellular symbionts exert their nutritional effect. It has been reported earlier that toward the end of larval life, in the prepupa, the mycetoms gradually are reduced in size. Breitsprecher (1928) described that during this period large masses of yeasts, even whole mycetocytes, enter the lumen of the intestine and are eliminated with the faeces without digestion taking place. In our experience, yeast cells are liberated from the mycetoms all through the growing period of the larva, and only relatively few of these cells are found in the faeces (Fig. 2). It is therefore possible that the host acquires vitamins by digesting the symbionts. The alternative, difficult to prove, would be a diffusion of vitamins from the symbionts into the cytoplasm of the mycetocytes and hence to the hemolymph of the host. This process would differ little from the well known phenomenon of an enrichment of any microbial culture medium by the synthesis in, and diffusion from, the cultured microbe.

Acknowledgment

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Summary

1. The shape and appearance of the yeast-like symbionts of *Lasioderma serricorne* F. and *Stegobium paniceum* L. have been described. The symbionts have been cultured in Hansen's solutions and on an agar medium.

2. The larvae of both species are relatively little affected by the absence of certain vitamins of the B-complex. After elimination of the symbionts these vitamins become absolutely necessary.

3. Sterilized larvae of either species have been successfully re-infected with the symbionts of the other host, which then retain their peculiar shape and mode of action.

4. Cultured symbionts exert the same growth promoting effect as dried brewers yeast when added to the food.

5. The symbionts are also sources of sterols for their hosts.

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