The data in Table 1, obtained by methods developed (*12*) to distinguish between such constituents as phosphatidyl ethanolamine, serine, choline, and sphingomyelin may provide a clue leading to the nature of the metabolic block that gives rise to prolonged alimentary lipemia in the atherosclerotic and to atherosclerosis. A fraction of this metabolic block may be the relative lack of ability of certain animal species to form lecithin. This conclusion is reinforced by the observations of Ahrens and Kunkel on the necessity of lecithin for clearing action (*4*) and by the correlation of the results reported in this paper with the species difference in the susceptibility to experimental atherosclerosis induced by cholesterol and other means. The data suggest that *certain* phospholipids are necessary for normal cholesterol breakdown or metabolism.


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† Postdoctoral fellow of the Life Insurance Medical Research Fund. Present address: Department of Physiology, University of Illinois College of Medicine, Chicago.

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## Similarities in Histochemical Differentiation of Insect Cuticle and the Walls of Parasitic Fungi


**A. Glenn Richards**

***Department of Entomology and Economic Zoology, University of Minnesota, St. Paul***


Incidental to a study of the pathology induced by the parasitic fungus *Herpomyces stylopygae* Spegazzini growing on the cuticle of oriental cockroaches (*Blatta orientalis* Linné) it was noticed that Mallory's triple stain gave a similar staining picture for the fungal walls and the insect cuticle. In both, the developing membrane first stains blue; later, parts of it change and stain red; still later, some parts become brown or black and refractory to staining. In insects this well-known sequence of changes occurs in areas called sclerites and the process is known as sclerotization (*1*). In this fungus all the walls remain undifferentiated (that is, stain blue) except the peculiar basal "shield" growing from the secondary receptacle. This shield undergoes changes paralleling those of cuticle undergoing sclerotization, as is shown in Fig. 1.

The genus *Herpomyces* belongs to the Laboulbeniales of the Ascomyceteae. Like ascomycetes in general,

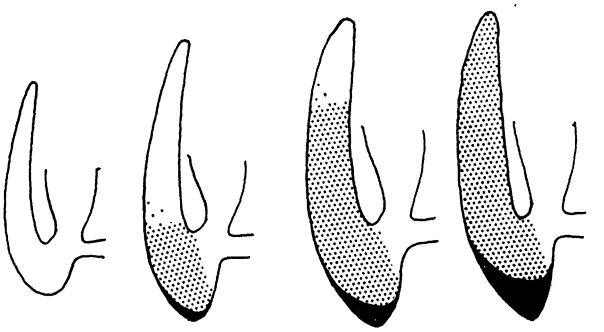

Fig. 1. Diagrammatic representation of color changes shown with Mallory's triple stain during differentiation of the basal "shield" of *H. stylopygae*. Sketched as seen in longitudinal section with "shield" on left and connection to secondary receptacle and rhizoid on right; cell protoplasts ignored. *Unshaded outline*, blue staining walls; *stippled areas*, red staining walls; *black areas*, amber to black walls refractory to staining.

its walls contain chitin instead of cellulose. Von Wettstein (*2*) failed to obtain a positive test for chitin in a species of *Laboulbenia* and tentatively stated that chitin was absent from this group. Ulrich (*3*) accepted this report as fact despite von Wettstein's clear reservation on such a conclusion because he had inadequate material for testing. The chitosan test for chitin (*1*) is positive with *Herpomyces*, although one has to be relatively gentle in applying the test or the walls will disintegrate and be lost (the same is true for the wing scales of certain moths). The walls are also positive for protein (Millon and ninhydrin tests).

Serial sections of the fungus show that the wall is composed of a relatively thick chitin-protein layer giving the afore-mentioned reactions and an extremely thin ($< 1\,\mu$ thick) surface layer which stains red or purplish with Mallory's stain.

These similarities suggested that some of the other color tests currently used in studies on insect cuticle be performed. The data may be summarized by saying that insect cuticle and *Herpomyces* cell walls are similar in these respects:

1) They have a thin surface layer staining red with Mallory's stain.

2) They have a thicker underlying layer containing chitin and protein.

3) They have the thicker layer showing the illustrated staining sequence during development of certain areas.

4) They have the brown or black color removed by treatment with hot NaOH or KOH solutions and by Diaphanol.

5) They are positive to the argentaffin test (ammoniacal $AgNO_3$), which in insect cuticle has been shown to be indicative of orthodihydroxyphenols involved in the sclerotization process.

6) They give the argentaffin reaction primarily in the

portions that will later stain red with Mallory's stain or be refractory to that stain (in *Herpomyces* primarily in the thin surface layer and in the "shield").

7) At certain stages they are positive for the $FeCl_3$-$Na_2CO_3$ test, which is a more specific reaction for orthodihydroxyphenols.

The two differ in that (i) the fungal cell wall is negative to staining with buffered osmic acid (*p*H 7.4) and with Black Sudan B, hence giving no evidence of having a waxy coating, and (ii) in the fact that whereas the green color given by $FeCl_3$, changing to red in $Na_2CO_3$, may be brilliant in insect cuticles, it is faint in the fungal walls.

Another species of *Herpomyces, H. ectobiae* Thaxter on the German cockroach (*Blattella germanica* Linné), is completely white in life. In serial sections its walls show only the blue color with Mallory's stain except for the thin reddish surface layer. This species, then, shows no parallel to the sclerotization process in insects. However, numerous insects have soft, unsclerotized cuticles during larval stages. The many other species of Laboulbeniales all grow as parasites on the cuticle of various living insects. Many of them have a dark or black "foot" appressed to the insect's cuticle. In view of the foregoing data it seems reasonable to suggest that the common blackening of portions of the wall of Laboulbeniales species may be based on reactions similar to those occurring in the host's cuticle. To be sure, tyrosinase activity is already well known in fungi and other plants; but whether the natural dark colors are due to condensation of indole-type structures, as with mushroom tyrosinase *in vitro* (*4*), or to the linkage of structural protein or protein-chitin chains by deaminated derivatives, as in insect cuticle (*1*), is not clear.

One might ask whether precursors for these processes are derived from the insect or synthesized by the fungus. Perhaps one might interpret the presence of numerous argentophile granules in the rhizoids penetrating the insect's cuticle as indicating that precursors may be obtained from the host, but the host has finished sclerotization before the fungus develops and at this stage shows little reaction to the argentaffin reagent. Such questions cannot be settled until someone learns how to cultivate these fungi, which are currently listed as obligatory parasites.

It seems to me that a closer comparison of data on chemical events in insect cuticles and fungal walls might well be valuable to both entomology and mycology. For instance, the work of Castle (*5*) on sporangiophore development illustrates a problem more favorably studied with fungal walls, whereas the relative ease with which large amounts of cuticle can be separated from protoplasm makes arthropod material more favorable for certain chemical studies.

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## Histamine Liberation *in vitro* and Mode of Binding of Histamine in Tissues

Allan L. Grossberg and Humberto Garcia-Arocha

*Department of Physiology, McGill University, Montreal, Canada*

In a recent paper on the intracellular distribution of histamine in dog's liver, Hagen (*1*) reported that a suspension in isotonic sucrose of the large granule fraction, which contains much of the bound histamine of the whole liver homogenate (*2*), releases its histamine when diluted with distilled water. During the course of an investigation of the mechanism of histamine liberation, we have employed a similar preparation and had independently made the same observation. We wish to report this confirmation of Hagen's work, together with additional observations that bear on the problem of histamine liberation.

The preparation employed is based on the original observation of Trethewie (*3*) that dog liver cellular fragments still retain bound histamine. Dog liver, perfused blood-free, is ground with sand in isotonic (0.32*M*) sucrose. The particulate fraction is brought down to 5000 *g* for 30 min in the cold, washed twice, and resuspended in the sucrose solution. The total histamine present in this fraction varies from 3 to 10 μg/g wet weight of the original tissue, and nonsedimentable histamine amounts to 5 to 10 percent of the total. Addition of 2 to 4 vol of distilled water immediately and quantitatively converts all the histamine to the nonsedimentable form, even at 0°C. In addition, the histamine is totally released by treating the suspension with 90 percent acetone in the cold, both in the presence and absence of a sufficient concentration of soybean trypsin inhibitor to eliminate the possibility that a trypsinlike enzymatic action is involved. Freezing and thawing the suspension quantitatively releases the bound histamine—an observation also reported by Hagen. Finally, addition of known lytic substances such as saponin, sodium taurocholate, and lysolecithin, at final concentrations of 5 to 200 μg/ml, rapidly and completely liberates histamine from the particles. It is noteworthy that the latter two of these substances have been reported to liberate histamine *in vivo* (*4, 5*). Hagen (*1*) observed that the surface-active compound octylamine (1 mg/ml) released histamine in his preparation.

The afore-mentioned actions take place at the *p*H (6.5) of the unbuffered sucrose suspension and at *p*H's up to 8.5, above which the suspension becomes increasingly less stable with respect to binding of