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.

References and Notes

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

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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

histamine. Upon addition of known basic histamine liberators such as 1,10-diaminodecane (DA10) and propamidine (*6*), or compound 48/80 (*7*), at final concentrations of 10 to 200 μg/ml, or of rat serum anaphylatoxin prepared according to Novy and DeKruif (*8*), histamine is progressively released from the particles. This liberation is temperature-, *p*H-, and concentration-dependent. The rate of release by 10 μg/ml 48/80 is usually sufficient to set free all the histamine within 60 min at *p*H 8 and 37°C. There is a much smaller, but measurable, rate of release in the controls. Doubling the liberator concentration approximately doubles the rate of release. The rate is 2 to 3 times less at *p*H 7, in the case of 48/80, and is even more markedly reduced at the lower *p*H in the case of DA10. The Q_{10} is approximately 2. These factors probably explain the failure by Hagen (*1*) and Copenhaver, Nagler, and Goth (*2*) to observe histamine release at room temperature by 48/80 and stilbamidine, respectively, in their unbuffered preparations.

All the foregoing observations have been repeated on a similar liver particle suspension (*9*) prepared by replacing the sucrose solution with Tyrode buffer, in which the histamine is less stably bound. In addition, a sucrose suspension of particles from sheep-liver capsule behaves in a similar fashion to the sucrose homogenate of whole dog's liver. Riley and West (*10*) have shown that in this capsular tissue mast cells are the predominant cellular elements.

The evidence cited here strongly suggests that histamine is not bound to a tissue component by a primary chemical bond but is most likely enclosed in a diffusible form within a mitochondrionlike particle. If this is so, the final step, in any series of reactions leading to the release of histamine, would be a change in the properties of the particle-cytoplasm interface, permitting the histamine to diffuse out. This change can be thought of most simply as a rupture or increase in the permeability of a membrane enclosing the particle. Our conclusion is thus in accordance with that independently reached by Hagen: histamine can be released by procedures that might be expected to damage a surface membrane.

It should be noted that, in the case of histamine release by the simple basic histamine liberators, all the components necessary for this action are present, after addition of the liberator, in the washed granular material obtained from the histamine-rich tissue. In the tissues examined by us, and in many other tissues, histamine appears to be located mainly in the mast cells (*10*), and it is attractive to suppose that the characteristic granules of these cells contain histamine and have a membrane that can be lysed by physical and chemical agents. The idea that such intracellular bodies are enclosed by a membrane that serves as a diffusion barrier is supported by permeability (*11*) and electronmicrographic (*12*) studies of liver mitochondria. In intact tissue, as Riley (*13*) has shown, the mast cells themselves are disrupted by the action of a basic chemical histamine liberator or of anaphylatoxin, presumably by an extension to the cellular membrane of the lytic action responsible for distintegration of the intracellular granules.

A complete report of this and related investigations will be published elsewhere (*14*).

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In vivo Activity of Pantothenylcyst(e)ine for Rats and Chicks

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Pantothenylcystine has been reported by Brown and Snell (*1*) to exhibit less than 2 percent of the activity of pantothenate for *L. arabinosus, L. casei,* and *S. carlsbergensis.* King and Cheldelin (*2*) and Brown and Snell (*3*) in studies comparing the activity of pantothenic acid conjugates for *A. suboxydans* found that pantetheine and pantothenylcysteine were more active than pantothenic acid or pantoic acid but somewhat less active than 4′-phosphopantetheine or coenzyme A. From these studies it was concluded that those derivatives containing a —SH group are, in general, considerably more active than the corresponding disulfide forms.

In view of the published data concerning the requirement for cystine in the biosynthesis of coenzyme A (*4*) and the microbiological activity of pantothenylcyst(e)ine, it seemed of interest to determine whether this latter compound would show biological activity in pantothenic acid-depleted animals. Recently Hoagland and Novelli (*5*) have shown that pantothenylcystine can be converted to pantetheine by treatment with a rat-liver supernatant. These findings are of interest in view of the data presented here, which show that pantothenylcyst(e)ine has little if any significant pantothenic acid activity in rats or chicks.

One-day-old White Leghorn S.C. chicks of mixed sex were prepared for the pantothenic acid assay as described by Thompson, Bird, and Peterson (*6*). In the two chick experiments reported, the birds were