normal-appearing nuclei. No effort was made at this time to study the growth requirements of the cells.

This method for preparing cell suspensions from insect tissue is reproducible and makes possible quantitative studies involving large numbers of cells. Conceivably it could provide monolayer cultures for viral research. We are hopeful that the liberated cells may dedifferentiate rapidly and be capable of growth in a less complex medium than is required, apparently, for explanted insect tissues.

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Protection of Fungi against Polyene Antibiotics by Sterols

While we were studying the structural chemistry of the polyene antifungal agent, filipin, the question arose of the possible mechanisms of its antifungal action (*1*). The presence of a conjugated polyene structure in filipin suggested that perhaps filipin interfered with the synthesis or the function of carotenoids in the fungi. In order to investigate this hypothesis, a mixture of carotenoids was obtained from carrots. The hexane extract, prepared according to the method of Loomis and Shull (*2*), was tested by the assay disc method on *Penicillium oxalicum* (*3*). The inhibitory activity of filipin was, indeed, completely prevented, and the organism grew normally in the presence of filipin plus the crude hexane extract.

Saponification of the hexane extract followed by chromatography on MgO : Hyflo Super Cel gave an active fraction which followed α- and β-carotene from the column. From this fraction a white material was obtained which was easily crytalized from methanol-water. This material was obviously not the usual type of carotenoid. Moreover, when crystalline preparations of α-carotene, β-carotene, or vitamin A were tested, they showed no activity at levels of 10 times that of filipin. The isolated material melted at 134.5° to 137°C and gave analytical data consistent with a $C_{20}H_{30}O$ structure. C-Methyl analysis completely ruled out the possibility that this material was a perhydrocarotenoid, for the material contained only 1.5 C-methyl groups per 20 carbons. An alternative possibility that the protecting agent might be a sterol was confirmed by positive Lieberman-Burchard and Salkowski tests. The analytical data are also in good agreement for sterols of the C_{29} series—that is, sitosterol and stigmasterol (*4*)—which have been isolated from carrots, if they are calculated for ½ H_2O of crystallization (*5*). $C_{29}H_{50}O \cdot ½ H_2O$: Calculated: C, 82.18 percent; H, 12.11 percent. Found: C, 82.01 percent; H, 12.46 percent.

The observation that the active agent is a sterol led to the testing of a number of such compounds for similar activity. Thus far, three fungi, *Penicillium oxalicum, Aspergillus niger,* and *Hansenula subpelliculosa,* have been used to study this phenomenon. The prevention of filipin inhibition of the *Penicillium* was observed in both the assay plate test and in liquid media. On agar plates, both carrot sterols and soybean sterols allowed growth of *P. oxalicum* to occur in the presence of filipin at a weight ratio of sterol to filipin of 0.5 : 1.0; *Hansenula* grew normally in shaken liquid culture at a ratio of 0.25 : 1.0. The effect of sterols on nine polyene antibiotics showed various degrees of protection, but no definite relationship between the number of unsaturated groups in the antibiotics and their vitiation could be established (*6*). Filipin and fungichromin inhibitions were most readily prevented, followed by amphotericin B, trichomycin, and rimocidin, while candicidin A, candicidin B, ascosin, and nystatin were only slightly affected. Unfortunately, only the purities of filipin and fungichromin were known so that the relative order of protection can only be tentatively suggested.

Of all the sterols examined thus far, cholesterol, ergosterol, sitosterol, stigmasterol, and to a slight degree, lanosterol, have been active in offsetting filipin inhibition of *H. subpelliculosa.* Ergosterone gives effective reversal of filipin activity; cholesterone is ineffective. None of the short-chain steroids that were tested had similar effects even at a ratio of steroid to filipin of 4 to 1. Apparent reversal occurs after long incubation periods, but preliminary data indicate the possible inactivation of filipin under these conditions.

The prevention of the antifungal activity of filipin by sterols has some interesting implications. Evidently, sterols play a much more important role in the growth processes of fungi than has hitherto been suspected. While a few microorganisms have been shown to require sterols for growth (*7–9*), our current studies indicate that such substances are probably essential metabolites for many fungi. This has not been recognized until now because these microbes are for the most part autotrophic for their sterol requirement. Except for *Labrynthula vitallina* var. *pacifica* (*9*) and *Saccharomyces cerevisiae* S C-1 when grown anaerobically (*7*), the need for this compound has not been demonstrated among the fungi.

The mechanism by which filipin and other polyenes inhibit fungi is intriguing. They might either prevent the synthesis of sterols which are necessary for growth or competitively replace the sterol as a cofactor of a reaction vital to the metabolism of the organism. If the synthesis of cholesterol is prevented, then the inhibition probably occurs at a stage beyond lanosterol formation (*10*). Squalene does not reverse the action of filipin on *Hansenula subpelliculosa* even at a squalene to filipin ratio of 20 : 1. Lanosterol reverses the antibiotic only very slightly at a lanosterol to filipin ratio of 4 : 1. This is a sharp contrast to cholesterol, which is active at a ratio of under 4 : 1 and probably at ¼ : 1.

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24 March 1958

Pyrrolidine Metabolism: Soluble γ-Aminobutyric Transaminase and Semialdehyde Dehydrogenase

A strain of *Pseudomonas* isolated by the enrichment culture technique with pyrrolidine as the sole carbon source has been found to catalyze the following reactions designated as γ-aminobutyric-

Table 1. Activity of γ-aminobutyric transaminase and semialdehyde dehydrogenase (in micromoles per hour per milligram of protein).

Medium*	Activity	
	Dehydrogenase†	Transaminase‡
Pyrrolidine	1.5	2.3
Aminobutyrate	1.3	1.7
Glucose	0.7	0.1
Glutamate	0	0

* Carbon source, 0.5 percent plus the following in grams per liter: K_2HPO_4, 1.5; NaH_2PO_4, 0.5; NH_4NO_3, 1; $MgSO_4 \cdot 7H_2O$, 0.2.
† Assayed by following the formation of DPNH spectrophotometrically (reaction 2) after the addition of cell-free extract to a system containing the following in micromoles per milliliter: potassium phosphate at *p*H 7.3, 100; DPN, 1; succinic semialdehyde, 0.2; mercaptoethanol, 5.
‡ Assayed by following the formation of DPNH spectrophotometrically (sum of reactions 1 and 2) after the addition of cell-free extract to a system containing an excess of the dehydrogenase and the following in micromoles per milliliter: potassium phosphate at *p*H 7.3, 100; DPN, 1; α-ketoglutarate, 1; γ-aminobutyrate, 1.

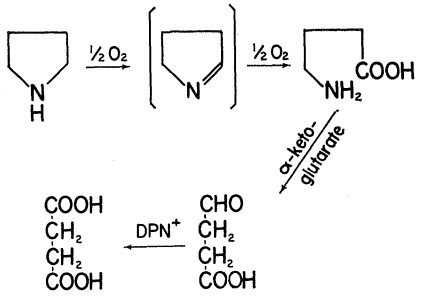
glutamic transaminase (reaction 1) and succinic semialdehyde dehydrogenase (reaction 2):

γ-Aminobutyrate + α-ketoglutarate ⇌ succinic semialdehyde + glutamate (1)

Succinic semialdehyde + TPN^+ ⟶ succinate + TPNH + H^+ (2)

The transaminase has been separated from the dehydrogenase activity and has been partially purified (85-fold) by protamine treatment, by ammonium sulfate and acetone precipitation, and by absorption and elution from calcium phosphate gel. The reaction appeared to be specific in that β-alanine, Δ-aminovaleric acid, or ornithine could not substitute for γ-aminobutyrate in reaction 1; pyruvate, oxalacetate, or α-ketovalerate could not substitute for α-ketoglutarate as amino group acceptor. The semialdehyde dehydrogenase (reaction 2) has been separated from the transaminase and has been partially purified (45-fold) by protamine and gel treatment and by ammonium sulfate and acetone precipitation. The reaction is specific for succinic semialdehyde. TPN is eight times as active as DPN at a concentration of 1 m*M* (*1*); malonic semialdehyde, glyoxalate, glycolaldehyde, and a variety of aliphatic and aromatic aldehydes were inactive. The reaction has not been experimentally reversed. The best preparations of these enzymes under the assay conditions outlined in Table 1 had specific activities of 4.5 and 3 μmole/min per milligram of protein for the transaminase and the dehydrogenase, respectively. A particulate preparation from brain had previously been found to catalyze a similar transamination (*2*). The description of a dehydrogenase from brain which catalyzes the oxidation of succinic semialdehyde is reported in an accompanying manuscript by Albers and Salvador (*3*).

That the formation of these enzymes depends on the carbon source employed in the culture medium is shown by the data presented in Table 1. Thus, in the absence of γ-aminobutyrate or pyrrolidine, little transaminase activity could be detected. Similarly, the dehydrogenase content is increased by growth on γ-aminobutyrate or pyrrolidine. Growth did not occur with 2-pyrrolidinone as the carbon source. The simultaneous induction (see *4*) of the transaminase and the dehydrogenase when growth occurs on pyrrolidine, as well as the apparently analogous primary degradation of proline (*5*) and hydroxyproline (*6*) suggests the following scheme for pyrrolidine catabolism:



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26 February 1958

Late Lower Silurian Fossils from Sillimanite Zone near Claremont, New Hampshire

J. B. Thompson has reported the discovery of fossils in the sillimanite zone of regional metamorphism in the extreme western part of Croydon Township, near Claremont, N.H. (*1*). To the best of our knowledge the Croydon occurrence of generically identifiable fossils in regionally metamorphosed rocks of the sillimanite zone is unique (for a summary of occurrences, see Bucher, *2*). The area has been mapped by C. A. Chapman, and the fossils are in rocks mapped by him as the upper part of the Clough formation (*3–5*). Detailed remapping by Thompson leads to the same stratigraphic interpretation as that arrived at earlier by Chapman but shows that the fossils are preserved in the nose of a major recumbent fold rather than in a homoclinal sequence as indicated by Chapman's geologic map (*4*, plate I).

The fossils are preserved as coarsely crystalline calcite in a matrix containing quartz, diopside, grossularite, and hornblende. Argillaceous rocks of the Littleton formation exposed about ¼ mile to the west contain almandite, staurolite, biotite, kyanite, and locally fibrous sillimanite. About ⅛ mile to the east, argillaceous rocks of the Littleton formation [mapped as Clough formation by Chapman (*4*, plate I)] contain almandite, biotite, and sillimanite. The sillimanite is in part in fibrous knots possibly pseudomorphous after kyanite. Some specimens contain staurolite, but it is less abundant than it is to the west. The fossil locality is thus interpreted as lying in the low-grade part of the sillimanite zone.

The fossils occur as a shell bed that has suffered a certain amount of current action, as is indicated by the disarticulated state of the brachiopods. The cardinalia of all the brachiopods are still well preserved, but fine external striae are barely discernible. Study of the fossils by Boucot shows the presence of the following: *Eospirifer* cf *E. radiatus, Plectambonites, Resserella* (= *Parmorthis*) cf *R. elegantula, Atrypa* cf *A. reticularis, Leptaena* cf *L. "rhomboidalis," Stricklandia* cf *S. lens ultima, Cyrtia* (?), unidentified rostrospiroid brachiopod, unidentified high-spired gastropod, *Porpites* (= *Paleocyclus*) cf *P. porpita* (*6*), unidentified zaphrentid tetracorals, *Heliolites,* and *Favosites.*

The presence of a smooth *Stricklandia* lacking outer plates suggests similarity to Williams' *Stricklandia lens ultima* (*7*, pp. 103–104). Williams records *S. lens ultima* from zones C_4 and C_5 of the Upper Llandovery in the Llandovery region (*7*, p. 129). The presence of *Porpites porpita* suggests a stratigraphic position near the top of the Upper Llandovery—that is, C_6—as is indicated by its occurrence in the lower Visby marl of Gotland (Hede, *8*) together with a plicated *Stricklandia* (*S. lirata*) and in 7_c of the Oslo region (*9*) together with *Stricklandia lirata. Porpites porpita* has not been recorded together with fossils of C_4 to C_5 age elsewhere, whereas unplicated or sparsely plicated species of *Stricklandia* do not occur elsewhere as high as C_6 or its equivalent. *Porpites porpita* is known in New York from the Upper Clinton Schroeppel shale (*10*, p. 350) and in