unbroken pods and ten samples from broken pods had no aflatoxins when harvested, an indication that pod and kernel damage are unimportant at that time and that toxins accumulate after harvest.

Determinations were made for the gross influence of kernel moisture on development of *A. flavus* and aflatoxins and on the development of the microorganisms (Fig. 1). Each point of these curves is a mean for two to five samples having similar moisture contents. Samples were collected from three locations. Aflatoxin concentrations were high where *A. flavus* was abundant, that is, when the moisture content was 23 to 34 percent; but at lower or higher moisture contents where *A. flavus* grew scantily there was little aflatoxin. The fungus was visible with the naked eye on 1 to 6 percent of the kernels that had a high aflatoxin concentration. But growth of the fungus was not visible in this way when aflatoxin concentration was low or absent. About 82 percent of the kernels with the highest moisture contents (38 to 43 percent) were invaded within 10 days by microorganisms. Unidentified bacteria and two fungi, *Rhizopus nigricans* Ehr. and *Fusarium oxysporum* (Schl.) Snyd. and Hans., were most prevalent. Two other fungi, *F. roseum* (Lk.) Snyd. and Hans. and *Macrophomina phaseoli* (Maubl.) Ashby, were less prevalent. Few organisms except *Aspergillus flavus* (Fig. 1) and *A. niger* van Tiegh. (Fig. 1) were isolated at 35 percent kernel moisture. Curves for these two fungi were parallel, although *A. flavus* was always more common than *A. niger*. A mucoraceous fungus accounted for nearly all of the "other" group in kernels having 30 percent moisture; species of *Penicillium* accounted for nearly all of the invaders in the "other" group at moisture contents below 30 percent. *Aspergillus glaucus* Link was the most prevalent fungus in kernels having 15 to 21 percent moisture, and kernels with less than 12 percent moisture were nearly free from fungus invasion.

The development of *Aspergillus flavus* and aflatoxin is regulated by competition with other fungi that invade the kernel (Table 1). *Aspergillus niger* was more competitive than *Rhizoctonia solani*, but both fungi limited development of the *A. flavus* when they were allowed to grow on peanut kernels for 48 hours prior to a challenging inoculation of *A. flavus*. Other experiments were made to determine whether the results of this experiment were due solely to competition or whether the common fungi that invade the peanut pods and kernels also might destroy aflatoxins. Results of these experiments (Table 2) indicate that the toxin is subject to fungal breakdown, although the amount broken down is related to initial concentration of aflatoxin.

Our results agree with results of McDonald and Harkness (*3*) which indicate that aflatoxins are rarely found in peanuts at the time of harvest. Our findings indicate that the mold growing on peanut kernel is analogous to the growth of molds in stored grains (*6*). Aflatoxins appear when kernel moisture and competitive factors favor development of *A. flavus* over other microflora on the kernel. The fungus apparently has a competitive advantage in broken pods, and it becomes established more rapidly than in pods with lesions inhabited by several competitors (*2, 4*). The growth of *A. niger*, which resulted in destruction of aflatoxins in culture experiments, and that of *A. flavus* were greatest at the same moisture contents. However, the data indicate (Fig. 1) that *A. flavus* always had a competitive advantage. Large amounts of aflatoxins are most likely to be elaborated when the kernel moisture is between 23 and 34 percent. Our results agree with those of McDonald and Harkness (*3*) who showed that aflatoxins can be essentially eliminated if peanuts are cured in mechanical driers. However, the lower limit of kernel moisture required for aflatoxin development must be determined exactly before recommendations for moisture contents that will prevent the formation of aflatoxin can be made with confidence.

LEE J. ASHWORTH, JR.*
Plant Sciences Department, Texas A&M University, College Station
H. W. SCHROEDER
Market Quality Research Division, Agricultural Research Service, USDA, College Station, Texas
B. C. LANGLEY
Texas Agricultural Experiment Station, Stephenville

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* Present address: U.S. Cotton Research Station, Shafter, Calif.

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Lysis of Pleuropneumonia-like Organisms by Staphylococcal and Streptococcal Toxins

Abstract. *Six strains representing three species of* Mycoplasma *were examined for susceptibility to lysis by staphylococcal and streptococcal toxins. All were sensitive to staphylococcal α-toxin, two to streptolysin S, and three to streptolysin O. The results support the concept that the limiting membrane of pleuropneumonia-like organisms is basically similar to those of many other cell types and provide additional evidence for the participation of cholesterol in cytolysis induced by streptolysin O.*

Protoplasts and spheroplasts of certain bacterial species undergo lysis on exposure to staphylococcal α-toxin or streptolysin S, agents which were thought earlier to act principally or exclusively on mammalian cells (*1*). These and other observations indicate that the plasma membrane is the site of action of the lytic staphylococcal and streptococcal proteins. If the membranes that enclose pleuropneumonia-like organisms (PPLO or *Mycoplasma*) are similar to those of bacteria and other cells, and there is substantial evidence that they are (*2*), then it seemed likely that PPLO would also prove susceptible to lysis by α-toxin, streptolysin S, or streptolysin O, or to some combination of them.

Parasitic strains of *Mycoplasma* (*M. gallisepticum* and *M. neurolyticum*) were cultivated in Chanock's medium (*3*) in the absence of agar and antibiotics; saprophytic strains (*M. laidlawii*) were cultivated in modified Edward medium (*4*), usually without addition of serum. The cultures (10 to 50 ml) were centrifuged at 25,000*g*

Table 1. Reduction in turbidity of strains of *Mycoplasma* (PPLO) in presence of bacterial toxins.

Test organism	Decrease in optical density in 30 min at 35°C*		
	Staphylococcal α-toxin (%)	Streptolysin S (%)	Streptolysin O (%)
	Parasitic		
M. gallisepticum A5969	12	15	41
M. neurolyticum KSA	17	2	14
M. neurolyticum A	47	30	13
	Saprophytic		
M. laidlawii A	78	0	0
M. laidlawii B	64	2	0
M. laidlawii B-15	66	0	0

* Corrected for 0- to 8-percent decrease in optical density of PPLO suspensions lacking toxin.

for 20 minutes, and the supernatant fluid was discarded. The sedimented organisms were suspended in sufficient 0.15*M* NaCl, buffered at *p*H 7.0 with 0.01*M* phosphate, to give an optical density of about 0.5 in a Cary Model 14 recording spectrophotometer at a wavelength of 500 mμ and with a 1-cm light path.

Staphylococcal α-toxin was purified as described (*4*). Streptolysin S was a purified product containing about 10,000 hemolysin units per milligram (*5*). Streptolysin O was partially purified (*6*) and was activated with cysteine (0.1 percent) before use. To 700 μl of PPLO suspension contained in a cuvette warmed to 35°C was added 50 μl of α-toxin, streptolysin S, or streptolysin O to give final toxin concentrations of 90, 50, and 50 μg per milliliter, respectively. After mixing, the optical density at 500 mμ was recorded continuously for 30 minutes at 35°C (Fig. 1).

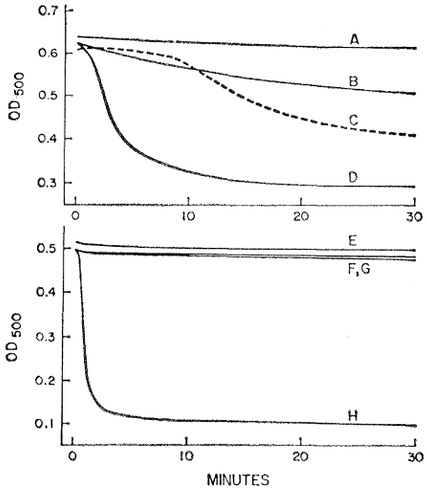

Fig. 1. Turbidity of PPLO suspensions as a function of time, in presence and absence of toxin. (*A*) *Mycoplasma neurolyticum* A only; (*B*, *C*, and *D*) same plus streptolysin O, streptolysin S, and staphylococcal α-toxin, respectively; (*E*) *M. laidlawii* A plus streptolysin O; (*F*) *M. laidawii* only; (*G* and *H*) same plus streptolysin S and staphylococcal α-toxin, respectively.

The combined results of tests with six strains of PPLO (Table 1) indicate qualitative and quantitative differences in sensitivity to a given toxin. Where a large decrease in optical density was recorded, visual examination revealed extensive clearing, and phase-contrast microscopic examination showed numerous ghosts and few intact PPLO; in mixtures showing small decreases in optical density, larger numbers of intact organisms and fewer ghosts were discernible. A decrease in optical density as small as 12 percent is interpreted as an indication of sensitivity to test toxin because of its reproducibility and because it differed substantially from the results obtained with control mixtures not containing toxin. Had different conditions of time, *p*H, and others been selected it is certain that decreases in turbidity greater than those found would, in some instances, be demonstrable.

The results show that all strains tested are susceptible to lysis by staphylococcal α-toxin, though not to the same degree, the saprophytic strains appearing to be somewhat more sensitive than the parasitic, a situation which is similar to that for simple osmotic lysis (*7*). In contrast to α-toxin, streptolysin S failed to lyse any of the saprophytic PPLO but caused partial lysis of two of the three parasitic strains. Apparently the receptor (or substrate) of streptolysin S differs from that of α-toxin, and presumably it is missing from the membranes of the saprophytic PPLO and strain KSA of *Mycoplasma neurolyticum*. Its identity remains to be established, but there is a hint that it may be phosphatidyl ethanolamine in bacterial membranes (*1*).

The toxicity of streptolysin O appears to depend upon its capacity to interact with membrane cholesterol, and it destroys many types of mammalian cells whose membranes contain cholesterol as a constituent (*8*). Protoplasts and spheroplasts of many bacteria lack sterols and are insusceptible to the lytic action of streptolysin O (*1*). Smith (*9*) and others have established the existence of two classes of PPLO as follows: (i) parasitic strains which require exogenous sterol for growth and which possess cholesterol as an essential membrane constituent, and (ii) saprophytic strains which do not require sterol for growth and which have membranes in which carotenol is present, possibly as the structural and functional analog of sterol, when grown in the absence of sterol. Strains of the first class are susceptible to lysis by streptolysin O while strains of the second are not (Table 1), thereby providing additional evidence for the concept that cholesterol is specifically involved in the cytotoxic action of streptolysin O. Razin (*2*) reported that *M. laidlawii* was sensitive to lysis by digitonin when grown in the presence of cholesterol but not when cultivated in its absence. Similar observations were made by Weber and Kinsky (*10*) regarding sensitivity of *M. laidlawii* to filipin, a polyene antibiotic which, like digitonin, appears to act by forming complexes with cholesterol. In our limited studies the presence or absence of serum as a source of cholesterol in Edward medium did not alter the insusceptibility of *M. laidlawii* to lysis by streptolysin O. Possibly, if not probably, results analogous to those of Razin (*2*) and Weber and Kinsky (*10*) would be obtained under other conditions. Finally, nothing appears to be known concerning susceptibility to bacterial toxins of bacterial L-forms as compared to PPLO. Conceivably one or more of the toxins examined could serve as reagents for distinguishing between the two classes of microorganisms.

ALAN W. BERNHEIMER
Department of Microbiology
MORTON DAVIDSON
Department of Medicine, New York University School of Medicine, New York

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Cell Proliferation in Hydra: An Autoradiographic Approach

Abstract. *Hydra cells take up substantial amounts of tritiated thymidine if the label is injected into an animal's gastric cavity shortly after feeding. Autoradiographic analysis shows that the isotope becomes incorporated into cell nuclei. The distribution of labeled nuclei probably represents the pattern of cell proliferation and is similar to the distribution of mitotic figures in histological preparations. The data show that cell proliferation is distributed along the body column and that there is no localized growth zone.*

Hydra is customarily used for studying morphological changes and regulation, yet little is known about the cellular growth patterns underlying these activities. Experimental studies on hydra have led Tripp (*1*) and, more recently, Brien and Reniers-Decoen (*2*) to postulate that growth and cell proliferation occur primarily in the subhypostomal (*3*) region. Such a growth pattern was largely deduced, however, and no direct analysis of the distribution of cell proliferation has been presented to support their thesis. A possible reason for this lack of analysis is the difficulty workers have experienced in finding mitotic figures in hydroid tissue (*4*). One approach would be to label dividing cells with tritiated thymidine and study their distribution by autoradiography, but previous attempts to label the nuclei of hydra (*5*) have been hindered by the relative impermeability of hydra to organic molecules in the external milieu (*6*).

In this report, I describe a successful method for administering tritiated thymidine to normal hydra so that the label becomes incorporated into dividing cell nuclei. To overcome the problem of permeability, thymidine solution is injected into the gastric cavity of hydra immediately after the animal has been fed *Artemia nauplii*. This presents the label directly to the digestive tissue. The colloidal nature of the mucus and partially digested food is essential in preventing the isotope from leaking out of the animal.

Hydra littoralis (*7*) were grown in the medium of Loomis and Lenhoff (*8*) with distilled water substituted for tap water. Culture density was maintained at one hydra per 15 ml of growth solution, the solution being renewed daily 1 hour after the animals were fed *Artemia nauplii*. The hydra were grown for 4 weeks before they were used in experiments, so that all animals had similar growth histories. A fine glass pipette was inserted through the mouth into the gastric cavity and 0.1 μl of tritiated thymidine (*9*) solution (0.1 μmole) was delivered by a 50-μl syringe (*10*). The average amount of isotope retained by the hydra 1 minute, 20 minutes, and 24 hours after injection was 89, 78, and 24 percent, respectively, as determined by monitoring the medium. This retention of substantial amounts of thymidine shows that the administration procedure was efficient.

Extended hydra were fixed in Lavdowsky's fluid (*11*) 24 hours after the thymidine was injected. Serial paraffin sections 5 μ thick were cut perpendicular to the long axis of the animals. The methods of Kopriwa and Leblond (*12*) were used for staining the sections and for the autoradiographic analysis. The sections were stained with Harris' hematoxylin, and the slides coated with Kodak KTB-3 liquid emulsion, exposed at 4°C, and developed in D-170. After exposure for 7 days, most of the labeled nuclei were associated with about 75 photographic grains each. The cytoplasm was relatively free of activity, indicating that little label was shunted into other synthetic metabolic pathways by either the hydra or its microbial flora.

The segment of radioautograph shown in Fig. 1 is taken from the middle stomach region. Incorporation

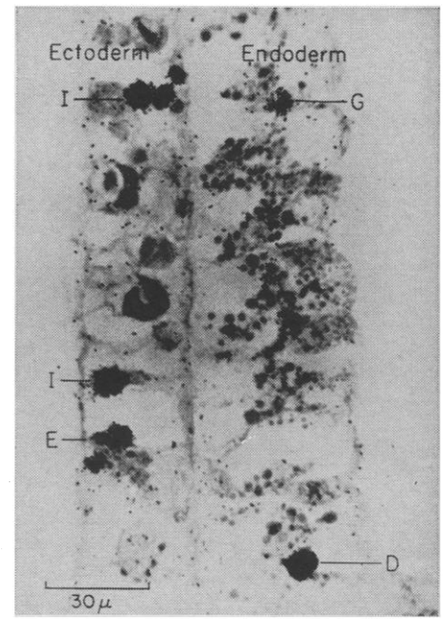

Fig. 1. Autoradiograph of hydra injected with triated thymidine. Endoderm (right) and ectoderm (left) are cut in cross section through the middle stomach region. Nuclei showing isotope incorporation in this photograph are: gland cell (*G*), digestive cell (*D*), interstitial cell (*I*), and epithelio-muscular cell (*E*).

of tritiated thymidine was observed in nuclei of all major types of cells distinguished in this study: digestive, interstitial, and gland cells in the endoderm; and cnidoblasts, interstitial, and epithelio-muscular cells in the ectoderm.

Labeled nuclei occurred at all levels

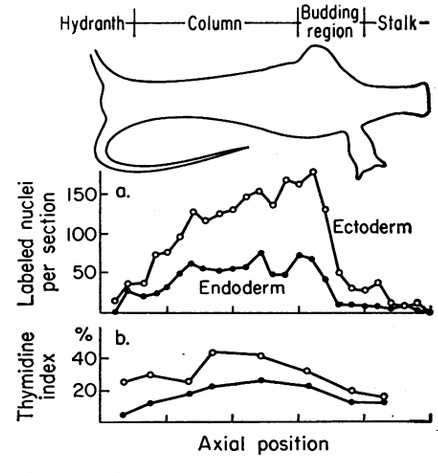

Fig. 2. Axial distribution of labeled nuclei in a hydra. Axial position (abcissa) is indicated by the drawing above the graph. Thymidine index is the percentage of nuclei which are labeled. Solid circles, endoderm; open circles, ectoderm. Counts of labeled nuclei were made on every 20th section. Thymidine indices were calculated for single sections at the positions indicated.