bias the data against the conclusion reported.

Rainwater can reach the base of the coral cap through various paths (Fig. 2). Under the normal pattern of intermittent showers, percolation of rainwater through the coral cap and interaction with aragonite results in a groundwater with a high ratio of strontium to calcium. We attempted to study this path of water movement. However, during heavy rains, surface runoff into sinkholes and fissure recharge to the water table limits appreciable interaction of groundwater with aragonite in the vadose zone of the coral cap. In addition, the ratio of strontium to calcium in groundwater for a well in a lower aragonitic terrace is somewhat reduced by low-strontium groundwaters which have percolated through higher, more calcitic terraces.

Thus, having reasons to believe that the efficiency estimates represent minimal estimates, we base our conclusions on six wells, one on the western nose (539P), and five on the south slope of Christ Church Ridge (629W, 16-15B, 15-2B and 15-8B). All have anomalously high strontium concentrations. Adjusted ratios of strontium to calcium in the water from these six wells average 34, in contrast to the ratio of 9 or less that would be expected if solution of aragonite were the only process acting in this area. We conclude that reprecipitation of calcium carbonate in the form of low-magnesium calcite is occurring concurrently with dissolution of aragonite above the water table. We estimate that the solution-reprecipitation process is operating at an efficiency greater than 90 percent.

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References and Notes

1. G. M. Friedman, *J. Sediment. Petrol.* **34**, 777 (1964).
2. R. G. C. Bathurst, in *Approaches to Paleoecology*, J. Imbrie and N. D. Newell, Eds. (Wiley, New York, 1964), pp. 357–376; J. D. Hudson, *Geol. Mag.* **99**, 492 (1962).
3. M. G. Gross, dissertation (California Institute of Technology, 1961); M. G. Gross, *J. Geol.* **72**, 170 (1964); S. M. Stanley, *Amer. Assoc. Petrol. Geol. Bull.* **50**, 1927 (1966); L. S. Land, dissertation (Lehigh University, Bethlehem, Pa., 1966); L. S. Land, F. T. Mackenzie, S. J. Gould, *Geol. Soc. Amer. Bull.* **78**, 993 (1967).
4. K. J. Mesolella, dissertation (Brown University, Providence, R.I., 1968); W. S. Broecker, D. L. Thurber, J. Goddard, Teh-Lung Ku, R. K. Matthews, K. J. Mesolella, *Science* **159**, 297 (1968).
5. R. K. Matthews, unpublished results.
6. J. C. Hudson and A. Newton, unpublished report (Agronomy Research Unit, Barbados Sugar Producers Association, Inc., Edgehill, Barbados, 1966).

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Lysosomal Stability during Phagocytosis of Aspergillus flavus Spores by Alveolar Macrophages of Cortisone-Treated Mice

Abstract. *Control mice and those treated with cortisone were exposed to aerosols of viable spores of* Aspergillus flavus. *Fifteen to 20 minutes later, animals were killed, and alveolar macrophages were obtained by tracheobronchial lavage. Electron-microscopic examination of these cells revealed that, whereas the lysosomes of control macrophages showed extensive attraction and fusion with the phagocytic membranes surrounding spores, the lysosomes of macrophages from animals treated with cortisone revealed little, if any, interaction. This diminished lysosomal response in forming phagocytic vacuoles may be important in the subsequent development of hyphal bronchopneumonia which frequently occurs in cortisone-treated mice exposed to spores of* A. flavus.

The increase in the incidence of severe systemic human infections with opportunistic fungi, such as *Aspergillus* (*1*), has prompted us to investigate the pathogenesis of pulmonary aspergillosis (*2, 3*). After a brief exposure to aerosols of viable spores of *A. flavus*, mice become highly susceptible to lethal hyphal bronchopneumonia if they have previously been treated with drugs such as cortisone or with cytotoxic agents considered to be associated with the development of fatal human aspergillosis (*2*). In contrast, normal control mice exposed similarly to *A. flavus* spores are resistant to this infection (*2*). This experimental model is being used in investigations of the cellular mechanisms involved in the enhanced susceptibility to secondary mycotic disease. Unlike the alveolar macrophages from exposed control mice, acid phosphatase is not released from the particulate fraction into the supernatant fraction of macrophages from cortisone-treated mice 8 hours after their exposure to spores (*3*). These data support the hypothesis (*4*) that corticosteroids may act by stabilizing lysosomal membranes. We now report ultrastructural observations which further support this hypothesis.

White female mice (Carworth Farms strain 1) weighing, on the average, 20 g were used. The experimental mice received 5 mg of cortisone acetate subcutaneously 2 days before they and untreated control mice were exposed to spores. Animals were exposed to aerosols of viable spores of *A. flavus* for 5 minutes in an exposure chamber (*3*). Mice were killed by aortic transection 15 to 20 minutes after removal from the exposure chamber. Alveolar macrophages were obtained by lavage of the lungs three or four times with 1.0 ml of either isotonic saline (0.89 percent) or sucrose (0.25*M*) through a 23- or 26-gauge needle inserted into the trachea (*3*). In the first experiment, washings obtained from 20 control mice exposed to spores were pooled for fixation, dehydration, and embedding, as were those from 12 animals treated with cortisone. In a second experiment, the washings obtained from 17 control mice and from 16 cortisone-treated mice were used similarly. In a third experiment, washings were obtained from cortisone-treated and control mice that were not exposed to spores. The washings obtained from each group were separately fixed in 2 percent phosphate-buffered osmium tetroxide at *p*H 7.4 and 0° to 4°C for 1 to 2 hours. Each milliliter of cell suspension was mixed in 1 ml of OsO_4 such that the final concentration of osmium was 1 percent. The cell suspensions were then centrifuged at 500 rev/min for 10 minutes, and the supernatants were decanted. The pellets were resuspended and dehydrated in a series of graded ethanols, treated with propylene oxide, and infiltrated in Epon 812. For each step in dehydration, it was necessary to resuspend and centrifuge the pellets. After infiltration, the cells were pipetted into No. 00 gelatin capsules and centrifuged at 1000 rev/min for 15 minutes. These capsules were then filled with Epon and polymerized in an oven at 45°C for 24 hours and at 60°C for 48 hours. Silver or gold sections cut with

glass knives on a Porter-Blum MT-1 ultramicrotome were collected on Athene 300-mesh copper grids and stained with a 5 percent solution of uranyl acetate, followed by lead citrate (*5*). All ultrathin sections were then examined by means of a Philips 100 B electron microscope.

Sections of cells from control and cortisone-treated mice showed that only a small percentage of alveolar macrophages had phagocytized spores during the 15- to 20-minute interval between exposure and killing. This is consistent with earlier observations with the light microscope (*3*).

The alveolar macrophages of control and cortisone-treated mice not exposed to spores appeared to be identical cytologically, as were macrophages of both groups of animals which had been exposed to spores but which had not phagocytized them. The cytoplasm of the alveolar macrophages of both groups contained numerous, well-distributed, variable-sized, membrane-limited bodies (lysosomes) which contained membranous as well as granular and amorphous debris (Figs. 2 and 3). These ovoid to elliptically shaped granules had a variable affinity for osmium.

The spores, whether intra- or extracellular, appeared similar, with a mean diameter of approximately 5 μm (Figs. 1–3). The central portion of each spore consisted of a dense homogeneous aggregate of deeply osmiophilic material usually devoid of detail at this resolution (Figs. 1–3). A roughly concentric translucent rim circumscribed the central dense portion. The periphery of the intra- and extracellular spores consisted of a deeply osmiophilic, variegated 0.5 μm rim of spore wall from which numerous osmiophilic bosselations protruded.

The spores phagocytized by alveolar macrophages of control and steroid-treated mice were usually surrounded, in part or totally, by a membrane (the phagocytic membrane). Within the membrane, a thin empty space was often encountered surrounding the spore. The phagocytic membrane of control alveolar macrophages (Fig. 2) also frequently encircled membranous, amorphous, or granular debris which appeared to be morphologically identical to the contents of cytoplasmic lysosomes. Lysosomes within control alveolar macrophages were observed (Fig. 2) to be fusing with the phagocytic membrane. In low-power electron micrographs, the lysosomes of control alveolar macrophages often appeared to be most densely aggregated in regions around the phagocytized spore with a concomitant relative decrease in number ("degranulation") in the remainder of the cytoplasm.

In contrast, the alveolar macrophages, from cortisone-treated mice that had phagocytized spores in vivo, had lysosomes which were more equally distributed throughout the cytoplasm despite the presence of spores within phagocytic membranes. Fusion or incorporation of lysosomes into the phagocytic membranes was only rarely observed in the alveolar macrophages obtained from cortisone-treated mice (Figs. 1 and 3).

Our ultrastructural observations on the phagocytic process in alveolar macrophages of control mice after exposure to *A. flavus* spores in vivo are similar to those reported for murine (*6*) and rabbit (*7*) alveolar macrophages.

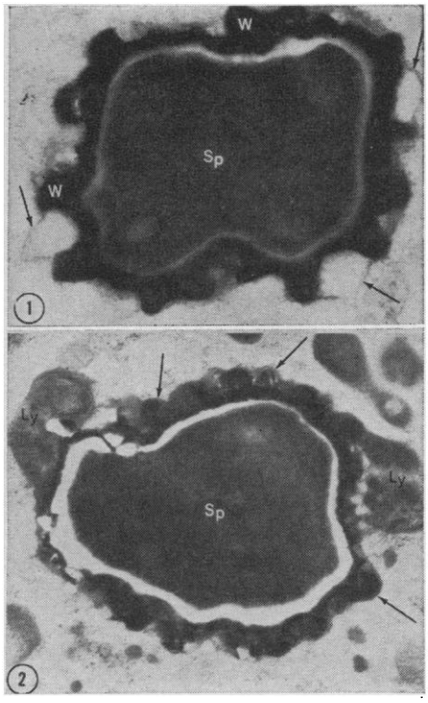

Fig. 1. Alveolar macrophage obtained from cortisone-treated mouse containing a phagocytized spore (*Sp*) of *A. flavus*. The central portion of the spore stains a dark gray, whereas the irregular projections of the spore wall (*W*) stain black. The entire spore is enclosed within a phagocytic membrane (arrows) (× 13,000). Fig. 2. Alveolar macrophage obtained from control mouse containing a phagocytized spore (*Sp*) of *A. flavus*. The space between the central part of the spore and spore wall is translucent. Portions of the phagocytic membrane can be seen (arrows). Two lysosomes (*Ly*) are fused with the phagocytic membrane (× 15,100).

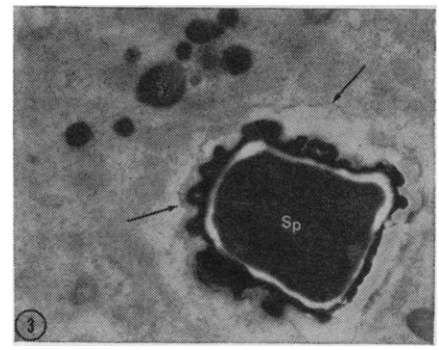

Fig. 3. Alveolar macrophage from cortisone-treated mouse containing a phagocytized spore (*Sp*) of *A. flavus*. The outer wall of the spore is irregular, and the entire spore is surrounded by a phagocytic membrane (arrows). Only few lysosomes (*Ly*) are present near the phagocytized spore (× 9880).

The differences in alveolar macrophages of control and cortisone-treated mice exposed to *A. flavus* spores noted in this study and an earlier one (*3*) can most easily be explained in terms of the hypothesis that glucocorticoids stabilize lysosomal membranes (*4*). Since cationic proteins with antibacterial activity have been reported in the granular fraction of polymorphonuclear leukocytes (*8*), it is possible that membrane stabilization could limit the interaction between such protective factors and spores phagocytized by alveolar macrophages of experimental mice. This stabilization of factors could explain the rapid intracellular spore germination noted in experimental alveolar macrophages as well as the decreased ability of these cells to limit spore growth in vitro (*3*). The diminished lability of the acid phosphatase, subsequent to phagocytosis of *A. flavus* spores, is also consistent with stabilization of lysosomes within alveolar macrophages of the experimental mice (*3*). It is conceivable that this early altered intracellular lysosomal response may be important in permitting spores to germinate during the pathogenesis of hyphal bronchopneumonia.

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References and Notes

1. A. G. A. Heffernan and S. P. Asper, Jr., *Bull. Johns Hopkins Hosp.* **118**, 10 (1966); G. P. Bodey, R. D. Powell, Jr., E. M. Hersh, A. Yeterian, E. J. Freireich, *Cancer* **19**, 781 (1966); P. P. Carbone, S. M. Sabesin, H. Sidransky, E. Frei, III, *Ann. Intern. Med.* **60**, 556 (1964); J. G. Gruhn and J. Sanson, *Cancer* **16**, 61 (1963); H. Sidransky and M. A. Pearl, *Dis. Chest* **39**, 630 (1961).
2. H. Sidransky and L. Friedman, *Amer. J. Pathol.* **35**, 169 (1959); H. Sidransky and E. Verney, *Lab. Invest.* **11**, 1172 (1962); H. Sidransky, E. Verney, H. Beede, *Arch. Pathol.* **79**, 299 (1965).
3. S. M. Epstein, E. Verney, T. D. Miale, H. Sidransky, *Amer. J. Pathol.* **51**, 769 (1967).
4. G. Weissmann and L. Thomas, *J. Exp. Med.* **116**, 433 (1962); G. Weissmann, *Biochem. Pharmacol.* **14**, 525 (1965); C. DeDuve, R. Wattiaux, M. Wibo, *ibid.* **9**, 97 (1962).
5. E. S. Reynolds, *J. Cell Biol.* **17**, 208 (1963).
6. Z. A. Cohn and E. Wiener, *J. Exp. Med.* **118**, 991 (1963); A. E. Karrer, *J. Biophys. Biochem. Cytol.* **7**, 357 (1960); *ibid.* **4**, 693 (1958).
7. E. S. Leake and Q. N. Myrvik, *J. Reticuloendothel. Soc.* **3**, 83 (1966); F. Shafer, B. J. Moberly, P. Gerhardt, *J. Infect. Dis.* **116**, 401 (1966).
8. H. I. Zeya, J. K. Spitznagel, J. H. Schwab, *Proc. Soc. Exp. Biol. Med.* **121**, 250 (1966).

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Deamino-oxytocin: Inactivation by Plasma of Women in Labor

Abstract. *Incubation of deamino-oxytocin with plasma obtained from women in labor reduced the potency of this analog of oxytocin when assayed on an isolated strip of mammary gland taken from a lactating rat. Plasmas of nonpregnant women had no detectable effect on this activity of deamino-oxytocin, and the effect of plasmas from women just previous to the beginning of labor was not significant. The original activities of the incubated deamino-oxytocin solutions could be restored by treatment with hydrogen peroxide. The inactivation may be caused by reductive cleavage of the disulfide bridge of deamino-oxytocin, this bridge being reformed by oxidation.*

Deamino-oxytocin (*1*) is an analog of the neurohypophysial polypeptide hormone oxytocin and, in contrast to oxytocin, does not possess a free amino group (Fig. 1). It has more powerful uterotonic (in rats), milk-ejecting (in rabbits), and vasodepressor (in fowls) activities (*2*) than the natural hormone does (*1*, *3*).

In humans, oxytocin appears to be the hormone responsible for milk ejection and to be implicated in the processes of labor. Yet deamino-oxytocin, when given to women in labor or puerperium, is even more potent than oxytocin in producing contractions of the uterus (*4*) and mammary gland (*5*).

These results, of great importance in attempts to clarify the mode of action of oxytocin, have furthermore led to an increasing interest in the possible use of deamino-oxytocin in obstetrics.

During human pregnancy an enzyme capable of inactivating oxytocin appears in the blood. It is apparently produced by the placenta (*6*). It has been called "plasma oxytocinase" and is an aminopeptidase of rather broad specificity (*7*), and still undetermined function. The enzyme produces a rupture of the ring of oxytocin by hydrolysis of the hemicystinyl-tyrosyl bond. Deamino-oxytocin, lacking an amino group, would not be expected to be attacked by this aminopeptidase, and indeed incubation with serum from women in labor does not affect the avian vasodepressor activity of deamino-oxytocin under conditions in which this activity of oxytocin is virtually completely destroyed (*8*).

There exists another "oxytocinase," which occurs in highest concentrations in the liver and kidneys and which appears to cleave the disulfide bridge in oxytocin by acting as a thiol oxidoreductase for disulfide bonds in protein. This action is followed by aminopeptidase degradation (*9*). This system, "tissue oxytocinase," is not specific to pregnancy, but there is evidence that it does occur in placental tissue. It is very much less stable than "plasma oxytocinase" and rapidly loses its activity at room temperature (*6*). There are reports of the finding of trophoblastic fragments in the general circulation in postpartum (*10*), which may mean that "tissue oxytocinase" of placental source could also occur in the blood, although this enzyme system is apparently not normally found extracellularly. Deamino-oxytocin, possessing a disulfide bond, should be susceptible to attack by a thiol oxidoreductase. The resultant dithiol-containing peptide would still presumably be resistant to amino peptidase attack. *S,S'*-Dihydrodeamino-oxytocin, deamino-oxytoceine, is much less potent than deamino-oxytocin as an avian vasodepressor agent (*11*). Therefore the formation of deamino-oxytoceine by the action of "tissue oxytocinase" on deamino-oxytocin could be detectable by biological assay.

We have investigated the effect of plasma of women in late pregnancy and labor on deamino-oxytocin, as expressed by changes in its biological activity, with care being taken to avoid the inactivation of labile enzyme systems.

Blood (20 ml) from the brachial vein of women in advanced pregnancy or active labor was collected in a cooled receiver. Immediately after extraction it was centrifuged at approximately 2°C for 10 minutes at 1000*g*, and the plasma was used at once. Plasma (2 ml) at 37°C was added to a mixture of barbital buffer (*p*H 7.9, 1 ml) and either oxytocin (*12*) (1 ml aqueous solution, containing approximately 2×10^{-3} μmole, with about 1 unit of milk-ejecting activity) or deamino-oxytocin (*12*) (1 ml aqueous solution containing approximately 2×10^{-3} μmole, with about 1 unit of milk-ejecting activity) at 37°C. A sample (1 ml) was taken from each mixture immediately after the addition of the plasma (zero time) and was added to a test tube containing water (3 to 4 ml) in a water bath at

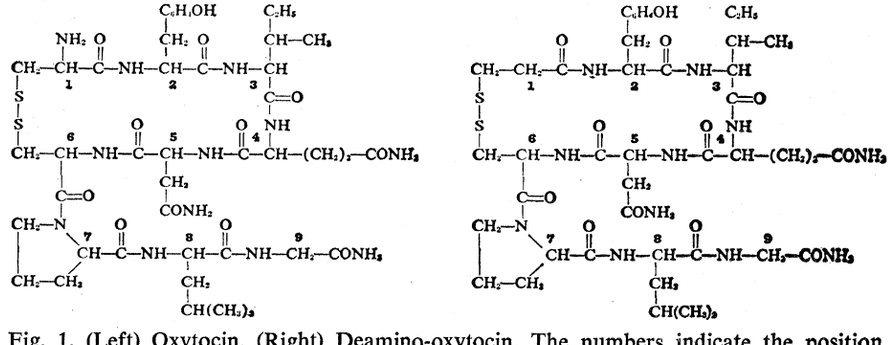

Fig. 1. (Left) Oxytocin. (Right) Deamino-oxytocin. The numbers indicate the position of individual amino acid residues in the structure.