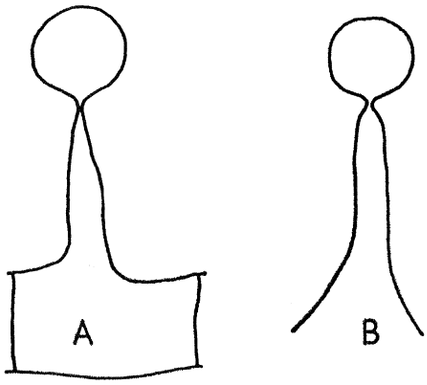

Fig. 1. Visible results of surface tension acting against an abstricting force. (*A*) Sterigma and young basidiospore of *Melampsora epitea* (camera lucida drawing from cytological preparation). (*B*) Late phase of splash in milk, droplet separating (*6*) (not to scale).

transverse blow from the bursting bubble must cause the near side of the sterigma to break first, which further invalidates Prince's explanation. The conidia of Entomophthoraceae are discharged by essentially the mechanism proposed by Prince, but under very different circumstances. The septum is very broad in proportion to the spore diameter, and the mechanical advantage is consequently relatively enormous. The broad base must also limit the directional error imposed upon a conidium by uneven rupturing of the conidiophore wall. Moreover, imperfect discharge in Entomophthorales, whose conidia are exposed to such secondary means of transfer as wind, rain, or insects, is much less serious than it would be in most higher basidiomycetes, whose undischarged spores are sheltered by folds, teeth, tubes, or gills.

Ten years ago, in another context, I published (*4*) drawings of a sterigma and young basidiospore of the rust *Melampsora epitea* beside a tracing of one of Edgerton's photographs of a droplet being abstricted from splashing milk (Fig. 1). The curves of minimal area exhibited by spore and sterigma—visible results of surface tension operating against an abstricting force—prove that the spore does not grow from a rigid sterigma but is thrust from a fluid one. Only when the ultimate shape has been achieved is it made permanent by deposition of a rigid wall in both spore and sterigma. Active streaming, which must either induce or be caused by electrical potential, occurs during spore formation; and the abstriction may be caused by like charges centered in the spore and below the base of the sterigma. If the repulsion that must exist during spore formation persists until the bubble bursts, we have an accurately directed discharge that satisfies requirements. When I stumbled on this possible explanation 2 years ago I discussed it with colleagues in several disciplines, in the hope of devising a method of testing it. (My mortification at needing 8 years to make this modest deduction is partly assuaged by the apparent failure of anyone else to make it.) Failure to devise a test and the fact that my explanation, like previous ones, did not account for the supposed droplet discouraged me from publishing. However, if we combine the bubble and repulsion mechanisms we have a unified theory that seems to fit the observed facts: the sterigma tip is snapped by abrupt bending, and the spore is regularly discharged in the appropriate direction and, in tube and gill fungi, for an appropriate distance. This discharge mechanism is so reliable that it has become the most distinctive feature of a large and ecologically important group of fungi.

The testing of the repulsion theory presents a challenge. A charge on the falling spores might be detected by observing their deflection in falling through an electric field, as was attempted by Buller (*2*); but as John Hart has pointed out to me, demonstration of a residual charge is inconclusive; for separation of a spore (with a membrane or polar molecules) from its sterigma would probably induce a small charge upon it.

In many Basidiomycetes the hymenium lines slender vertical tubes or covers closely spaced gills. It is important that the spores should not be thrown too far and should not drift laterally before they fall free of the hymenium. A persistent charge of the same sign as that upon the hymenium would help to prevent such accidents.

One inevitably wonders how such an elaborate mechanism arose. When the fungi first left their ancestral aquatic habitats it was vital to replace their now largely functionless motile spores with effective dispersal agents (*5*). At first the spores were probably dispersed largely by rain or spray; but, if the fungi were to penetrate all terrestrial niches successfully, air-borne spores were essential; and forcible discharge is strongly adaptive in facilitating aerial dispersal. The repulsion mechanism is thus an expected extension of the process of spore formation on a slender sterigma. It is not so simple to visualize the evolution of the bubble mechanism. Gas may have been originally a chance metabolic byproduct, which occasionally burst irregularly through the primary membrane of the spore. If the rupture even occasionally served to break the sterigma tip, perfection of the bubble mechanism by further mutations would be expected; for maintenance of the repulsive charge consumes energy, and a device that ensures more prompt release of the spores at maturity than enzymatic dissolution of the wall could provide would be highly adaptive.

D. B. O. SAVILE
Plant Research Institute, Central Experimental Farm, Ottawa, Canada

References and Notes

1. L. S. Olive, *Science* **146**, 542 (1964).
2. A. H. R. Buller, *Researches on Fungi* (Longmans, Green, London, 1909–1924), vols. 1–3.
3. A. E. Prince, *Farlowia* **1**, 79 (1943).
4. D. B. O. Savile, *Mycologia* **46**, 736 (1954).
5. Evolutionary problems encountered by the fungi in penetrating terrestrial niches are discussed in volume 3 of *The Fungi*, G. C. Ainsworth and A. S. Sussman, Eds. (Academic Press, New York), in press.
6. Tracing from D'A. W. Thompson, *On Growth and Form* (Macmillan, New York, ed. 2, 1948), plate facing p. 390 (phot. H. E. Egerton).

4 December 1964

Glutathione as an Inducer of Feeding in Ticks

Abstract. *Ticks engorged solutions containing reduced glutathione as readily as blood, provided that the tonicity of the solutions was adjusted to that of plasma. Among substances tested this effect was specific for the tripeptide. Glutamic acid inhibited the feeding response.*

The tick *Ornithodoros tholozani* (Laboulbene and Megnin) is the vector of Asiatic human relapsing fever in the Near East. Its hosts are various mammals, both wild and domestic, as well as man. From the first nymphal stage the tick normally feeds every few months and can survive several years of starvation. Since blood is the only fluid imbibed by ticks, the question arises as to what substance or substances initiate the feeding response.

Ornithodoros tholozani can be easily fed on blood through an artificial membrane made of parafilm stretched on a glass ring. The ticks probe through the membrane into the solution provided it is warmed to 38°C, but they do not im-

bibe the liquid unless it contains appropriate stimulants. In our experiments, about 80 percent of the nymphs that had been starved for 2 years fed within 2 hours through such a membrane on sheep blood. Almost the same response was obtained when whole blood was substituted by a hemolysate of washed erythrocytes. No feeding took place on water or saline isotonic with blood.

The factor which stimulates the feeding response in the blood-sucking mosquito *Aedes aegypti* L. is adenosine triphosphate (ATP) (*1*). However, $10^{-2}M$ or $10^{-3}M$ ATP with or without the addition of 0.15*M* NaCl was refused by the ticks, and another stimulant had to be sought.

The red cell hemolysate was not utilized by the ticks after it had been subjected to extensive dialysis. Furthermore, when the hemolysate was dialyzed against an equal volume of saline for 48 hours, the diffusate was readily taken up by the ticks. The diffusate lost most of its activity after heating to 100°C for 15 minutes. Thus we inferred that a thermolabile compound of low molecular weight is at least partly responsible for the feeding response. Among the various red-cell constituents which we considered to have suitable properties, the tripeptide glutathione was chosen as a likely candidate. When glutathione (*2*) at a concentration of $10^{-2}M$ in saline, *p*H 7, was offered to the ticks, it was engorged rapidly. As a typical example, a 5-mg nymph imbibed 30 mg of this fluid in 10 minutes. To ascertain that the effect of the reduced glutathione was not due to an impurity we conducted the following test. To the solution of glutathione a slight excess of 5,5′-dithiobis (2-nitrobenzoic) acid was added. This compound combines specifically with sulfhydryl groups (*3*). After this treatment no feeding took place until addition of excess glutathione restored its original concentration. Table 1 shows that a significant response could be elicited also by a $10^{-3}M$ glutathione solution, but not by a $10^{-4}M$ solution. The concentration of glutathione in mammalian blood is of the order of $10^{-3}M$ (*4*). Thus the decreased response of the ticks to this concentration might indicate that other substances present in blood have an additive or sinergistic effect.

Table 1. The feeding response of *O. tholozani*. Abbreviations: GSH, reduced glutathione.

Compound	No. of experiments	No. of ticks	No. feeding (%)
Whole blood	5	139	78
Hemolyzed red cells	6	131	59
Distilled water	2	40	0
0.15*M* NaCl	3	100	0
0.3*M* sucrose	3	60	0
$10^{-2}M$ GSH*	2	40	5
$10^{-2}M$ GSH†	2	40	85
$10^{-2}M$ GSH‡	12	266	64
$10^{-3}M$ GSH‡	3	60	22
$10^{-4}M$ GSH‡	2	40	0
$10^{-2}M$ GSH‖	2	40	43

* In water. † In 0.3*M* sucrose. ‡ In 0.15*M* NaCl. ‖ In 0.15*M* KCl.

Table 2. Effect of glutathione analogues and some related amino acids on the GSH feeding response. Results expressed as the percentage of the total number feeding on the compound; $10^{-2}M$ GSH was added to each compound. Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; GABA, γ-amino butyric acid.

Compound*	No. feeding (%)
Control (GSH alone)	64 ± 3.6†
L-Glutamic acid	22 ± 5.4
D-Glutamic acid	28 ± 7.3
Glutamine	52 ± 6.9
GABA	56 ± 4.6
l-cysteine	59 ± 5.1
S-methyl-glutathione	60 ± 6.7
GSSG	71 ± 4.5
Glycine	71 ± 5.1

* All analogues were tested at $10^{-2}M$. † Standard error of the mean, calculated on the basis of results with at least 6 groups of 20 ticks each.

The tonicity of the solution was of paramount importance. Optimal results were obtained with solutions whose osmotic pressure was isotonic with that of blood. The nature of the solute did not seem to play a decisive role since both the nonelectrolyte sucrose and NaCl induced a similar response, while KCl was less effective. On the other hand, the response to glutathione was highly specific. No feeding response was obtained with oxidized glutathione, *S*-methyl-glutathione (*5*), or with any of the constituent amino acids: glycine, cysteine, or glutamic acid. Only glutamic acid, in either the L or D configuration, exerted considerable inhibitory action when used at concentrations equal to that of the glutathione present (Table 2). Since neither glutamine nor γ-amino-butyric acid produced significant antagonistic action, one can deduce that the glutamic acid moiety plays an important and specific role in the attachment of glutathione to its chemoreceptor site.

Other ticks, the common fowl tick *Argas persicus* (Oken) and the eyeless tampan *Ornithodoros moubata* (Murray), were also induced to feed by glutathione, but unlike *O. tholozani* a definite though small percentage of these populations also imbibed saline alone.

The specificity of glutathione in activating the sucking response of the tick bears a striking resemblance to the role of this tripeptide in the well-known case of the feeding reflex of *Hydra littoralis* (*6*). This is further emphasized by the inhibition exerted by glutamic acid (*7*). Although the sulfhydryl group (SH) of glutathione is not essential for its action on the *Hydra* receptor (*8*), blocking the SH by the methyl group renders the tripeptide ineffective as a stimulator of feeding for ticks. However, it is also possible that, as in the case of *Hydra* (*9*), other substances not related to glutathione will be found which have similar effect. In any case, our investigation may widen further the evolutionary significance of glutathione-activated receptors beyond the hydrozoan group (*10*).

RACHEL GALUN
S. H. KINDLER
Israel Institute for Biological Research, Ness-Ziona, Israel

References and Notes

1. T. Hosoi, *Nature* **181**, 1664 (1958); R. Galun, Y. Avi-Dor, M. Bar-Zeev, *Science* **142**, 1674 (1963).
2. Schwarz, BioResearch, Inc., New York, N.Y.
3. G. E. Ellman, *Arch. Biochem. Biophys.* **82**, 70 (1959).
4. E. C. Albritton, *Standard Values in Blood* (Saunders, Philadelphia, 1952), p. 108.
5. Synthesized by Zion Chemicals, Yavne, Israel.
6. W. F. Loomis, *Ann. N.Y. Acad. Sci.* **62**, 209 (1955).
7. H. M. Lenhoff and J. Bovaird, *Nature* **189**, 486 (1961).
8. E. E. Cliffe and S. G. Waley, *ibid.* **182**, 804 (1958).
9. H. Forrest, *Biol. Bull.* **122**, 343 (1962).
10. H. M. Lenhoff and H. A. Schneiderman, *ibid.* **116**, 452 (1959).
11. The skillful technical assistance of Mrs. H. Yordan is gratefully acknowledged.

24 November 1964

Exovagination of Newt Endoderm: Cell Affinities Altered by the Mesodermal Inducing Factor

Abstract. *Implantation of a highly purified mesodermal inducing factor in the blastocoel of early gastrulae results in the spreading of endoderm over the ectoderm. The effect can be explained by a change in cell affinities in the ectoderm, initiated by the mesodermal factor.*

"A central problem of cytodifferentiation is the identification and characterization of control factors extrinsic to the cell and the elucidation of the mechanisms by which these impinge upon the cell's inner controls" (*1*).