The plants, after exposure for 48 hours to ^{14}C, were grown for an additional 96 hours in the light in a normal atmosphere to allow for ^{14}C translocation, incorporation, and respiration. Symbiont respiration was calculated by attributing the difference in the evolution of carbon (milligrams of ^{14}C per gram of carbon) between symbiotic and nonsymbiotic roots to symbiotic respiration. It was assumed that the symbionts of doubly infected plants had a ratio of respired carbon to biomass carbon similar to that determined for singly inoculated plants.

Plant shoots contained slightly less than half of the added label (Fig. 1). Roots accounted for 15 to 22 percent, and below-ground respiration accounted for 31 to 34 percent. The mycorrhizal fungi incorporated 1 percent and respired 3 percent of the ^{14}C assimilated. Older plants with a greater weight of mycorrhizal fungi would utilize greater amounts (*3*). Nodules of nonmycorrhizal plants infected with *Rhizobium* incorporated 2 percent of the tracer while respiring 5 percent. Nodules of mycorrhizal hosts incorporated 3 percent of the products of photosynthesis but respired 9 percent of the ^{14}C fixed. The weights of the shoots and roots of plants containing rhizobium and rhizobium plus mycorrhizal symbionts were lower than those of control plants or of plants infected with mycorrhizal fungi only. These weight differences, however, were not statistically significant. The increased CO_2 assimilation in the presence of the symbionts indicates that the plant may have been able to compensate, in part, for the needs of the microbial partners. This was investigated by measuring $^{14}CO_2$ fixation rates during an 8-hour exposure, followed by immediate harvesting of the plant materials (Table 1). The CO_2 fixation rate of the mycorrhizal and rhizobial plants was 7 percent higher per unit weight of shoots than that of the control. Mycorrhizal-rhizobial plants incorporated 16 percent more ^{14}C than the controls.

Symbiotic nitrogen-fixation rates were increased by mycorrhizal infection because of an increase in nodule weight (88 mg of nodules per gram of root for rhizobial roots compared to 144 mg of nodules per gram of root for doubly infected plants). Nodular tissue on alfalfa roots has been found to increase after inoculation with mycorrhizal fungi (*6*). These fungi are thought to exert their effect primarily by increasing phosphorus uptake. Since some phosphorus was added to the nonmycorrhizal treatments in this experiment, other nutrients also may have been involved (*7*). The extra carbon required in the presence of the mycorrhiza was offset to a large extent by higher nitrogen and carbon dioxide fixation rates (Table 1). Discussion of the carbon requirement for nitrogen fixation and other nutrient uptake by symbiotic associations is somewhat academic if the possibility that the plant can have altered photosynthetic rates in the presence of the symbionts is not considered.

Table 1. The $^{14}CO_2$ fixation (milligrams per gram of shoot carbon per hour) and $^{15}N_2$ fixation (milligrams per gram of nodule) by symbiotic and nonsymbiotic 4-week-old faba beans.

	$^{14}CO_2$ fixation (mg g^{-1} shoot carbon $hour^{-1}$)	$^{15}N_2$ fixed	
		Total (mg)	Rate (mg g^{-1} of nodule)
Control	17.4		
Mycorrhizal	18.8*		
Rhizobial	18.2†	0.78	16.2
Mycorrhizal-rhizobial	20.2*	1.06†	15.8

*$P < .05$. †$P < .10$.

Our estimates of carbon flow through root-microbial systems in soil were based on the premise that the symbionts did not significantly alter root respiration without altering root weight. Increased plant cytoplasm in fungal-infected root cells (*8*) and increased respiration of nodular tissue in the presence of bacteroids (*9*) have been noted. In our study, 4 percent of the respiration was unaccounted for in the presence of the two microbial symbionts, indicating some interaction between the symbionts. This, however, would not affect our data on the incorporation of ^{14}C into symbiont tissue, on total underground respiration, or on the relative rates of photosynthesis. The physiological interaction of host, fungi, and bacteria controls the response of the plants to microbial infection. An understanding of the various interactions and nutrient flows in such symbiotic associations should make feasible the selection, genetic manipulation, and management of each or all of the three components.

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References and Notes

1. C. A. Atkins, D. F. Herridge, J. A. Pate, *Isotopes in Biological Dinitrogen Fixation* (International Atomic Energy Association, Vienna, 1978), p. 211.
2. J. D. Mahon, *Plant Physiol.* **60**, 817 (1977).
3. R. M. N. Kucey and E. A. Paul, in preparation.
4. F. R. Warembourg and E. A. Paul, *Plant Soil* **38**, 331 (1973).
5. E. A. Paul and R. Johnson, *Appl. Environ. Microbiol.* **34**, 263 (1977).
6. S. E. Smith and M. J. Daft, *Aust. J. Plant Physiol.* **4**, 403 (1977).
7. L. H. Rhodes and J. W. Gerdemann, *Soil Biol. Biochem.* **101**, 361 (1978).
8. G. Cox and P. B. Tinker, *New Phytol.* **77**, 371 (1976).
9. J. S. Pate, D. B. Layzell, C. A. Atkins, *Plant Physiol.* **64**, 1083 (1979).
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Ureaplasma urealyticum Incriminated in Perinatal Morbidity and Mortality

Abstract. *Perinatal morbidity and mortality are associated with colonization of the chorionic surface of the placenta by* Ureaplasma urealyticum *or* Mycoplasma hominis *or both. These organisms are more strongly associated with unfavorable gestational outcome than group B streptococci.* Chlamydia trachomatis *does not appear to be important in the etiology of reproductive casualties. The mechanisms linking the mycoplasmas to perinatal disorders and death are not clear but merit investigation.*

The causes of perinatal morbidity and mortality in humans are not clearly defined. Nebulous concepts such as "small for dates infants," "low birth weight," and "placental insufficiency" are often invoked, but are nonspecific or elusive as to etiology. Premature birth is the most common antecedent of infant death, and premature labor remains unexplained. Because the placenta is the active interface between mother and fetus, it is the appropriate organ to study for clues to the causes of abnormal pregnancies.

Ureaplasmas in the female genitourinary tract have been related to low birth weight (*1*), infertility (*2, 3*), and spontaneous abortion (*4, 5*). Some investigators have found these organisms to be a greater threat to gestational outcome when isolated from the endometrium than from the cervix (*6*). We report that colonization of the chorionic surface of the placenta by *Ureaplasma urealyticum*

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and *Mycoplasma hominis* is even more strongly associated with abnormal outcome of pregnancy.

We cultured the chorionic surfaces of the placentas of 572 infants born at Brigham and Women's Hospital from November 1978 to June 1980. The infants were selected from three categories: perinatal deaths, intensive care infants, and control infants. A perinatal death was defined as a stillbirth or death from 20 weeks of gestation to 28 days after birth. An intensive care infant was one who weighed 2200 g or less at birth, was less than 36 weeks in gestational age at birth, or required neonatal intensive care for at least 48 hours. A control infant was a normal full-term infant delivered on the same day as a birth that eventuated in perinatal death. When a death occurred on a day other than the birth date, the placenta of a normal full-term infant born on the date of the death was cultured as the control. All specimens were numbered so that cultures could not be associated with the category of the infant.

Cultures were taken after carefully peeling away the amnion to avoid microbial contaminants acquired during passage through the birth canal. Two Rodac contact plates were applied directly to the exposed chorionic surface, one containing A7 medium (*7*) for the isolation of *U. urealyticum*, the other, Hayflick medium (*8*) for the isolation of mycoplasma species. Both plates were incubated by the Fortner method (*9*). Ureaplasmas and mycoplasmas were then identified (*9*). Three swab cultures were also taken. One was rubbed across the surface of two blood agar plates, one of which was incubated by the Fortner method, the other in a CO_2 atmosphere for the recovery of group B streptococci. The second swab was streaked on Hayflick medium and incubated aerobically for *M. pneumoniae*. The third was immersed in sucrose and phosphate transport medium (*10*) for inoculation for *Chlamydia trachomatis* in 1- to 4-day-old McCoy cells treated with cytochalasin B. Bacteria were identified by the method of Lennette *et al.* (*11*).

The highest rate of isolation of *U. urealyticum* and *M. hominis*, either separately or combined, was obtained in cultures from intensive care infants (Table 1 and Fig. 1). The second highest rate of isolation was obtained in cultures from the group of perinatal deaths. Analysis with contingency tables demonstrates that these rates are significantly different from the control rates for both microorganisms either singly or combined. The highest significance was found for *U. urealyticum* isolations considered alone ($P = .0005$). *Mycoplasma pneumoniae* was not isolated. Group B streptococci were isolated from placentas in 9 percent of the perinatal death group and 4 percent of the intensive care infants. The differences between these and the control rate (4 percent) are not statistically significant.

Table 1. Recovery of *Ureaplasma urealyticum* and *Mycoplasma hominis* from the chorionic surfaces of the placentas of perinatal death, intensive care, and control infants.

Category of infant	Number of placentas positive for microorganism (percent)		
	*U. urealyticum**	*M. hominis**	*U. urealyticum* and *M. hominis* combined
Perinatal death ($N = 125$)	24 (19)†	3 (2)‡	2 (2)§
Intensive care ($N = 293$)	78 (27)†	18 (6)‡	15 (5)§
Control ($N = 154$)	17 (11)	2 (1)	1 (1)
Total	119 (21)	23 (4)	18 (3)

*Includes combined isolations. †$P = .0005$. ‡$P = .03$. §$P = .02$.

Chlamydia trachomatis was not isolated from any placenta. These organisms may be present in the genitourinary tracts of men and women but do not appear to colonize the chorionic surface of the placenta.

Because of the increased prevalence of sexually transmitted infections, it is vital to know which of the diverse agents implicated in human genitourinary tract infections can influence perinatal morbidity and mortality. *Ureaplasma urealyticum* and *M. hominis* are both found in the male and female genitourinary tracts, and our results indicate that both are associated with neonatal illness, prematurity, and death. The mechanisms by which these organisms are linked to unfavorable outcome of pregnancy merit identification. Increased awareness of this association may permit effective preventive measures to be adopted. This is especially important because of the ubiquity of sexually transmitted diseases and the grave personal and social burdens of the reproductive casualties with which they are associated.

The presence of *U. urealyticum* has been correlated with subacute focal inflammation of the endometrium (*12*). The firm attachment of *U. urealyticum* to spermatozoa has also been described (*13*). These findings suggest that *U. urealyticum* may be present at the time of conception and implantation. It has been reported (*14*) that microorganisms can also be introduced by coitus during pregnancy. Such observations indicate that the introduction of microorganisms from the genitourinary tract can adversely affect perinatal survival.

Dische *et al.* (*5*), in a study of four pregnancies resulting in three spontaneous abortions and one perinatal death, implicated infections of the fetal membranes with *U. urealyticum* or *M. hominis*. These microorganisms were also isolated from the lungs of two fetuses and the liver of one. They concluded that the fetal infections followed placental involvement.

The placenta is intimately involved in the growth, development, and well-being of the fetus. Placental enzymes complement those of fetal origin in sustaining

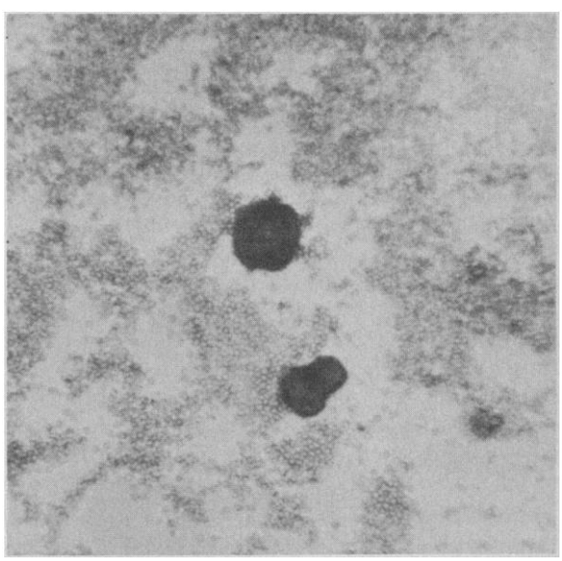

Fig. 1. Colonies of *Ureaplasma urealyticum* on a Rodac contact preparation of an area of placenta freshly stripped of amnion. The infant associated with this placenta was 1 week premature, weighed 2015 g at birth, and required intensive care.

and supporting essential gestational processes and optimal secretion of gestational hormones. The placenta is the organ of transport of fetal nutrients and wastes. As an interface between mother and fetus, it shelters the fetus from physical adversity and immunological rejection; it may also serve more complex defense mechanisms. Microorganisms in the placenta may conceivably impair these multiple functions to the detriment of the fetus.

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References and Notes

1. P. Braun, Y. H. Lee, J. O. Klein, S. M. Marcy, T. A. Klein, D. Charles, P. Levy, E. H. Kass, *N. Engl. J. Med.* **284** (No. 4), 167 (1971).
2. R. B. Kundsin and S. G. Driscoll, *Surg. Gynecol. Obstet.* **131**, 89 (1970).
3. H. Gnarpe and J. Friberg, *Am. J. Obstet. Gynecol.* **114**, 963 (1972).
4. R. B. Kundsin, S. G. Driscoll, P. L. Ming, *Science* **157**, 1573 (1967).
5. M. R. Dische, P. A. Quinn, E. Czegledy-Nagy, J. M. Sturgess, *Am. J. Clin. Pathol.* **72** (No. 2), 167 (1979).
6. B. Stray-Pedersen, J. Eng, T. M. Reikvam, *Am. J. Obstet. Gynecol.* **130**, 307 (1978).
7. M. C. Shepard and C. D. Lunceford, *J. Clin. Microbiol.* **3**, 613 (1976).
8. L. Hayflick, *Tex. Rep. Biol. Med.* **23** (Suppl. 1), 285 (1965).
9. R. B. Kundsin, A. Parreno, S. Poulin, *J. Clin. Microbiol.* **8**, 445 (1978).
10. *Guide to the Laboratory Diagnosis of Trachoma* (World Health Organization, Geneva, 1975), p. 25.
11. E. H. Lennette, E. H. Spaulding, J. P. Truant, *Manual of Clinical Microbiology* (American Society for Microbiology, Washington, D.C., ed. 2, 1974).
12. H. W. Horne, A. T. Hertig, R. B. Kundsin, *Int. J. Fertil.* **18**, 226 (1973).
13. H. Gnarpe and J. Friberg, *Nature (London)* **245**, 97 (1973).
14. R. L. Naeye, *N. Engl. J. Med.* **301**, 1198 (1979).
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Toxicity, Odor Aversion, and "Olfactory Aposematism"

Many plants and animals are chemically protected against predation by slow-acting systemic poisons present in their tissues and body fluids. These compounds are commonly bitter tasting but nonvolatile, and hence odorless. Some examples are well known: nicotine in tobacco plants (*Nicotiana* spp.), morphine in the opium poppy (*Papaver somniferum*), quinine in the cinchona tree (*Cinchona officinalis*), and strychnine in *Strychnos* spp. Less familiar examples include emetine in the roots of *Uragoga ipecacuanha* (*1*), emetic steroids in fireflies (*Photinus* spp.) (*2*), and cantharidin in meloid beetles (*3*).

Often the organisms involved are identifiable by odors unrelated to the toxins. There is no evidence to indicate that the odors themselves, at their natural concentrations, are intrinsically repellent to predators or play any direct role in chemical defense. In fact, no satisfactory biological explanation appears to have been advanced to account for such odors.

Palmerino *et al.* (*4*) have reported that if laboratory rats first exposed to a neutral odor while drinking sugar water are subsequently made ill, the odor becomes conditioned as a drinking deterrent. This result suggests that the characteristic odors of poisonous plants and animals may have evolved as the olfactory concomitants of bitter, odorless toxins and that they may function as conditioned stimuli for the deleterious effects of the ingested poisons, should the bitter taste itself be insufficient to deter the predator. Experienced predators would thereby be warned to search for food elsewhere. Warning by odor may appropriately be called olfactory aposematism, in analogy to the well-known alternative forms of aposematism shown by animals that warn their predators by visual or acoustic means (*5, 6*).

Odor, visual appearance, sound, taste, and tactile properties can all be thought of as elements of an "aposematic gestalt" that a predator makes use of to "key out" and appraise a potential prey item. The prey's odor, visual appearance, and sound would normally be the first of these elements encountered by the predator. Because it is obviously to the advantage of the prey to discourage the predator at the earliest possible stage of their interaction, one would expect all three modalities—odor, coloration, and sound—to enter into the elaboration of aposematic signals in nature. In plants, in which acoustic aposematism is nonexistent and visual aposematism relatively rare (at least of the vegetative parts), olfactory aposematism may well be the primary form of warning.

Visual mimicry is a well-studied consequence of the ability of predators to learn to avoid distasteful prey on the basis of appearance and coloration (*5*). If, as might be inferred from Palmerino *et al.* (*4*), predators can learn an avoidance response based on odor alone, then one can easily envision the evolution of imitation of warning odors by palatable species (Batesian mimicry) and convergence of odor in unrelated distasteful species (Müllerian mimicry). Such olfactory mimicry has indeed been invoked to account for apparent similarities in odor in certain chemically protected insects (*7*). The increasingly sensitive analytical techniques now available to the natural product chemist may make it possible to determine whether such presumed chemical mimicry has a basis in fact. It is particularly tempting to predict that olfactory mimicry, both Batesian and Müllerian, should be commonplace in plants.

The preceding is not meant to imply that odors are invariably aposematic or that olfactory aposematism can be achieved only through de novo evolution of warning chemicals. Odor is a consequence of the chemical emission that characterizes all forms of life, and most biological odorants are undoubtedly without signal function. Aposematic odors, one might imagine, could in many instances be no more than odors of incidental origin that have only secondarily, under appropriate predation pressure, and with or without chemical elaboration, taken on a communicative role. Pheromones, it has been hypothesized, could in some cases have had a comparable origin (*8*).

We also do not mean to imply that olfactory aposematism can occur only in association with slow-acting toxins. Noxiousness manifests itself in many ways in organisms, as through distastefulness, contact irritancy, pugnaciousness, and possession of defensive glands or mechanical weaponry. Body odors could take on a warning function in all such cases.

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References

1. M. Grieve, *A Modern Herbal* (Dover, New York, 1971).
2. T. Eisner, D. F. Wiemer, L. W. Haynes, J. Meinwald, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 905 (1978).
3. E. Kaiser and H. Michl, *Die Biochemie der tierischen Gifte* (Deuticke, Vienna, 1958).
4. C. C. Palmerino, K. W. Rusiniak, J. Garcia, *Science* **208**, 753 (1980).
5. H. B. Cott, *Adaptive Coloration in Animals* (Methuen, London, 1957).
6. D. C. Dunning and K. D. Roeder, *Science* **147**, 173 (1965); W. M. Masters, *Behav. Ecol. Sociobiol.* **5**, 187 (1979).
7. M. Rothschild, *Trans. R. Entomol. Soc. London* **113**, 101 (1961).
8. E. O. Wilson, *Sociobiology* (Belknap Press, Cambridge, Mass., 1975), p. 229.

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