References and Notes

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* Present address: Chalmers University of Technology, Gothenburg, Sweden.

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Strain of Mycoplasma Associated with Human Reproductive Failure

Abstract. *A strain of mycoplasma not previously described has been isolated from the chorion, decidua, and amnion of a patient who sustained a spontaneous abortion during the middle trimester. The fetal membranes exhibited an inflammatory reaction, but no evidence of other infectious agents, bacterial or viral, was noted. The T strain identified is not a classical mycoplasma; it differs in growth and nutritional requirements from the T strains previously characterized.*

Inflammatory reactions observed in the fetal membranes associated with many spontaneous abortions and premature births have been unexplained bacteriologically (*1*). In a series of such specimens, mycoplasma species were sought with the use of two techniques. The standard Hayflick medium (*2*) was inoculated and incubated under microaerophilic and aerobic conditions in a search for the classical, large-colony mycoplasma strains. Shepard's low-*p*H medium (*3*) was inoculated for identifying small-colony T strains and incubated under microaerophilic conditions. Blood-agar cultures were inoculated and incubated under microaerophilic and aerobic conditions for bacterial flora. A microaerophilic atmosphere on all media was accomplished with the Fortner plate method (*4*). All cultures were incubated at 37°C.

A strain of mycoplasma which does not resemble any strains previously described was recovered from chorion, amnion, and decidua of a spontaneous abortion during the middle trimester. This strain grew only on Shepard's medium and not on Hayflick's and had the colonial morphology of a T strain 20 to 40 μ in diameter, but it differed from previously reported T strains (*5*) in its slower growth on primary isolation and its fastidious nutritional requirements. In primary cultures, colonies could be visualized only after 4 days of incubation, were irregular in shape, had a deeper-staining central core, and accepted the Dienes' stain up to 2 weeks after isolation on the original medium. Shepard's broth did not support subcultures. A new broth (developed by Shepard) supplemented with urea and containing a phenol red indicator did support subcultures of this strain (*6*). The membranes cultured on blood agar under both aerobic and microaerophilic atmospheres showed no bacterial growth, thus eliminating the consideration that L forms had been induced from bacterial forms by penicillin present in the medium on which the initial isolation was made. Colonies of the newly encountered mycoplasma also grew on ascitic agar (*7*) which contains no penicillin. Lung and liver cultures of the fetus were negative for mycoplasmas. The liver showed no growth on blood agar. A few colonies of diphtheroids grew from culture of the lung.

Histologically, the decidua showed extensive necrosis and subacute inflammation; the fetal membranes and umbilical cord vessels were severely inflamed (Figs. 1 to 3). The infection of the membrane appeared to be of long duration. Unusual sclerosis of placental villi was observed. Acute inflammatory exudate, apparently aspirated and swallowed by the fetus (Fig. 4), filled the lumens of the bronchi and stomach. There was no evidence of subjacent tissue reaction. No organisms were identified in sections stained with hematoxylin and eosin, Giemsa, periodic acid-Schiff, or Gram stains. The placentitis did not resemble that which accompanies such antenatal viral infections as rubella, cytomegalovirus disease, herpes, vaccinia, or varicella. No inclusion bodies were found.

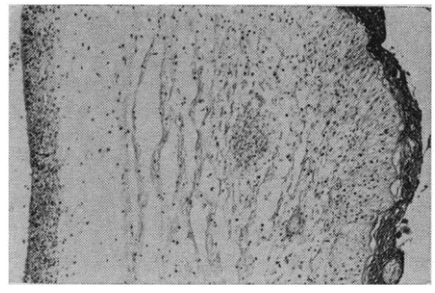

Fig. 1. Fetal membranes, amnion to the left. Necrosis and inflammatory infiltration are evident. (× 45)

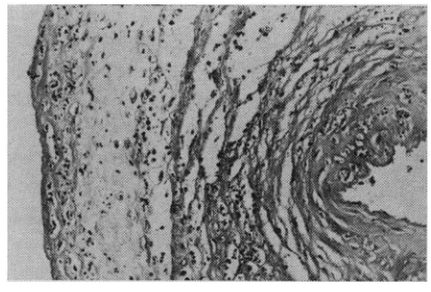

Fig. 2. Fetal membranes, amnion to the left. The amniotic epithelium is necrotic. Polymorphonuclear leukocytes are scattered in the chorionic membrane and amniotic connective tissue. Karyorrhexis of these cells is evident. (× 45)

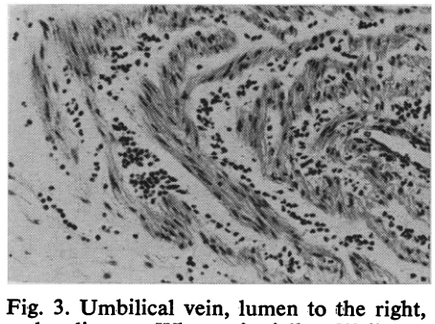

Fig. 3. Umbilical vein, lumen to the right, and adjacent Wharton's jelly. Well-preserved polymorphonuclear leukocytes lie between muscle bundles of the media and in the otherwise hypocellular contiguous stroma. (× 167)

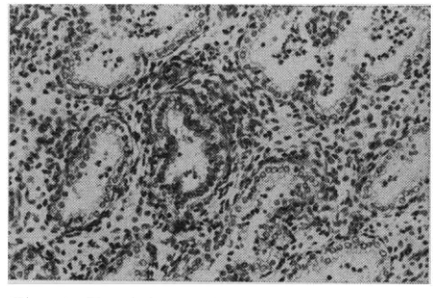

Fig. 4. Fetal lung. A suspension of polymorphonuclear leukocytes and proteinaceous debris lies within bronchiolar lumens. The immaturity of the fetus is indicated by the broad cellular septa. (× 167)

The patient, a 27-year-old woman in generally good health, whose first four pregnancies were uneventful, had expelled the female fetus 5 days after spontaneous rupture of membranes, in the 17th week of gestation. The placenta was retained, thus requiring manual removal. There was no fever or other systemic evidence of infection. Subsequent to isolation of the mycoplasma, the patient and her husband returned for study. The same small strain of mycoplasma was isolated from a cervical swab and from urine sediments of both husband and wife. Cultures of endometrial biopsy, the husband's urethra, and throats of husband and wife were negative for mycoplasmas.

Subsequent to this finding, the same strain was recovered from membranes of three of six spontaneous abortions or premature births and from cervical cultures of five of ten women with a past history of repeated spontaneous abortions. *Mycoplasma hominis I* was isolated from placental tissues of another case of spontaneous abortion. Cervical cultures of the mother in this case were also positive for *Mycoplasma hominis I*.

Shepard confirmed our isolation of the newly encountered mycoplasma from the fetal membranes and photographed typical colonies (Fig. 5). He suggested naming this mycoplasma the Boston T strain until further investigations to determine its appropriate taxonomic position have been made.

RUTH B. KUNDSIN
Department of Surgery, Peter Bent Brigham Hospital and *Harvard Medical School, Boston, Massachusetts*
SHIRLEY G. DRISCOLL
Department of Pathology, Boston Hospital for Women, and *Harvard Medical School*
PEN-MING L. MING
Department of Pathology, Peter Bent Brigham Hospital

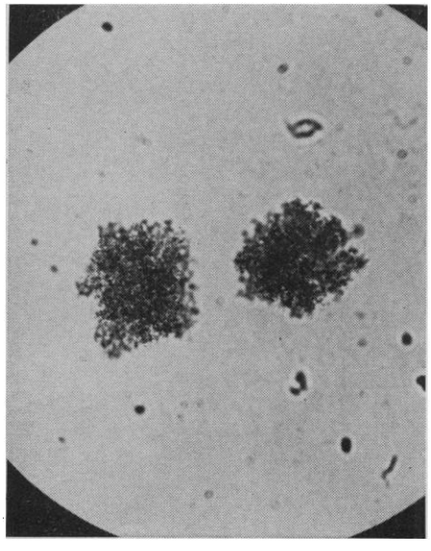

Fig. 5. Highly characteristic agar colonies of the Boston T strain of mycoplasma isolated from a case of human spontaneous abortion. The colonies were successfully propagated on tryptic digest agar, *p*H 6.0, containing 20 percent of normal horse serum. The initial incubation period was 48 hours at 36°C, followed by further incubation at room temperature for 28 days. Colonies are 30 μ in diameter. Wet-stained agar preparation (Dienes' method). (× 533) [After M. C. Shepard]

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Anatomical Connections between Medial and Lateral Regions of the Hypothalamus Concerned with Food Intake

Abstract. *No anatomical connections have yet been demonstrated from the ventromedial ("satiety") to the lateral ("feeding") areas of the hypothalamus. Lesions were induced with goldthioglucose in mice in the ventromedial region, including the arcuate and ventromedial nuclei. With the Fink-Heimer stain for degenerating axons, fiber connections between these two areas were demonstrated.*

Much attention has been given to the role of the hypothalamus in regulation of food intake. In a variety of species, notably the rat (*1*) and the mouse (*2*), lesions in the ventromedial nucleus (VMN) of the hypothalamus have produced hyperphagia and obesity, while lesions in the lateral hypothalamic area (LHA) have led to a cessation of eating (*3*). On the basis of these findings, a dual mechanism has been postulated in the regulation of food intake: a "satiety center" in the VMN and a "feeding center" in the LHA. The physiological and theoretical evidence in favor of this theory has been reviewed (*4*). The theory was based on the assumption that direct connections exist between these areas with impulses from the VMN inhibiting neural activity in the LHA. For example, impulses from the VMN to the LHA may cause a feeding animal to stop eating. By use of the Nauta-Gygax silver method (*5*), sparse fiber connections between the LHA and the medial zone including the VMN have been demonstrated (*6*). Because this method tends to suppress the impregnation of fine-caliber degenerating fibers, the LHA-to-VMN connections may well be more massive than Nauta's experiments showed (*6*). As yet, no neural pathways oriented in the opposite direction, that is, connections from VMN to LHA, have been found.

We now report evidence of direct fiber connections between the medial and lateral regions of the hypothalamus. Demonstration of such connections was made possible by the Fink-Heimer method (*7*), which provides a stain for degenerating axons particularly effective in the demonstration of degenerating axon terminals. Basically, this method is a modified combination of the original, nonsuppressive, terminal-degeneration method of Nauta (*8*) and the Nauta-Gygax suppressive method (*5*).

In our experiments, 20 female albino mice (Charles River) were injected intraperitoneally with the chemical compound goldthioglucose which has been shown to produce lesions in the ventromedial region of the hypothalamus (*9*) with a direct relation between lesion size and dosage. The injected mice weighed between 20 and 25 g, and the concentration used was equal to 0.5 mg per gram of body weight, which is approximately one-third of the lethal dose.

In several experiments with the use of the Fink-Heimer method, a survival time of 3 to 4 days after injection was found to be optimum for the demonstration of axonal degeneration. At this time, the animals were killed with an overdose of ether, and their hearts were