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.

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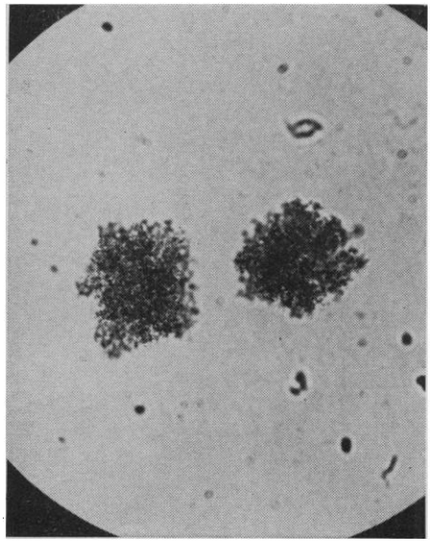

Fig. 5. Highly characteristic agar colonies of the Boston T strain of mycoplasma isolated from a case of human spontaneous abortion. The colonies were successfully propagated on tryptic digest agar, *p*H 6.0, containing 20 percent of normal horse serum. The initial incubation period was 48 hours at 36°C, 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

perfused with physiological saline solution and then with a neutralized 10-percent formalin solution. The brains were removed from the skulls, fixed in a 10-percent formalin solution for approximately 1 week, and soaked in a 30-percent sucrose solution for 3 to 4 days (*7*). Brains were then sectioned in either a frontal or sagittal plane at 25 μ on a freezing microtome and collected successively in nine vials containing a dilute formalin solution (1.5 percent concentration).

With the Fink-Heimer method, sections in vials 1, 4, and 7 were stained in accordance with procedure I, while those in vials 2, 5, and 8 were stained as in procedure II. For cytological examination of the lesions, sections in vials 3, 6, and 9 were stained with the Nissl method (*10*). Degeneration of fibers of passage and terminal axon arborizations, including synaptic end structures, was identified microscopically and recorded in projection drawings of selected sections.

To obtain optimum results in staining degenerating axons and their terminals in the hypothalamus, a slight modification of the procedures of the Fink-Heimer method was advantageous. In procedure I, step 3, the impregnation time was extended to 12 to 16 hours. Furthermore, it was advantageous to eliminate step 4. In procedure II, step 4, the concentration of silver and pyridine was increased to 0.375 percent, and the impregnation time was extended to 40 to 48 hours.

The Nissl stain showed that the lesion produced by goldthioglucose was located in the ventromedial region of the hypothalamus and included all the arcuate nucleus, the ventrocaudal half of the VMN, and the ventral tip of the dorsal premammillary nucleus. The rostral half of the VMN did not seem to be involved in the lesion.

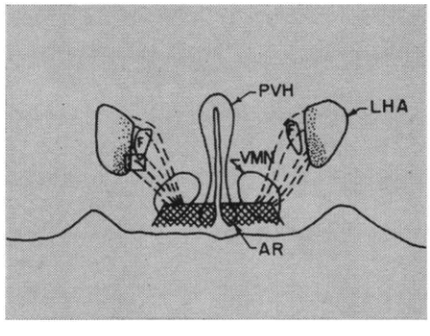

Fig. 1. Degenerated fiber tracts leading from medial region and terminating in lateral hypothalamic area; lesion (cross-hatched area), degenerated fibers (coarse stipple), and terminal degeneration (fine stipple). *AR*, arcuate; *F*, fornix column; *LHA*, lateral hypothalamic area; *PVH*, periventricularis hypothalami; *VMN*, ventromedialis hypothalami. Enclosed area on left is shown in Fig. 2.

The staining procedures showed degenerated axons extending from the site of the lesion in a dorsolateral and slightly rostral direction and terminating in the medial region of LHA. Most fibers passed slightly ventral to the fornix column, whereas those coming from the most dorsal boundary of the lesion in VMN passed just dorsal to the bundle; some isolated fibers seemed to traverse the fornix bundle directly. It was difficult to determine whether these mediolateral connections that passed ventral to the fornix column originated in the arcuate nucleus or the VMN. Those fibers that passed dorsal to the fornix seemed to originate from the VMN. Terminal degeneration was lightest in the rostral and caudal regions of LHA and heaviest in its middle region. No terminal degeneration was seen at all in the lateral half of LHA. These results are illustrated semidiagrammatically in Fig. 1. Figure 2 shows terminal degeneration near the fornix bundle.

To determine whether fiber connections between the medial and lateral areas of the hypothalamus could be stained with the Nauta-Gygax suppressive method (*5*), ten additional female albino mice, weighing between 20 and 25 g, were injected in similar manner to that described above. However, five of these mice were killed 4 days after injection, whereas the other five were killed 10 days after injection. Selected sections were stained either with the Fink-Heimer method or the Nauta-Gygax suppressive method.

In those killed 4 days after injection, sections stained by the Nauta-Gygax suppressive method (*5*) showed only sparse evidence of axon degeneration; no signs of terminal degeneration could be found in LHA. By contrast, adjoining sections prepared by the Fink-Heimer method (*7*), showed an abundance of terminal degeneration as described above. In those killed 10 days after injection, the Nauta-Gygax suppressive method revealed numerous degenerated axons extending from VMN to LHA, again without impregnation of degenerated axon terminals. In the same cases, however, the Fink-Heimer method also failed to demonstrate terminal degeneration. These experiments

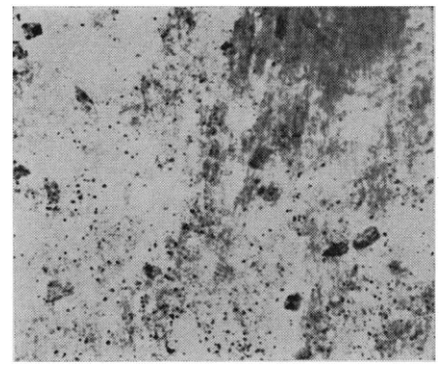

Fig. 2. Terminal degeneration in and near LHA. Fornix is at upper right.

corroborate Heimer's (*11*) conclusion that the suppressive Nauta-Gygax method is generally unable to demonstrate degenerating axon terminals, whereas the Fink-Heimer method impregnates degenerating axon terminals within certain temporal limits which in our experiment appear to extend around survival of 3 to 4 days.

Our experiments, by showing evidence of direct fiber connections from the medial to the lateral hypothalamic area, furnish some anatomical basis for the long and widely held concept that the medial hypothalamic ("satiety") region can act as an inhibitor of the lateral hypothalamic ("feeding") area.

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