MYCOSIS OF THE BOVINE FETAL MEMBRANES DUE TO A MOULD OF THE GENUS MUCOR.

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An accurate knowledge of the number and variety of living organisms which may invade the uterochorionic space during pregnancy in bovines and multiply enough to set up localized or general disease of the chorion can only be gained by a cumulative study of the pregnant uterus before discharge of the fetus. After expulsion of the latter, adhesion of the placenta, the loss of pathological fluids held between uterine wall and chorion, the soiling of the placenta by the bedding, or its destruction by the cow, all stand in the way of an elucidation of those still unknown agencies of disease which may be of far more significance than is credited to them. The relation of \textit{Vibrio fetus} to abortion may be considered as established. Occasionally \textit{Bacillus pyogenes} is present in such numbers in the organs of the discharged fetus that tentatively it may be regarded as a primary agent in certain cases.\(^1\)

Among a considerable number of pregnant uteruses obtained from abattoirs, the writer found one which deserves a brief description. The uterus was brought intact to the laboratory and the following facts were ascertained.

\textit{Case 182.}—When the wall of the uterus was cut through there was found between uterine mucosa and chorion (uterochorionic space) a considerable amount of a turbid fluid full of small flakes. Over a region of the chorion about 20 cm. in diameter, corresponding with or resting on the left shoulder and thorax of the fetus, the cotyledons, five in number, are separated from the uterine wall so that the latter

can be lifted away from the fetal membranes over this area. The pedicles of these cotyledons (maternal caruncles) are short, blunt projections of the uterine wall, varying in size, in color grayish with some hemorrhagic spots. The affected cotyledons are enlarged, the margins much thickened, and rolling cup-shaped over into the central necrotic portion. The chorion between these detached cotyledons is beset with yellowish gray, slightly elevated plaques, 2 to 4 mm. in diameter and a fraction of a mm. thick, producing a coarse goose-skin appearance. The subchorionic edematous tissue appears as a glassy, gelatinous layer 1 to 2 cm. thick. In one horn the chorion, over an area 6 cm. square, is beset with grayish to yellowish plaques containing minute hemorrhages. The fluid in the allantois at both horns is clear. The amniotic fluid contains a fairly abundant amount of fecal matter. The cervix of the uterus is normal and the external os is tightly closed and provided with the normal mucous plug.

The fetus is 25 inches long and normal, with the following exceptions. There is edema of the sheath of the umbilical vein within the abdomen, also slight edema of the capsule of the kidneys, and of the interlobular tissue of the lungs. The rumen contains considerable yellowish (fecal) matter, but the fourth stomach contents are still clear and colorless.

Although the gross appearances did not correspond closely to those found in the presence of Bacillus abortus, the case was considered one of infectious abortion due to this bacterium, until examination of the scrapings of the cotyledons showed a branched mycelium and no bacteria of any description. Cultures on slanted agar, sealed with sealing-wax and unsealed, of material from the surface of the chorion and from the amniotic fluid developed a mould. Cultures from the lungs and meconium of the fetus also contained only a mould, while those from the fourth stomach, spleen, liver, and one kidney remained sterile. Subsequent study of the moulds from placental and fetal cultures showed them to be identical and belonging to the genus Mucor.

The absence of Bacillus abortus from the cultures was confirmed by the results of inoculation of guinea pigs with the fluid exudate in the uterochorionic space, with amniotic fluid, and with contents of the fourth stomach of the fetus. The guinea pigs killed after 26 days
were normal and cultures remained sterile. Films from the chorion and the fluid exudate showed no bacteria of any kind. In the necrotic masses scraped from the cotyledons, a branched, non-septate mycelium was detected.

Tissues fixed in Zenker's fluid from various diseased and normal regions of the placenta were studied in sections in order to determine the relation of the mould to the tissues and the character of the tissue reaction. Although a variety of dyes was used, the mycelium stained very poorly and only careful focusing with a partly closed condenser made it possible to identify the filaments and trace them as far as the section permitted.

The remnants of the caruncles projecting from the uterine wall were densely infiltrated and covered with masses of polynuclear leucocytes. Among these masses the branched mycelium could be traced, penetrating them in various directions. The corresponding cotyledons with the sloughed off portions of the caruncles still embedded in them presented a variety of changes, consisting chiefly of necrosis of most of the tissue and masses of polynuclear leucocytes. Traces of the original tissue appeared in islands of villi, densely filled with blood corpuscles. Branched mycelium could be seen penetrating the necrotic and purulent foci. It varied somewhat in diameter and was more or less angular and dilated in places. Most of the filaments appeared empty, at least the staining did not reveal any contents. No fruiting bodies, either in the form of sporangia or chlamydomomes, were found.

As stated above, the intercotyledonous areas of the affected region of the chorion were beset with hemispherical elevations about 2 to 4 mm. in diameter, which gave the membrane a coarse goose-skin appearance. The sections showed that over the elevations it was deprived of its epithelium and the subjacent, bared zone filled with dense masses of nuclear debris which projected somewhat and gave the surface its nodular outline. This lesion is similar to that found in the bared chorion when Bacillus abortus is the agent, as well as in cases associated with Vibrio fetus. It would seem as if any injury to or destruction of the epithelium leads to a movement of leucocytes towards the surface, under which they gather in dense groups and disintegrate.
The invasion of the fetal membranes by a mould presupposes some primary focus in the body of the dam, whence spores might enter the circulation and break through into the fetal cotyledons. Unfortunately nothing is known of the dam, since the cow was slaughtered in the routine work of the abattoir and only the pregnant uterus reserved. If the above view of the source of the infection is correct it would imply the existence of moulds in the lungs of the cow where the oxygen requirements are sufficient for the production of sporangia and spores.

The literature on pathogenic mucors is very meager as contrasted with that on Aspergillus. Lichtheim isolated from moistened bread two species pathogenic for rabbits after intravenous injection of fairly large doses of spores, Mucor rhizopodiformis and Mucor corymbifer. The chief loci of germination and growth were the kidneys, the lymphoid tissue of the intestines, and the mesenteric nodes. Paltauf soon afterwards found a mucor in focal lesions of the cerebrum, cerebellum, lungs, pharynx, and ileum of a man 52 years old. The mucor was not cultured. The dimensions given indicate that he had a much smaller type under observation than the types mentioned above. Lindt isolated two additional species from moistened, incubated wheat bread which were pathogenic for rabbits after intravenous injection of spores. These were named Mucor pusillus and Mucor ramosus, both distinguishable from one another and from the species cultured by Lichtheim on account of the shape and dimensions of the spores. Hückel isolated a mucor from a cerumen plug taken from the ear of a patient 28 years old. This he identified with Mucor corymbifer.

**Biological and Pathogenic Characters.**

The mould was readily cultivated at incubator temperature on ordinary nutrient agar, although an addition of 1 per cent dextrose increased the vigor of the growth. At 70°F. the growth was much...
slower. It presented the following characters. A dense, branching, silken, whitish mycelium spreads over the surface and, in tubes, penetrates vertically for a distance of 5 to 10 mm. into the depths of the agar. The mycelium varies from 4 to 10μ in diameter. It contains a slightly refractile, coarsely or finely granular cytoplasm. The branching of the mycelium was at no time observed to follow any definite scheme. It evidently depended on the condition of the substrate.

In addition to this adherent layer of interlacing filaments an abundant aerial woolly mass of filaments appeared within 48 hours. These were found empty and often collapsed later on, probably as a result of drying. Rhizoids, or radiations of short filaments from centers of growth on the agar surface, were found, from which fruiting hyphae arise to bear at the free end the sporangia. These sporangiophores and the rhizoids can be distinguished from the vegetative mycelium by their brownish tint under a low power. The rhizoid gives origin to a short trunk which soon divides irregularly into a small number of sporangiophores.

The sporangia, barely visible to the unaided eye, become a grayish brown color as they ripen and appear almost black under a low power in transmitted light. They are subspherical, measuring transversely about 80μ and vertically about 64μ, although smaller heads occur. The columella seen after discharge of the spores projects as a spherical body from the funnel-shaped, expanding end of the supporting hypha. The slightly brownish spores are spherical and measure about 4.5μ, although some as small as 4μ and as large as 5μ may be seen.

The mycelium on the agar surface may form in places two transverse septa close together. The intervening walls of the filaments bulge and the segment thus isolated and containing a granular cytoplasm forms a rather thick wall showing later on a double contour. The filaments on both sides of this new body shrivel. In rare instances a series of such segregated and encysted masses of cytoplasm may form. These bodies, usually denominated chlamydospores (Gemmen, Dauerzellen), were quite numerous in all cultures. No zygospores were seen at any time. An examination of the literature indicates that Lichtheim's Mucor rhizopodiformis comes nearest to
the species under investigation, although there are certain differences such as a colorless, slightly larger spore (5 to 6 μ) in Lichtheim's culture.

No special studies were made upon the behavior of this species under different environmental conditions. This much was frequently observed, however, that the mould is very capricious in the production of sporangia. Apparently the same conditions at one time lead to a rapid, copious formation of sporangia with ripening of spores in 2 to 3 days, in another to none. The obvious interpretation is that slight unrecognized differences in the environment exercise a decisive influence. Evidently oxygen is an important factor, for the sealing of tubes, which reduces the oxygen tension through absorption of the oxygen by the culture medium and by the mould during the early stages of growth, interferes with spore production.

To determine whether spores of the mould would germinate and develop a mycelium in the organs of rabbits, the growth from a dextrose agar culture was thoroughly stirred about in sterile bouillon and filtered through five layers of sterile fine bandage material to remove fragments of mycelium and clumps of spores. The resulting feebly clouded, lightly brownish fluid was found with only isolated spores. Doses of 1.5 and 0.5 cc. were injected into an ear vein of two rabbits weighing 2,218 and 2,404 gm. respectively. The rabbit receiving the smaller dose was in appearance normal until the 8th day, when it was chloroformed. The weight had dropped from 2,404 to 2,286 gm. The spleen was a trifle large and congested. It was beset with numerous subcapsular, 0.5 mm., isolated or agglomerated, whitish, slightly projecting nodules. Both kidneys show the same kind of nodules, about 1 to 5 mm. apart. Some of these correspond to opaque whitish streaks passing through the medulla and continuing in slenderer radial lines to the pelvis. In the latter no changes are evident. Scattering nodules occur in the liver and one in a Peyer's patch. The appendix is free. Several mesenteric nodes contain large necrotic foci. In one kidney nodule, crushed between slides, branching mycelium could be traced for some distance. Bits of spleen, liver, and kidney tissue placed in agar slants developed a rich mycelium within 24 hours.
The second rabbit died unexpectedly on the 7th day. No disturbance of health had been noticed but the weight at death was only 1,740 gm. Lesions much smaller than in the first rabbit were found in spleen and kidneys. The spleen was small and pale. The cause of death was probably some intercurrent influenza, since the lungs were generally congested and edematous and the nostrils ringed with dry crusts. There was, however, no similar disease among the older stock animals and it may be that the resistance had been lowered by the mould.

Sections of tissue of the first rabbit fixed in Zenker's fluid presented a number of interesting details which can only be briefly enumerated. The lesions of the kidneys in the cortex were made up of dense collections of polynuclear leucocytes within areas in which the interstitial cells had also proliferated. The affected area included one or several glomeruli and the tubules immediately surrounding them. The origin of the lesion, whether in a glomerulus or the tubules, could no longer be determined, as the original structures were barely identifiable. The focus thus begun could be traced downward, the associated tubules being filled with masses of partly disintegrated cells, chiefly polynuclears. These plugs extend to the tip of the papilla. Here the pelvis contains a mass of polynuclears completely surrounding the papilla. Branched mycelium could be seen in all the involved tubules. It was most abundant in the cellular masses of the pelvis. The changes in the spleen were similar to those in the cortex of the kidney. In the mesenteric nodes the foci were so numerous as to coalesce and involve more than half the node. Mycelium was specially abundant. In the liver two kinds of lesions were present, collections of polynuclear leucocytes taking the place of destroyed liver tissue and much smaller foci composed of multinucleated cells, some closely resembling those of the tubercle. No formed elements could be made out within these giant cells, but it is probable that they contained mould spores. Microscopic foci were not found in the lungs although all the injected spores must have passed through these organs.

These two inoculations serve to place this mucor among Licht-heim's so called pathogenic species. There are certain minor differences between his results and the present, which might disappear in
larger series. The slightly lower pathogenic effect when compared with Lichtheim’s tests may be due to the fact that the inoculations were made after the mould had been kept 23 months on culture media.

The source of the infection in the cow can only be conjectured. The two widely distributed mucors, not pathogenic for rabbits, *Mucor mucido* and *Mucor racemosus*, occur frequently in dairy products, and it is probable that the species under consideration may be found there and thus represent a possible source of the infection.

**SUMMARY.**

A mucor, closely resembling Lichtheim’s *Mucor rhizopodiformis*, was isolated from the diseased chorion of a cow and from the lungs and digestive tract of the fetus. No other microorganisms were detected. The mucor was demonstrated in teased preparations from the fresh cotyledons as well as in sections of tissues suitably hardened. It produced focal lesions in rabbits following the intravenous injection of spores. The condition of the amniotic fluid and the contents of the rumen of the fetus justify the inference that premature expulsion was impending.