A CHARACTERISTIC LOCALIZATION OF BACILLUS ABORTUS IN THE BOVINE FETAL MEMBRANES.

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PLATES 20 TO 22.

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While making a study of the diseased membranes in cases of infectious abortion of cattle, the writer came upon a peculiar and characteristic habitat of Bacillus abortus Bang, in the epithelial covering of the chorion.

This layer of cells which faces the epithelial covering of the uterine mucosa and is in intimate contact with it covers the intercotyledonous areas of the chorion and is continuous with the epithelium of the villosities of the cotyledons which dip into the depressions of the maternal caruncles. The general character of the cells may be seen in Fig. 4. The cells vary somewhat in height. The vesicular nucleus is round or oval in outline and the chromatin appears as minute 1 μ spheres against the nuclear membrane. The free border of the cytoplasm appears frequently in the form of blunt finger-like or conical projections, giving the surface a fimbriated appearance. In the specific infectious disease of the fetal membranes these cells, either individually or in series, are densely filled with minute bacilli. The invasion is recognizable under a low power in that the cytoplasm of the affected cell assumes a blue color when the section is stained in eosin-methylene blue (Fig. 1). High powers resolve this tint into fine, short, rod-like bodies (Figs. 2 to 6). The bacilli do not lie on the cell or in the ectoplasm but fill the cell body entirely. When the microscope is raised or lowered the cytoplasm appears filled in all optical sections.

The host cells are more or less altered as to size and condition of nucleus. Some cells are still normal in size and the nucleus is recog-
nizable as a vesicle with contained chromatin (Fig. 4). In others the nucleus is compact, pyknotic. The cell body becomes enlarged and it may measure up to 40 $\mu$ in diameter (Fig. 6). Vacuolation of the cytoplasm is not uncommon (Fig. 3). Fetal membranes at the end of the normal period of gestation which are macroscopically without pathological changes have been found uniformly free from such cell contents. The same is true of fetal membranes from cases of abortion associated with spirilla from which Bacillus abortus is not obtainable, either in cultures or through guinea pig inoculation. In all cases in which the invasion of the epithelial cells was detected this feature was associated with positive cultures of Bacillus abortus, or the characteristic guinea pig disease after inoculation, or with both tests positive. Simple cover-slip preparations from the exudate or the necrotic villi were frequently sufficient to demonstrate the presence of infected epithelia. Besides the chorionic epithelium, the epithelial cells at the margin of the cotyledons and those of the outermost villi of the latter have been found invaded (Figs. 2 and 5). The bulk of the villi though undergoing profound changes are as a rule free from nests of bacteria.

That the cell localization described above is a regular occurrence wherever B. abortus is active may be gathered from the scanty literature by an interpretation of certain statements made, although none of the authors to be cited made an attempt to determine the precise source of the clumps of bacilli. Thus Bang in 1897 describes the bacteria in films from the fetal membranes as either free or in dense clumps which appeared to have been formed inside cells. The following statement occurs in the British report: "In many places the bacilli are collected into dense groups or colonies. Some of these groups look as if they were bounded by a cell membrane and give the impression of being contained inside tissue cells." It is also stated that: "It is an easy matter to identify the characteristic clumps of abortion bacilli in microscopic preparations made from the uterine exudate discharged immediately before or after abortion." In the Appendix we read as

4 British report, p. 6.
5 British report, p. 15.
6 British report, Appendix, p. 27.
follows: "... so far as the membranes and swabs are concerned a positive diagnosis was based on the presence of the specific microbe in the typical clump form because these materials were always very impure and it is not possible to identify scattered abortion bacilli in a mixture of bacteria." Zwick and Zeller\(^7\) refer to bacilli in large numbers in uterus exudate and state that cells may be filled with them. Fig. 3 illustrates very well what these writers probably refer to in most instances. In some cases, however, they may have seen leucocytes which at times are quite numerous and densely filled with bacilli. The latter are probably set free from the detached and disintegrated epithelial cells and taken in by leucocytes.

The mode of invasion of the epithelium by bacteria manifesting no true motility but only Brownian motion may be tentatively explained by assuming that the bacteria entering the uterochorionic space by way of the blood vessels in the uterine wall adhere to the ectoplasm and are rubbed into the substance of the cell by the pressure exerted by the uterine wall on the chorion. Once in the cytoplasm the bacteria find it a favorable medium for multiplication and a protection against phagocytosis. An alternative explanation would be to ascribe ameboid activities to the chorionic epithelium, for which theory at present no adequate proof exists.

It may be maintained that the cell parasitism is not an active disease process but rather secondary to it and operating only on cells partly or wholly devitalized. This interpretation has been kept in mind in the study of the various cases. When the disease is so far advanced that expulsion of the immature fetus occurs, the pathological material may fail to furnish an unequivocal answer to the above question. In several cases, however, in which a living calf at full term had fetal membranes in the early stages of infection, and in one slaughtered cow, the appearance of the invaded cells and the irregular distribution of the infection make it probable that they had been invaded while still in a normal condition (Figs. 2, 4, and 5).

The other types of epithelia, such as those of the uterine mucosa and of the amnion, have not been found invaded. The uterine epithelium resembles in its columnar appearance that of the chorion, but the cytoplasm forms a smooth, level, uninterrupted surface. The amniotic epithelium is of the flat, squamous type.

\(^7\) Zwick and Zeller, Arb. k. Gsundhsamte., 1913, xliii, 1.
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SUMMARY.

The significance of this invasion of the chorionic epithelium from the standpoint of pathogenesis cannot be properly evaluated until a more complete history of the successive localizations of Bacillus abortus has been obtained. It is safe to assume that this particular cell parasitism is but one of a series of localizations and centers of multiplication in the fetal membranes although evidence points to it as perhaps the earliest stage in which the organism gains by rapid, unchecked multiplication a considerable advantage over the host. The local destruction of an epithelial covering by an infectious agent when other miscellaneous infectious agents are absent may or may not be of much importance, for it would depend on the regenerative activity of the epithelium, the tendency to the gathering of injurious transudates, and the toxic substances associated with the bacilli.

It is probable that localizations also occur in the walls of the blood vessels of the chorion. Thus far only one case of this kind has been observed. The fusiform connective tissue cells of the adventitious coat of a blood vessel 0.8 mm. in diameter were completely replaced by clumps of minute bacilli. Since there is usually a slight perivascular cell infiltration in the diseased placenta this localization may be largely responsible for the circulatory disturbances which lead to death of the fetus. The case referred to may be but a greatly exaggerated illustration of the action of Bacillus abortus in the walls of the blood vessels where they are too few in number at any one time to be identified. It is known, 8, 9 that in the guinea pig disease with pronounced lesions Bacillus abortus is demonstrated only with great difficulty because of its scarcity.

The more or less specific localization and multiplication of bacteria within cells not having phagocytic functions have thus far been demonstrated in leprosy, syphilis, and in a disease of mice recently described by Tyzzer 10 who found an active invasion of both liver cells and intestinal epithelium by a bacillus. In cells to which phagocytic powers have been ascribed the specific localization of certain bac-

teria is well known. Thus tubercle bacilli occur within the endothelial
cells of the tubercle. Leprosy bacilli have been found within a
variety of cell groups. Mouse septicemia bacilli occur regularly
within certain phagocytic cells of the blood.\textsuperscript{11} Recently bacteria
have been found attached to the cilia of the respiratory tract in per-
tussis by Mallory and Hornor,\textsuperscript{12} in a form of guinea pig pneumonia
by the writer.\textsuperscript{13} Actual occupation of epithelial cells followed by
active multiplication of the invaders and destruction of the cell has,
however, been frequently demonstrated for the sporozoa. That it
may occur more often among bacteria is highly probable. Rapidity
of multiplication and cell destruction or invisibility or both may
stand in the way of a satisfactory demonstration.

EXPLANATION OF PLATES.

PLATE 20.

\textbf{Fig. 1.} Placenta of Heifer 203, which gave birth to a small but apparently
healthy calf. Period of gestation unknown. The epithelium covering the chorion
is enlarged, irregular in outline, the cytoplasm stained blue, and the nuclei are
pyknotic. The cytoplasm of all is densely packed with bacilli not recognizable
at this magnification. \textit{B. abortus} was isolated from the placenta through guinea
pigs. 10 days later the calf, having scoured somewhat, was killed and \textit{B. abortus}
was isolated from small foci of bronchopneumonia through guinea pigs. $\times 125$.

\textbf{Fig. 2.} Placenta, Cow 146, aborted. A marginal villus of a cotyledon cut
transversely and showing two epithelial cells \textit{in situ} densely filled with bacilli.
One cell is enlarged, projecting, the other very flat, cut transversely, and showing
as a slender, nematode-like body. The nucleus of this latter infected cell is
shown. $\times 1,000$.

\textbf{Fig. 3.} Film made from placental exudate, Cow 298, aborted. Alkaline
methylene blue. The epithelial cell is enlarged, vacuolated, and filled with
bacilli. $\times 1,000$.

\textsuperscript{11} Koch, R., Untersuchungen über die Aetiologie der Wundinfektionskrank-
heiten, Leipzig, 1878.


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PLATE 21.

Fig. 4. Placenta, Cow 171. Fetus obtained in utero after slaughter. The chorionic epithelium is shown in situ. One densely infected cell in the center, others, right and left. The cell nuclei are no longer of normal appearance but the cells themselves are only slightly swollen. × 1,000.

Fig. 5. Placenta, Cow 91, aborted. A short blunt fetal villus from the margin of a cotyledon is shown with all of the covering epithelium filled with bacilli. × 1,000.

PLATE 22.

Fig. 6. Placenta, Cow 127, which gave birth to a full term calf. The epithelial cells of the chorion are slightly lifted away from their normal base. The cells are much larger than normal, the nuclei pyknotic. All are densely packed with bacilli. × 1,000.

Guinea pigs inoculated with placental tissue of all the above cases, ground up and suspended in salt solution, became diseased and yielded pure cultures of B. abortus. In several cases cultures were obtained directly in spite of the soiled condition of placenta.
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