ON THE METHOD OF PROPAGATION OF THE BACILLUS OF STEFANSKY INOCULATED INTO THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO [1]

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We have studied histologically the method of propagation of the Stefansky bacillus inoculated on the chick embryo with a view to obtaining a culture. These inoculations were made by a technique already described by one of us. The favorable development of the culture has been proved by a series of successive transfers.[2] The specimens for examination were removed at intervals after inoculation which varied from 24 hours up to the 10th day.

From the histological point of view the chorioallantoic membrane consists of a mesenchymal layer clothed by an epithelium which is uni- or pleuri-stratified at different points. In the 24 hours following the inoculation one finds on the surface of this epithelium a magma constituted in part by autolyzed cells and in part by masses of bacilli, the whole representing the material removed from the rat and inoculated into the egg. There is observed at the place of contact of this magma a reaction of the allantochorionic epithelium which is manifested by multiple stratification. Occasional rare bacilli have penetrated into the interstices of this epithelium, where they are found either isolated or in groups of four or five. The mesenchyme seems intact; no reactional element exists.

From the second day after the inoculation, although the epithelium of the superficial layer does not seem to contain more bacilli than before, and in any case none has passed beyond the depths of the epithelial barrier, one is surprised to observe in some instances the presence of Stefansky bacilli within the mesenchyme. It seems evident that they can only have come there by way of the blood vessels.

In the following days one observes progressive penetration of the bacilli into the depths of the epithelial covering layer. They accumulate in the intercellular spaces, where they consti-

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[1] Reprinted in approved translation from the *Annales de l'Institut Pasteur* **76** (1949) 535-538 (June).

[2] SOEUR MARIE-SUZANNE. *C. R. Soc. Biol.* **142** (1948) 35.

tute tiered masses joined one to another by strands. Finally, breaking through the limiting layer of this epithelium, they invade the mesenchyme (Fig. 1 A, B, and C).

This penetration occurs not only at the point of inoculation but often at a considerable distance, which leads to the assumption that the bacilli, at the same time they are penetrating into the depth, also spread along the surface and later penetrate at distant points of the epithelial layer.[3]

From the third or fourth day the bacilli which penetrated into the mesenchyme by way of the epithelium, spread out and are found either within cells or apparently free in the spaces of the mesenchymal network; and give rise, often in considerable numbers, to eosinophilic cells marking the reaction of the infected organism.

In the following days one can see, developing progressively, masses of mesenchymal cells the density of which is in contrast with the delicate reticular structure of the surrounding tissue (Fig. 2). Bacilli are found in these masses, but they also exist apart from them, more or less isolated in the open reticular tissue. In the denser zones, where the bacilli are more numerous than elsewhere, one also finds after the fourth day, mixed with the bacilli, clear maroon-colored granulations which seem probably to be of bacillary origin, signifying destroyed bacilli. In any case, it seems that one can say that the fifth day marks the moment when the invasion of the mesenchyme is complete.

By the seventh day these cellular nodules are enlarged and appear as large plaques made up of cells with a clear nuclei, the voluminous protoplasmic bodies of which are crammed with bacilli which stain intensely by the Ziehl method (Fig. 3). It is curious to see that these cells, whatever may be their "charge" of bacilli, present no sign whatever of nuclear or protoplasmic alteration. A harmonious cohabitation between the cells and the bacilli seems to have been established.

These homogeneous infiltrative masses are clearly individualized and visible even under low magnification in the midst of

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[3] In short, the penetration of the bacilli cultivated on the chorioallantoic membrane seems to progress by two routes: one occurs, by progressive stages, through the epithelial coat; the other occurs by the way of the blood stream and permits an earlier invasion of the mesenchyme tissue. One is led to think that this second way derives—if one may so express it—from the first. One knows in fact that the epithelium of the allantochorion is in very intimate contact with capillary vessels which sometimes seem to be intra-epithelial. It is thus that the bacilli get into the circulating blood.

the mesenchymal tissue, which remains of normal appearance. At the limit between the two there is a very narrow transition zone where one sees, forming a sort of limiting zone, a layer of eosinophiles, either grouped or isolated. Also in the same region, the blood capillaries are surrounded by a thickened perithelium which is loaded with bacilli. Finally, one frequently sees in the apparently normal mesenchymal tissue isolated, rounded cells which also contain very numerous microbes. These bacilli are often granular or rod-shaped; there is no reason to confuse them with the granulations of the reactional eosinophilic cells which also exist, in large numbers, disseminated in the mesenchyme.

Some of these cells are certainly mobile, and perhaps one should attribute to them a role of gradually disseminating the numerous bacilli which they contain. In short, the bacilli which reach the mesenchyme by the direct intra-epithelial route or indirectly by the way of the capillaries, seem to be disseminated by the intermediation of round mesenchymal cells which swarm throughout the mesenchymal layer.

In the transfers, the hyperplastic reaction of the epithelial coat is much less intense than that of the primary inoculation. This fact doubtless permits more rapid penetration of the bacilli through this epithelium, for the invasion of the mesenchyme itself seems to be much earlier. In consequence, the dense cellular nodules within the mesenchyme seem also to form much more rapidly.

DESCRIPTION OF PLATE

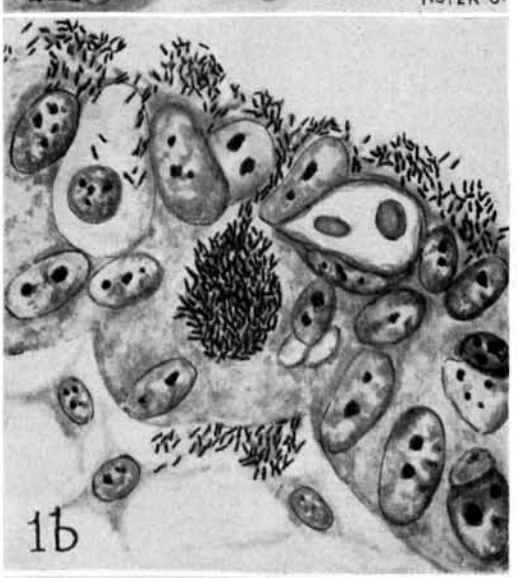
PLATE 13.

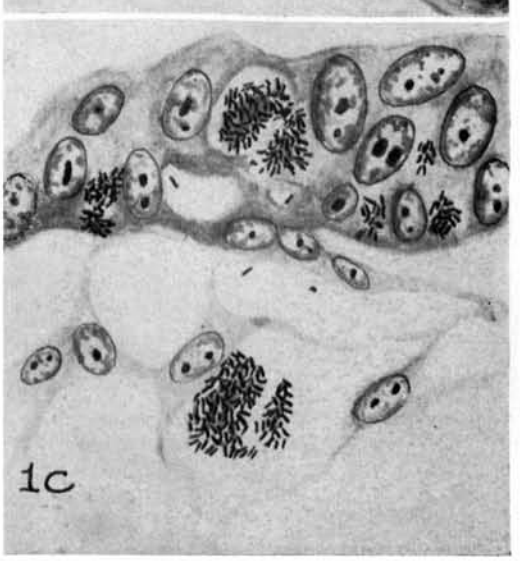
FIG. 1, a-c. Successive stages of the transepithelial migration of the Stefansky bacillus through the epithelium of the chorioallantoic membrane of the chick embryo, and the invasion of the mesenchyme.

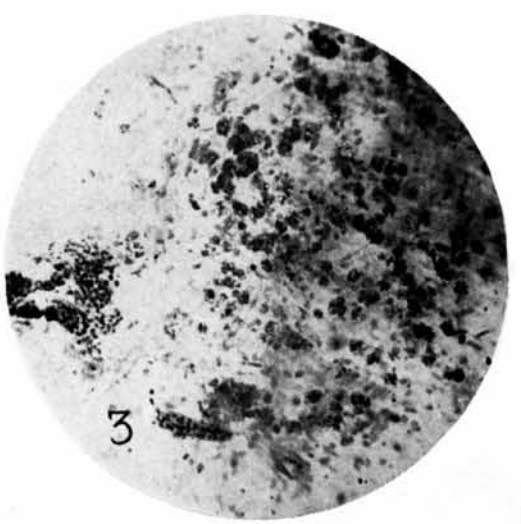
FIG. 2. Subepithelial nodule of the mesenchyme.

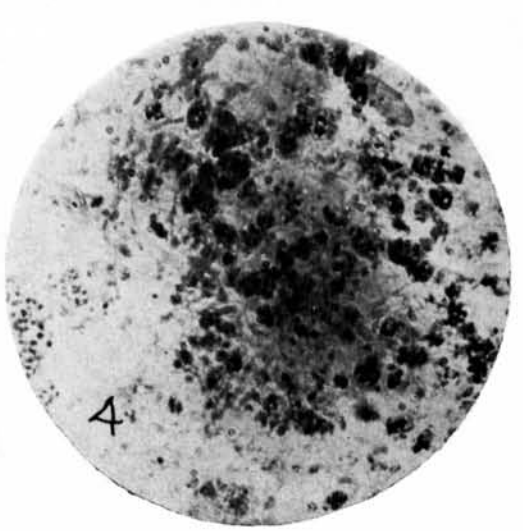
FIG. 3. Mesenchymal cells filled with bacilli; a detail of the constitution of the nodule.

FIG. 4. Same, in higher magnification.









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