ABILITY OF BACTERIA OF THE GENUS Proteus TO BEHAVE AS INTRACELLULAR PARASITES

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The behavior of bacteria of the genus Proteus, differing in a number of features of pathogenicity was studied in a culture of chick embryonic fibroblasts. The ability to produce lysis of rabbit's, sheep's, and human erythrocytes and the ability to produce hyaluronidase and proteolytic enzymes were first studied for all the strains selected. Ability to behave as intracellular parasites was most marked among the Proteus mirabilis group, which possessed all the features studied. The Proteus vulgaris group, with similar properties, exerted a cytopathogenic action without penetrating into the cell. Other bacteria of the Proteus genus, which did not possess all the features indicated, had no cytopathogenic action on the culture of chick embryonic fibroblasts.

The role of bacteria of the genus Proteus in human pyogenic infections is accepted by many authorities [6, 7, 11]. However, no criteria have yet been established which would enable the independent role of Proteus in a pathological process to be correctly assessed, and the pathogenesis of infection by the organisms of this group has not yet been adequately studied.

Investigations by other workers [5, 9, 10] have demonstrated the possibility of differentiation of pathogenic and nonpathogenic bacteria in human and animal tissue cultures, and evidence of the ability of virulent bacteria to behave as intracellular parasites has also been obtained [2, 3, 13, 15-17].

Since there are few publications devoted to the behavior of bacteria of the genus Proteus in tissue culture [1, 11], it was decided to investigate the connection between the group of properties of these organisms which can be interpreted as criteria of their pathogenicity (hemolysins, proteolytic enzymes, hyaluronidase) and the character of interaction with a culture of chick embryonic fibroblasts.

EXPERIMENTAL METHOD

Altogether 28 strains of Proteus were studied. For each strain, the biochemical species (P. mirabilis or P. vulgaris) was determined on the basis of fermentation of sugars and indole production from protein, hemolytic activity was studied against rabbit's, sheep's and human erythrocytes by Matusis's method [6], and the production and degree of activity of proteolytic enzymes were studied by the method of Gubarev and Zastavnaya [4] and of hyaluronidase by Smirnova's [8] modification of McLean's method.

The cultures were subdivided into three groups: 1) 10 strains of P. mirabilis possessing the whole series of properties studied; 2) 10 strains of P. vulgaris with the same properties in vitro; 3) a control group of 8 strains of P. mirabilis and P. vulgaris with none of the above-mentioned features.

A primary trypsinized culture of chick embryonic fibroblasts was obtained by the usual method and grown on cover slips in test tubes in medium without antibiotics. A 24-h culture of Proteus in a dose of

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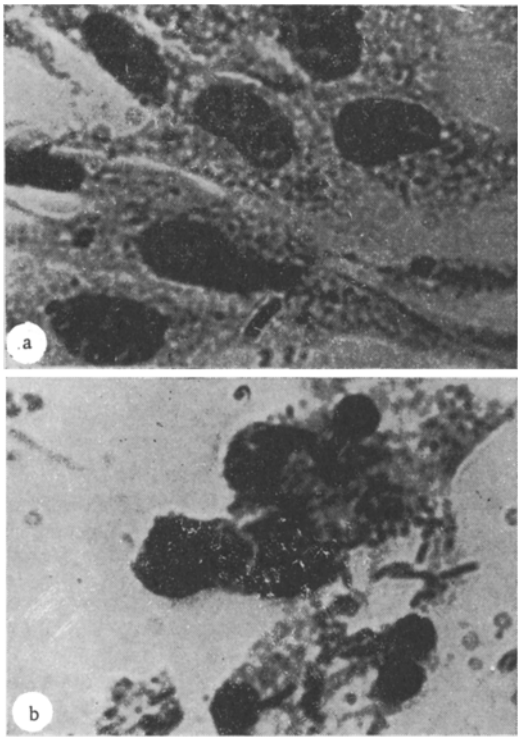

Fig. 1. Control (uninfected) culture of chick embryonic fibroblasts (a) and a culture 6 h after infection with Proteus of group 1 (b). Romanowsky–Giemsa, 350×.

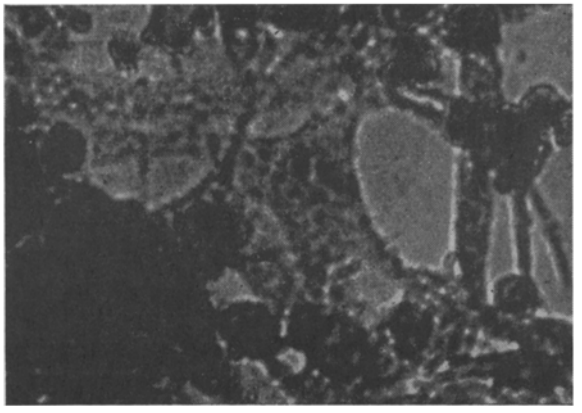

Fig. 2

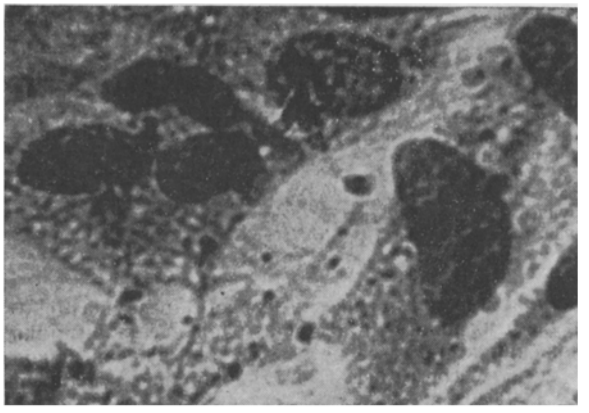

Fig. 3

Fig. 2. Culture of chick embryonic fibroblasts two days after infection with Proteus of group 2. Romanowsky-Giemsa, 200 ×.

Fig. 3. Culture of chick embryonic fibroblasts two days after infection with Proteus of group 3. Romanowsky–Giemsa, 350×.

100,000 bacterial cells was used for infection. At various times after infection (3, 6, and 12 h, 1, 2, and 4 days) the specimens were washed, fixed, stained by the Romanowsky–Giemsa method, and photographed. Uninfected tissue cultures were studied simultaneously. Altogether 434 tubes containing a culture of chick embryonic fibroblasts were used in the experiments.

EXPERIMENTAL RESULTS

The uninfected tissue cultures retained their normal cell structure and their ability to proliferate throughout the period of observation (Fig. 1a).

All the strains of group 1 were able to behave as intracellular parasites in the chick embryonic fibroblasts. Within 3 h after infection, microorganisms had penetrated inside the cells and started to proliferate there. After 6 h, cytopathic changes began to appear in the cells: the cytoplasmic processes were destroyed, the nuclei became round in shape, and underwent pycnosis, bacterial cells were visible in the cytoplasm, and their marked polymorphism with the presence of giant forms demonstrated that they were reproducing (Fig. 1b). Death of the tissue culture was complete after 1-2 days. Remnants of nuclei against the background of cytoplasm packed with bacteria could be seen in the specimens.

Among the strains of group 2 Proteus only two were found to behave as intracellular paracytes, and their behavior in the tissue culture was similar to that of group 1. The remaining strains, although unable to exist intracellularly, nevertheless had effects on the chick embryonic fibroblasts. These strains modified the tissue culture cells from outside without penetrating into them, and at later periods. Toward the end of the period of observation they gave rise to a focal pathogenic effect, among areas of intact tissue (Fig. 2).

The strains of group 3 of Proteus not only had no cytopathic action on the culture of chick embryonic fibroblasts, but they themselves died on contact with it. The cells infected with these strains were essentially indistinguishable from uninfected tissue cultures throughout the period of observation (Fig. 3).

Bacteria of the genus Proteus, like other bacteria of the Enterobacteriaceae family [12, 14, 17], were thus shown to be capable of behaving as intracellular paracytes in chick embryonic fibroblasts. This property was most marked in the case of P. mirabilis, which possesses hemolytic, hyaluronidase, and proteolytic properties. The ability of P. vulgaris, with the same properties, to propagate intracellularly is limited, but these organisms exert their cytopathic action on the tissue culture from outside. Strains of Proteus without this series of characteristics can be regarded as nonpathogenic in their action on tissue cultures.

The differences in the behavior of the Proteus strains with identical properties in vitro suggests that this series of features of pathogenicity is not confined to the properties which were studied in this case, and that ability to behave as intracellular parasites is determined by unknown factors which, in the case of P. mirabilis, are as a rule encountered in conjunction with the characteristics studied in these experiments. In addition, the results are evidence of the multiplicity of pathogenicity factors among bacteria of the genus Proteus.

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