ELECTRON-MICROSCOPIC DATA ON THE METHOD OF PENETRATION OF SHIGELLAS INTO THE INTESTINAL EPITHELIAL CELLS

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Penetration of freshly isolated virulent shigellas (Sh. sonnei) was studied during the first few hours after infection of ligated loops of rabbit small intestine. The shigellas first adhered to the glycocalyx of unchanged epithelial cells. Later the glycocalyx disappeared at the sites of their adhesion, the microvilli swelled and died, and the bacteria then penetrated into the cytoplasm of the epithelial cells. During their penetration a hollow was formed in the exposed cell membrane and this also surrounded bacteria penetrating into the apical part of the cytoplasm. However, this membrane ruptured almost immediately, and the bacteria subsequently multiplied in the cytoplasm from which they were not separated. This mechanism of penetration into the epithelial cells is evidently specific for shigellas.

The pathogenic properties of the shigellas (agents of dysentery) are based on their ability to penetrate into the cytoplasm of epithelial cells, to multiply in ulceration [2]. This has been shown by the study of natural dysentery in monkeys and in experiments in which animals were infected in various ways. Electron-microscopic investigations with guinea pigs and monkeys with experimental dysentery [8, 10-12] and after conjunctival infection of guinea pigs [5, 13] have shown that shigellas penetrating into the epithelial cells can lie freely and grow in the cytoplasm without being confined by membranes. The same observations have been made after infection of monolayer cell cultures with shigellas [8].

The earliest stages of interaction between shigellas and epithelial cells and the mechanism of penetration of shigellas into the intestinal epithelial are still unknown. Such investigations are very difficult to carry out by enteral infection of animals.

The method used in the experiments described below was that suggested for the study of cholera vibrios [7] and also used to study shigellas [1, 6] – injecting the bacteria into ligated loops of rabbit small intestine. With this model any accurately measured number of microorganisms can be used to infect the isolated loop of intestine and the sites of interaction between the microorganisms and the epithelial can easily be found in the earliest stages.

EXPERIMENTAL METHOD

Rabbits were kept without food for 48-72 h and anesthetized with hexobarbital intravenously; after laparotomy several loops of small intestine 10 cm in length were ligated (avoiding damage to the blood vessels), separated by intact segments 5 cm long. An injection of 2-3 billion shigellas in 5 ml of a 6-h broth culture of a freshly isolated virulent strain of Shigella sonnei, grown with shaking (125 agitations per minute) was given into each loop. The loops of bowel were removed under intravenous anesthesia 30 min and 1, 2, 3, 6, 9-10, and 12 h after infection and small pieces were fixed in glutaraldehyde with the addition of

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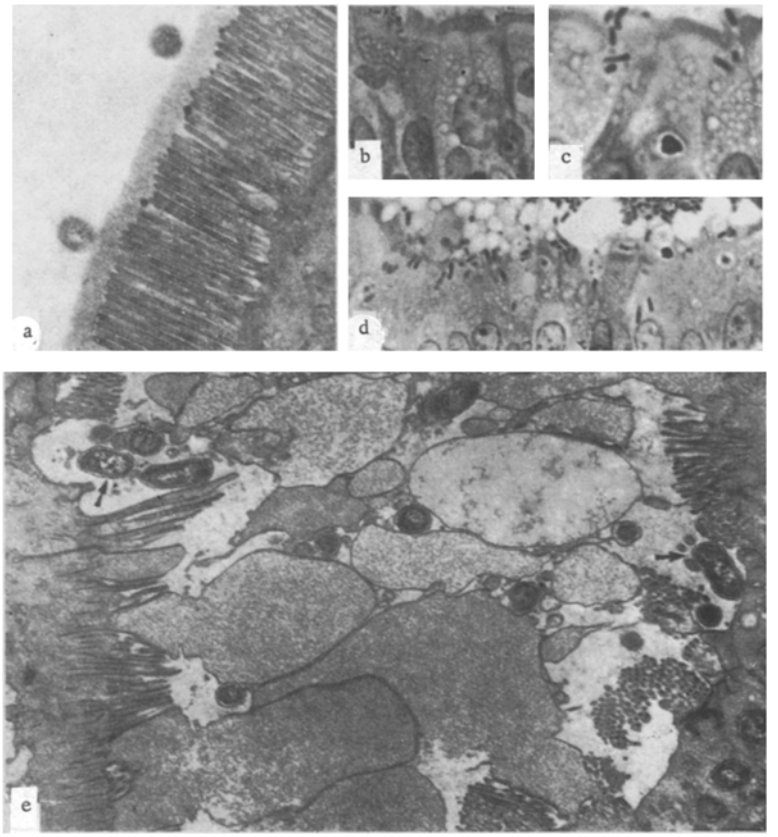

Fig. 1. Penetration of shigellas into cytoplasm of intestinal epithelial cells: a) 30 min after infection: penetration of shigellas into layer of glycocalyx covering microvilli (8000 ×); b,c,d) 2 h after infection: attachment of shigellas to surface of epithelial cells with destruction of brush border and penetration of bacteria into cytoplasm of cells ("thick" sections; toluidine blue; b) 1070 ×, c) 1600 ×, d) 1070 ×); e) 2 h after infection: same areas in the electron microscope – swelling and death of microvilli, shigellas on exposed cell membrane (arrows) and in cytoplasm of cells (bottom left) (4800 ×).

thiophosphamide and osmium tetroxide to detect the glycocalyx [3] and then embedded in Epon. Sections 1 μ in thickness were stained with toluidine blue and examined in the light microscope; ultrathin sections were stained with lead citrate and uranyl acetate and examined in the JEM-5g or JEM-7 electron microscope.

EXPERIMENTAL RESULTS

Histological examination of the thick sections showed that 30 min after infection the bacteria appeared to be adherent to the surface of some epithelial cells. After 1-2 h in many places the shigellas were attached to the epithelial surface. In these places the brush border was absent and the bacteria found in the cytoplasm of the cells (Fig. 1b, c, d), some of which were dividing, were also absent. Later both the number of infected epithelial cells and the number of bacteria in their cytoplasm increased. As a result of destruction of the infected cells defects appeared in the epithelium and the leukocytic response increased. By 9-12 h after infection the mucous membrane was covered with a purulent exudate, thickly infiltrated with leukocytes and the epithelium itself was frequently destroyed over a wide area.

The electron-microscopic investigation showed that after 30 min the microorganisms were lying freely in the lumen but some of them were in close contact with the layer of the glycocalyx covering the microvilli (Fig. 1a). By 2 h many more bacteria were seen on the surface of the epithelial cells. In these places the glycocalyx was absent, and some microvilli had changed into large vesicles projecting into the lumen (Fig. 1e). These vesicles, filled with homogeneous finely granular contents and surrounded by a

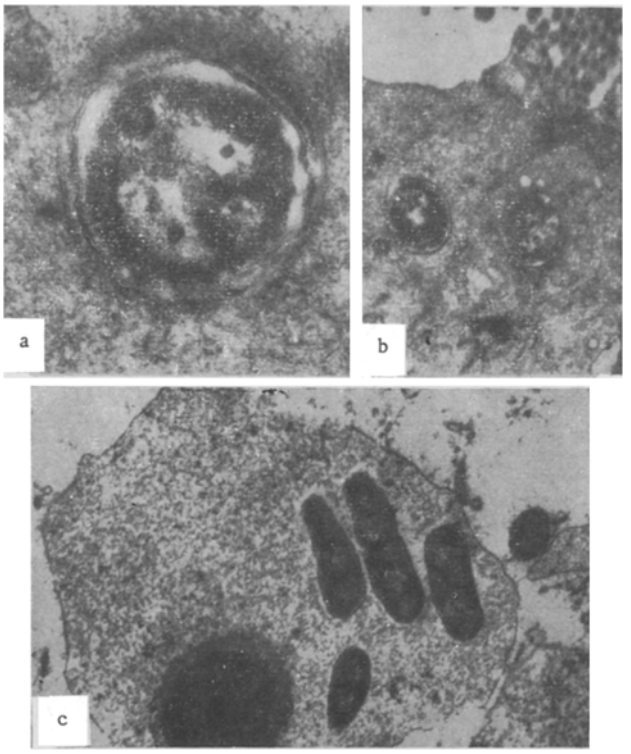

Fig. 2. Shigellas in cytoplasm of intestinal epithelial cells: a) 2 h after infection: on right and left remnants of a triple membrane surrounding a bacterium can be seen (70,000 ×); b) 2 h after infection: on the right remnants of the membrane can still be seen in some places, on the left a free-lying bacterium (18,000 ×); c) 12 h after infection: dividing bacteria lie freely in the cytoplasm of an epithelial cell, desquamated into the lumen of the intestine, with a pycnotic nucleus (13,000×).

triple cell membrane, also were observed in the lumen. In some places the microvilli were absent and the bacteria lay directly on the plasma membrane of the cells which formed a depression in these areas (Fig. 1e). Disturbances of the integrity of the cell membrane were not found although shigellas were seen not only on the surface, but also in the cytoplasm of the cells. In the apical zones of the cytoplasm a triple membrane could sometimes be seen closely wrapped around them, but more usually it was distinguishable only in certain areas (Fig. 2a,b). However, in the deeper parts of the cytoplasm around bacteria penetrating there (some of them dividing) no membranes could be detected.

Later, in the infected epithelial cells, the microorganisms formed groups with nothing separating them from the surrounding cytoplasm; the electron density of which was reduced after 12 h. Numerous desquamated epithelial cells with a pycnotic nucleus and structureless cytoplasm, containing free-lying bacteria (Fig. 2c), were present in the intestinal lumen. Polymorphs, present in large numbers in the lumen of the intestine and in the tissues of the mucous membrane itself, also contained bacteria in their cytoplasm. In such cases, however, the shigellas (singly or in small groups) were contained in vacuoles surrounded by membranes (phagosomes), containing a certain quantity of electron-dense material (probably a product of the lysosomes), and many of the bacteria in these phagosomes were evidently destroyed.

These investigations thus showed that shigellas in the intestine initially adhere to the glycocalyx of completely unchanged epithelial cells. The glycocalyx then disappears in these places and highly distinctive changes take place in the microvilli, leading to their death. The bacteria then penetrate into the cytoplasm of the epithelial cells. During their penetration, depressions are formed initially in the exposed cell membrane, which also surrounds bacteria entering the apical part of the cytoplasm. However, this membrane evidently begins to break up almost at once and the bacteria subsequently multiply in the cyto-

plasm, from which they are not separated. This mechanism of penetration into the epithelial cells is specific for shigellas, for invasion by salmonellas [9, 10], for instance, follows a different course. According to the available data, salmonellas do not induce these changes in the microvilli, and inside the cells as a rule they are surrounded by membranes and do not multiply, but are simply transported through the epithelium. The special features distinguishing the pathogenic properties of different agents thus affect even the earliest stages of their interaction with the intestinal mucous membrane. These observations also confirm observations on intraepithelial parasitization of shigellas, made with other models, as the basis of their pathogenic properties.

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LITERATURE CITED

1. N. R. Vasser and Yu. E. Polotskii, Zh. Microbiol., No. 7, 66 (1972).
2. M. V. Voino-Yasenetskii, Arkh. Pat., No. 11, 3 (1970).
3. Ya. Yu. Komissarchik and E. S. Snigirevskaya, in: The Structure, Function, and Reactivity of Cells [in Russian], Leningrad (1973).
4. L. S. Nosova, M. N. Trofimova, L. E. Sal'nikova, et al., Tsitologiya, 14, No. 6, 783 (1972).
5. K. Tenner, P. Rats, and B. Sheren', Arkh. Pat., No. 11, 12 (1970).
6. H. G. Arm, T. M. Floyd, J. F. Faber, et al., J. Bact., 89, 803 (1965).
7. S. N. De and D. N. Chatterje, J. Path., 66, 559 (1953).
8. H. Ogawa, Acta Path. Jap., 20, 267 (1970).
9. A. Takeuchi, Am. J. Path., 50, 109 (1967).
10. A. Takeuchi, Current Topics Path., 54, 1 (1971).
11. A. Takeuchi, S. B. Formal and H. Sprinz, Am. J. Path., 52, 503 (1968).
12. A. Takeuchi, H. Sprinz, E. H. Labrek, et al., Am. J. Path., 47, 1071 (1965).
13. W. Wessl and P. Racz, Virchows Arch. Path. Anat., 342, 109 (1967).