MORPHOLOGY AND IMMUNOLOGY

PENETRATION OF THE SKIN BY MICROORGANISMS

COMMUNICATION I. EXPERIMENTAL DATA ON THE PASSAGE OF MICROORGANISMS THROUGH SEMIPERMEABLE MEMBRANES

A. V. Lizgunova

From the Department of Microbiology (Head – Active Member of the AMN SSSR V. D. Timakov) of the N. I. Pirogov Second Moscow Medical Institute

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The ability of microorganisms to pass through the skin, and the conditions for this have interested research workers for a long time. According to reports in the literature the passage of microorganisms through the skin is limited by processes of self-cleansing taking place in that tissue; it is also connected with the whole complex of protective properties of a biological nature which proceed inside the skin.

The variety of conditions limiting the passage of microorganisms through the skin led us to undertake the solution of this problem in stages.

The majority of authorities [1, 3, 4, 8 and others] associate the penetration of the skin by microorganisms in man with some form of trauma to the epithelium – cuts, scratches or other injuries. A far smaller number of workers assert that the outer layer of the human skin, if undamaged, does not present a sure defense against the organisms causing disease [5-7].

We undertook to investigate in the first place the part played by the epidermis in the process of entry of various microorganisms through the skin.

We obtained the epidermis from cadaver skin by the method of A. V. Pshenichnov and B. I. Raikher [2]. At predetermined areas of the skin burns were produced by means of hot compresses. The epidermis formed a blister and was streched over a specially prepared glass cannula with a wide portion, and the narrow portion of the cannula was passed through a cork stopper which closed a flask in such a way that the lower edge of the membrane was in contact with the nutrient medium in the flask. Through the narrow end of the cannula was injected a 24-hour culture of Vibrio metchnikovii in peptone water, in a volume of 0.5 ml; every day thereafter for the next 3-5 days a microbiological examination was made of the peptone water contained in the flask.

The results differed in accordance with the area of the skin from which the epidermomembrane was obtained. Where the epidermis was taken from the upper third of the skin of the leg, then all the cultures in peptone water and the subcultures on alkaline agar showed the presence of the vibrio; it can be assumed that here a part was played by the porosity of the membrane resulting from the presence of hairs on the skin of the leg. On the other hand, the epidermomembrane from skin of the posterolateral area of the abdomen, devoid of hairs, was impermeable to the vibrio.

However, dead epidermis of human skin, with its biological properties modified by the high temperature, could not give results of a reliable physiological order. For this reason we subsequently used the skin of a living frog (in spring) for the investigations.

Each frog was decapitated immediately before the experiment, and the skin of the trunk removed aseptically. Pieces of skin of the required dimensions were cut out and stretched over the wide portion of the glass cannula, which was then introduced into the flask of peptone water. Depending on the aim of the experiment the skin was stretched with either its epithelial or its serous surface uppermost. A 24 hour culture of the microorganism was introduced inside the glass tube.

All the membranes of frogs' skin, stretched with the epithelial and serous sides uppermost, and a control cellophane membrane were impermeable to V. metchnikovii in observations lasting 8 days.

Control experiments with a membrane of 3 layers of gauze showed that in the conditions appertaining, the vibrios which passed into the flask of peptone water were capable of surviving in it for 2-3 days or more.

Later experiments with Bacillus mirabilis, using suitable nutrient media for this organism, showed that if placed on the epithelial surface of the frog's skin, it will pass through it in under 24 hours. If B. mirabilis is placed on the serous surface of the skin, it could be found in the nutrient medium only after daily observation for 16 days. However, this period of time cannot serve as proof of passage of B. mirabilis through the skin of the frog, for after 16 days processes of autolysis might have begun to occur in the skin as a result of disturbance of its physiological requirements. In control experiments with a cellophane membrane, B. mirabilis was found in the nutrient medium beyond the membrane after 48 hours.

A further control was a 1% solution of trypan blue. This solution was poured on to the epithelial surface of the skin and the cellophane membrane, which were then placed in flasks containing petone water and tap water. In neither liquid was the dye found after 8 days of observation.

Thus, living frogs' skin and cellophane were both impermeable to V. metchnikovii and trypan blue. Only B. mirabilis was able to surmount both obstacles – dead or living – and in the latter case, in the direction from the epithelial to the serous surface.

The numerous epidemiological observations indicating the possiblity of leptospiral infection through the skin, and consequently a passage of leptospires through it, led us to carry out a series of experiments by the method already described, with leptospires of 2 strains – BShch and Dubna 4. As a nutrient medium for the leptospires we used the ordinary Vervoort-Wolfe medium prepared with the addition of buffer solutions. In 50% of the experiments the skin was stretched with its epithelial side uppermost and in the same number, with the serous surface uppermost.

The experiment with leptospires was carried out only if not less then 10-15 specimens were found in a field of vision in the culture.

Inspection of the nutrient medium for the presence of leptospires was carried out with dark-ground illumination 2 hours after introduction of the culture and daily thereafter for 18 days. The presence of living, motile leptospires was recorded.

The results of experiments with the skin of winter frogs showed that leptospires could pass through it when its epithelial surface was uppermost; this was seen in the flask after only 2 hours, in a number of one in 2-3 fields of vision (see Table).

Leptospires did not pass through the skin of these frogs turned with the serous side uppermost, nor through the cellophane membrane. However, leptospires were found in a number of one in 2 fields of vision at the other side of a control membrane of 3 layers of gauze.

Leptospires did not pass through the skin membranes of summer and spring frogs with the epithelial or serous side uppermost; they could begin to appear in the nutrient medium (when nonmotile) on the 11th-18th day as a result of breaking of the membrane by the onset of autolysis.

It follows from the foregoing that the living skin of summer and spring frogs is impermeable to leptospires both from the epithelial to the serous layer and vice versa. The skin of winter frogs was found to be permeable to leptospires in the direction from the epithelial to the serous surface.

In later experiments by the same method we tested the permeability of the skin of summer and spring frogs to the following microorganisms: 1) Staphylococcus aureus; 2) Staphylococcus citreus; 3) Bacillus anthracoides and 4) Escherichia coli. Permeation or absence of microorganisms was checked by daily cultures for 5-7 days on suitable elective and differential media for each of the above microorganisms. The experimental results showed that not all the microorganisms pass through the skin of the living frogs to an equal degree. This unequal permeation

Permeability of the Membrane From the Skin of the Frog to Leptospires and Microorganisms

Membranes	Leptospires							Microorganisms			
	time of observation							time of observation – from 1 to 7 days			
								name of microorganisms			
	2 hr	1 days	2 days	1–10 days	11 days (rupture of mem.)	1–17 days	18days (rupture of mem.)	St. aureus	St. citreus	E. coli	B. anthracoides
	winter frogs			summer frogs							
1	2	3	4	5	6	7	8	9	10	11	12
Skin of the frog from the outer surface	1/2 1/3	1/2 0	0 0	0 0	many/1 many/1	0 0	many/1 many/1	55,5	16,6	16,6	100
Skin of the frog from the inner surface	0 0	0 0	0 0	0 0	many/1 many/1	0 0	0 0	0	22,4	5,5	5,5
Cellophane	0	0	0	0	0	0	0	0	33,3	0	0
Gauze	1/2	1/2	1/2	many/1	many/1	many/1	many/1	100	100	100	100

Notes. 1) $1/2$, $1/3$ – one per 2-3 fields of vision, many/1 – many in one field of vision. 2) In columns 9-12 the intensity of permeation is expressed in percent.

of microorganisms through the skin depends primarily on its position, i.e. whether it has its epithelial or its serous suface uppermost. There is no doubt that the degree of permeation of microorganisms through the skin is also connected with the distinctive features of the microorganisms themselves. Nevertheless the same microorganisms passed from the epithelial to the serous surface much more easily than in the opposite direction.

It is seen from the Table that the positive results during permeation of microorganisms from the epithelial to the serous surface varied from 16.6 to 100%. The greatest ability to pass through the skin in this direction was shown by B. anthracoides (100% permeation), next followed Staph. aureus (55.5% positive results). Staph. citreus and E. coli each had equal ability to pass through the skin. Passage of microorganisms from the serous to the epithelial surface took place with much greater difficulty, and the number of positive results did not exceed 22.4%. The greatest fall in the intensity of permeation in this direction was shown by B. anthracoides and Staph. aureus.

This difference in the power of permeation cannot be attributed to the difference in the size of the microorganisms (the smallest and largest microorganisms pass with equal ease). Neither can this phenomenon be explained by the presence or absence of motility in the microorganisms or by their different resistance to physical and chemical agents.

The factors facilitating the permeation of the skin by B. mirabilis, B. anthracoides, Staph. aureus and leptospires may be extremely diverse, and their role in this process requires further study.

The control gauze membrane was found to be permeable to all the microorganisms used in the work: Staph.

aureaus and citreus, B. anthracoides, E. coli, leptospires and V. metchnikovii. Cellophane allows the passage of B. mirabilis and Staph. citreus only; the remaining microorganisms (E. coli, B. anthracoides, Staph. aureus and leptospires) did not pass through cellophane under the above experimental conditions.

SUMMARY

The ability of epidermis to serve as a barrier to microbes is preserved to a certain extent on the epidermomembranes prepared by Pshenichnov's method from the skin of human cadaver.

The barrier function of live uninjured frog's skin varies with respect to different microbes. Thus, B. anthracoides, Staphylococcus areus, B. prodigiosus and leptospires penetrate the frog's skin with comparative ease in the direction from the epithelial surface to the serous one. Penetration of microorganisms in the opposite direction either does not take place at all or is more difficult.

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