PENETRATION OF MICROORGANISMS THROUGH THE SKIN

COMMUNICATION II. THE STUDY OF THE CONDITIONS AND MODES OF PENETRATION OF MICROORGANISMS THROUGH THE SKIN IN MICE

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The present investigation was undertaken inorder to study the effect of different physiological conditions on the possibility of penetration of microorganisms through the skin. Experiments were carried out on mice. Under these circumstances we took into consideration the age of the animals, their nutrition and seasonal influences. The experiments were not duplicated on other animals, because we know from the literature that the reaction of the epithelial cells does not depend on the thickness of the epidermis. "Both in the multilayered epithelium of the human skin or of the skin of the mouse's paw, and in the two- and four-layered epithelium of the skin of the dorsum of the rabbit or mouse, the processes take place in the same way" [2].

EXPERIMENTAL METHOD

The work was carried out on different groups of mice: one group consisted of mice weighing 15-18 g, another of mice weighing 19-22 g. In selecting the animals it was taken into consideration that the group of smaller mice grew on an inferior diet (with a much smaller milk content). Later, however, both during and after the experiments, in the course of observations lasting 2-3 months on the mice, the diet of both groups was the same, and remained unchanged until the end of the observations.

Besides the weight and diet, the external appearance of the animals was taken into consideration. In the group of smaller mice, disheveled and sluggish animals were selected, and in the group of larger mice, animals with smooth, glistening fur, and active and lively in their behavior, were included.

Using the different groups of mice, we tried to reproduce the experiments with them at different times of the year. The smaller animals weighing 15-18 g and 2-2½ months old were used for the experiments in winter and autumn and sometimes in the early spring, when it was still cold (March). The larger mice weighing 19-22 g and 3-3½ months old were included in the experiment only in the very warm time of the year – June, July and August. The selection of the animals in accordance with the conditions of their growth, with their weight and external appearance was thus sufficient to enable differences in their biological activity to be discerned, and the additional factor of seasonal influences created even greater variations in the sensitivity of the animal to infection.

Proof of the penetration of microorganisms through the skin was the development of infection with a fatal termination due to the microorganisms introduced. This required the use of microorganisms pathogenic to mice: Salmonella typhimurium, Pneumococcus I, Bacillus anthracoides, Streptococcus haemolyticus and Bacterium pyocyaneum.

Outcome of Infection through the Skin

Name of microorganism	Summer-fortified groups of mice				Winter-enfeebled groups of mice			
	days between infection and death	death+isola. specific causative	death - specific causative	total no. of surviving mice (in%)	days between infection and death	death+isola. specific causative	death - specific causative	total no. of surviving mice (in%)
B. anthracoides	10	5%	—	95	2—47	84%	5%	11
B. pyocyaneum	11—12	—	7%	93	—	—	—	—
Str. haemolyticus	1	—	3%	97	3—24	93%	—	7
Salmonella typhi murium	—	—	—	100	21	—	5%	95
Pneumococcus	2—5	10%	20%	70	15—45	90%	10%	—
Pneumococcus I on scarified skin	2—5	40%	—	60	—	—	—	—
Pneumococcus I in association with hyperthermia	3—5	45%	35%	20	—	—	—	—

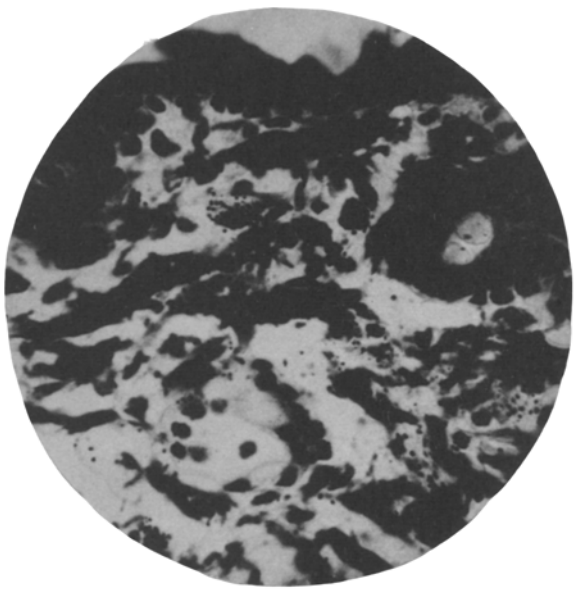

Fig. 1. Penetration of streptococci into the epithelium and subcutaneous cellular tissue of a mouse in experimental infection through the skin. The mouse was killed immediately after the experiment.

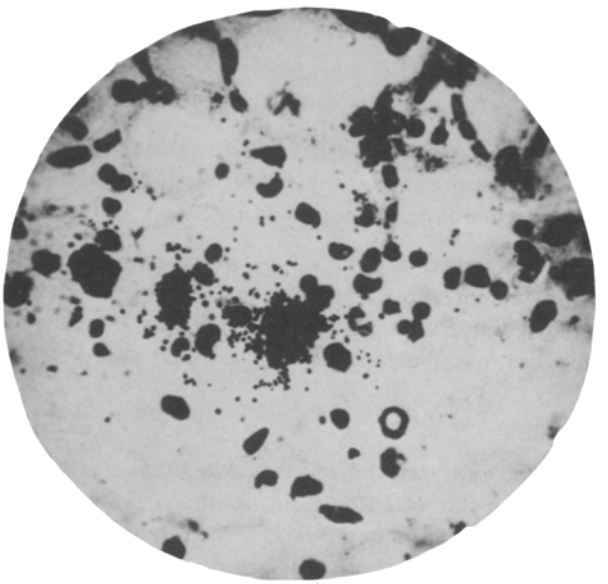

Fig. 2. Penetration of microorganisms into the loose connective tissue of a mouse after infection through the skin. The mouse was killed immediately after the experiment.

All the microorganisms used in the experiments were cultivated on liquid nutrient media. In order to prove that the infectious agent entered the body in fact through the skin, a method was devised which excluded infection of the animals by any other route. In order to ensure fixation, each mouse was secured to an individual Leitz stage, and by means of a screw, the tail was clamped to the stage with a metal plate, and lowered into a narrow precipitation tube filled with a 24-hour culture of the microorganism. The animals were kept in this position for 2 hours.

The tail of each experimental mouse was then carefully taken out and dried.

In parallel experiments, after the conclusion of the exposure, the tail of each mouse was quickly cut off aseptically at a level considerably above that reached by the culture. The stump was immediately cauterized,

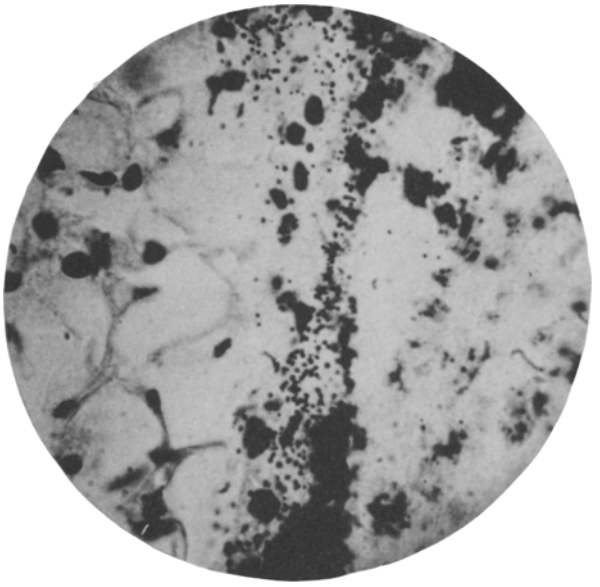

Fig. 3. Penetration of microorganisms into the tissue space after infection of a mouse through the skin. The mouse was killed immediately after the experiment.

which prevented bleeding. By cutting off the tail in this way there could be no doubt that subsequently we had to deal only with microorganisms penetrating into the blood and lymph through the skin during the time of exposure.

The results of the experiments in accordance with either scheme showed that no difference was observed in the onset and development of the infection or in its absence.

The trauma inflicted on the animal during the operation of removal of the tail was checked by control experiments in which the tails of mice were lowered into sterile broth. Observations for a period of 2 months showed that the percentage of fatal issues in these conditions varied between 0 and 2.

Performance of the experiments with different species of microorganisms was accompanied by subcutaneous infection of the animals of the same group with the same organisms. The mice included in the experiment were previously checked to ensure that they were not carriers of pneumococci.

Besides reproducing infection with a fatal issue, experiments were carried out in order to discover if it was possible for microorganisms to penetrate the skin and to determine the rate of their appearance in the blood and organs. In these experiments we used cultures of Pneumococcus and of Bacillus mirabilis.

The tails of the mice were immersed in tubes containing cultures of the microorganisms for times varying from 10 minutes to 2 hours. At the expiry of the predetermined time, the mice were released from the stages and killed at different periods: some after 1-2 hours, others after 1-6 days. The cadavers were immediately examined, and their organs and the blood were investigated under the microscope and bacteriologically.

EXPERIMENTAL RESULTS

The experimental results showed that Bacillus mirabilis has a considerably smaller ability to penetrate through the skin of a living animal than Pneumococcus: Bac. mirabilis, being a saprophyte, showed only 2.38% of penetration through the skin, whereas Pneumococcus, possessing a high degree of pathogenicity toward mice, without producing a fatal issue, showed 26.32% of penetration through the skin of the mice. Under the same conditions, the intensity of penetration of the pneumococcus was 11.1 times greater than that of Bac. mirabilis.

The total number of mice used in the experiments was about 2500. In each section of the experiments there were about 200 mice.

The summarized results of the main experiments, carried out with the strains of Streptococcus haemolyticus, Bac. anthracoides, and Bact. pyocyaneum, given in the table, show that in the groups of the larger mice in summer conditions a low mortality was observed, and between 93 and 97% of the animals survived.

The smaller mice (15-18 g), experiments with which were carried out in the autumn and winter, died in larger numbers. As a result of infection with Bac. anthracoides under these conditions, their survival rate was only 11%, and of infection with Strep. haemolyticus this percentage fell to 7.

In the experiments using a culture of Salmonella typhimurium, the larger mice in the warm spring months showed maximum resistance to this microorganism: after the usual exposure for 2 hours, the number of surviving animals was equal to 100%. Infection of the 15-18 g mice with the same culture in the cold winter months resulted in 5% of fatal issues without isolation of the specific causative agent. Hence it follows that the undamaged skin of both the more and the less resistant groups of mice was impermeable to such pathogenic microorganisms as Salmonella typhimurium, which in natural infection penetrate the body via the mouth.

Experimental infection with pneumococci, using both the larger and smaller mice, showed high susceptibility to this infection – a fatal issue with isolation of the specific causative agent was found among the larger mice in the summer time in 10% of cases, and among the smaller mice in wintertime, in 90%.

Preliminary trauma to the skin of the tail by means of scarification increased the mortality of the larger animals in the summer time to 40%, and in experiments in association with hyperthermia the mortality reached 45%.

The ability of microorganisms to pass through the skin of mice was confirmed also by histological methods of investigation of 200 animals. The aim of this particular investigation was to obtain some idea, although only relative, of the paths of penetration of microorganisms from the epidermis to the layers of the true skin, and of the rate of their movement along this path. For this purpose we selected a culture of Streptococcus haemolyticus, Griffith's type I and Lancefield's group A. In order to prevent excessive maceration of the epithelium, we used the exposure of one hour as established above.

Histological examination of the skin of the tails of the mice at the end of the specified period of time showed the presence of microorganisms, having penetrated beneath the epidermis into the dermis. The relatively small number of coccoid forms in the tissue of the dermis suggested that in the period of 2 hours the streptococci had passed beyond the limits of the skin and would have to be sought in the lymphatic glands. In subsequent experiments the exposure was increased to 18-22 hours, and both the skin of the tails and the inguinal lymphatic glands were subjected to histological examination. A study of the histological preparations from this series of mice revealed not only penetration and distribution of streptococci in the dermis, but also in other tissues, and also enabled the biological reactions of the tissues in response to the introduction of microorganisms to be followed to some extent. The most informative patterns were obtained in mice killed immediately after the experiment; from them the following facts were determined:

a) groups of cocci were found under isolated desquamated epithelial cells, in the thickness of the epithelium and close to the hair follicles (Fig. 1);

b) microorganisms were found in the subcutaneous cellular tissue in groups, and granulocytes were seen, containing phagocytosed microorganisms (Fig. 2);

c) cocci were abundantly distributed in the tissue interspaces in the muscle in the perivascular lympahatic spaces and in the perivascular tissue of the blood vessels (Fig. 3).

In many histological preparations biological reactions to the introduction of microorganisms could be seen: signs of proliferation of the endothelium of the vessels and leucocytic thrombi in the smaller vessels. No microorganisms could be found in the tissue of the lymphatic glands during this period of observation.

It may be concluded from the findings described that the function of the undamaged skin as a factor in the defense of the body against microorganisms is confirmed by the histological investigations.

SUMMARY

Factors protecting the skin from microbic penetration are clearly seen on the example of several groups of large mice where the percentage of survival ranges from 70 to 100%; the mortality rate rises only with scarification of the skin and overheating of the body. The protective properties of the skin in smaller mice decreases sharply and the percentage of the animals survivingdrops to 7-11. The above factors showed their maximal protective properties with regard to Salm. typhimurium. Although this microbe was highly pathogenic when administered to mice per os, subcutaneously or intraperitoneally, it was unable to penetrate through the skin.

Histological investigations demonstrated that uninjured epithelium possesses powerful protective properties against microorganisms, which are able to penetrate under it only when it is desquamated or through the hair and sebaceous follicles.

With the microbes penetrated into the mice skin the immunological reactions are the most intense during the first 24 hours and even during the first few hours after infection.

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