ON THE MORPHOLOGY OF EXPERIMENTAL STREPTOCOCCUS INFECTION IN RABBITS WITH A PRIMARY INTRADERMAL FOCUS

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In the literature available to us we did not encounter morphological works dealing with the study of experimental streptococcal infection, and, in particular, of primary foci in the dynamics of their development.

The present work is an attempt to study the local tissue changes arising with the intradermal injection of streptococci. The investigation was conducted microbiologically and on the basis of morphological complexes, and during it we turned our attention to the dynamics of the process. The internal organs were also morphologically studied to elucidate the effect of a local streptoroccal focus on them.

EXPERIMENTAL METHOD

The experiments were carried out on 65 rabbits, 2,000 – 2,900 g in weight. For the inoculation of the animals we used a virulent culture of hemolytic streptococcus group A, Griffith's serological type I (R-phase), obtained from the Division of Microbiology of the Institute of Experimental Medicine, Akad. Med. Nauk SSSR. Eighteen- to twenty-hour cultures of streptococcus, in dishes containing 5% blood agar, were washed off with a small quantity of physiological NaCl solution, carefully mixed by shaking for 1-2 minutes, and diluted with physiological saline in accordance with an optic standard. The culture of hemolytic streptococcus, in the corresponding dilution, was injected into rabbits intradermally in a volume of 0.1 ml. In order to select the optimal infecting dose we set up a series of experiments with different concentrations of the microbial bodies (50,000, 500,000 and 5,000,000). Under the conditions of our experiments a concentration of 5,000,000 cells in the inoculating dose was shown to be optimal. With this dose a clearly manifested focus was formed after 72 hours. Before the animals were inoculated the number of viable organisms in the infecting dose was determined; it usually consisted of 40-50% of the calculated dose. The lateral surfaces of the rabbits were carefully depilated. Two to four foci were created in each rabbit, i.e., 1-2 foci per side, in immediate proximity to the paws. The foci were investigated immediately after inoculation, and then after 2, 4, 8, 24, 36, 48, 60 and 72 hours and 5 days. At the indicated intervals the animals were sacrificed by means of air emboli.

Sectioned portions of the skin containing the foci, stretched out on a cardboard frame, and regional lymph nodes (axillary and inguinal) were fixed in formalin, or, in the last experiments, in Carnu's solution. In 26 rabbits the heart and kidneys were subjected to morphological examination at different intervals of the experiment, and, in certain animals, the periarticular tissue, liver, and spleen as well. The resected portions were imbedded in paraffin and studied after being stained with hematoxylin-eosin, by the method of Van Gieson, and with thionine; in the last series of experiments (12 rabbits, interval after inoculation 36, 48, 60 and 72 hours) we used Schiff's periodic acid reagent (PAS). For the microbiological investigation the foci were sterilely resected in their entirety, cut into tiny pieces with scissors, and ground in physiological saline with a pestle; following this the corresponding dilutions were prepared and seedings were made to dishes containing 5% blood agar for a tally of the number of colonies growing out.

EXPERIMENTAL RESULTS

Macroscopically, after 1 hour (10 foci) and 2 hours (20 foci) following the inoculation, the site of injection of the streptococci differed very little from the surrounding skin; only in certain cases was there observed a small thickening. With microscopic examination one's attention, in both intervals, was called to a disintegration of the collagen fibers and a rounding of the fibroblasts. Scattered leukocytes were noted in three rabbits, in the dermis under the epithelium, after only one hour. After two hours, against the same background of connective tissue loosening and rounding of the fibroblasts, there were, as a rule, scattered leukocytes in considerable numbers, predominantly under the epithelium and around the vessels. We did not succeed in bacterioscopically showing the microbes at these intervals, with the exception of one case, where after only 2 hours focal accumulations

of leukocytes were observed between the bundles of collagen fibers. In this case the microbes were distributed in both the focal accumulations of leukocytes and in the surrounding tissue.

In the site of injection of the streptococci, after 4 and 6 hours (10 rabbits, 20 foci), a minimal thickening of the skin and a hyperemia were inconstantly observed. With histological investigation leukocytes were seen dispersed throughout the entire dermis against the background of connective tissue loosening, predominantly under the epithelium, and along the path of vessels and hair follicles.

The lumens of vessels in the areas of leukocyte accumulation were filled with leukocytes, and their endothelium was swollen. Six hours following the injection of the streptococci a more considerable leukocytic infiltration was observed; the foci were coarser, and the leukocytes were arranged in bands along the route of collagen fiber bundles. Clumps of disintegrating leukocytes were encountered, in which macrophages were admixed. At these intervals the microbes, as a rule, could still not be detected bacterioscopically. The exception consisted of 3 rabbits: in 2 of them, after 4 hours, single, poorly stained, cocci were noted; in only one case was there demonstrated a large number of streptococci, situated in the foci of leukocytic accumulations.

Diffuse thickening and hyperemia of the skin were observed at the sight of injection of the streptococci in the majority of animals 8 hours after the inoculation (10 rabbits, 10 foci). With microscopic examination the same alterations were detected, but, in addition, against this background there were encountered, in many rabbits, rather large foci or leukocyte accumulation between the bundles of collagen fibers.

Twenty-four hours after injection of the streptococci, in all the rabbits (10 animals, 10 foci) except one, dense, slightly elevated infiltrates were noted at the site of injection. Upon microscopic investigation, large foci of leukocytic infiltration were discovered. As in the foregoing experimental interval, the leukocytes were arranged as dense masses between the bundles of collagen fibers, but areas were encountered where they lay in a continuous mass, in which there were already undifferentiated connective tissue fibers. The majority of leukocytes were in a state of disintegration; in two cases streptococci were discovered, both lying free and phagocytized.

Thirty-six hours (20 rabbits, 20 foci) and 48 hours (20 rabbits, 20 foci) after the inoculation, circumscript foci of thickening were formed at the site of injection of the streptococci in the majority of animals, 0.2 to 1 cm in diameter. Upon histological investigation, circumscript foci of leukocutic infiltration were present in almost all the animals, in the form of one single, or several small, foci, presenting themselves as generating abscesses. In several places the border of the abscesses wss very clear, but lacking marked proliferative alterations on the side of the surrounding tissue. In 6 cases streptococci were detected in the leukocytic accumulations.

At the interval of 60 hours following the injection of streptococci (20 rabbits, 20 foci) in almost all cases there was noted, at the site of injection, slightly raised, sometimes pale, sometimes hyperemic foci of thickening up to 1 cm in diameter. Upon microscopic investigation the foci of leukocyte accumulation were shown to be larger than in the previous intervals; in many cases they reached the muscular layer of the skin. The changes in the tissue surrounding the abscesses were the same as in the previous intervals of the experiment. The bundles of collagen fibers in the foci were either preserved in the form of isolated fragments or were completely absent, and the entire basic mass of the focus consisted of disintegrating leukocytes and macrophages.

Seventy-two hours after the inoculation (28 animals, 28 foci), the foci at the site of injection of the streptococci were larger, reaching up to 1.5 cm, many of them with an ulceration at the surface. Upon histological investigation, formed abscesses were discovered in half the cases. In isolated cases a dense macrophagal-leukocytic infiltrate extended to the muscular layer of the skin. The deep veins of the skin were thrombosed. The epithelium over the abscesses became necrotic. Streptococci were discovered in the disintegrating mass of the exudate in 2 cases. In the remaining cases the foci were approximately the same as the form they had taken in the preceding intervals. Proliferative changes were absent or were extremely weakly manifested. The development of granulation tissue about the periphery of the abscesses was observed, in isolated cases, only after 120 hours following the inoculation (10 rabbits, 20 foci).

Testing the skin with the periodic acid- Schiff reaction, we did not detect any expressed changes in the connective tissue at the different experimental intervals. In the regional lymph nodes of the cutaneous foci of injury the changes were inconstant, irregular, and weakly manifested. Enlargement and hyperemia of the lymph nodes was observed in many of the rabbits, but these alterations did not depend on the interval of the experiment. Upon microscopic investigation, the lymph nodes, during the early intervals, did not differ from the nodes in the control experiments (4 rabbits). Indeed, only after intervals of from 48 to 120 hours were changes discovered in the lymph nodes in 18 of the animals, generally corresponding to the extent of the changes in the skin. Forty-eight hours after the injection of the streptococci there was noted a dilatation of the marginal sinuses, an accumulation of light, large cells and isolated leukocytes in them, and occasional disintegrating cells. After 60 and 72 hours clearly expressed dissociation of the cells was observed in the sinuses. In several cased disintegration of the lymphocytes was noted in the follicles. After 120 hours we observed a decrease in the size of the follicles, a dilatation of the sinuses, and filling of the latter with large cells. In the marginal sinuses, macrophages were encountered with an

Number of Microbes in the Foci According to the Intervals of the Investigation

No of the experiment	Intervals of the Investigation (in hours)							
	immediately following inoculation	2	4	8	24	36	48	60	72
1	3.2×10^3	44.8×10^3	165×10^3	---	---	---	---	---	---
2	---	108×10^3	240×10^3	430×10^3	1.5×10^6	---	---	---	---
3	---	---	---	---	---	2.1×10^6	4.7×10^6	6×10^6	4.3×10^6

adiposity of the protoplasm and with phagocytized products of nuclear decomposition. Microbes were not detected in the lymph nodes bacterioscopically.

The data of the series of experiments is presented in the table, illustrating the dynamics of an intradermal streptococcal focus in rabbits as studied by microbiological investigation.

Thus, we see that immediately after the inoculation the number of viable microbes in the focus diminishes considerably. Beginning from 2 hours an intensive multipliation of the microbes is observed, attaining its maximum 60-72 hours after the injection of the streptococci.

The results obtained permit characterizing the focus of infection arising, under our experimental conditions, in the skin of the rabbit by the dynamics of its development.

It merits attention that up to 72 hours inclusively, according to both the morphological and microbiological data, the local process progresses. It is interesting that under the applied experimental conditions proliferative changes on the part of the tissue surrounding the abscess were either absent or extremely weakly manifested in the early intervals (up to 72 hours), and only after 120 hours following the injection of streptococci, when the microbes were not dispersing and, morphologically, the process had abated, was a proliferative reaction observed. Upon morphological examination of the internal organs, dystrophic changes were discovered in the heart, kidneys, and liver. The absence of an increase in these changes with a prolongation of the intervals in the experiment speaks for the generation of the dystrophic processes not being related to an effect of the streptococcal infection. In several rabbits hyperplasia of the follicles or the pulp of the spleen was observed, again without relation to the experimental intervals. Changes in the spleen in the form of a disintegration of the lymphocytes, characteristic for this infection, were not noted. Under the given experimental conditions no other changes were observed in the internal organs as a result of the streptococcal infection, especially according to the treatment of the sections with the periodic acid- Schiff reagent. This testifies to the mild course of this infection, which is also corroborated by the changes developing in the intradermal foci.

SUMMARY

The development of experimental intracutaneous streptococcus foci (178 in number) in 65 rabbits was subjected to a complex (morphological and bacteriological) investigation; the effect of these foci upon the condition of internal organs was also studied. Group A (first serological type) streptococcus culture was administered according to Griffiths. The experiments lasted from 1 to 120 hours. Local infectious processes progress up to the time when the abscesses are formed – 72 hours. A significant proliferative reaction around these foci is noted only after 120 hours, when it is already impossible to isolate the microbes from the foci. There were no changes in the internal organs connected with the presence of the streptococcus foci.