Bacteriopyknosis is a Morphological Manifestation of Bacterial Death in the Organism

A. A. Pal'tsyn, E. G. Kolokol'chikova, N. V. Chervonskaya, A. K. Badikova, I. A. Grishina, and E. P. Popova

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Rats were immunized with *Pseudomonas aeruginosa*. A suspension of bacteria of the same strain was mixed with a sufficient amount of serum from the immunized rats to produce agglutination. This bacteria-serum mixture was injected intramuscularly to intact rats. Rapid dissolution of bacteria (during 1-3 h) was noted in the intercellular space, where microbial cells were reduced to pyknomorphic detritus. The formation of detritus from destroyed bacteria was demonstrated by detecting the radioactivity of the detritus in cases where animals were infected with bacteria labeled with tritium. The phenomenon described is a mechanism of bacterial dissolution in the organism differing from phagocytosis and complement-dependent lysis.

Key Words: *antibodies; wound infection; immunology of bacterial infections*

Knowledge of how microbial cells die in the organism of homoiothermic animal is essential for combating bacterial infections. Two bactericidal factors are now associated with antibody action, namely extracellular lysis by complement [8] and intracellular killing and digestion by phagocytes [4,6,7]. Both mechanisms can be initiated and boosted by antibodies unable themselves to kill bacteria [5,9]. In the present article we describe one more mechanism of the killing and breakdown of bacteria, one that depends on antibodies, occurs in the intercellular space, is morphologically manifested by bacteriopyknosis, and, at least in some cases, acts faster and is more effective than complement or phagocytes.

MATERIALS AND METHODS

Outbred rats weighing 220-280 g were infected with a 24-h *Pseudomonas aeruginosa* (strain 453, L. A. Tarasevish State Research Institute for the Standardization and Control of Biomedical Preparations) culture flushed off agar. In some experiments microbial cells were labeled with ^3H prior to administration. For this purpose 100 μCi/ml ^3H-uridine were added to agar. Control animals were injected with 0.3 ml of bacterial suspension in Hanks' solution in a concentration of 8×10^9 cells/ml to the right *m. gastrocnemius*. The same dose of bacteria agglutinated by homologous immune serum in a volume of 5-10% of the bacterial suspension was administered to experimental animals. For preparation of the immune serum rats were injected i.m. with a *P. aeruginosa* (strain 453) culture containing 2×10^9 cells/ml in Hanks' solution 3 times at 7-day intervals. Blood for serum preparation was taken 7-60 days after the third injection of bacteria.

Animals were killed under ketamine anesthesia 1, 2, 3, 8, and 20 hours and 1, 2, 3, and 15 days after infection. The primary foci were studied by electron microscopy and electron autoradiography. The latter was performed in two ways: 1) contamination with bacteria preliminarily labeled *in vitro*; 2)

Department of Pathological Anatomy, Laboratory for the Prevention and Treatment of Bacterial Infections, A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, Moscow. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

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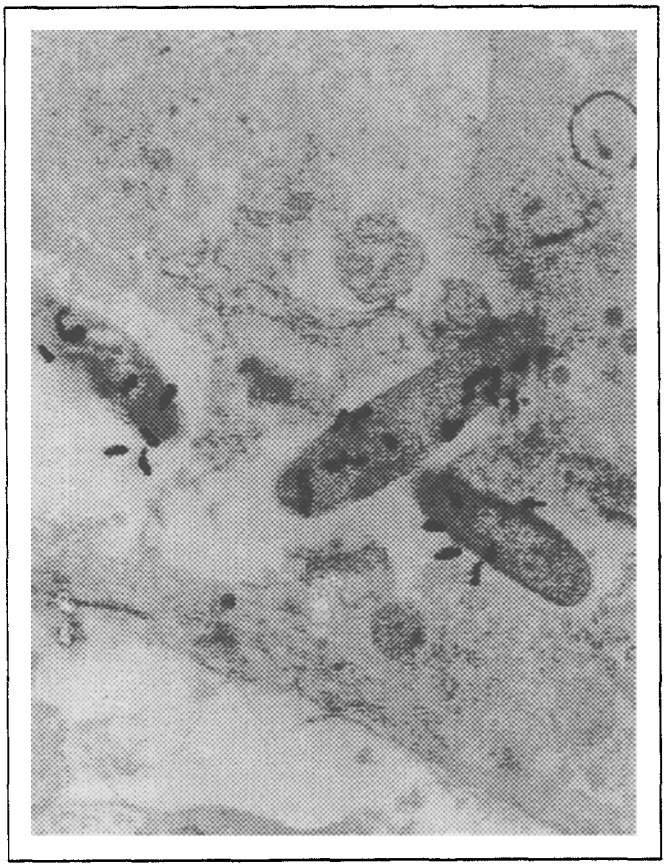

Fig. 1. Primary focus of control animal incubated with ^{3}H–uridine 20 h after infection. Bacteria situated among necrotic masses preserved a normal structure and the ability to synthesize RNA. ×20,000.

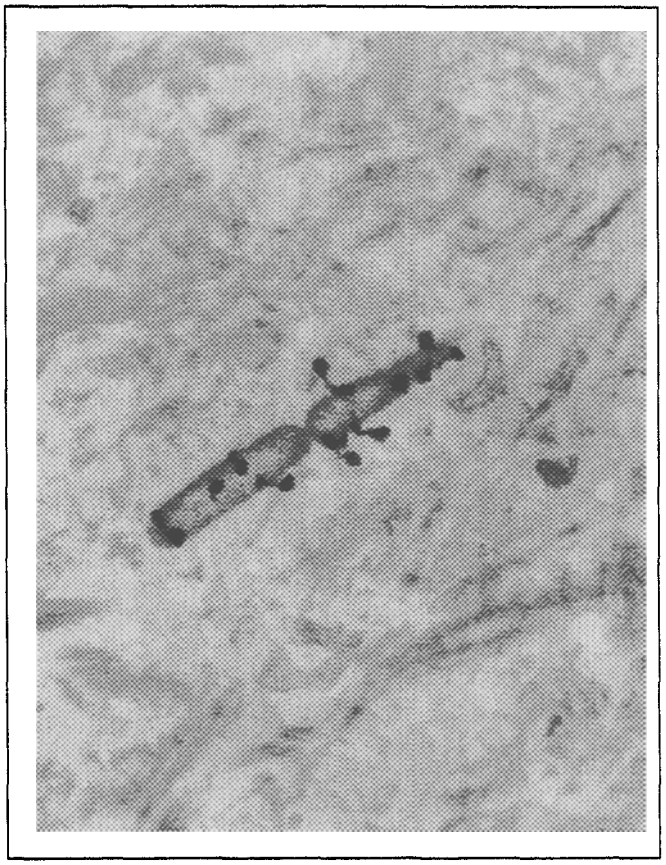

Fig. 2. Primary focus of experimental animal incubated with ^{3}H–uridine 1 h after infection. A few bacterial cells retained the native structure and capacity for RNA synthesis; they are embedded in amorphous electron–dense detritus and are devoid of label. ×15,000.

incubation of tissue pieces from a primary focus with ^{3}H-uridine. Live bacteria or their RNA-containing decay products were the sole labeled object in the preparations of primary foci obtained by the first variant of electron autoradiography. Even the shortest stay of labeled microorganisms in the organism, 1 h, is rather long (by microbiological standards) and comparable to the lifetime of one generation. During this time the concentration of labeled atoms decreases in live bacteria due to the RNA turnover and in the destroyed microorganisms via a partial breakdown of all cell components, including RNA. As a result, the content of the label drops so much in samples that it cannot be detected by routine electron microscopic autoradiographic technique. For this reason the method previously proposed [2] to improve the sensitivity of electron autoradiography was used in this variant. The principle of this modification consists in coating photoemulsion onto a thick (20-30 μ) section of material. The thick section contains markedly more labeled material than an ultrathin section of the same object. Thus, the relatively dense accumulation of silver grains can develop above a thick section even for a low concentration of labeled atoms. For visualization of this accumulation ultrastructurally, a thick section coated with a layer of emulsion is cut perpendicularly to its plane into ultrathin sections. In traditional radioautography a slide is viewed through the emulsion layer. The latter is invisible in a good preparation and only silver grains are seen above the structures which are the source of radiation. In our modified radioautographs the emulsion layer is situated above the tissue and is seen well. Labeled atoms can be found only in those structures which are no farther than 1 μ from the emulsion layer (the mean run of ^{3}H β-particles is 1 μ). Silver grains do not cover a labeled structure, but are situated directly above it or somewhat to the side of it not farther than the mentioned distance. Such a disposition of silver grains may be unusual, but it does not indicate the source of radiation less reliably than overlapping in the test material, thus achieving the goal of radioautographic analysis. The second variant of electron radioautography was carried out according to the routine technique [3].

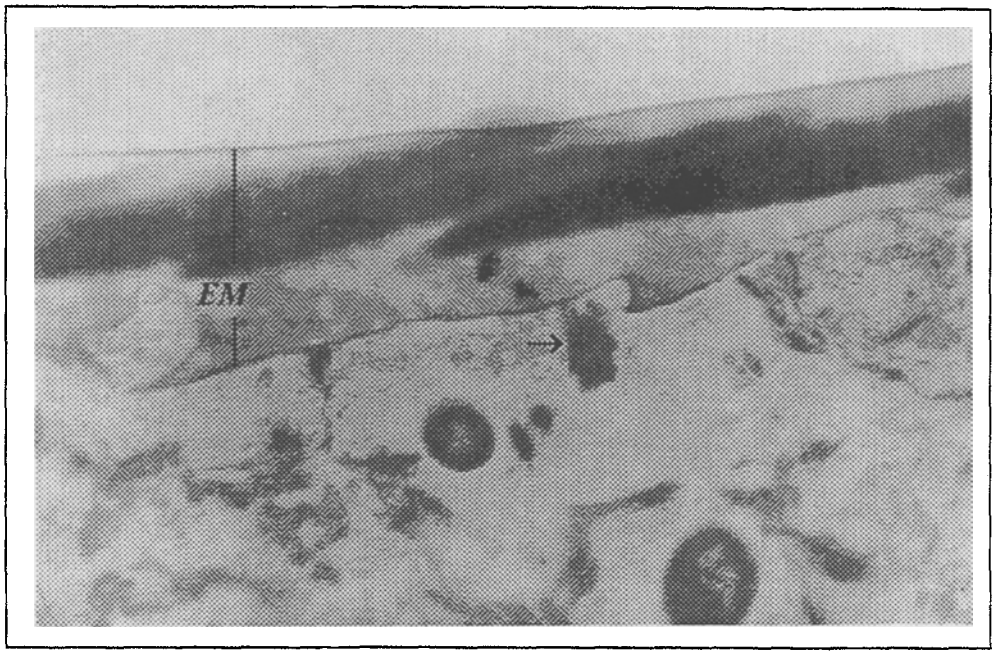

Fig. 3. Primary focus of experimental animal. Infection was performed with labeled bacteria. Fixation was carried out 1 h later. An ultrathin section of the radioautograph was cut vertically. Silver grains are seen in the emulsion layer (*EL*) in the vicinity of the detritus (arrow). ×20,000.

The effect of the immune serum on viability of *P. aeruginosa in vitro* was studied by radioautography and photometrically according to the accumulation of acid metabolites in the culture medium. The technique and results of these experiments were described in detail earlier [1].

RESULTS

In the control group 12 animals out of 15 died during the course of 15 days after infection. The rats lost from 20 to 70 g of their weight, were less active, and developed a cough, rhinitis, and conjunctivitis during this period. The limb with the primary focus swelled acutely and the rat could not put weight on it. The skin above the primary focus necrotized 3-4 days later and an ulcer developed. Acute plethora, hemorrhages, leukocytic infiltration, and extensive zones of necrosis of the muscle fibers and connective tissue were noted in the primary foci of control animals. An abundance of bacteria was found between normal and dystrophic muscle fibers in zones of necrosis or inside necrotized cells. For 3 days after infection the majority of bacterial cells showed no signs of destruction and were able to incorporate ^{3}H-uridine (Fig.

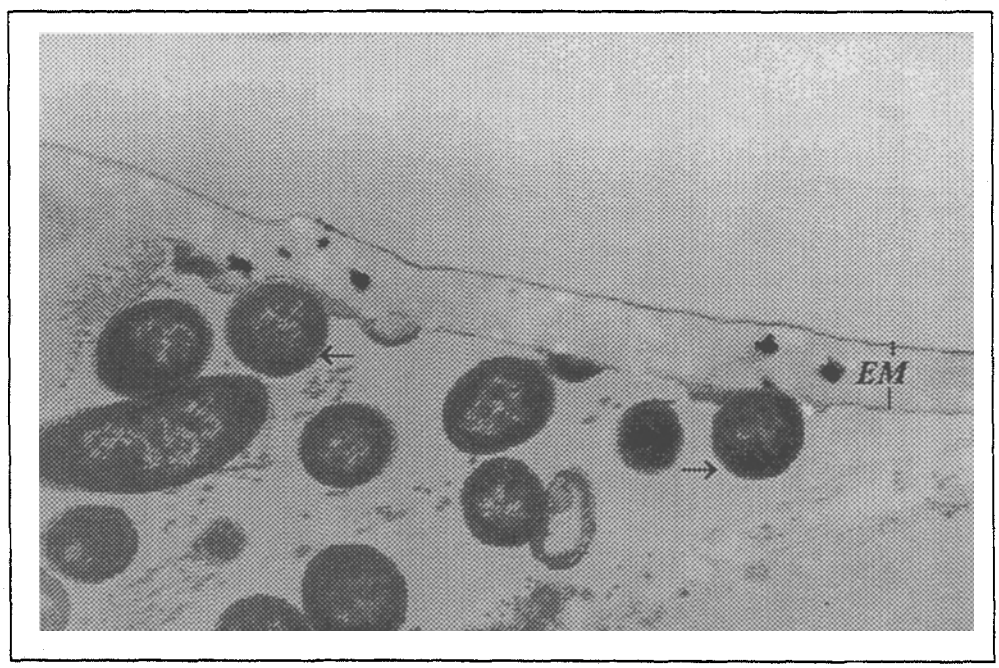

Fig. 4. Primary focus of cont-rol animal. Infection was performed with labeled bacteria. Fixation was carried out 1 h later. An ultrathin section of the radioautograph was cut vertically. Silver grains are seen in the emulsion layer (*EL*) close to some bacterial cells (arrows). ×20,000.

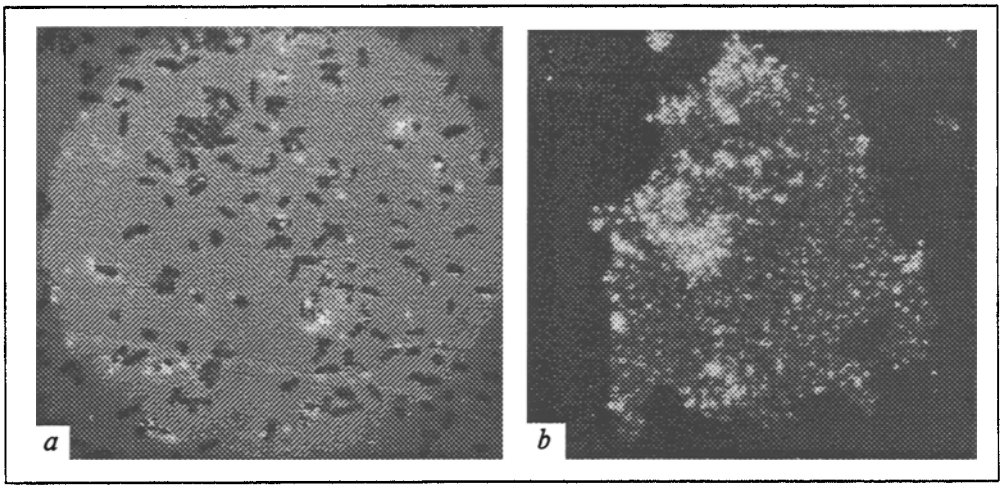

Fig. 5. Viability of *Pseudomonas aeruginosa* coupled with antibodies *in vitro*. ^{3}H–Uridine is incorporated into the bacteria incubated with normal (*a*) and immune agglutinator (*b*). In the radioautograph silver grains were photographed in reflected light and appeared as white rather than black dots. In the agglutinate (*b*) the label is distributed less evenly than in the dissociated bacteria (*a*). However, a marked reduction of the label concentration was not found in the agglutinate, i.e., in the reaction with antibodies. ×1000.

1). Phagocytosis of bacteria by neutrophils was often observed and many phagocytized microbes were viable.

The animals of the experimental group did not die; they gained 5-40 g in weight (23 g on average) over 15 days; the rats retained the usual motor activity and had no inflammatory changes in the eyes or upper respiratory tract. Edema of the limb with the primary focus was considerably less pronounced than in the control; ulcers did not develop at the site of the primary focus and the function of the extremity was preserved. Two weeks after infection the limb was similar to an intact one according to visual examination and palpation. No abscesses formed at the site of bacterial injection. Extensive leukocytic infiltrates were not found in the primary foci of experimental animals. Neutrophils were consistently present among bacterial conglomerates, but they were sparser than in infiltrates and the structure of conglomerates was as described below. As early as 1 h after infection only some microbial cells had a normal morphology, while the remainder became transformed into amorphous electron-dense masses (Fig. 2). We termed the formation of such detritus from bacterial cells bacteriopyknosis. If the inoculation was performed with bacteria labeled previously, the pyknotic masses contained label (Fig. 3) as well as morphologically intact bacteria in the experiment and control (Fig. 4). When ^{3}H-uridine was administered to the tissue of an already formed primary focus, the label was incorporated only into bacterial cells with the native structure and pyknotic masses were free of label (Fig. 2). These findings suggest that pyknotic masses consist of destroyed and coagulated bacterial cells. Coagulation developed quickly and 3 h after infection there were no bacteria with normal structure. Phagocytosis of microbes by neutrophils was very seldom found. The absence of an abscess in the primary focus 15 days after infection confirms the limited nature of phagocytosis in the experimental animals. Neutrophils noted in bacterial conglomerates were of abnormal appearance in experimental animals and differed markedly from those in the control, from neutrophils of the purulent wound, or from neutrophils phagocytizing bacteria *in vitro*. In such preparations neutrophils degranulated extracellularly and they did not contain either phagosomes with remains of bacteria or tissue fragments, but wide electron-transparent cavities free of granules were formed, sometimes with a grainy content.

The dissolution of bacteria with the production of pyknomorphic masses develops under the influence of antibodies only in the organism. No marked bacteria loss was found in a test tube where *P. aeruginosa* was reacted with antibodies even after 24 hours. The addition of immune serum resulted in the formation of a relatively large agglomeration if the bacterial concentration in the test tube was high enough (8×10^9 cells/ml). In this case not lysis but just some degree of decrease of bacterial viability was found as compared to the control (the addition of normal serum). Such a reduction in vital activity was due to the slowed rate of diffusion of substances in the massive agglomerations (Fig. 5, *a*, *b*), as was shown elsewhere [1].

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METHODS

Assessment of the Efficacy of Treatment of Hemangiomas

Yu. F. Isakov, V. N. Fedorova, V. V. Shafranov, A. V. Butorina, E. E. Faustova, S. S. Bykovskaya, and N. V. Fomicheva

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Assessment of the aftereffects of cryoexposure and ultrahigh-frequency cryoexposure on hemangioma tissue of various types, cavernous and squamous, showed a higher cryogenic effect in hemangiomatous tissue preexposed to ultrahigh-frequency waves. A quantitative criterion is proposed for assessing the efficacy of the studied methods of exposure.

Key Words: *mechanical parameters of the skin; hemangiomas*

Recent reports demonstrate the efficacy of treating children with hemangiomas by cryogenic and ultrahigh-frequency (UHF) cryogenic methods [6,8]. This study was undertaken to develop quantitative biomechanical criteria for comparing the efficacy of hemangioma treatment by two methods: cryogenic and UHF-cryogenic.

MATERIALS AND METHODS

An acoustic analyzer of tissues, a device permitting the assessment of biomechanical properties of the skin by recording the rate of propagation of ground waves (5-6 kHz frequency), was used for objective quantitative evaluation of the skin [5].

Hemangiomas were treated by local destruction with a device of a priming type by the standard method [4]. A combined method of UHF-cryodestruction was used for the treatment of cavernous hemangiomas. The presence of cavernous hemangiomas with a substantial subcutaneous part, often of a complex localization, which had failed to be cured by other methods was an indication for applying this method. Hemangiomas were exposed to a UHF electromagnetic field using a contact source in the physiotherapeutic mode at 1.2-1.5 W/cm^2 power for 1 to 5 min, depending on tumor activity. The period of subsequent cryodestruction of irradiated hemangioma was 1 to 3 min. Hemangiomas up to 1 cm thick were irradiated with a Luch-2 device, those thicker than 1 cm with a Pilot device. If the wavelength and attenuation coefficient are known, the depth of freezing can be estimated, which, with the above sources, was 1.3-3.7 cm. The procedure was repeated after 25-30 days, if necessary. The rate of ground waves was measured before exposure and after thawing in the

Department of Surgery, Russian State Medical University, Moscow

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