Bacterial Localization: The Use of an Autographic Method for the Study of Infectious Processes in Mice

The eradication of infections by means of antibacterial agents depends on a knowledge of the distribution of various pathogenic microorganisms; this is essential for a clear understanding of infectious states and for an evaluation of host-parasite interactions. Traditional methods of investigations are based on bacterial counts in various homogenized organs; this method is time-consuming and cumbersome, and furnishes only average values of the organs examined. Attempts have been made to overcome this difficulty by using whole-animal autoradiography in mice infected with labelled microorganisms[1,2]. This method furnishes a comprehensive picture in a single animal but it has a poor degree of reliability in time[3]. There are two reasons for this: the first is radioactivity due to by-products of both living and dead microorganisms; the second is the rapid dilution of radioactivity caused by multiplication of microorganisms. To overcome these difficulties we employed a modification of the autographic method[4-6].

Materials and methods. Germ-free and normal Swiss albino mice were i.v. infected with 0.5 ml of a 6 h broth culture of *Staphylococcus pyogenes aureus* (NTCC 8369) or *Escherichia coli* (ATCC 4157) or *Klebsiella pneumoniae* (ATCC 9590) containing approximately 10^9 cells per ml. Mice were killed, and frozen under sterile conditions; 300 µ sections were cut at −12 °C, carefully unrolled on to the surface of a 2% agar gel (agar 2% in 0.1 *M* buffer phosphate pH 6 according to Soerensen), and then irradiated with an UV-lamp for 1 min. This procedure kills any superficial microorganisms displaced during sectioning procedures. Sections were incubated at 37 °C (*S. pyogenes aureus* and *E. coli*) or at 25 °C (*K. pneumoniae*) for 18 h. After incubation the sections were carefully covered with a thin liquid film of a solution of triphenyltetrazolium chloride (750 µg/ml) in a saturated aqueous Na_2HPO_4 solution. Living microbes assumed a red colour; sections were photographed by transparency.

Results and discussion. Figure 1 shows the distribution of Staphylococci 2 h after infection. Their presence in liver, spleen and lungs is evident and there is also a moderate renal excretion. Our results agree with those obtained by BONVENTRE and IMHOFF[1-3] using autoradiography, and with the observations of ROGERS[8]; i.v. injected bacteria appear to be taken up chiefly by the reticuloendothelial system where they generally survive for some days. Figure 2 represents an *E. coli* infected 'germ-free' female mouse at the 16th day of pregnancy, killed 2 h after infection. The general distribution is similar to that shown in Figure 1; the placentae appear heavily infected, whereas the fetuses and the amniotic fluid are sterile. This indicates a possibility of using this method for evaluating the relationship between maternal and fetal infections. Figure 3 shows a mouse 48 h after infection with *Klebsiella pneumoniae*. Microorganisms are present almost exclusively in liver and spleen; microorganisms present in the intestine belong to the normal intestinal flora.

This figure demonstrates that it is possible to evaluate bacterial distribution and fate even several hours after infection. We found good correspondance between the degree of microbial infection revealed by our method and the number of microorganisms found by the plate bacterial count method. The degree of sensitivity is high: we had no difficulty in observing bacterial distribution even in mice infected with as few as 500 cells (dilution 1×10^{-6}

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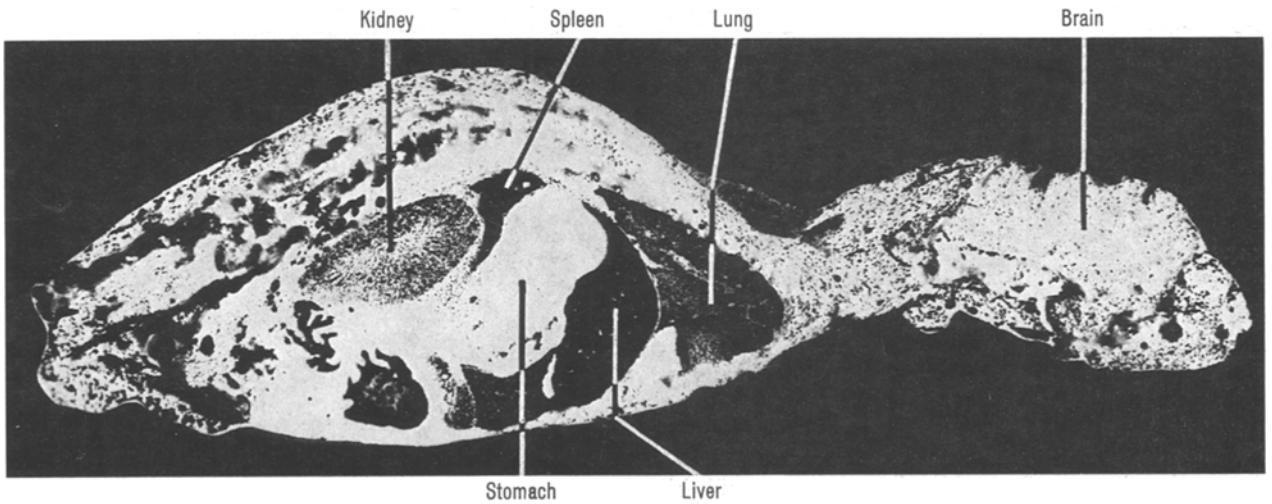

Fig. 1. Whole-body sagittal section of a normal mouse 2 h after i.v. infection with *Straphylococcus pyogenes aureus* (50×10^9 microorganisms). Vital staining with triphenyltetrazolium. The dark areas contain numerous living microbes; the white areas do not contain living microorganisms. × 2.5.

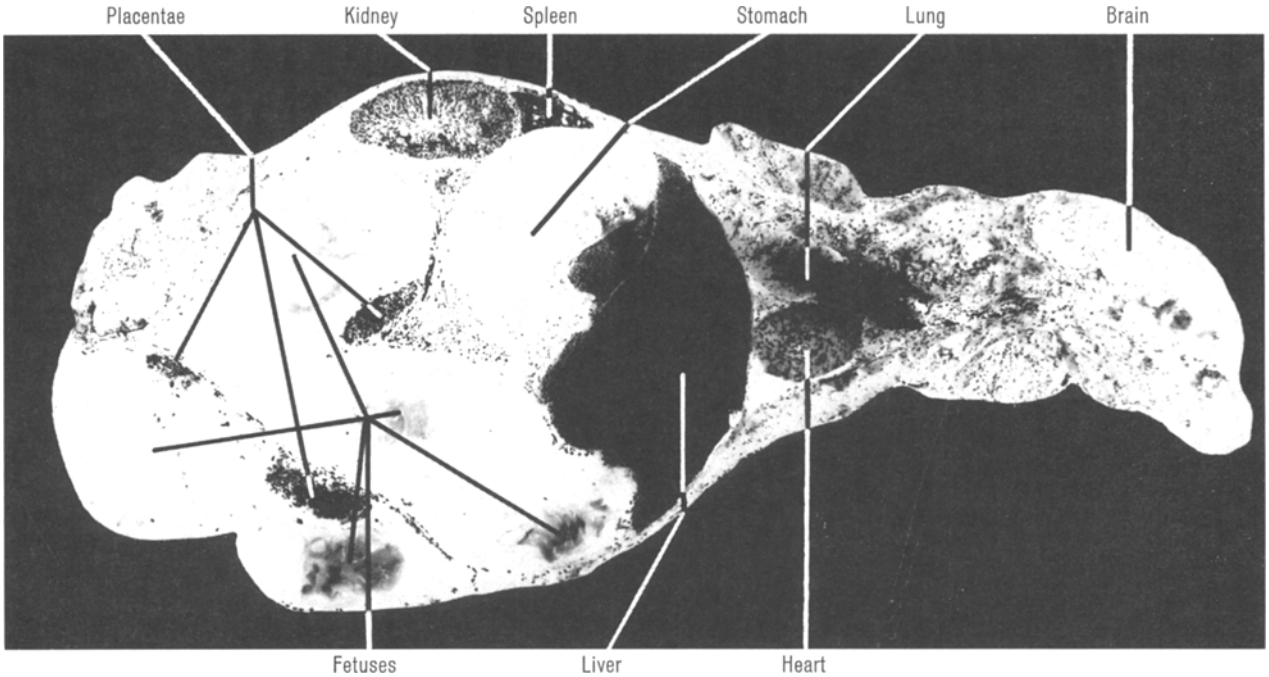

Fig. 2. Whole-body sagittal section of a pregnant germ-free mouse 2 h after i.v. infection with *E. coli* (50×10^9 microorganisms). Vital staining with triphenyltetrazolium. × 1.8.

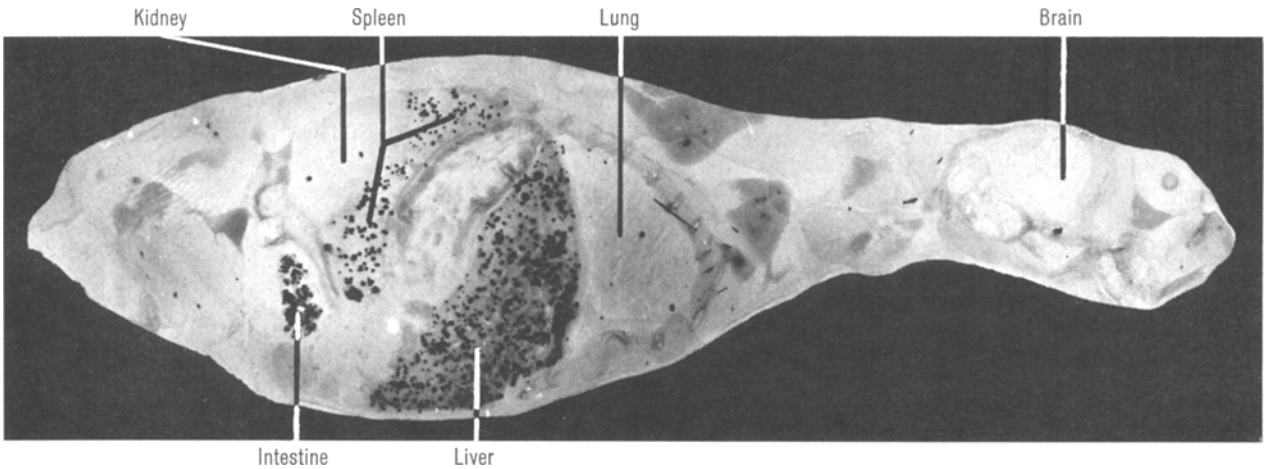

Fig. 3. Whole-body sagittal section of a normal mouse 48 h after i.v. infection with *K. pneumoniae* (50×10^9 microorganisms). Vital staining with triphenyltetrazolium. × 2.5.

of a 6 h microbial culture in liquid medium). Infected sections were also subcultured in order to verify that the microorganisms present were identical with those injected. In our experience the autographic method is a practical tool for obtaining reliable information about bacterial distribution in infected mice. The study of this distribution may bridge the wide gaps which still exist in our knowledge of the pathogenesis of infection. The method shows the distribution only of living microorganisms and therefore furnishes a true map of their distribution. It possesses the advantages typical of whole-body sections, principally a general view of the microbial tropism on each experimented animal.

Riassunto. Il metodo autografico è stato impiegato per la valutazione della distribuzione dei germi. Sezioni sagittali complete di topi precedentemente infettati sono state incubate e i germi vivi sono stati rivelati mediante colorazione vitale con trifeniltetrazolio. Le risultanti mappe dettagliate della distribuzione batterica permettono di penetrare il meccanismo dei processi difensivi dell'ospite e le proprietà invasive del germe.

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