8 h after Sindbis i.v. inoculation and afterwards fell off more quickly from serum than urine.

It appears likely, therefore, that urinary interferon derives directly from the plasma pool and its high specific activity is reached through the selective renal glomerular filtration. Indeed i.v. administered interferon has been detected in the kidney[8]. The urine/serum (U/mg) ratios suggest that some of the urinary interferon is derived, after 8 h, directly from the kidney and these data are consistent with the finding that inhibitory activity in the kidney is low and delayed[2]. Nevertheless the slower tailing off of the urinary interferon curve may also be accounted for by a small residue of urine in the bladder.

The possibility that urinary interferon could be due to a trauma of the bladder after catheterization has been excluded by the absence of blood in the urine and by collecting the urine only once, from 3 rabbits, 10 h after the virus inoculation. Again urinary interferon had a specific activity of 674 (U/mg) as compared to 4.7 in the serum of the same group of rabbits.

The data here reported suggest that levels of blood interferon are the result of complex equilibria of circulating interferon with tissues and body fluids and with simultaneous leakage of this protein into the urine. In this respect interferon behaves like lysozyme[12] or the sex-dependent α_2-globulin in the rat[13]. An important site of interferon breakdown may occur also in the intestine as it appears for serum albumin[14,15].

When this work was completed, we learnt that OH[16] has also found interferon in some body fluids and a small amount in urine. The discrepancy with our results may be due to the fact that OH-induced interferon production by typhoid endotoxin, and it is known[17] that this interferon has a higher molecular weight than the virus-induced one and is likely to be largely retained by the kidney filter.

From these results the conclusion is drawn that interferonuria represents an excellent condition for obtaining highly purified interferon. Clearly the dynamic and the metabolic fate of interferon will be more directly traced by isotopically labelled pure interferon[18,19].

Riassunto. È stato dimostrato che l'inoculazione endovenosa di virus Sindbis nel coniglio provoca formazione di interferone che viene liberato nel sangue ed escreto con le urine. L'interferone urinario sembra derivare quasi completamente dal siero, ha alti titoli ed ha le stesse proprietà fisico-chimiche e biologiche dell'interferone sierico.

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PRO EXPERIMENTIS

Whole Body Sagittal Technique in Mice Employing a New Bio-Autographic Method: Its Utility for the Evaluation of the Body Distribution of Antibacterial Drugs

Body distribution of antibacterial drugs is usually evaluated by measuring tissue levels using bacteriological or scintillometric methods: these, however, are troublesome and time consuming and require a large number of animals to obtain true average values. Progress was achieved by the autoradiographic method, using whole body sagittal sections of mice, which allows more rapid and accurate assay[1-5]. This method however requires time (generally not less than 4 months) to give reliable results; moreover, it allows a correct evaluation of distribution only in the period immediately following administration for the genesis of labelled active or inactive metabolites: therefore contemporaneous chromatographic trials are essential. For the purpose of obtaining a distribution pattern closer to the effective chemotherapeutic distribution, a method has been developed in our laboratory[6], based on the direct inoculation of thin, whole body sections of mice previously treated with antibacterial drugs, and on the direct observation of the live microbe by vital staining with tetrazolium salts.

Methods. Swiss albino mice, treated with antibacterial drugs and killed at various hours after the administration, are used; they are frozen on a Leitz microtome type 1300 and cut at $-10\,^{\circ}C$ into sections of 300 μ. Sections are

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placed on the surface of agar medium in Petri dishes and partially dried at 37 °C for 1 h; the section is then inoculated by means of a platinum loop with *Staphylococcus pyogenes aureus* NTCC 8369 (dil. 1:10 of a 6 h culture in broth with a final content of $1.5 \cdot 10^8$ germs/ml), or other suitable gram-positive or gram-negative microorganisms, incubated for 18–20 h at 37 °C and covered with a thin liquid film of a solution of triphenyltetrazolium chloride (750 µg/ml) in a saturated aqueous Na_2HPO_4 solution. The live microbe rapidly assumes a bright red colour due to intracellular precipitation of red triphenylformazan[7]. Areas where a bacteriostatic level of antibiotic has been attained are germ-free and therefore colourless by transmitted light, while areas with a lower antibiotic level (and therefore at different degrees of bacteriostasis) show a red colour of different intensity, proportional to the number of live microbes. Similar results are obtained using other tetrazolium salts. Control sections with untreated mice always show a uniform bacterial growth. Photographs are taken using a Leitz Aristophot apparatus with a Summar *f*8 objective.

Results. Figure 1 shows a whole body sagittal section of a novobiocin-treated mouse. The lack of diffusion phenomena is demonstrated by the visualization of fine structural details such as diaphragm, pleura and pericardium. Tetracycline distribution in bones is shown in Figure 2. The picture is similar to that obtained using the Wood light, but the dose of chlortetracycline is 10 times less than that necessary for UV-light fluorescence. With UV-light fluorescence, moreover, the existence of an inactive fluorophore common to active or inactive tetracycline creates some doubts about the interpretation of the 'true' antibacterial distribution. Figure 3 shows part of a section of a penicillin-treated mouse and demonstrates differences between blood and tissue levels. The blood found in the section of aorta, hepatic and coronary vessels and heart cavity is free of active antibiotic, while the liver, muscles and the myocardium demonstrate the presence of antibiotic in high concentration.

Discussion. A method is described which may be employed for quick and reliable evaluation of tissue distribution of an antibacterial drug. The results may be photographed in detail. The advantages of this technique over the autoradiographic method are: (1) information is more rapidly obtained on the relationship between distribution and persistence in tissues of antibacterial drugs; (2) tissue levels represent a true antibacterial picture, with simultaneous evaluation of the antibiotic molecule and of the various active metabolites; (3) a general view is obtained of the passage of antibiotics across various physiological barriers (blood-brain barrier, placenta barrier and occasionally blood-eye and blood-synovia) or pathological barriers (blood-abscess or blood-necrotic infected area) for correct information on the possible chemotherapeutic value of a drug.

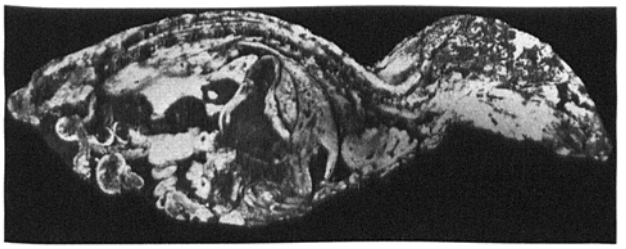

Fig. 1. Whole body sagittal section of a mouse 2 h after the i.v. administration of 10 mg/kg of novobiocin. Inoculation with *Staphylococcus pyogenes aureus* and vital staining with triphenyltetrazolium hydrochloride. Actual size.

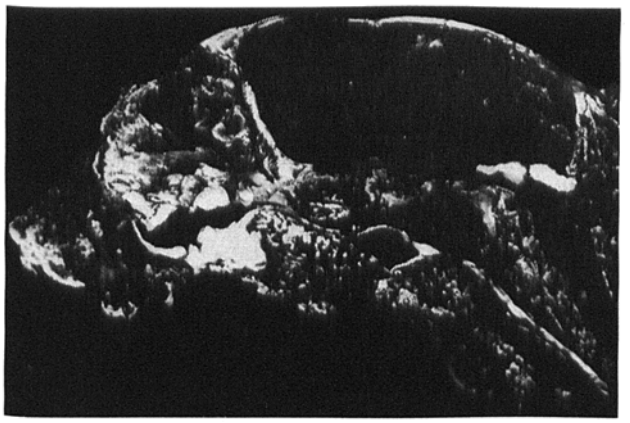

Fig. 2. Detail of a whole body sagittal section of a mouse orally treated with 10 mg/kg of chlortetracycline hydrochloride. Inoculation with *Staphylococcus pyogenes aureus* and staining with triphenyltetrazolium hydrochloride. Magnification × 5.2.

Fig. 3. Detail of a whole body section of a mouse treated i.p. with 10 mg/kg (10.580 IU/kg) of the hydroiodide of penicillin G. diethylamino ethyl ester. Inoculation with *Staphylococcus pyogenes aureus* and staining with triphenyl-tetrazolium hydrochloride. Magnification × 3.2.

Riassunto. È stato studiato un metodo per lo studio della distribuzione di farmaci antibatterici basato sull'insemenzamento diretto con svariati germi di sottili sezioni longitudinali di topo previamente trattato con antibatterici; la presenza di germe vivo viene rilevata per mezzo di una colorazione vitale con il cloridrato di 2,3,5-trifeniltetrazolio.

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