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Received March 11, 1954. P.S.E.B.M., 1954, v85.

Autoradiographical Distribution and Excretion Studies with S^{35}-Labelled Penicillin. (20948)

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Previous investigations of the pharmacology of penicillin have depended in general on bacteriological methods of penicillin estimation. In the present investigation autoradiography has made possible a more detailed localization of the radioactive substances in histological sections, so that the distribution and fate of penicillin in normal mice could be studied. Some preliminary observations have also been made on the distribution of penicillin in diseased tissue, and on the inhibitory effect of probenecid* on the excretion of penicillin by the kidneys.

Methods. Radioactive (S^{35}-labelled) penicillin was prepared biosynthetically according to methods similar to those described by Smith and Hockenhull(1) but using stirred bottles and aeration. Crystalline sodium penicillin was obtained via the ethylpiperidine salt. Paper chromatography showed almost entirely benzylpenicillin. The histological technic was complicated by the solubility of penicillin in water and fixation fluids. The labelled penicillin was injected intravenously in a tail vein. A dose of one millicurie per g of body weight was employed, which corresponds to 8 μg of penicillin per g of body weight. Animals were killed at different times after injection. In most cases the entire body of the mouse was rapidly frozen in isopenthane at —170°C. The frozen mice were mounted on large stages and allowed to reach —10°C, when they were sectioned. All of the sections were dried at —10°C. In some of the mice small pieces were taken from organs and treated by the usual freeze-drying methods with subsequent paraffin-embedding and sectioning at room temperature. Contact autoradiographic technic was used with exposure for some weeks in iron presses at —10°C.

Results. Distribution in normal tissues. Penicillin is rapidly distributed to the tissues from the blood stream. In all cases the relative distribution of penicillin concentration in the various tissues was similar from 5 minutes after the injection until the penicillin began to disappear from the tissues.

Fig. 1 shows the distribution of penicillin in an adult mouse 20 minutes after injection. The numbers indicate the relative degrees of radioactivity in the organs as a percentage of that of the blood. These were obtained by measuring on a dried section with a mica-window G-M tube, when the whole section, except for the measured area, was covered.

The greatest penicillin concentration is found in the kidneys, which are responsible for the elimination of most of the penicillin, but the liver is not to be disregarded as an excretory organ, as can be seen from the high activity in the biliary system. All the activity in the lumen of the intestine appears to come from the biliary system. The subcutis

* Probenecid is the generic name for *p*-(di-n-propylsulfamyl)-benzoic acid supplied under the trademark 'Benemid' (U.S.A.) and 'Probecid' (Sweden).

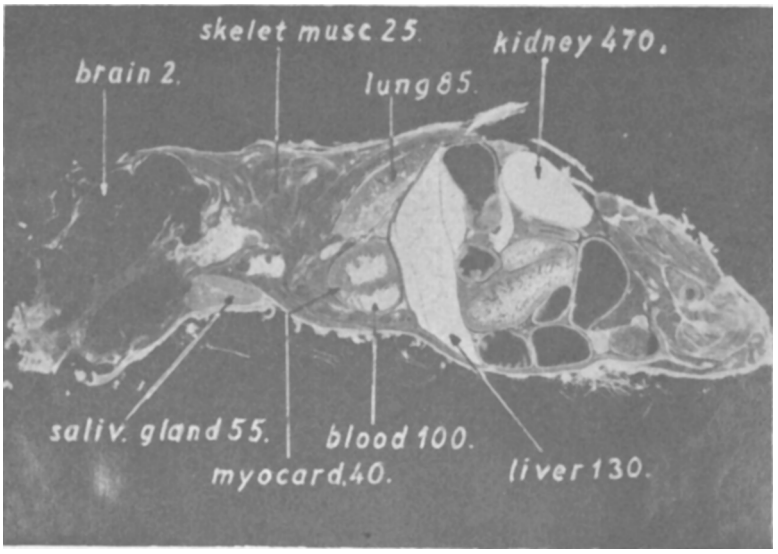

FIG. 1. Autoradiograph showing the distribution of labelled penicillin in an adult mouse 20 min. after intravenous injection. The numbers refer to the penicillin concentration as percentage of the blood concentration.

and the lungs show a high concentration of penicillin, while it is markedly lower in skeletal muscle. The penicillin concentration in compact bone is low, in the brain very low, and still lower in the cerebrospinal fluid.

Fig. 2 (autoradiograph of a pregnant mouse 30 minutes after an intravenous injection) shows that the activity is high in the uterine wall, rather low in the fetus, and almost nonexistent in fetal fluids. In addition, it appears that the fetal brain contains essentially less penicillin than the other parts of the fetus, indicating that the blood-brain barrier is functioning at this early stage.

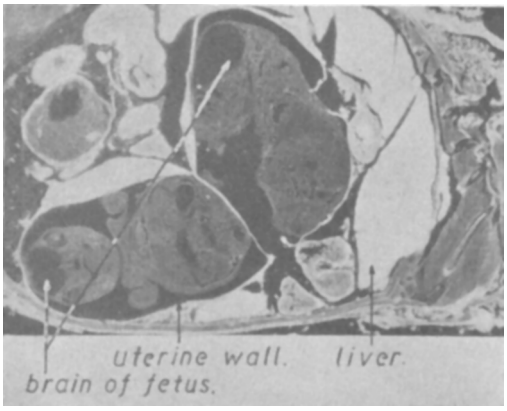

FIG. 2. Autoradiograph showing the uterus with two fetuses in a pregnant mouse. White areas correspond to high penicillin concentration.

Fate of penicillin in body. After 1½-2 hours most of the penicillin is excreted, but some activity is still present in the kidneys, bladder, liver, and intestines. Penicillin also disappears rather slowly from the cerebrospinal region and the eyes. Rowlands *et al.* (2) measured the excretion of intramuscularly administered radioactive penicillin in the urine of anesthetized cats. They showed a recovery in the urine of 100% by measurement of the radioactivity with a G-M tube. A large correction for self-absorption in the samples was necessary. The present investigations on mice were based on the opposite principle. The penicillin remaining in the body, and not that excreted in the urine was measured. The results are in agreement with those of Rowlands *et al.* in that neither penicillin nor its possible breakdown products remain in the tissues. On the other hand, my results show that some radioactivity terminates in the alimentary tract, despite the parenteral administration of the penicillin in mice, which is at variance with Rowlands' results in cats.

Fig. 3 shows an autoradiograph of a mouse 5 hours after intravenous injection. It is obvious that radioactivity has disappeared

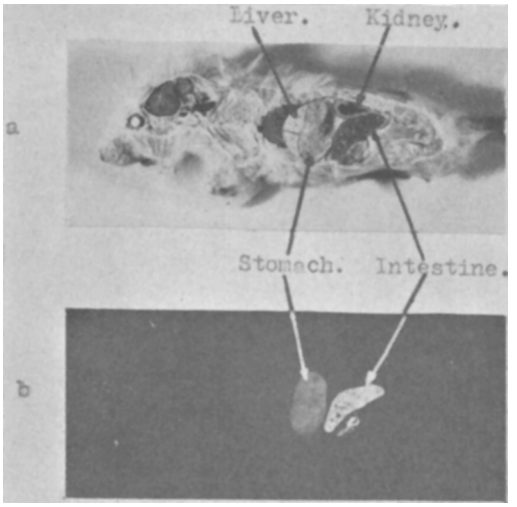

FIG. 3. a) Photograph of the section surface of a mouse killed 5 hr after intravenous injection of labelled penicillin. b) Autoradiograph of (a).

from all tissues, even the kidneys and liver, while the activity excreted via the bile ducts to the intestines is not reabsorbed to any large extent but remains in the intestinal lumen. There is also some activity in the stomach, which could have emanated from the penicillin excreted in the saliva. (This autoradiograph was produced by bringing the even surface of the frozen block into direct contact with the film. As S^{35} produces rays of only a short range, pictures of relatively good resolution could be produced in this simple manner.) Throughout the experiments intravenous injections have been used to obtain rapid distribution and to avoid any inactivation of penicillin during resorption from an intramuscular injection site.

Beyer(3) in his monograph on penicillin pharmacology states: "Inactivation of penicillin in the body is of uncertain significance in the overall elimination of the compound." According to most of the information available, up to 100% of parenterally administered penicillin is excreted in a still biologically active state. In view of this, it does not appear probable that inactivation in the body can seriously alter the distribution picture in a study of this kind. If a physico-chemical separation of possible breakdown products from intact labelled penicillin in body fluids and tissue extracts could be carried out, it might be possible to give a more definite answer to the question of inactivation.

Pathological tissues. Tissue changes due to infectious agents can, to a great extent, affect the ability of penicillin to reach the bacteria. Fig. 4 shows the distribution of penicillin 30 minutes after injection in the case of a subcutaneous abscess of natural origin. The contents of the abscess were of caseous character. The penicillin has apparently passed through the abscess membrane but has not reached the center. (The concentration of penicillin in the subcutis is always relatively high.) The experimental animal received probenecid before the injection of penicillin.

Site of action of probenecid. There are a number of compounds that block the renal excretion of penicillin on a competitive basis. Probenecid is at present the most promising of these agents. Renal clearance tests(4) have shown that probenecid can reduce penicillin excretion in the urine to about 20% of normal, which is interpreted as inhibition of the tubular secretion while glomerular filtration continues as usual.

Fig. 5 shows an autoradiograph of the

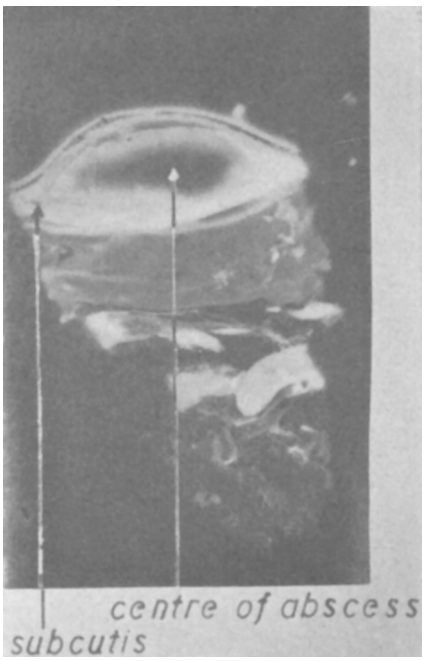

FIG. 4. Autoradiograph showing the ability of penicillin to pass an abscess membrane.

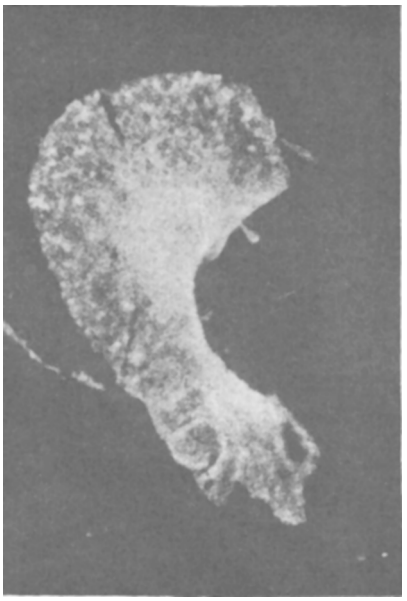

FIG. 5. Autoradiograph of a kidney of a mouse that received probenecid before labelled penicillin.

kidney of a mouse that received 0.2 mg of probenecid per g weight *per os* 2 hours before the injection of penicillin. The glomeruli are visible against a background of relatively penicillin-free tubuli, while in the case of animals that received only penicillin the renal tubuli show a very high degree of activity.

Summary. Radioactive S^{35}-labelled penicillin has been injected into mice and its distribution in the various tissues of the body studied by autoradiographical technics. The results correlate well with the observations that have been made using the microbiological assay for penicillin. In the kidneys the concentration is always, and in the liver generally, higher than in the blood, but in other organs during the whole course of distribution and excretion it is lower than in the blood. In the lungs the concentration nearly approximates that of the blood, while in most tissues it is considerably lower. It is lowest in the central nervous system. The method here described can be applied to the study of penicillin distribution in diseased tissues. Preliminary observations have shown the diffusion of labelled penicillin into abscesses, and the difference in distribution of penicillin in the kidneys under the influence of probenecid and after the administration of penicillin alone.

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Received March 11, 1954. P.S.E.B.M., 1954, v85.

Plasma Lipoprotein Changes Following Thermal Injury in the Dog.* (20949)

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Following burn injury, there is consistently a rise in the α-globulin fraction of plasma in human beings, dogs, and various other animals, as shown by electrophoretic studies (1-4). The fact that, in dogs, the α-globulin fraction contains all of the plasma lipoproteins(5,6) suggested to us that these lipoproteins might be considerably altered by thermal injury. Further support for such a relationship comes from chemical fractionation studies(7).

In the present investigation, the lipoproteins were isolated from the plasma of burned dogs and characterized by flotation in an analytical ultracentrifuge. Marked changes in the lipoprotein fractions were shown to

* These studies aided by contract between Office of Naval Research, Department of the Navy, and the University of California.