RADIOBIOLOGY

Autoradiographical Detection of Tritium-labelled Inulin in the Kidney

By perfusion tests performed on the kidneys of rabbits which had previously been intravenously injected with inulin (100 mgm./kgm. body-weight), it has been shown that inulin was deposited in the kidney tubule cells[1]. These observations were recently confirmed by Balint and Forgacs[2]. Other authors, however, did not agree with my conclusions[3,4].

The autoradiographical detection method seemed suitable to us to obtain further information about the distribution pattern of inulin in the kidney. By means of this technique using tritium-labelled inulin we investigated the kidneys of rats and rabbits.

Alkali-stable inulin was labelled with tritium gas according to the method of Wilzbach[5] (Graul and Hundeshagen[6]). This preparation was dissolved in water and evaporated three times. The final product was obtained by crystallization from an aqueous solution with a mixture of ethanol–ether (v/v, 1/1). Its specific activity was 71·5 μc./mgm. or 286 c./mol. (assumed molecular weight: 4,000), and was alkali-resistant like the initial product. In solution the substance obtained gave a negative reduction test, and after hydrochloric hydrolysis it gave a positive Selivanoff reaction. This is sufficient guarantee that the inulin molecule had not been altered by labelling.

Five mgm. of tritiated inulin was injected under direct vision into the renal artery of an anæsthetized rabbit. After 5 min. the kidneys were removed and fixed in formalin. Transverse sections were examined by the autoradiographical method[6], using an exposure time of 2–9 months. After developing the photochemical layer, the tissue sections were stained with hæmatoxylin–eosin.

Figs. 1 and 2 represent autoradiograms obtained. Over the cells of the proximal and distal tubules there is an obvious blackening, which in other fields appears still more intense, and is even visible in some instances over the tubule lumina.

We obtained corresponding results in rats injected intravenously with tritiated inulin (50 mgm./kgm. body-weight). In these experiments the kidneys were removed after 10 and 20 min. The autoradiograms were not as intensively marked as in the above-mentioned tests, but generally a blackening over the tubule cells was clearly visible.

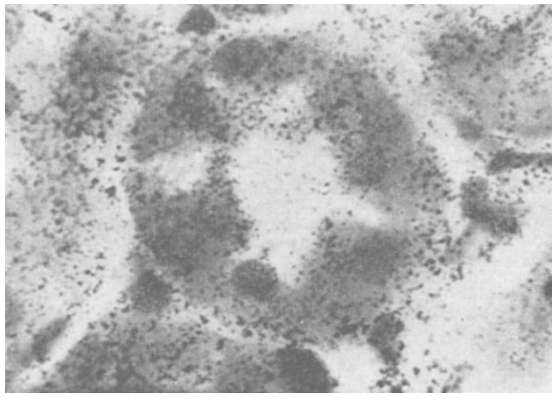

Fig. 1. Autoradiogram of a rabbit kidney 5 min. after injection of 5 mgm. tritiated inulin into the renal artery. Exposure time, 6 months. Proximal tubule. (× *c.* 900)

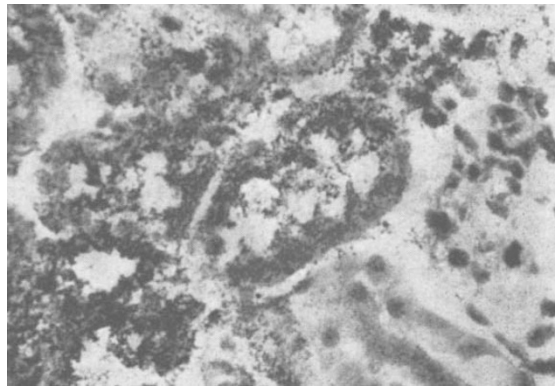

Fig. 2. Experiment similar to Fig. 1. Distal tubule, same as Fig. 1. (× *c.* 360)

The results of these autoradiographical investigations on kidneys with tritiated inulin confirm our opinion that inulin is deposited in the tubule cells of the kidney. No information about the mechanism of this process was obtained.

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Experimental Allergic Encephalomyelitis in Irradiated Rats

Lipton and Freund[1] have produced experimental allergic encephalomyelitis in the rat by intracutaneous injection of homogenized homologous rat brain mixed with paraffin oil and tubercle bacilli. Lumsden[2] reported earlier that in the rat experimental allergic encephalomyelitis can be induced only by injecting the antigen mixture using the intracutaneous and not the subcutaneous route.

If the concept of radiation sickness as an auto-immune disease[3] is valid, then the question may be put whether it would be possible to induce experimental allergic encephalomyelitis in irradiated rats.

In the concept of auto-immune reactions of irradiated animals, which is being reported elsewhere[3], it is necessary to presume that antibody-producing cells undergo a change, that is, they mutate, thus becoming foreign to their own organism. In that manner these cells become antigenically stimulated by the organism's own cells—both normal and those which had also undergone a mutation. Antigenically stimulated cells should be radio-resistant, since immunity already acquired is not impaired by irradiation. By absorbing radiant energy, the antibody-producing cells mutate until they acquire a structure which, according to the clonal selection theory[4],