EFFECT OF FREQUENCY OF ACTION OF A PATHOGENIC FACTOR ON KIDNEY TISSUE: AUTORADIOGRAPHIC STUDY

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The kidney tissue of mice was investigated autoradiographically by means of thymidine-H^3 under normal conditions and after single and repeated injections of mercuric chloride at intervals of 1 day and 1 week. Noninbred male albino mice (26) weighing 25-30 g were used in the experiments. In the control mice (5) the index of labeling in the epithelium of the proximal tubules was relatively higher. After the more frequent action of the pathogenic factor on the kidney, more cells synthesizing DNA appeared in it. It is postulated that the correlation existing between the number of labeled epithelial cells of the proximal tubules and the number of flattened cells lying beyond their basement membranes is a distinctive index of the regenerative changes in the tissue of the damaged kidney.

Key words: regeneration; frequency of injury; kidney.

The histological and histochemical changes developing in the kidney tissues under the influence of mercuric chloride are sufficiently well known [1, 4-7, 10]. However, only a few investigations into the effect of pathogenic factors on kidney tissue have been carried out by the method of autoradiography [8,9].

It was decided to investigate the relations between degenerative and regenerative changes in the kidney by means of autoradiography after exposure to the action of an irritant applied at different frequencies.

EXPERIMENTAL METHOD

Altogether 23 male albino mice weighing 25-30 g were used in the experiments. The animals were divided into 3 groups. The experimental mice received subcutaneous injections of mercuric chloride, each in a dose of 15 mg/kg. The mice of group 1 received 1 injection, those of group 2 received 2 or 3 injections at intervals of 1 day, and those of group 3 received 2 or 3 injections of mercuric chloride at intervals of 1 week. The control group consisted of 5 mice. Two animals from each group (except the control) received an intraperitoneal injection of thymidine-H^3 in a dose of 1.5 μCi/g body weight on the 3rd-7th day before the last injection of mercuric chloride. The animals were killed 1 h after injection of the isotope. Pieces of kidney were fixed in 10% neutral formalin and embedded in paraffin wax. To prepare the autoradiographs sections 6-8 μ in thickness were used. After dewaxing, the sections were coated with type M (NIKFI) emulsion and exposed in a refrigerator at 4-6°C for 30 days in light-proof containers. Development was carried out by the method recommended by NIKFI (metol-hydroquinone developer, fixation in 30% hyposulfite solution), and the sections were stained with hematoxylin and eosin.

Autoradiographs of the kidney were studied in the light microscope giving a magnification of 1000 times. Cells were taken as labeled if no fewer than five tracks were present above their nuclei. About 4000 cells were counted in each section: epithelial cells of the proximal and distal tubules, cells of the glomeruli, and flattened cells under the capsule of the kidney and beyond the basement membrane of the tubules were distinguished.

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TABLE 1. Relative Percentage of Labeled Cells in Different Parts of the Nephron

Group	Time of expt.	Cell types			
		Subcapsular	Proximal tubules	Distal tubules	Lying beyond basement membrane of tubules
Control	—	0,02	0,3	0,05	0,07
1	Three days after 1st injection	0,2	0,65	0,12	0,7
	One week after 1st injection	0,1	0,6	0,05	0,5
2	Three days after 2nd injection	—	0,12	0,07	0,1
	One week after 2nd injection	0,05	1,2	0,1	0,82
	Three days after 3rd injection	0,05	0,6	0,05	0,35
	One week after 3rd injection	0,05	0,67	—	0,2
3	Three days after 2nd injection	0,27	0,22	0,12	0,45
	One week after 2nd injection	0,12	0,17	0,05	0,25
	One week after 3rd injection	0,05	0,2	—	0,27

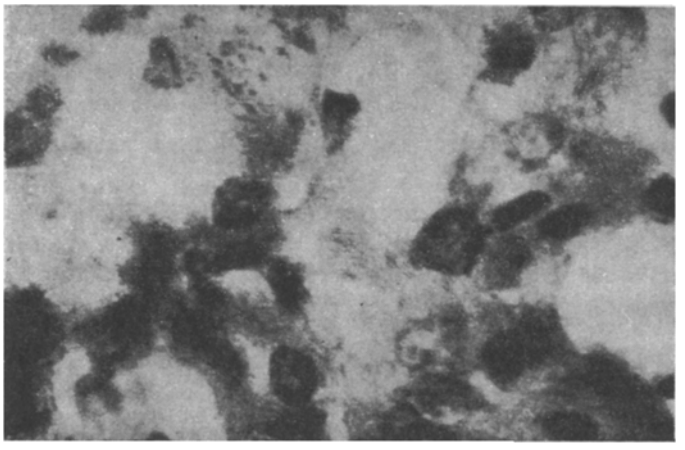

Fig. 1. Autoradiograph of mouse kidney after 1 injection of mercuric chloride: cell of proximal tubule, labeled with thymidine-H^3. Type M (NIKFI) photographic emulsion. Exposure 30 days. Stained with hematoxylin and eosin. 630×.

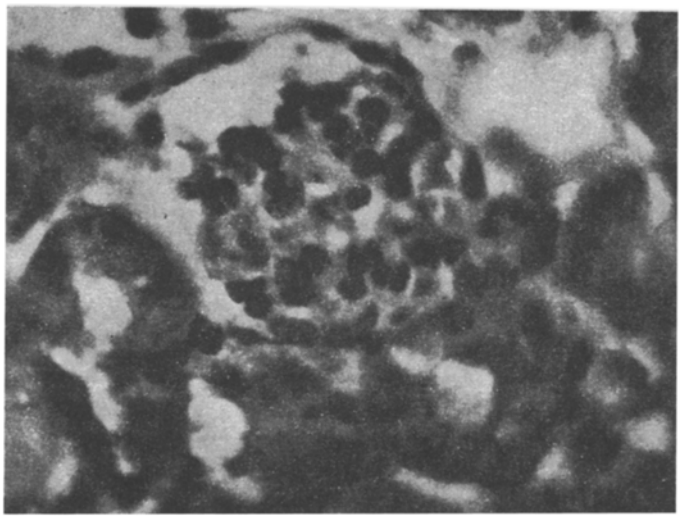

Fig. 2. Autoradiograph of kidney after 1 injection of mercuric chloride: solitary cells of a glomerulus labeled with thymidine-H^3. Type M (NIKFI) photographic emulsion. Exposure 30 days. Hematoxylin-eosin, 630×.

EXPERIMENTAL RESULTS

As the results given in Table 1 show, the labeling index in mice of the control group was low. It was relatively high in the proximal tubules, whereas in the distal tubules, glomeruli, and other structures the number of labeled cells was negligible or they were absent.

After 3 days to 1 week the number of labeled cells in the animals of group 1 was increased (Fig. 1). Solitary cells with tracts above the nuclei also appeared in the glomeruli (Fig. 2), but these were not observed in the control animals.

In the animals of group 2, 3 days after the 2nd injection of mercuric chloride a marked decrease in the number of labeled cells was found in the epithelium of the proximal tubules (half the number in the control, a quarter the number found at the same time after the first injection). However, after 1 week the labeling index of the cells of the proximal tubules was 4 times higher than the control. Three injections of the poison into the animals of this group did not produce such marked fluctuations in the number of cells with tracks above the nuclei. Nevertheless, the labeling index in the epithelium of the proximal tubules and in the flattened cells lying beyond their basement membrane was higher than the control although it was lower than in the animals of group 1.

In the mice of group 3, receiving injections at intervals of 1 week, the decrease in the labeling index of the cells of the proximal tubules became appreciable 1 week after the 3rd injection. Meanwhile the number of labeled flattened cells beyond the basement membrane of the proximal tubules remained above the control in every case.

The results of these observations showed that in mice receiving mercuric chloride more often the number of labeled cells was greater than in the animals receiving the poison at longer intervals. In other words, during the more frequent action of the pathogenic factor on the kidney, more cells synthesizing DNA appeared in it. A similar phenomenon has been observed in the liver after administration of carbon tetrachloride [2, 3].

Depending on the rhythm of action of the mercuric chloride the number of labeled flattened cells lying beyond the basement membrane of the renal tubules varied. These variations were always synchronous with changes in the labeling index in the epithelium of the renal tubules. The type of these cells also changed: they became more oval and larger. These cells and their correlation with the number of labeled cells in the tubules can be considered to be a distinctive indicator of regenerative changes in the tissue of the damaged kidney and they reflect the course of regeneration.

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