PATHOLOGY

Sex Differences in Renal Toxicity of Mercury in the Rat

A PRONOUNCED sex difference was found to exist in the renal tubules of rats injected intravenously with minimally toxic doses of bichloride of mercury. Mercury, in toxic doses, produces an acute tubular necrosis in a wide variety of experimental animals and man^{1,2}. Histologically, when minimally toxic doses are given to rats, the lesions are confined to the straight portions of the proximal tubules.

Eighty-five male and female Sprague-Dawley rats weighing 125–200 g were used. Water and food were given *ad libitum*. Eight control rats, 4 male and 4 female, received an intravenous injection of 0.2 ml. of isotonic saline. Of the remaining 77 rats, 6 were excluded from the results because at the time of the mercury injection, some was administered subcutaneously instead of intravenously. The experimental group consisted of 71 rats, 40 males and 31 females. Each received 0.4 mg mercury (as the bichloride salt in water) per kg body-weight injected intravenously into a tail vein. The concentration of mercury was 0.4 mg/ml. of water and the total injected volume never exceeded 0.2 ml. The rats were anaesthetized with ether, bled, and killed 24 h after the mercury injection. Autopsy was performed immediately. The kidneys were examined macroscopically. Thin midline sections were taken and placed in 10 per cent formalin buffered to pH 7 with phosphate. The specimens were embedded in paraffin, sectioned at 5 μ and stained with hæmatoxylin and eosin.

The results are summarized in Table 1. The male rats developed a rather severe coagulative necrosis of the straight portion of the proximal tubules in the inner zone of the renal cortex. Females, receiving the identical dose of mercury, showed little or no tubular necrosis.

When lower doses of mercury were injected in a similar manner (0.3 mg/kg), 70 per cent of the males developed some degree of necrosis of the proximal tubule, while females given the same dose showed no necrosis.

Table 1. INCIDENCE OF PROXIMAL TUBULAR NECROSIS, STRAIGHT PORTION AFTER INTRAVENOUS INJECTION OF 0.4 MG/KG MERCURY

Amount of necrosis*	Males	Females
0 (controls)	4	4
0	2	18
1+	11	13
2+	12	0
3+	9	0
4+	6	0
Total	44	35

* 0, No necrosis; 1+, occasional cells necrotic; 2+, $\frac{1}{2}$ – $\frac{1}{3}$ cells necrotic; 3+, $\frac{1}{2}$ – $\frac{2}{3}$ cells necrotic; 4+, nearly all cells necrotic.

Eschenbrenner and Miller noted a sex difference in the response of kidneys of mice to oral administration of chloroform³. Male mice developed renal necrosis and kidneys of female mice were spared. This has been well documented by others^{4–6}. Sex differences in the effects of mercury on the renal tubules of rats have not, to our knowledge, been previously described. Morphological differences between the kidneys of male and female rats do not seem to exist as they do between male and female mice³. However, it is well known that significantly more protein is excreted by the kidneys of male than female rats. The differences in the morphology of the mouse kidney and protein excretion in the rat may be either directly or indirectly related to sex hormone effects.

Sex differences exist in the amounts of proximal tubular necrosis produced by minimally toxic doses of mercury in the albino rat of the Sprague-Dawley strain. For a given

dose injected intravenously, the male is much more susceptible.

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Human Breast Carcinoma examined by the Fluorescent Antibody Technique

ANTIBODIES to malignant tumours have been demonstrated by various means including the Schultz Dale technique¹, the intradermal inoculation of patients with extracts of their own carcinomas², complement fixation tests³, and the agar-precipitin diffusion plate technique of Ouchterlony⁴. Heterologous systems have usually been used. The main use of fluorescent antibodies in cancer research has been to compare the antigenic structure of normal and malignant cells, but recently Nairn *et al.*⁵ have detected globulin on the surface of cancer cells following procedures designed to raise the antibody titre. We describe here an attempt to demonstrate autologous antibodies to human breast carcinomas, of varying degrees of malignancy, by Coons's fluorescent antibody technique using a double layer staining method.

The procedures used are those described by Coons *et al.*⁶ and Nairn *et al.*⁷. Sections were cut in a cryostat from blocks of cancer tissue snap-frozen immediately after operation and stored at -20°C . The sections were fixed by immersion in 95 per cent ethanol for 30 min at room temperature, rinsed in phosphate-buffered saline at pH 7.1, and incubated with the patient's serum for 30 min. After washing in buffered saline the sections were stained for 30 min with anti-human globulin labelled with fluorescein isothiocyanate which had been absorbed with liver powder to reduce non-specific staining. Some, but not all, of the labelled anti-human globulin was passed through a 'Sephadex G 25' column to remove unbound fluorescent material. After staining the sections were washed in buffered saline and mounted in glycerol-saline and examined with a Reichert Zetopan microscope with Binolux fluorescent equipment. This apparatus permitted the easy identification of tumour by its phase contrast image and its subsequent examination by fluorescent light. This was very important because non-specific staining of the tumour stroma produced a variety of artefacts easily mistaken for positive staining of tumour cells.

Control sections were treated with unconjugated anti-human globulin serum before staining. Two preparations of conjugated anti-human globulin were used, one of rabbit anti-human globulin conjugated with fluorescein isothiocyanate according to the method described by Chadwick and Fothergill⁸ and a second preparation supplied by Stayne Laboratories of goat anti-human globulin also conjugated with fluorescein isothiocyanate. These conjugated antisera agglutinated red blood cells sensitized to incomplete anti-D serum at dilutions up to 1 : 50 and stained nuclei of sections of rat liver treated with serum containing anti-nuclear factor. No precise titration was attempted.

No specific fluorescence of tumour cells was observed in 24 breast carcinomas. The addition of complement, increasing the incubation times or temperature, or using unfixed sections of tissue did not affect the results. It is concluded that specific antibodies fixed to tumour cells or