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Immediate Effects of Continuous Beta-Irradiation to the Kidneys

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Summary. Renal response to irradiation was examined over the course of 24 h in an animal preparation of continuous renal irradiation. Continuous beta-irradiation to the renal parenchyma was carried out in $C_{57}Bl_6$ mice by application to the surface of both kidneys of a ^{32}P-impregnated anion exchange resin yielding an initial beta surface dose of ~30 cGy/min and ~2.3 cGy/min at a 2 mm depth. This technique proved easily reproducible and highly effective in causing immediate renal damage. In a kinetic study, the blood urea nitrogen (BUN) values of 5 week old mice exposed to continuous renal irradiation rose significantly (2 h: 21 ± 4; 4 h: 33 ± 4; 6 h: 42 ± 5; 16 h: 65 ± 8; and 24 h: 62 ± 5 mg%) while that of sham-operated animals (provided with a nonradioactive resin) remained below 17 mg% throughout the entire observation period. When mice of various ages were exposed to continuous renal irradiation over a 16 h period, the functional changes were shown to be inversely correlated with the age of the animals (BUN at 5 week: 65 ± 8; 8 week: 51 ± 6; and 11 week: 20 ± 2 mg%). Thus, our results indicate that continuous beta-irradiation to the kidneys promptly causes severe age-related renal functional deficits. This novel approach may offer an alternative to the current methods of investigation of radiation-induced renal damage.

Key words: Radioactive phosphorus, Acute renal failure, Radiation-induced renal damage, Radiation nephritis.

Introduction

The radionuclide ^{32}phosphorus (^{32}P) is particularly suited for topical administration because of its ability to deliver high energy beta emissions over a short tissue range. Past therapeutic experience with topical use of the nuclide in man has been generally limited to the application of ^{32}P-impregnated sheets to various superficial skin lesions [8, 11, 14]. Experimentally, the direct application of the nuclide to the external surface of various lymphoid organs has proved highly effective in depleting both circulating and static lymphoid cell populations [3, 6, 7]. Recently, we have explored the possibility of applying ^{32}P to the renal cortex for the purpose of inducing chronic renal failure in small rodents. The potential experimental use of the radionuclide prompted this preliminary study in mice to determine the immediate changes in renal function subsequent to topical ^{32}P exposure.

Materials and Methods

Animals and Husbandry. Four weeks old male $C_{57}Bl_6$ mice were obtained from Charles River Breeding Laboratories (St. Constant, Qué.). The animals were allowed to acclimatize for at least 7 days prior to surgery. All mice were fed water ad libitum and a standard high fat pellet ration.

Preparation of Radioactive Strips. A sodium ^{32}P-phosphate solution pH 7.0, kindly supplied by Merck-Frosst Co. (Pointe Claire, Qué., Canada) was diluted with distilled water to give 2 ml aliquots containing 100 μCi ^{32}P-phosphate.

Binding of ^{32}P to a resin template was obtained according to the technique described by Svobodová et al. (1971). Briefly, 6 x 8 mm strips of polyethylene-backed anion exchange resin (Polygram Ionex-25, Mackerey-Nagel Co.) were suspended individually at room temperature in the radioactive aliquots. Average time allotted for radioactivity uptake by the resin was 16 h.

2 x 4 cm pieces of 0.9 mm thick lead foil were utilized to back the radioactive resin strips in order to restrict irradiation solely to the renal parenchyma. The lead backing was precoated overnight with a silicone polymer (382 Medical Grade Elastomer, Dow Corning Co.) to prevent accidental lead intoxication.

Calculation of ^{32}P Resin Uptake. At the end of the incubation period of the resin strip into the ^{32}P solution, the liquid phase was harvested from each tube by careful decantation and the radioactivity from each resin strip and its corresponding ^{32}P solution was determined. The percentage ^{32}P uptake by the resin was calculated as:

$$\frac{\text{cpm strip}}{\text{cpm (strip + } ^{32}\text{P solution)}} \times 100.$$

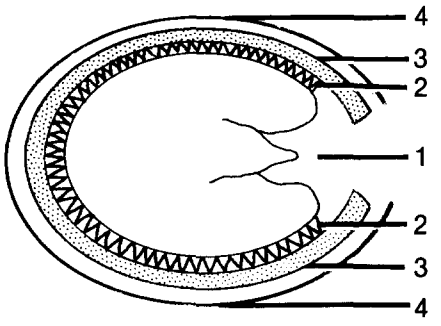

Fig. 1. Schematic diagram of a transverse kidney section illustrating the surgical arrangement for beta-irradiation *1*, renal hilum; *2*, tissue adhesive; *3*, ^{32}P-impregnated resin; and *4*, lead backing

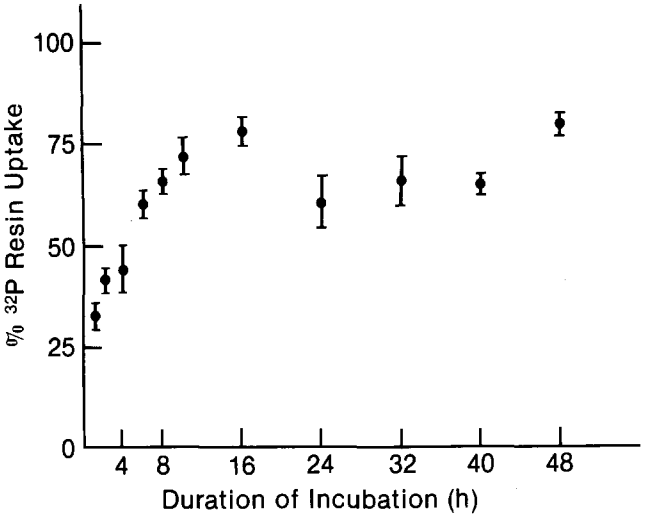

Fig. 2. Kinetic of ^{32}P uptake by resin at pH 7.0. The ordinate represents the percentage of ^{32}P removed from the liquid phase at the indicated times. Mean values of six samples are shown. *Bars* indicate S.D.

Surgical Procedure. Mice were anesthetized with ether. The left kidney was exposed through a paraspinal incision and immobilized. Radioactive strips were fastened to the dorsal, ventral and lateral kidney surfaces with a thin layer of tissue adhesive, 2-cyano-acrylate (Histoacryl Blue, B. Braun, Melsungen, A.G.) A total of three strips was used for each kidney. Siliconized lead backing was shaped to enclose the preparation completely. The kidney was then replaced into the renal fossa. The subepidermal tissue layers were approximated with silk sutures and the skin was secured with clips. The entire procedure was then repeated on the right kidney for a total operating time of approximately 20 min. The final surgical arrangement is illustrated in Fig. 1. Sham-operated animals receiving non-radioactive resin strips were otherwise subjected to the same surgical procedure.

At the time of sacrifice blood was obtained for analysis of urea nitrogen concentrations, by IL-9 Autoanalyzer (Instrumentation Laboratory Inc, Lexington, Mass.).

Statistical Analysis. Where applicable, data are expressed as mean ± S.D. Student's *t* test for unpaired observations was used to evaluate results for their statistical significance. A p value less than 0.05 was considered significant.

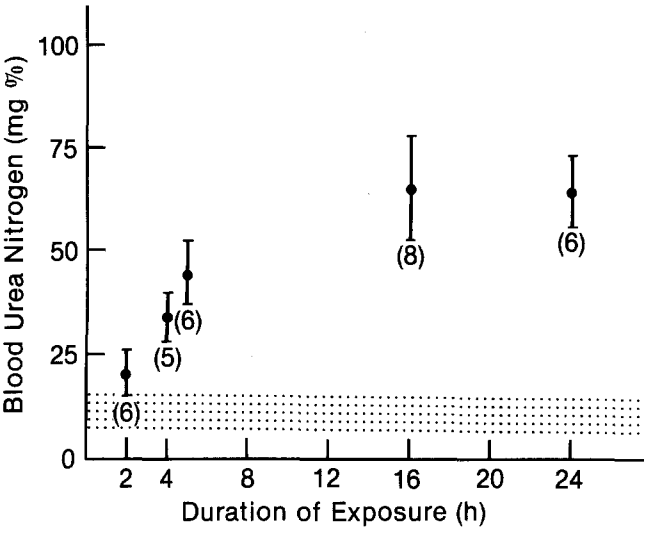

Fig. 3. Time course of continuous beta irradiation-induced renal damage. BUN values of 5 weeks old $C_{57}Bl_6$ mice at varying times after the application of ^{32}P-impregnated resin strips to both kidneys. Details of the experiments are given in the Results. Mean values are shown. *Bars* indicate S.D. *Numbers* in parentheses represent the numbers of animals studied at each time interval. The *shaded area* at bottom indicate the BUN values from sham-operated animals

Results

1. ^{32}P uptake by Resin. A constant amount of resin (6 x 8 mm strip) was incubated at room temperature with a constant amount of ^{32}P (100 μCi/2 ml) for various times. Figure 2 illustrates the ^{32}P intake by the resin, of which nearly 80% of ^{32}P present was taken up in less than 24 h. This typical kinetic curve shows a steep uptake rate during the initial 10 h. The uptake reached its endpoint at approximately 16 h. We attached no significance to the slight fluctuation in uptake observed afterwards. Rather than ^{32}P leakage from the resin, a sampling variation can be postulated. For reasons of convenience, however, we used a 16 h incubation period throughout.

2. Time Course of Continuous Beta Irradiation-Induced Renal Damage. This experiment was conducted in 5 weeks old male mice. Continuous beta-irradiation to both kidneys was achieved by the topical application of ^{32}P-impregnated resin strips. Groups of animals were sacrificed at varying times following the surgical application of the radioactive strips. Results of these experiments are shown in Fig. 3. From the initial studies it is clear that continuous beta-irradiation to the kidneys has a pronounced effect on renal function. As early as 4 h after onset of the exposure, the BUN values of irradiated mice were significantly higher than that of sham-operated littermates.

3. Age Sensitivity to Continuous Beta Irradiation-Induced Renal Damage. Irradiation experiments based on the same principle as that described in the paragraph above were

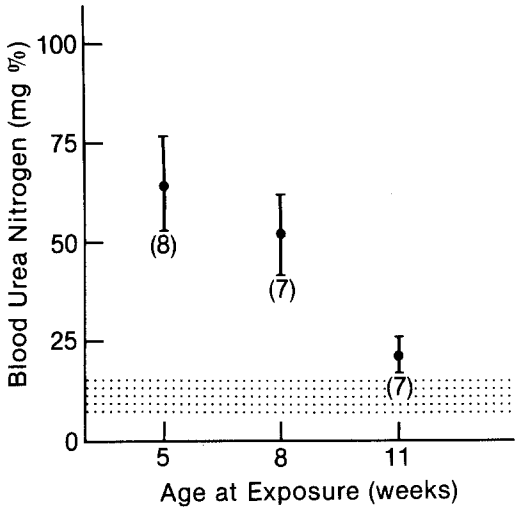

Fig. 4. Age sensitivity to continuous beta irradiation-induced renal damage. BUN values of $C_{57}Bl_6$ mice of varying ages 16 h after the application of ^{32}P-impregnated resin strips to both kidneys. Details of the experiment are given in the Results. Mean values are shown. *Bars* indicate S.D. *Numbers* in parentheses represent the numbers of animals of each age studied. The *shaded area* at the base of the figure indicate the BUN values from sham-operated animals

carried out. In this case however, male mice of varying ages were submitted to continuous beta-irradiation to both kidneys with ^{32}P-impregnated resin strips for 16 h. The animals were sacrificed and the degree of radiation-induced renal damage was assessed functionally. Results of the experiment are shown in Fig. 4. It can be seen that an inverse correlation exists between the age at exposure and the degree of renal damage observed.

Discussion

In this study, continuous beta-irradiation with ^{32}P, a method originally developed for the treatment of skin tumors, was used to induce renal damage in mice. Standardization of the method of preparation of the ^{32}P-impregnated resin can be established readily and the application of the resin to the kidney surface is quick, relatively easy to perform and well tolerated by the animals. Restriction of the irradiation to the renal parenchyma is achieved successfully with lead backing. Moreover, once in place the resin strips can be removed easily.

Phosphorus-32, which is a beta emitter with a maximum energy of 1.7 MeV and a half-life of 14.2 days, was used as the radiation source. The calculation of the beta dose rate was based on tables by Rossi and Ellis [13] with values for resin thickness, t, and average absorption coefficient in tissue, μ, of 0.15 mm and 4 cm^{-1} respectively. A concentration of ~11 mCi/cm^3 of the radioactive substance in the resin yielded an initial beta surface dose rate of 30 ~cGy/min. At depths of 1 and 2 mm of tissue the initial dose rate was 4.6 cGy/min and ~2.3 cGy/min respectively. Since the longest kidney irradiation time (24 h) was short compared to ^{32}P half-life, an assumption was made that the dose rate was constant during irradiation and the total kidney dose was obtained by multiplying the initial surface dose rate with the irradiation time.

Irradiation to the renal parenchyma is thought to initiate a sequence of events, including mitosis-linked cell death and mitotic delay that culminates in the biological expressions of renal failure and morphological changes. In the animal preparation used here functional changes were detected as early as 2 h reaching a plateau by the 16 h of exposure. Longer follow-up studies conducted in our laboratory indicate that 5 weeks old male $C_{57}Bl_6$ mice will not survive the irradiation dose applied here for longer than 10–15 days.

The kidney is another example of an organ that appears to be resistant to radiation, at least as judged by morphological studies. Dosages in excess of several thousands of roentgens are required for renal changes to occur. Similarly, kidney damage is generally not apparent following ^{32}P administration. Ten days following a single injection of ^{32}P (250 μCi) in mice, only occasional shrinking of epithelial cells lining the renal tubules was observed; the degenerative effects on the tubules were maximal between 13–15 days with early evidence of regeneration [16]. No changes were seen up to 50 days later. Grad and Stevens (1950) found no changes in the kidneys of rats and tumor-bearing mice 2 h to 9 days following a single large dose of ^{32}P (0.51–4.0 mCi). 32 of 48 patients with various blood dyscrasias given repeated i.v. injections of ^{32}P (cumulative dose 4.5–69.8 mCi) showed evidence of vascular and tubular changes [10]. The most characteristic histological changes were thickening of Bowman's capsule with only rare glomerular basement membrane involvement. Hyperemia, swelling, vacuolation and degeneration of the epithelium of the tubules, especially in the convoluted tubules, were also present.

What accounts for the apparent differences between the results of the present study and those of earlier works? Several possible mechanisms can be suggested to account for our results. The simplest of these is that the surgical procedure is in itself capable of causing renal damage. This possibility, however, seems unlikely for all sham-operated animals survive the procedure indefinitely. However, our results do not exclude the possibility that surgical trauma combined with beta-irradiation may adversely affect renal function.

A second possible mechanism to account for the reduction in renal function observed shortly after the beginning of continuous beta-irradiation is the young age of the animals at the time of exposure. Further inspection of the data in Fig. 4 shows that elevated BUN levels from ^{32}P-irradiated mice of the $C_{57}Bl_6$ strain gradually decrease with increasing the age of the animals. A well known exception to the lack of abnormalities in the kidney with moderate amounts of radiation is the enhanced susceptibility of developing kidneys to radiation injury. Likewise,

renal tissue undergoing compensatory hypertrophy is highly radio-sensitive [12]. Whole-body exposure to sublethal doses of ionizing radiation during the neonatal period results in the formation of abnormal renal glomeruli [1, 2, 9]. The primary result of the radiation injury is a failure to form the normal number of nephrons. In addition, extensive damage to forming nephrons produced dysplastic glomeruli and other glomeruli which are arrested in their development. This extensive injury is compounded by the development of intercapillary glomerulosclerosis later on [1, 5].

Alternatively, the mode of irradiation itself may be an important contributing factor to the immediate renal damage observed in the animal preparation used here. Much of our knowledge concerning irradiation-induced renal damage derives from studies using single or fractionated X-ray exposure. It is possible to speculate that continuous irradiation to the kidneys induces renal modifications notably different in their nature and severity from that observed following single or fractionated X-ray exposure. The technique described here offers a relatively easy, accurate and reproducible method for analysis of continuous irradiation to the renal parenchyma. In that context, further studies are needed to demonstrate the applicability of this technique to the study of low level continuous renal irradiation.

In summary, this study demonstrates that continuous beta-irradiation to the kidneys in the mouse induces early severe functional deficits. Studies in progress are verifying whether this method of continuous bilateral kidney irradiation is effective in producing chronic renal failure in small laboratory animals.

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