RELATION BETWEEN THE SITE OF RADIATION DAMAGE AND THE FUNCTIONAL CONDITION OF THE ORGAN

COMMUNICATION I.

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In connection with pathological, anatomical, and clinical observations, the question as to the relationship between the degree of damage to an organ and the level of its activity has often been discussed [1]. However, until now no experimental investigations have directly confirmed that such a relationship exists. Nevertheless, the problem may well be of considerable theoretical and practical importance, and it is for this reason that the present work has been undertaken.

The disease chosen for study was radiation sickness in which morphological changes occur throughout the body, but their extent varies greatly from one organ to another. Consequently, in this case the attempt could be made to control the extent of the damage by varying the level of activity of the organ by pharmacological means.

The most convenient organs to study were the kidneys, which usually suffer comparatively little damage in acute radiation sickness [2,4]. Damage to the renal parenchyma under appropriate experimental conditions might constitute a convincing demonstration of the truth of the idea under test.

Except for observations made on tissue cultures of the chicken mesonephros [3], we have found no reports of investigations of this kind. In the experiments referred to, secretion of the tubules was induced by adding phenol red or magnesium sulfate to the drinking water. It was found that epithelium which was secreting rapidly was more than usually sensitive to the action of x-rays. These investigations are extremely important, but they do not decide the problem of the localization of the radiation damage in whole organisms under the control of many regulatory systems.

METHOD

We chose diuretics which would exert a selective action on different parts of the nephron. These requirements were most nearly met by theophylline and mersalyl. Theophylline causes a dilatation of the glomerular capillaries, and consequently increases filtration, while mersalyl exerts its principal action on the epithelium of the tubules.

Numerous experiments have been carried out to determine the maximum dose of these drugs which can be tolerated when given intraperitoneally, and the time of their maximum effect. The irradiation was timed to occur at this period.

The experiments were carried out on 400 mice of both sexes weighing 20-25 g, and on 40 guinea pigs weighing 400-450 g, using a double control.

In each experiment, one part of the animals of the same sex and weight was submitted to irradiation only, and the other received only the diuretic. For postmortem examination, the animals were killed by destroying the medulla oblongata.

Irradiation of the whole animals was carried out using an RUM-3 apparatus, used under the following conditions: voltage 180 kv, current 10 ma, filter 0.5 mm Cu and 1 mm Al, intensity 45-48 r/ minute, focusing distance 30 cm for mice and 40 cm for guinea pigs, dose 1400 r (measured by a Gris dosimeter). For irradiation, each mouse was placed in separate cardboard box measuring 7 x 4.5 x 4.5 cm, and the experimental and control animals were irradiated simultaneously. Each guinea pigs was irradiated separately in a plywood box measuring 15 x 10 x 9 cm.

RESULTS

All the irradiated animals developed acute radiation sickness. In evaluating the results, attention was paid to the time of survival after irradiation, and to the condition of the renal parenchyma.

Animals irradiated during the period of maximum activity of the diuretic survived for 2-4 days, while controls lived 3-6 days. Consequently, in most cases, increased renal function reduced survival time by about one third.

The symptoms of severe damage were constant in both groups. Usually the fur became disshevelled by the 2-4th day, there was a photophobia, diarrhea, and the animal died 12-24 hours after the onset of clinical symptoms.

Physical signs were few and constant. The animals were emaciated, the stomach was empty, the intestine was distended by fluid and by the gaseous contents, there were sometimes hemorrhages into the intestinal mucosa, liver, lungs, testes and skin. Histological study revealed the typical picture of acute radiation sickness, the only difference between the experimental and the control group being in the condition of the renal parenchyma.

In the first experiment, in which theophylline was given 30-45 minutes before irradiating, there was considerably increased renal damage in 91% of the animals. Because of some variability in the times at which the animals died, it was possible to follow the changes occurring in the kidneys. At first there was a marked and frequently uneven engorgement of the glomeruli with blood. They increased in size, and the vascular plexus lost its usual clear outline.

Somewhat later there was a change in the structure of the vessels walls, they became swollen, lost their structure, and became basophil. The endothelial cells began to necrose, and most of the nuclei showed a clear central zone and an accumulation of large grains of chromatin beneath the membrane. The number of nuclei in a glomerulus became reduced from the normal 50-60 per section 10-12 μ thick, to 12-15.

The lumen of the blood vessels was lost through the great increase in the thickness of the walls, and many glomeruli lost their structure (Fig. 1). Sometimes the cavity of Bowmans capsule was enlarged and filled with an amorphous oxyphil mass. In animals which survived longer, necrosis of some of the loops of the glomeruli occurred, together with thrumbosis of some of the vessels. The glomeruli were considerably enlarged, and the outline of the vascular plexus was lost in an amorphous mass of strongly basophil thrombi .

There were numerous hemorrhages around the glomeruli. Necrosis of the arterial walls followed by formation of thrombi was found in the stroma of the renal cortex. Other glomeruli, usually fewer in number, preserved their normal structure. In the epithelium of the main portions of the tubules, varying degrees of dystrophy could be seen. Necrotic changes were common in the tubules when there was extensive thrombosis of the vessels.

In the second experiment, in which mersalyl was injected 45-90 minutes before irradiating, renal damage occurred in 86 % of the animals. The renal parenchyma exhibited a characteristic change. The glomeruli retained their normal structure, and only in places could some swelling and loss of structure, be observed together with basophilia of the vessel walls and necrosis of individual endothelial cells. In some cases there was vascular engorgement of separate loops of the glomeruli. In the stroma, zones were found where the arterioles had necrosed, and the walls had lost their structure.

The main portions of the tubules were greatly thickened through a swelling of the epithelium, which almost filled the lumen. Many of the cells had indistinct outlines and a granular cytoplasm, and there were

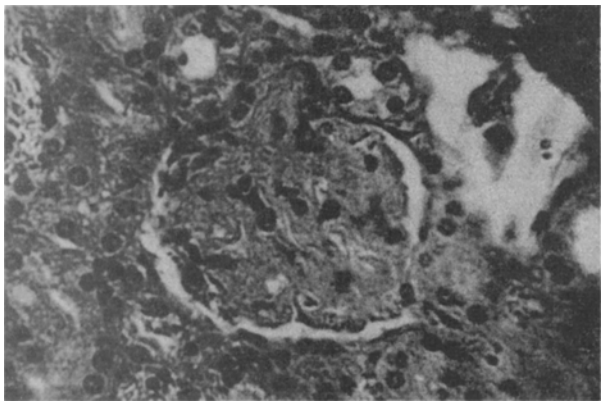

Fig. 1. Renal changes following the injection of theophylline and general radiation. Swelling and loss of structure of glomerular vessel walls, disappearance of nuclei. (Van Gieson's stain. Magnification, eyepiece 10 x, objective 60 x).

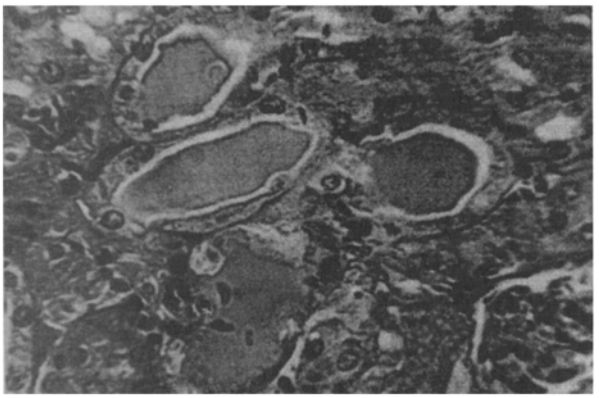

Fig. 2. Renal changes following injection of mersalyl and radiation. Cylinders staining with picric acid present in lumen of canals. (Same stain. Magnification eyepiece 12 x, objective 45 x).

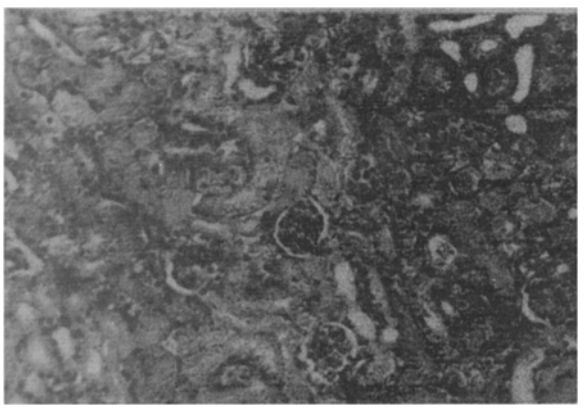

Fig. 3. Changes in the glomeruli and tubules following injections of mersalyl and exposing the whole animal to radiation. Glomeruli intact, epithelium of the main portions of the canal is necrotic. Same stain, eyepiece 10 x objective 20 x .

no nuclei, these having broken up into an amorphous mass, or into separate clumps. In many cases the visceral layer of the capsule was considerably swollen, and many of the cells had died.

By use of various methods and stains (polarization microscopy, use of osmium, Sudan, neutral red, Nile blue) it has been shown that in the dying epithelial cells and in the mass of dead cell remnants which fills the lumen of the tubules, there is a small amount of fat, most of it neutral. The main portion of the detritus consists of both small and large droplets, staining dark with osmium and yellow-brown with Sudan. With Van Gieson's stain, the cytoplasm of the epithelium reveals small uniform masses which stain deeply with picric acid. In the necrotic areas, these inclusions are larger and present in greater numbers, and in parts they fill the lumen of the canals, and finally form numerous cylinders (Fig. 2).

In the control groups of both experiments, which received only the radiation, the renal damage was restricted to necrosis of isolated cells of the endothelium of the glomeruli; there was a very slight swelling of the vessel walls and very slight degenerative changes of the epithelium of the tubules. In another group of control animals, which were given diuretics only, and which were used for determining the dose, no morphological changes occurred in the renal parenchyma. Thus, the trend of the results indicates clearly that the shortness of the survival time of the experimental animals results from radiation damage to the renal parenchyma, leading to functional disturbances.

The most important result was that which showed the influence of diuretics on the extent of the renal damage, and its dependence on the time of their injection. Thus, when theophylline was used, the maximum change in the renal parenchyma was observed when it was injected 30-45 minutes before irradiation. If the period of irradiation was extended up to 60-90 minutes, renal damage was considerably reduced. Finally, if the irradiation was given 15 minutes after the theophylline injection, the kidneys were not affected.

Mersalyl was effective in increasing the severity of the radiation sickness only when the irradiation was given less than 45 minutes after the injection, and the maximum effect was observed after 60-90 minutes, but was sometimes maintained for longer periods. The period of maximum effectiveness with respect to increasing the radiation damage of the renal parenchyma coincided exactly with the period of maximum diuretic action, as

determined in preliminary experiments. The results afford a clear illustration of the critical importance of the functional condition of the nephron during irradiation.

It now becomes necessary to explain the reason for the failure in 11.5% of the experiments, when no renal damage followed irradiation, and in 8% of the experiments when the injection of diuretics produced no effect, or in the 3.5 % of the animals where the changes in the nephron were better shown in the control animals. These results are probably due to giving the irradiation when the kidneys were not functioning at their maximum efficiency, or, in other cases, due to irradiating the control animals at a time when the kidneys happened to be particularly active.

Microscopical investigation demonstrated well-marked radiational renal damage in the animals which received injections of diuretic substances. The site of maximal change in the neurone depended to a remarkable extent on the particular diuretic used. Thus, theophylline causes, principally, damage to the vascular portion of the nephron. With mersalyl, however, these is a striking difference between the main portions of the tubules, which necrose, and the glomeruli, which retain their structure (Fig. 3).

Detailed studies of radiational damage to the epithelial portion of the nephron have demonstrated characteristic features. It appears that the damage to the tissues leads to the formation of harmful lipoprotein complexes giving an unusual reaction with Sudan (pale yellow-grey color). Possibly, this particular kind of necrosis is confined to radiational damage.

In the experiments described, it may be assumed that the site of the damage was determined by the functional condition of the kidney at the time of irradiation.

SUMMARY

A study was made of the relationship between local radiational damage and the functional condition of the kidney. Kidneys were chosen for investigation, because they usually do not suffer much in radiation sickness. Function was stimulated pharmacologically.

The experiments were performed on 400 mice and 40 guinea pigs. Total irradiation was timed to coincide with the period of maximal effect of the diuretics. An RUM-3 apparatus was used, at 180 kv, 10 ma, filter 0.5 mm Cu and 1 mm Al, focal length 30 cm for mice and 40 cm for guinea pigs, dose 1400 r.

When the kidneys are stimulated to function at an abnormally high rate, the result is to confine the radiation damage to the kidney parenchyma. When theophylline is given, the vascular portion of the nephron is affected, and with mersalyl it is the epithelial region which is damaged; the effect obtained depends on the diuretic mechanism.

LITERATURE CITED

[1] I. V. Davydovskii, Pathological Anatomy and the Pathogenesis of Human Diseases*. 2 (Moscow, 1958).

[2] N. A. Kraevskii, Outlines of the Pathological Anatomy of Radiation Sickness* (Moscow, 1957).

[3] R. Chambers and G. Cameron, Radiology. 37, 186-193 (1941).

[4] Sh. Warren, Arch. Path. 34, 1070-1084 (1942).

* In Russian