Histological Changes Produced by a Single Large Injection of Radioactive Phosphorus (P$^{32}$) in Albino Rats and in C3H Mice*

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Numerous reports have appeared, describing the therapeutic use of radioactive phosphorus (P$^{32}$) in polycythemia vera (5–7, 10, 11, 15, 19, 22, 23), leukemia (4, 5, 7, 9, 13–16, 19, 22–25), and other conditions (10, 12, 22). However, there have been few systematic investigations of the effects of radiophosphorus on the histological appearance of organs and tissues (1, 8, 21). An attempt was therefore made to discover the immediate effects of P$^{32}$ on cytological structures and the location of the effects throughout the body by means of histological studies.

METHODS

Twenty-two normal albino rats and eight C3H mice bearing mammary tumors were injected subcutaneously with a single large dose of P$^{32}$ administered as phosphoric acid without carrier. The dose administered to each animal, the time after injection when the animal was sacrificed, and other details are described in Table 1. The animals were killed with chloroform, and tissues were fixed in Heidenhain’s Susa and stained with hematoxylin–eosin for histological examination. Attempts were also made to localize the administered P$^{32}$ in the tissues by means of the Geiger counter and the autographic technic, but these results will be published separately.

RESULTS

In general, the effects of P$^{32}$ were most evident in causing damage and destruction in the nuclei of cells. The nuclear changes consisted chiefly of pyknosis and karyorrhexis of mitotic nuclei. Metabolic nuclei appeared to enlarge, to become pale and vesicular, and to contain prominent eosinophilic nucleoli. In the cytoplasm, changes in the ability to take up stain and vacuolation were seen in damaged and recovering cells, and some loss of polarity of cell constituents; e.g., in the position of the Golgi apparatus in the intestinal epithelium.

Little change was observed in those structures which might be grouped as structural elements. Thus, no effect was noted on the fibrous elements of connective tissue, nor on the cellular elements (fibroblasts, mast cells, macrophages, etc.). The myofibrils of muscle also showed no change. The effects on bone were not examined in this investigation, but Bloom noted some dead cartilage cells in the epiphyseal plate of a P$^{32}$-injected mouse (2). Little change was observed in cartilaginous tissue of our animals, either in the chondrocytes or in the perichondrium. The epidermis showed no evidence of damage, but pyknosis and nuclear dust were visible in the cells of the hair root follicle as early as 1 day after injection and as late as 9 days.

The cerebrum and the cerebellum showed no changes, and nervous tissue in general appeared very resistant to P$^{32}$.

A slight inflammatory reaction in the lungs was seen 12 and 24 hours after injection, as indicated by a sticking of neutrophils and other white blood cells to the endothelium of small venules in the lung tissue, but with little infiltration of the lung parenchyma. No changes in the bronchioles or trachea were noted in our animals.

No changes were seen in the kidneys of these animals, in any parts of the nephron, or blood vessels, or in the bladder.

Among the tissues which synthesize secretions, the exocrine glands were very resistant. Thus, no radiation damage was visible in the salivary glands of the animals examined, in the lacrimal, or in the accessory male sex organs.

The only change that may be said to have occurred in the pancreas due to the administration of P$^{32}$ is the appearance of pyknotic acinar cells which were widely scattered throughout the tissue. However, in the rat this reaction is not specific for

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P32, but occurs in response to other types of trauma as well.

No alterations were noted in the histological structure of the liver except in one mouse 10 days after injection of the radioactive phosphorus. Necrosis of the cells around the central veins was visible, and the sinusoids were abnormally wide. The damaged cells had indistinct outlines—a bright eosinophilic homogeneous cytoplasm and pyknotic and karyorrhectic nuclei.

### TABLE 1

<table>
<thead>
<tr>
<th>Molluscums of P32</th>
<th>No. of animals</th>
<th>Sex</th>
<th>Time interval between injection and sacrifice</th>
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<td>1.0</td>
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<td>10 minutes</td>
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<td>1</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>1</td>
<td>M</td>
<td>1 hour</td>
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<tr>
<td>1.4</td>
<td>1</td>
<td>M</td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td>1</td>
<td>F</td>
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</tr>
<tr>
<td>4.0</td>
<td>1</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>1</td>
<td>M</td>
<td>1 day</td>
</tr>
<tr>
<td>1.25</td>
<td>1</td>
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<td>1.6</td>
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<td>F</td>
<td></td>
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<td>F</td>
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</tr>
<tr>
<td>4.0</td>
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<td>M</td>
<td></td>
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<td>F</td>
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**CSH Mice**

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<tr>
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</tr>
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<td></td>
</tr>
<tr>
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<td>1</td>
<td>48 days</td>
</tr>
<tr>
<td>1.3</td>
<td>1</td>
<td>7 days</td>
</tr>
<tr>
<td>1.8</td>
<td>1</td>
<td>9 days</td>
</tr>
</tbody>
</table>

* All rats weighed 50-60 gm. except those injected with 4.0 mc.—which weighed 140 gm.
† 60-gm. rat which received two doses of P32—one of 2.0 mc. at 0 hours, and another of 2.0 mc. at 11 hours—and was sacrificed at 19 hours.
† All mice were adult and weighed 50-60 gm.

In the endocrine glands, no changes were observed in the pituitary, thyroid, or adrenal glands. However, in the gonads, although no changes occurred in cells in the metabolic state, functioning as hormone producers, some radiation effects were observed in the cells undergoing mitotic and meiotic divisions. Thus, in the testis, the Leydig and Sertoli cells remained unaffected. Only the germinal cells were damaged and these not until 8 days after P32 injection. In one rat, the changes involved a few spermatogonia and some primary spermatocytes. In some instances, these spermatocytes showed very abnormal meiotic figures and also nuclear irregularities in the resting forms such as binucleatism, with two separate nuclei, or two nuclei joined by strands, giant cells, and other freakish structures. Some spermatocytes also were dead and pyknotic. In other cases, there was some chromatolysis of nuclei of spermatocytes. There were only minimal changes in the rest of the male genital system.

The first unequivocal changes in the histology of the ovary appeared 24 hours after P32 administration. In the graafian follicles, numerous cells throughout the granulosa were pyknotic and degenerating; large droplets of nuclear and cytoplasmic debris floated free in the liquor folliculi. In follicles where the destruction of the granulosa was almost complete, the whole follicle had a slightly collapsed and irregular outline, the basement membrane becoming indistinct in places. Some of the smaller follicles showed similar changes in the granulosa; others just beginning to undergo involution and atresia showed none. Primary follicles, newly formed corpora lutea, and interstitial cells remained unaffected at 24 hours. However, at 48 hours some very small follicles showed some pyknotic changes. No changes were found in the germinal epithelium or in the uteri.

The injection of P32 had no damaging effect on the normal mammary tissue of CSH mice bearing mammary tumors; however, the effect of this radioactive isotope of phosphorus on the spon-
FIG. 2.—Section of the thymus of a rat which received 2.0 mc. of P³², 2 hours before sacrifice. Low magnification. At this magnification the thymus is normal in appearance. The dark staining cortex is packed with lymphocytes, the lobules are large. Microscopically, scattered pyknotic lymphocytes are found in the cortex.

FIG. 3.—Section of the thymus of a rat which received 2.0 mc. of P³², 12 hours before sacrifice. Low magnification. Signs of P³² damage are seen in this thymus. The size of the individual lobules, the thickness of the cortex, and its staining intensity are much reduced because of a marked reduction in the number of lymphocytes present. Microscopically, almost all the lymphocytes are seen to be pyknotic.

FIG. 4.—Section of the thymus of a rat which received 2.0 mc. of P³², 48 hours before sacrifice. Low magnification. Radiation damage has resulted in a loss of all distinction between cortex and medulla. The dark-stained areas represent groups of completely pyknotic lymphocytes, and the remainder, the reticular supporting tissue of the thymus and some resorbed fat tissue.

FIG. 5.—Section of the thymus of a larger rat which received 4.0 mc. of P³², 3 days before sacrifice. Low magnification. Practically no lymphocytes remain in the thymus; only a small amount of reticular and reduced fat tissue is found.
The changes seen in the lymph nodes were first seen 24 hours after P³² administration, at which time there was a great loss of lymphocytes from the entire gland, and particularly from the nodules, while many of the remaining lymphocytes showed pyknosis or karyorrhexis. From the second to the ninth day, the nodular structure was entirely absent, but masses of macrophages and monocytes were seen between the large, pale reticular cells. In the thymus (Figs. 2–5) the destruction of lymphocytes was even more evident, and the effects were observed earlier (at the 12-hour interval). Some loss of lymphocytes in the malpighian corpuscles of the spleen was also noted 12 hours after the injection of P³². The changes seen in the subsequent intervals are essentially the same as those seen in the other lymphatic organs.

In the gastro-intestinal tract, the effect of P³² on the esophagus was negligible. In the stomach, mitoses normally occurring at the bases of the gastric pits among the mucous neck cells were replaced by pyknotic cells 24 hours after the injection of P³². However, the other cell types of the epithelium were not affected. Small areas of hemorrhage were observed in the juxta-pyloric region.

The most striking changes seen in the gastro-intestinal tract were noted in the epithelium of the small intestine (Figs. 6–13). Two hours after P³² injection, the only change visible was found in the crypts, where an occasional cell with a pyknotic nucleus could be seen among the numerous mitoses which occur normally. By 12–24 hours, the crypts were full of pyknotic nuclei, and the few mitoses which were apparent showed abnormalities (Fig. 8). The total number of cells in the crypts was reduced. The Paneth cells remained normal in appearance at this time interval, but the goblet cells were much enlarged, pale, and vesicular, with several large eosinophilic nucleoli. These abnormal cells occurred throughout the crypts, and a few were also found forming the epithelium at the base of the villi. Above these, the villus cells were normal, except that the goblet cells were much distended with mucus.

At 48 hours there were already some signs of recovery, since a few normal mitoses started to appear in the middle portion of the crypts (Fig. 9). Pyknoses, however, were still very numerous, especially in the lower part of the crypts, but the number of such damaged cells was reduced. Enlarged cells with unusually pale nuclei and large nucleoli could be seen in the crypts and as high as halfway up the villi. The Golgi apparatus of these cells did not appear in the normal position which is immediately apical to the nucleus.

At 72 hours, the thickness of the mucosa was definitely greater. However, the picture seen at this time was far from normal. The total number of cells in the crypts was much decreased; there was often a wide lumen, and the epithelium was thin and wavy in outline. The cells varied in height from the normal columnar to low cuboidal—the total size, however, being much increased. The nuclei were often large and pale, and many showed three or four very large nucleoli. Some giant cell forms and binucleate cells were present, and the goblet cells were very numerous. The epithelium of the villi was also very much thinned and the total number of cells reduced (Figs. 6 and 7). In the ileum the enlarged goblet cells caused a marked bulging, since the epithelium between them was low cuboidal in form, with very large, pale, round nuclei (Fig. 18). Goblet cells were not present in the duodenum, but marked degenerative changes were seen in the other cells of the villi. The cells were irregularly shaped and the nuclei often shrunken, especially toward the tips of the villi. These villus cells had a finely vacuolated cytoplasm, which was also most obvious toward the tips of the villi (Fig. 11). However, in spite of the very abnormal picture 3 days after the administration of P³², the picture of the whole mucosa after 8 days had returned completely to normal.

Other changes occasionally noted in the small intestine were edema and engorgement of the ves-
Fig. 6.—Section of the duodenum of a rat which received 1.0 mc. of P³², 5 minutes before sacrifice. Low magnification. In this normal duodenum, note the height of the villi and crypts, also the thickness and regularity of the epithelial cells.

Fig. 7.—Section of the duodenum of a rat which received 4.0 mc. of P³², 3 days before sacrifice. Low magnification. The damaging effects of the P³² radiation are shown by the great decrease in the over-all thickness of the mucosa. The epithelial cells are very much reduced in height, and their nuclei are widely and irregularly spaced.

Fig. 8.—Section of the duodenum of a rat which received 4.0 mc. of P³², 24 hours before sacrifice. High magnification. Several cross sections of the bases of the crypts of Lieberkuhn show a complete absence of mitotic figures, which are replaced by a large number of pyknotic cells, indicated by arrows. The Brunner’s glands below the crypts appear completely normal; they do not normally show mitoses.

Fig. 9.—Section of the duodenum of a rat which received 1.25 mc. of P³², 48 hours before sacrifice. High magnification. Recovery from P³² damage in the crypts is shown by the presence of several normal mitoses, indicated by the arrows. However, some pyknotic cells are also seen, and there is a thinning of the whole epithelium with a relative increase in the number of goblet cells.
Fig. 10.—Section of a villus tip from the duodenum of a rat which received 2.0 m.c. of P32, 5 minutes before sacrifice. High magnification. The epithelium of the villus has retained its normal appearance. The cells are tall columnar in form, closely packed and the nuclei evenly placed.

Fig. 11.—Section of a villus tip from the duodenum of a rat which received 4.0 m.c. of P32, 8 days before sacrifice. High magnification. The damaging effect of the P32 is shown by the extreme thinning and irregularity of the epithelium. The cells show very variable shapes, dark shrunk, irregular nuclei and a fine vacuolization throughout their cytoplasm (shown clearly at the tip of the central villus). The striated border is present and unchanged, but follows the very irregular outline of the epithelium.

Fig. 12.—Section of the villus tip from the ileum of a rat which received 2.0 m.c. of P32, 2 hours before sacrifice. High magnification. The epithelium of the villus has retained its normal appearance. The cells are tall columnar in form, closely packed and the nuclei fairly evenly placed.

Fig. 13.—Section of the villus tip from the ileum of a rat which received 4.0 m.c. of P32, 3 days before sacrifice. High magnification. The damaging effect of the P32 is shown by the changes in the epithelium. The goblet cells appear tremendously swollen with mucus secretion. The chief cells are now very widely spaced and have a low cuboidal form, with rounded nuclei and a fine vacuolization of their cytoplasm.
sels of the submucosa and a reduction in the number of lymphocytes present in the villus. Macroscopically, some small hemorrhagic areas were observed. The Brunner’s glands of the duodenum were not damaged.

In the colon there was pyknosis of a few cells in the crypts, and on recovery the number of goblet cells appeared to be increased.

DISCUSSION

The histological changes brought about by the administration of large doses of P32 to animals were very similar to those produced by intense treatment with x-rays (26). It has been shown previously that the effects of large doses of x-rays were either direct or indirect (17). The experiments with P32 reported here suggests that the changes observed in gastro-intestinal tract, mammary tumors, ovary, hair follicles, and bone marrow were due to a direct impact of the rays. This assumption is supported by unpublished results which show that these structures are among those with the highest concentration of P32 in the body. It is also known that these tissues have a high mitotic rate, which may render them particularly sensitive to ionizing radiations.

On the other hand, the lesions of the thymus and lymphatic system may be due to a nonspecific reaction such as is observed in many types of stress resulting in a release of adrenal hormone, which then acted on the thymic-lymphatic system. Thus, Leblond and Segal (17) have demonstrated that thymic involution did not occur in intensive x-radiated adrenalectomized rats, if the thymus was shielded from direct destruction by the rays. However, such involution took place in intact animals, even if the thymus was protected. Examination of reports (20) of thymic involution due to massive x-radiation of hypophysectomized rats with hypofunctioning adrenals revealed that no precautions were taken in these experiments to shield the thymus against direct destruction by the rays. However, it is not unlikely that an additional direct effect of the rays of P32 may have contributed toward the destruction of the thymus, an organ with a high P32 content. The occasional hemorrhagic areas noted in the stomach might also be due to an indirect effect.

The changes observed in the villi of the small intestine can be explained as follows: a rapid cell formation takes place in the crypts, from which new cells move up into the villus epithelium. These are later extruded into the intestinal lumen from the villus tip (18). P32, by destroying dividing cells in the crypts, reduces the supply of cells to the villus, presumably without decreasing the cell loss from the tip. As a result, a decrease in the total cell number would occur with a subsequent distortion of villus architecture (compare Figures 6, 10, and 12, with Figures 7, 11, and 13).

The dose of P32 may be of some importance in determining the type of histological change obtained. Thus, Bloom (1) administered P32 to mice in smaller doses than those reported here and did not observe any damage in the thymico-lymphatic system, Peyer’s patches, or ovary. However, in his animals there was damage in the duodenum, bone marrow, and testis. The Paneth cells of the ileum also increased in number and size 15 days after the administration of the radioactive phosphorus (3). Platt, who used still smaller doses administered repeatedly to humans for many months, observed “fibrosis, hyalinization of collagen, and a vascular alteration characterized by thickening and hyalination of small blood vessels” (21). However, in addition to the dose administered, species differences and the time in which P32 was allowed to act may also play a part in the different responses obtained.

CONCLUSIONS

Twenty-two normal young male and female albino rats and eight adult female C3H mice received a single large dose of radiophosphorus (P32) and were sacrificed from 2 hours to 9 days later.

The cellular destructions consisted mostly of pyknosis and karyorrhexis of mitotic nuclei and enlargement of metabolic nuclei. The cytoplasm of damaged cells showed vacuolation and reduced staining ability.

The destructive effects of P32 were most marked in lymphatic organs and bone marrow, small intestine, granulosa of ovarian follicles, and mammary tumors.

In the crypts of the small intestine, massive degeneration and pyknosis of dividing cells resulted in thinning of the epithelium of crypts and villi, probably because no new cells were supplied from the crypts to balance the continual loss of cells by extrusion normally occurring at villi tips (18).

The mammary tumors showed many scattered pyknotic cells at 24 hours after injection. At 48 hours, large blood lakes had formed without much tissue damage. At 9 days, most of the tumor was necrotic and liquefied.

Lesser changes were noted in stomach and colon, in the seminiferous tubules of testis, and in the hair follicles.

Little or no damage was observed in salivary glands, pancreas, liver, genito-urinary system, endocrine glands (except the ovary), brain, muscular and connective tissue.
ACKNOWLEDGMENTS

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