from French Indochina, were not sensitive to most antibiotics in doses smaller than 100 units or μg per ml test fluid. Organisms resistant to neomycin or terramycin were not encountered. The number of strains tested with prodigiosin is small but the results are encouraging.

Summary. Fifty-three recently isolated strains of *V. comma* were tested against 10 antibiotics. The results showed a wide variation of susceptibility to these drugs, according to the geographical origin of the vibrios.

Received May 11, 1951. P.S.E.B.M., 1951, v77.

Some Early Nuclear Effects of Ionizing Radiation.* (18753)

SHIELDS WARREN, MARGARET W. HOLT, AND SHELDON C. SOMMERS.
From the Cancer Research Institute, New England Deaconess Hospital, Boston, Mass.

The cellular changes which follow ionizing radiation have been most extensively investigated in relation to mitoses. It is noted that within 12 minutes after exposure to heavy doses, mitotic inhibition may occur, and the chromosomes involved show clumping, fragmentation and lagging(1). Nuclear chromatin degenerates and giant nuclei may result. These relatively temporary effects are seen particularly in the mitotic prophase, and the different susceptibilities of various cell types is well established. Cytoplasmic alterations such as vacuolization and massing of the Golgi network and fragmentation are observed, but major interest still attaches to nuclear changes. Various mechanisms have been invoked in explanation. The idea that ionizing radiation prevents formation of desoxyribose nucleic acid from ribose nucleic acid, thus inhibiting chromosome synthesis(2), has also some support from investigation of chemical agents which inhibit prophase onset and are hence termed "radiomimetic." According to Dustin(3) these agents produce sequentially (a) cytoplasmic swelling, (b) alteration of nuclear membrane, (c) accumulation of thymonucleic acid on nuclear membrane with fragmentation and disappearance of all chromosomes, (d) spherical massing of basic protein and ribonucleoprotein, with persisting nucleolus, then (e) rapid fragmentation with extrusion of debris from the cell.

Present studies. As a preliminary to histochemical study of this problem, a series of rats injected intraperitoneally with large doses of P^{32}† were killed at hourly intervals. Lethal radiation effects consisting of nuclear pyknosis and fragmentation were usually observed in lymphoid tissues, intestinal crypts, bone marrow and gonads. In contrast to and at times preceding this lethal type of radiation reaction, nuclear swelling was observed in many tissues. In those sensitive cells mentioned above it proceeded to cell death, whereas in other organs such as liver, kidney and perhaps brain, nuclear swelling disappeared within 24 hours. Thus the more radioresistant tissues appeared to show a nonlethal reversible radiation effect.

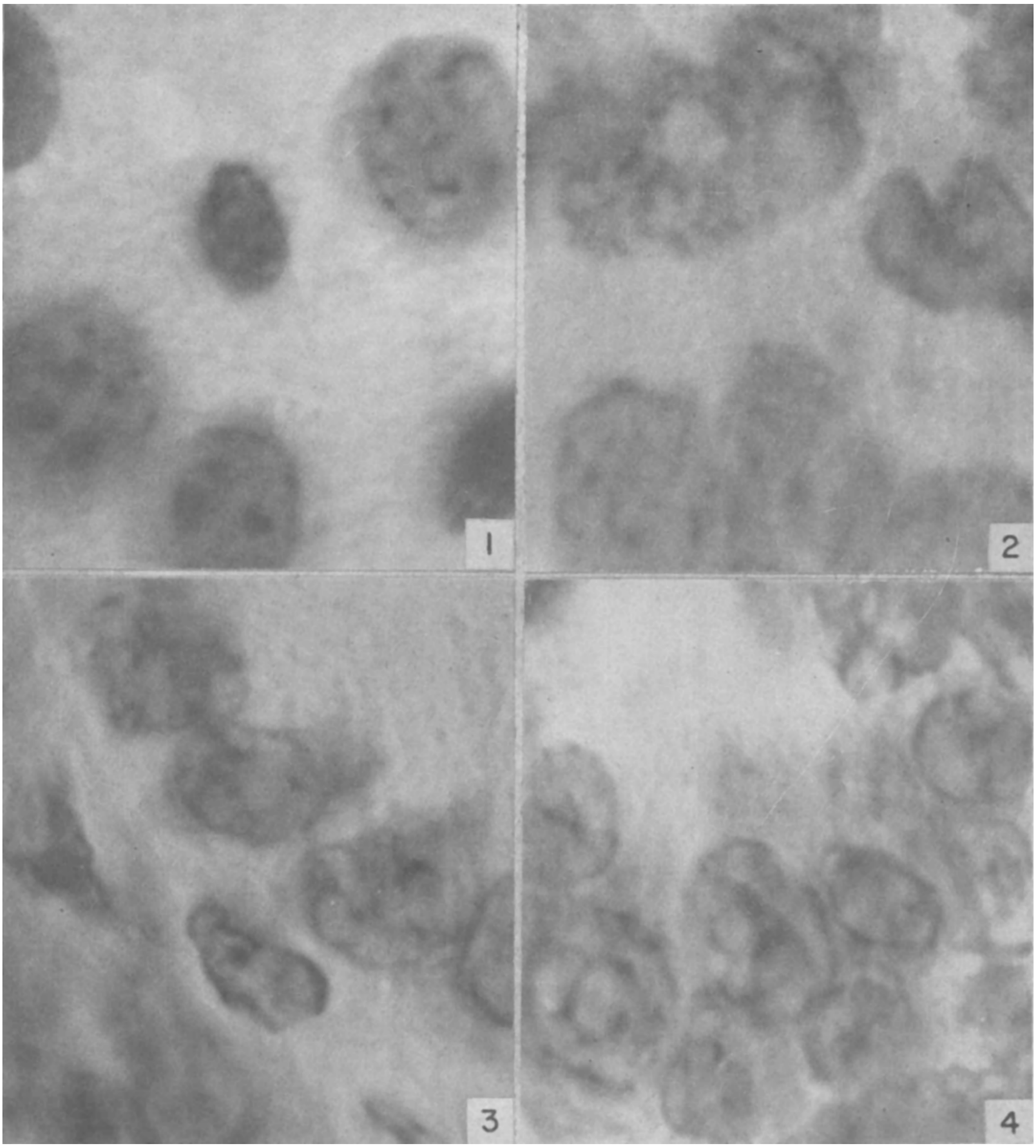
With the conventional formalin- or Zenker-fixed paraffin embedded tissues, however, nuclear detail is not adequately preserved to permit of an accurate study and interpretation of such relatively slight nuclear alterations. The simplified freezing-drying technic devised in this laboratory for autoradiography(4), and since used with success in histochemical problems(5), has proved useful in this situation. Feulgen stains of normal and irradiated tis-

* This work was done under U. S. Atomic Energy Commission Contract AT (30-1)-901 with the New England Deaconess Hospital.

1. Warren, S., *Arch. Path.*, 1942, v34, 443.
2. Koller, P. C., *Brit. J. Radiol.*, 1947, Suppl. No. 1, 84.
3. Dustin, P., *Nature*, 1947, v159, 794.

† The P^{32} used was obtained from the Oak Ridge National Laboratory.

4. Holt, M. W., Cowing, R. F., and Warren, S., *Science*, 1949, v110, 328.



Frozen-dried, dry-mounted tissues, photographed in polarized light to reduce halation. Feulgen stains, ×3000.

FIG. 1. Nuclear details of normal mouse liver cells, showing vacuoles. Dark chromatin masses present are often eccentrically vacuolated on microscopic examination.

FIG. 2. Untreated mouse sarcoma #180. Nuclear vacuoles are numerous and prominent in sarcoma cells, producing the lacy pattern shown.

FIG. 3 and 4. Epithelial nuclei of small intestinal crypts, 3½ hr after subcutaneous injection of 249 μc of P^{32} into a normal 65 g rat. Large irregular intranuclear vacuoles are observed, irregularly stretching nuclear membranes. Cytoplasm is abnormally coarse and thready.

sues were examined from groups of rats and mice, some of which were exposed to 200 KV X-radiation; or the 90-120 MEV neutron beam, with an ionizing component reduced below the LD_{50} by means of lead shields, from the Harvard cyclotron; or P^{32} injection. Slides were prepared by the dry mounting

5. Holt, M. W., Sommers, S. C., and Warren, S., *Anat. Rec.*, in press.

method which prevents loss of water soluble substances. Such preparations have excellent cytologic detail surpassing that of ordinary histologic technic and comparable to the appearance of living cells in phase-contrast microscopy. The advantages of specific staining and relative permanence can be achieved.

Nuclei of normal mouse liver (Fig. 1) kidney, and untreated mouse sarcoma 180, among other tissues, contain numerous vacuoles covered with Feulgen-positive material, and the nucleoli are often eccentrically vacuolated. Nuclear membranes are seen as sharp smooth lines. Nuclear vacuoles of neoplastic cells are larger and relatively numerous (Fig. 2).

Soon after radiation, for example 3½ hours after 249 μc of carrier-free P^{32} injected subcutaneously into rats, striking nuclear changes occur, which are more conspicuous in radiosensitive cells. Epithelial nuclei of intestinal crypts develop giant clear vacuoles surrounded by irregular layers of Feulgen-positive material. The nuclear membranes are irregularly thinned and stretched by the clear vacuoles within, which apparently explode from the nuclei with lethal effect (Fig. 3,4). The nucleolar vacuolization is likewise abnormally exaggerated. Relatively radiosensitive neoplasms such as mouse sarcoma 180 acquire similar intranuclear vacuoles after irradiation, as for example by neutron beam of cyclotron origin. The size of the vacuoles produced exceeds those of any normal tissue examined. Both in contrast to the appearance of conventional histologic preparations of radiated tumor and to unirradiated tumor prepared by freezing-drying every nucleus shows vacuolation (Fig. 5,6).

Less extreme but equally significant intranuclear vacuolization occurs within 24 hours in radioresistant cells such as those of liver. Enlarged clear vacuoles distend the nucleus or push against the blurred nuclear membranes and are apparently extruded. The nuclear membranes appear rough, stain unevenly, and become fibrillar and poorly defined in places. A majority if not all liver cells are affected (Fig. 7, 8). About 24 hours after injection of P^{32}, the non-lethal nuclear radiation effects tend to subside in liver, but with persistence of an occasional nucleus with vacuoles and relatively less content of Feulgen-positive material.

Changes observed by others(2,6), and by ourselves both in conventional and frozen-dried tissue preparations, include a moderate general increase in nuclear volumes not involving vacuolization, decreased Feulgen staining of nuclear chromatin, sap and membranes, and cytoplasmic swelling. Chromosomes in mitoses stain abnormally densely, and appear clumped and unusually "sticky" in later phases of cell division.

Later radiation effects have not as yet been investigated with this technic.

Discussion. The major investigations into radiation effects upon cells have been concerned with lethal alterations of sensitive cells, such as those of lymphoid, hematopoietic and gonadal tissues. To understand the acute radiation syndrome(7) with its signs of general toxemia, it may be well to extend our studies of the less marked nonlethal changes observed in physiologically important tissues such as the liver. The newer freezing-drying and histochemical technics now available make possible the demonstration of relatively slight morphologic changes, which, as in the liver, may accompany important alterations in metabolic activity.

While processes of equal importance to the nuclear synthesis of nucleic acid compounds may remain undiscovered, the present study confirms the belief that radiation adversely affects the ribose and desoxyribose nucleic acid components which are Feulgen-positive. At this time, it seems logical to ascribe some radiation effects on mitoses to interference with production and metabolism of such Feulgen-positive nuclear constituents.

Summary. Frozen-dried, dry mounted Feulgen stained preparations of irradiated rat and mouse tissues were employed to study early cytologic changes after ionizing radiation. Fine nuclear details are preserved by the freezing-drying method. Lethal radiation effects observed in radiosensitive cells include

6. Platt, W. R., *Arch. Surg.*, 1947, v43, 1.
7. Warren S., and Bowers, J. Z., *Ann. Int. Med.*, 1950, v32, 207.

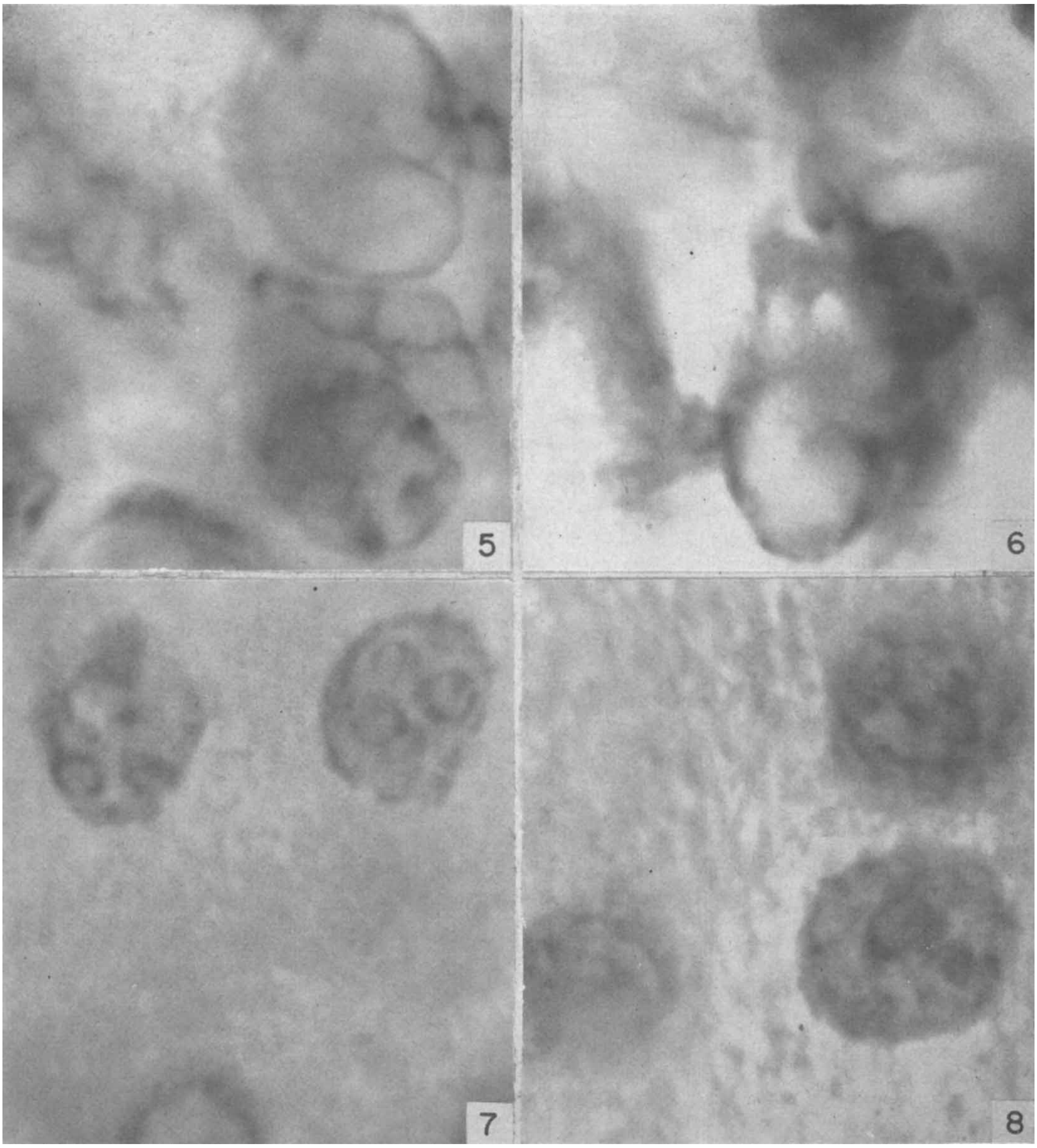


FIG. 5 and 6. Mouse sarcoma #180, 24 hr after sublethal exposure to cyclotron beam consisting primarily of neutrons of the 90 to 120 MEV range. Giant intranuclear vacuoles are uniformly present, with extreme nuclear swelling. Some nuclei are composed almost wholly of one or two vacuoles.

FIG. 7 and 8. Liver cells, 3½ hr after subcutaneous injection of 249 μc of P^{32} into a normal 65 g rat. Nuclei contain sharply outlined vacuoles, some touching nuclear membranes. Cytoplasm appears abnormally coarse and vacuolated.

formation of large intranuclear vacuoles with stretching of nuclear membranes. Nonlethal changes in radioresistant tissues comprise smaller intranuclear vacuoles, decreased Feulgen stain of nuclear chromatin and sap, and loss of regular sharp staining of nuclear membranes. Such changes in liver cells may contribute to the acute radiation syndrome. Histochemical technics may provide a better understanding of tissue changes after ionizing radiation.

Received May 7, 1951. P.S.E.B.M., 1951, v77.