ROENTGEN RAY INTOXICATION.

III. Speed of Autolysis of Various Body Tissues after Lethal X-Ray Exposures.

THE REMARKABLE DISTURBANCE IN THE EPITHELIUM OF THE SMALL INTESTINE.

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The question of Roentgen ray injury and certain modifications of intracellular ferments has been reviewed by Hall and Whipple (1). We must admit that the various methods of studying intracellular ferments are at present very crude and probably give mere hints as to what goes on within the cell. These ferments are accustomed to their normal environment which is very carefully balanced by the presence of the circulating fluids which remove waste products and furnish necessary supplies to the cells. The reaction of these ferments in a mass of dead or dying cells cut off from circulatory influences may or may not have any significance as to the true physiological function of these ferments.

We have submitted much evidence of true cell injury involving both nucleus and protoplasm following x-ray exposures. We submit below evidence to show that cell autolysis in the epithelium of the small intestine is profoundly modified by x-ray exposures. Some investigators go so far as to claim that primary injury of cell ferments is a fact and is in itself responsible for subsequent reactions and cell death. But how can one tell that this modification of cell autolysis is due to a primary ferment influence or some other physiological disturbance which influences the ferments secondarily? When the investigator gives this explanation of primary cell ferment reaction, he is indeed in distress when he attempts to explain the great differ-
ences in the effect of the x-rays on different cell structures. Our methods permit of a distinction in cell and nuclear structure between these cells which react so very differently to the x-rays. But present methods do not permit us to recognize a great multitude of individual cell ferments in the parenchymatous organ cells. Compare the cells of the bone marrow, intestinal mucosa, pancreas, and liver. They are all rich in ferments but they react very differently indeed to the x-rays. One must be very conservative in the analysis of data concerning intracellular ferments and deductions drawn from such experiments.

It was pointed out by Hall and Whipple (1) that the intestinal epithelium in radiated dogs was subject to rapid postmortem autolysis, so much so, that a few hours after the death of the radiated animal very little of the epithelium could be demonstrated. These workers also indicated that the crypt epithelium was more abnormal in this respect than the tip epithelium. With these observations in mind, a series of autolysis experiments was undertaken using sections of organs from the series of dogs described in Paper II. In this series an attempt is made to demonstrate the various changes in cell autolysis observed at various periods following a lethal x-ray exposure.

**Method.**

A series of dogs was exposed to 350 to 480 milliampere minutes of radiation diffusely over the abdomen. These experiments are described in the preceding paper. Dogs were sacrificed under chloroform or ether anesthesia at various periods of 2, 24, 48, 72, or 96 hours after exposure to radiation. A set of sections of each organ for control is fixed immediately in 10 per cent formaldehyde. Five sets of sections, each section about 3 to 5 gm. in weight, are cut from each organ immediately after death of the animal. Each section is placed, while still warm, in a bottle containing approximately 150 cc. of warm 0.9 per cent sodium chloride solution previously saturated while warm by shaking for an hour with an excess of chloroform. All the bottles are then warmed to 37°C., corked, placed in an incubator at 37°C., and the tissues allowed to autolyze. A set of sections is removed at 4, 8, 10, 12, and 24 hours respectively and immediately fixed in 10 per cent formaldehyde. There is no putrefactive odor.
present in the autolysis bottles, even after 24 hours autolysis of intestinal sections, indicating that the growth of putrefactive bacteria has been inhibited by the chloroform and suggesting that the autolysis is due to the endogenous ferments. There are no free chloroform globules present in the autolysis bottles, it being completely held in solution. The odor of chloroform is still quite strong after 24 hours autolysis. Several sets of experiments were done with sodium chloride solution without chloroform, but these were found to be very irregular, probably due to variation in the outgrowth of bacteria.

Organs from three normal unirradiated dogs are treated to the same routine and used as controls.

**Histological Method.**

The autolysis sections are studied with regard to the effect of radiation upon the rapidity of autolysis *in vitro*. Gradations of autolysis are based upon the intensity of staining of the nuclei, the integrity of the cell outline and the cell contents, and the complete digestion of the cell itself. In designating changes for the purposes of constructing curves indicating the speed of autolysis, the following criteria are used:

- 0 means no autolysis perceptible.
- A or + means cell nuclei are pale and washed out, as compared with the control of that organ—slight autolysis.
- B or ++ means nuclear structure is lost, run together, or indistinct—well advanced autolysis.
- C or +++ means the nuclear outline is shadowy and indistinct. There may appear to be nothing left but nuclear debris in the space occupied by the cell—marked autolysis.
- D or T means total autolysis; the nuclei are gone, though the basement membranes are usually intact. At this stage, there is nothing left but pale fibroblasts, polymorphonuclears, endothelial cells of the blood vessels, and the fibrillar intracellular matrix.

It would seem that these arbitrary periods of autolysis are irregular in sequence but these periods were established by preliminary experiments and seemed to us to give the required information. In plotting these findings, it also serves our purpose to consider the individual stages (or extent) of autolysis and the individual period of
autolysis as equal and numerically progressing units upon the coordinates. It is evident that if all of the constants are equal and presented in the same manner, the experimental results are being compared upon a satisfactory basis. Therefore, although all of our units are arbitrary, they are constant throughout and any consistent variation in the plotted curves may be considered as demonstrating changes produced by the experimental procedure (exposure to radiation).

EXPERIMENTAL OBSERVATIONS.

All the histological material for this study was fixed in 10 per cent formaldehyde and stained by the usual hematoxylin-eosin method, using paraffin sections. About 600 slides were studied and reviewed at least three separate times as unknown specimens to prevent any bias from knowledge of sequence of events in autolysis periods. The grades of autolysis have been established and described in the preceding paragraph. Curves of the various tissue reactions were established in form similar to Text-figs. 1 and 2. These many charts cannot be published but the statements made below are based on a study of such charts and sections. Much of the more important data can be studied in Table I.

We may note that certain cells common to organs and tissues are not much, if at all, disturbed by these long x-ray exposures. Such are fibroblasts and mature connective tissue cells, endothelial cells and macrophages, muscle tissues, and certain white blood cells. All these tissues are resistant to autolysis in the control and radiated animals and may appear as very little changed even by the long 24 hour period of autolysis at 37°C. Some, but not all, of these cells are supposed to be poor in ferments.

The more specialized cell types of the abdominal viscera are our chief concern. In the gastrointestinal tract, we differentiate between the tip epithelium which covers the villus and the crypt epithelium which is more of the secreting cell type. In the spleen and mesenteric lymph nodes, we have made a rather broad distinction between the pulp and the Malpighian bodies; in the pancreas between the isles of Langerhans and the remainder of the parenchyma. In the kidney, the proximal convoluted tubules are contrasted with the straight
### TABLE I.

**Autolysis during Periods of Hours as Indicated.**

<table>
<thead>
<tr>
<th></th>
<th>Hrs. autolysis</th>
<th>Control</th>
<th>2 hrs. after x-rays</th>
<th>24 hrs. after x-rays</th>
<th>48 hrs. after x-rays</th>
<th>72 hrs. after x-rays</th>
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Degree of autolysis indicated as follows (see complete description under Histological method): 0 means zero autolysis. A means slight autolysis. B means well advanced autolysis. C means marked autolysis. D means total autolysis.
tubules and the glomeruli, mainly because of their difference in autolysis; in the liver, the parenchyma cells are contrasted with the bile ducts.

In the normal intestine, the autolytic digestion of the mucosa starts in the tips of the villi and progresses fairly slowly and evenly to the bases of the crypts. Even at the end of 24 hours, the crypt epithelium may be fairly well preserved while the tip epithelium is almost totally autolyzed. In the radiated animal, on the other hand, the crypt epithelium autolyses at a greater speed than the tip epithelium and is practically completely autolyzed in 10 to 12 hours or earlier, while even after 12 hours of autolysis the tip epithelium in the same animal may be fairly well preserved (compare Text-figs. 1 and 2).

Discussion of Experiments.

Normal Autolysis.—Three normal dogs are sacrificed under anesthesia. Sections of abdominal viscera are autolyzed at body temperature in 0.9 per cent sodium chloride solution saturated while warm with chloroform as described above. The usual course of digestion is from without inward and the progress is steady and continuous. The digestion in the gastrointestinal sections progresses from the villi to the bases of the crypts. Comparisons are made of the penetration of the autolysis as well as of the progress of cell digestion.

Stomach: The chief and parietal cells of the gastric mucosa are more susceptible to autolysis than are the tip cells. The former are autolyzed rapidly and are totally digested at the end of 10 hours. The tip cells autolyze somewhat more slowly and are completely digested in 24 hours.

Duodenum, jejunum, ileum, and colon are quite similar in the type of their autolysis. The tip epithelium is the first to autolyze. In these control experiments the time may vary from 10 to 24 hours before completion of the digestion. Considerable variation is to be noted in this reaction of the epithelium of the villi. The autolysis of the crypts is usually delayed 4 to 8 hours, after which it proceeds regularly though usually not to completion in 24 hours (Text-figs. 1 and 2).
S. L. WARREN AND G. H. WHIPPLE

Spleen pulp is found to autolyze 4 to 8 hours faster than the Malpighian bodies, which, as a rule, are not completely autolyzed by 24 hours. Their centers are fairly well preserved at 12 hours. The pulp at this time is usually pretty well digested.

Pancreas digests pretty well as a unit. The islands of Langerhans autolyze on the average 2 to 4 hours more slowly at first than the parenchyma but later the speed varies considerably so that they may totally digest in 10 hours, or only partly digest in the full 24 hours.

Kidney convoluted tubules are very sensitive to autolysis. Usually they are completely digested in 8 or 10 hours. The remainder of the kidney parenchyma consisting of the straight and collecting tubules and the glomeruli is more slowly digested, the full 24 hours usually being required for their complete autolysis.

Liver parenchyma and bile capillaries autolyze as a unit.

Autolysis of Radiated Organs.—Seven normal dogs are exposed to large amounts of radiation, given diffusely over the abdomen, and sacrificed at definite periods after exposure. Autolysis specimens are studied (as above) in regard to the progress of digestion in the specialized cells of the various organs.

Stomach epithelium, though the speed of autolysis may seem to have increased slightly, is digested well within normal limits. This is true for the whole series of dogs which were killed 2, 24, 48, 72, and 96 hours after radiation.

Intestinal epithelium shows a striking change from the controls. The speed of autolysis of the tip epithelium in each case is practically within normal limits. The great change has occurred in the speed of the autolysis of the crypt epithelium. The curves in Text-figs. 1 and 2 illustrate this very well.

The speed of autolysis of the crypt epithelium is rapid and must be due to the immediate effect of radiation upon these cells. The most pronounced increase in the speed of autolysis occurs within the first 2 hours after exposure to radiation. The change produced in these cells, or what is left of them, is permanent up to the time of the death of the animal 4 days later. Even after a lapse of 4 days, these cells autolyze faster than the normal crypt epithelium. These curves correspond remarkably well, indicating that the increase in autolysis is due to a change in the cell protoplasm which persists
at least for 4 days after radiation. In all of the animals exposed to radiation, the crypt epithelium of the small intestine is completely autolyzed in 12 hours in the duodenum, ileum, and jejunum, while in the experiments with normal animals, the crypt epithelium is rarely autolyzed completely in 24 hours and usually the autolysis is only well advanced by that time.

*Colon* shows an increase in the speed of autolysis of the crypt epithelium and possibly of the tip epithelium, though the latter is less pronounced. The effect of the radiation seems to decrease or wear off slightly, for the speed of autolysis is somewhat slower and approaches the normal in the 3rd and 4th days after exposure to radiation. This may be connected with the fact that there is also relatively little injury to the colon, anatomically or histologically. The most pronounced increase in the speed of autolysis in the colon epithelium occurs, therefore, within the first 48 hours.

*Spleen* shows an apparent increase in the speed of autolysis, especially in the first 24 hours after exposure to the x-rays. The 72 hour curve is within normal limits, while both the 48 and the 96 hour curves show an increased speed in autolysis.

*Pancreas* shows a slight but definite increase in the speed of autolysis, especially during the first 48 hours after exposure. The 96 hour curves seem to indicate also that the speed of autolysis is increased, but the 72 hour curves are irregular as in the spleen series. The islands of Langerhans autolyze as a rule somewhat faster than the pancreatic parenchyma.

*Kidney* does not show changes in the speed of autolysis after exposure to radiation. The convoluted tubules are totally autolyzed in 8 hours. The other tubules and the glomeruli, while autolyzing more slowly, apparently digest at the normal rate. This would suggest that radiation has little effect upon the kidney.

*Liver parenchyma* shows a definite increase in the speed of autolysis, with a maximum in the specimens removed 2 hours after the radiation. The 24 and 48 hour experiments show less increase above normal.
DISCUSSION.

Many observers have studied ferment changes \textit{in vitro} in normal organs or tumor tissues exposed to x-rays before the unit period of autolysis. Much of this work has been reviewed by Hall and Whipple and need not be discussed. Our experiments indicate that the spleen, liver, and pancreas taken from a radiated animal will show a more rapid autolysis than similar tissues from control normal animals under similar conditions of autolysis. The kidney shows no such change by the methods used. We may recall that the spleen shows obvious histological injury after exposure to the x-rays, but such changes are lacking in the liver, pancreas, and kidney.

The stomach and colon are much more resistant than the small intestine, but we shall publish experiments to show that large and permanent gastric and colon ulcers may be produced by the x-rays.

The \textit{duration of the injury} done the body cells is of much interest to us. We note that abnormal autolysis of the spleen, liver, and pancreas is most striking within 24 hours after the radiation. After 48 hours, there is a distinct decrease in this reaction as observed in dogs killed 72 and 96 hours following the lethal dose of x-rays. In the small intestine, it is easy to demonstrate the abnormal autolysis in dogs even 96 hours after the x-ray exposure. This indicates in a sense that these cells are suffering from a persistence of the injury exerted by the x-rays, even during the 4th day after the exposure. It is significant that the clinical symptoms always reach a crisis on the 4th day. If this period is survived, the dog usually goes on to recovery.

We may note in passing that the cell necrosis and injury in the intestine as in the skin is associated with a minimal wandering cell reaction. This point deserves further study. We shall record also the fact that ulcers caused by the x-rays are as stubborn in healing in the stomach and intestine as is so well recognized in the skin.

SUMMARY.

Exposure to large doses of x-rays will cause notable increase in the speed of autolysis of the crypt or secretory epithelium of the dog's small intestine. These changes can be demonstrated readily in
material obtained from dogs killed 2, 24, 48, 72, or 96 hours after the initial radiation (Text-figs. 1 and 2).

In the radiated dogs the secretory crypt epithelium of the small intestine autolyses first and the epithelium of the villi last, while the reverse is true in the normal control small intestine. These abnormalities of autolysis associated with lethal Roentgen ray exposures can be demonstrated for the small intestine over the whole 4 day period subsequent to radiation.

The colon shows little change and the stomach no demonstrable changes in autolysis under like conditions. The kidney likewise is negative.

The spleen, lymph glands, liver, and pancreas show a moderate increase in speed of autolysis in tissues taken from radiated animals within 48 hours of the initial exposure.

What the significance of this disturbance of cell ferments in the intestinal mucosa may be, we cannot pretend to say. At least these observations strengthen one's confidence in the profound functional disturbance of this important intestinal epithelium—a disturbance which we believe is responsible for the clinical abnormalities and fatal intoxication.

BIBLIOGRAPHY.