THE PATHOLOGICAL ANATOMY OF HYDRAZINE POISONING.¹

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PLATE XXVII.

In a recent article in the Journal of Biological Chemistry² is reported a study of the influence of hydrazine upon the intermediary metabolism in the dog, by Professor F. P. Underhill and I. S. Kleiner, in which it is shown that although this poison produces extensive anatomical alterations in the liver of dogs, yet it causes little change in the partition of urinary nitrogen and sulphur, beyond that due to the starvation from which animals poisoned with this drug suffer as they always refuse food while under the influence of the drug. During these studies tissues from the poisoned dogs were kindly placed at my disposal by Professor Underhill for histological examination, and I also poisoned and examined the tissues of a few other dogs, as well as of cats and guinea pigs. A summary of the results of this histological study has been included in the article mentioned, but it has seemed that the histological changes observed are so remarkable and of such importance that they deserve a somewhat more extensive description and consideration.

Hydrazine, NH₂—NH₂, is not a substance that is likely to become so commonly used that it will be of general toxicological importance, although the phenyl derivative, phenyl hydrazine, has long been known to have sometimes had poisonous effects upon those who have used it extensively in studying sugars by the methods devised by Emil Fischer. However, the lesions produced by hydrazine are of such a nature that this drug promises to have a field of usefulness in certain metabolic and pathologic investiga-

¹Received for publication April 27, 1908.
²Jour. of Biol. Chem., 1908, iv, 165.
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In these experiments the sulphate of hydrazine has been used, and the general effects are described by Underhill and Kleiner as follows: "The researches of Borissow, of Pohl, and of Poduschka have demonstrated the relatively great toxicity of this compound, and have defined the series of manifestations following its introduction into the body. With doses of 0.1 gram of hydrazine sulphate per kilo of body weight subcutaneously injected, vomiting is observed, which is succeeded by extreme restlessness. There is augmentation of the heart beat which later falls far below the normal, and respiratory difficulty is accompanied by general paralysis. At this stage a short period of coma usually ensues which terminates in death. The entire cycle of events is completed within a very few days. Coincident with the symptoms noted above is the appearance in the urine of variable quantities of protein and bile pigments, together with appreciable amounts of allantoin crystals. The liver appears to suffer fatty metamorphosis, and autolysis of various tissues of such experimental animals leads to the presence of significant quantities of allantoin in the digestion mixtures."

Organs from eleven dogs, two cats and four guinea pigs poisoned with hydrazine have been studied, and the anatomical changes resulting have been found to be quite constant. The poison is remarkable in that it affects only the liver, so far as the histological evidence shows, leaving all the other organs unaffected. In the liver the changes are very constant, although the degree of change has not always been found to vary directly with either the size of the dose or the duration of the poisoning, presumably because of individual variations or inconstant conditions in the dogs examined. The changes consist essentially in a fatty degeneration of the cytoplasm of the liver cells, which begins in the center of the lobules and progresses outward, until in dogs dying from three to six days after being poisoned the process has extended to involve extensively all but the peripheral cells of each lobule, and no cells are present that do not contain more or less visible fat. Only the cytoplasm of the cells is primarily affected by the poison, the nuclei remaining intact until long after the cytoplasm has become a barely distinguishable network of protoplasmic granules and threads inter-
spersed between closely packed droplets of fat; when the degeneration has reached this degree the nucleus may begin to stain faintly, enlarge, become vacuolated, and disappear, as if suffering from lack of nutrition on account of the quantity of fat surrounding it. After a time the cells in the center of the lobule lose not only their cytoplasm, but the fat that has replaced it also disappears, so that nothing remains of the liver cells in the center of the lobules but an occasional badly degenerated cell or a practically naked nucleus; the place of the cells is usually taken by compensatory dilatation of the capillaries. Often there is some fatty change in the epithelial cells of the bile ducts, but the stroma cells and the stellate cells of Kupfer are entirely unaffected. Occasionally there are present in the bile vessels homogeneous bile-stained plugs. There were never observed instances of primary destruction of the nucleus, and the features of the ordinary central necrosis of the liver produced by bacterial poisons are entirely lacking; nothing like focal necrosis has been observed, and there is little or no tendency for leucocytic accumulation in the liver.

When stained with Sudan III, the vacuolization of the liver cells is seen to be due entirely to fat, which accumulates as minute droplets, packing the cytoplasm of the most affected cells so full that it seems to be one mass of fat. The nucleus of even the most fatty cells usually appears unaltered, and retains its position in the cell, surrounded on all sides by fat. In all marked cases there are practically no liver cells that do not show more or less fat, but the demarcation between the greatly affected central portion and the less affected peripheral portion is usually quite sharp, because of a sudden transition from cells packed with fat to cells containing but a few droplets. Fat droplets are also found frequently in the epithelium of the bile ducts, but not in the cells of the stroma and blood vessels. In those cases in which the effect of the poison has been most marked, so that the parenchyma cells in the centers of the lobules have disappeared almost entirely, the fat will be found occupying the intermediate zone of the liver lobule, the cells of the vessels and stroma left in the center of the lobule being practically free from fat, and the amount of fat in the peripheral cells being small in amount in contrast with the middle zone; in such speci-
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mens the histological picture is very striking, the orange Sudan III stain forming a solid band, surrounded on each side by the hematoxylin-stained nuclei of the surviving liver cells.

Hydrazine does not seem to affect organs other than the liver, although occasionally minute hemorrhages into the lung or a small amount of bloody pulmonary edema can be found. In one dog considerable hemorrhage was found in the medulla of one adrenal, and in the same dog there were numerous small interstitial hemorrhages into the pancreas with several typical foci of fat necrosis in the immediate vicinity of the organ. The lungs and kidneys are usually congested, but show no microscopic changes. In most of the dogs the brain, heart, gastro-intestinal tract, pancreas, adrenals, lymphatic system and generative organs have been examined without any changes whatever being found. Fatty changes were never found in the myocardium or kidney by special staining. Apparently hydrazine does not affect the red corpuscles, for the liver cells and the spleen and lymph glands were quite free from abnormal pigmentation. Hyaline thrombi or fibrinous deposits were never observed in even the most altered livers.

The general features of hydrazine poisoning may be shown by giving a typical protocol, as follows:

Dog A. Weight 10.83 kilos; very fat, part pug, male. January 15, 11 A. M., I injected 0.4 grm. hydrazine sulphate in 20 c.c. water, subcutaneously in left flank. The dog was a trifle indisposed the next day, but recovered by the end of 48 hours. January 18, 11 A. M., I injected 0.8 grm. hydrazine into the right flank. At this time the dog seemed perfectly well. January 19.—The dog was found dead in the cage, having apparently died about 6 A. M.

Autopsy.—Subcutaneous tissue at the site of injection is pink, but shows no evidence of infection or acute inflammation. Cultures from this point and from the heart's blood gave no growth. Liver was very yellow, friable in consistency, and weighed 335 grams. The centers of the lobules were red, but the rest of the lobule was strikingly yellow. The veins were full of blood. Heart showed no evident changes; the cavities were full of dark blood which was not coagulated. Spleen showed no gross changes. Kidneys were very much congested, with the cortical vessels particularly injected. Stomach showed some slight congestion of the mucosa, but no other changes were found here or in the rest of the gastro-intestinal tract. Lungs contained a great amount of blood but showed no definite areas of hemorrhage or consolidation.

Histological Examination.—Liver. The central part of each lobule when stained with hematoxylin and eosin shows a great decrease in the intensity of staining, this condition involving fully four fifths of the length of the cords.
in most cases; in some lobules only a few cells at the corners remain approximately normal. This change affects almost solely the cytoplasm, the nuclei staining with nearly as great intensity near the center as at the periphery. The cytoplasmic change consists of a high degree of vacuolization which reduces the cytoplasm to a coarse network, enclosing vacuoles in size varying from droplets as large as the nucleus down to minute granules; the affected cells are somewhat increased in size. There are no cells, even at the corners of the lobules, that do not show some vacuoles. There can be found no leucocytic accumulation, no congestion, and no connective tissue proliferation. In the central area the liver cells are entirely absent, except for occasional extremely degenerated cells with but minute shreds of cytoplasm remaining; there also are many nuclei that resemble liver cell nuclei, some of which stain faintly and some appear normal. Sections cut in agar, and stained by Sudan III and hematoxylin, show each lobule to consist strikingly of three zones of about equal width. The central zone contains neither liver cells nor fat droplets that are readily recognized, but consists chiefly of surviving stroma and capillary cells, with an occasional degenerated liver cell or liver cell nucleus. The intermediate zone is extremely fatty, rather sharply defined from the central zone, and less so from the peripheral zone. Here the cytoplasm of practically every liver cell is so densely packed with fat that the individual granules cannot be distinguished; but no matter how fatty the cell the nucleus is almost always clear and well stained. Occasionally the droplets fuse and force the nucleus to one side, but more often it is central and surrounded with fat. The peripheral zone differs solely in that the fat droplets are of smaller size and less abundant, so that much of the cytoplasm can be distinguished. Some of the small bile ducts contain fat droplets in the epithelium.

Kidney shows considerable congestion, especially in the pyramids but there are no changes in the epithelium. No fat droplets are found in sections stained by Sudan III.

Lung shows many of the alveoli full of blood, which contains no fibrin and no excess of leucocytes.

Myocardium shows no changes. No fatty changes present in sections stained with Sudan III.

Adrenal shows extensive hemorrhage in the center of the medulla, but no leucocytic accumulation and no thrombosis.

No changes found in any of the other organs and tissues.

When the animals are given enough hydrazine to make them quite sick, and are then allowed to recover, the process of repair of the liver lesions seems to consist simply in a gradual decrease in the amount of fat in the cytoplasm until the cells resume their normal appearance, which requires two or three weeks. The cytoplasm of such cells has been found extensively vacuolated, but not reacting for fat, so that there is apparently some intracellular edema following removal of the fat. This recovery of greatly degenerated cells shows how specifically the poison affects the cytoplasm.
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without injuring seriously the nucleus, and without destroying the vitality of the cell. No evidence of regenerative proliferation of the liver cells and bile capillaries, or connective tissue overgrowth, which are so characteristic of the recovery from acute yellow atrophy, has ever been observed.

Summary.—Hydrazine seems to be a poison with an almost specific effect upon the cytoplasm of the parenchymatous cells of the liver, for when the poison is given subcutaneously this tissue alone shows evident structural alterations, although equal or greater amounts must reach other organs and tissues. It seems to have remarkably little effect upon other than hepatic cells, and does not cause any appreciable destruction of red corpuscles; slight hemorrhages are occasionally produced, but much less than by other poisons with a similar effect upon the liver. It attacks only the cytoplasm of the liver cells, never affecting the nucleus primarily, and causes a profound fatty metamorphosis of the type commonly referred to as “fatty degeneration.” In this respect it resembles phosphorus, from which it differs in two important particulars. Hydrazine attacks first the cells in the center of the lobules, while phosphorus shows its first and most marked effects upon the peripheral cells; and secondly, phosphorus usually causes marked fatty changes in the myocardium, the kidneys, and indeed throughout the body, whereas the effects of hydrazine seem to be limited almost absolutely to the liver. The unknown poisons of acute yellow atrophy and eclampsia, and most of the bacterial poisons, attack first and chiefly the nuclei of the liver cells, in contrast to the strictly cytoplasmic effects of hydrazine. Phosphorus also affects the nuclei more than does hydrazine. On this account the recovery of the liver to normal after hydrazine poisoning is remarkably rapid and complete, there being no permanent anatomical alteration after recovery from a most severe non-fatal poisoning.

As a poison for use in experimental studies of hepatic metabolism, hydrazine would seem to commend itself over phosphorus on account of its more selective action upon the liver. In the maximum sublethal doses given for experimental purposes it will destroy fully as large an amount of liver tissue as will phosphorus; but there will always be left a considerable amount of liver tissue in a fair
state of preservation, and presumably functionally competent, whichever poison is used. When recovery of the experimental animal is desired the return of the liver to normal will probably be more rapid and more complete after hydrazine than after phosphorus.

To the pathologist the peculiar action of hydrazine presents many interesting problems. In the first place, why is it that hydrazine shows its effects first in the center of the lobules, while so similar a poison as phosphorus attacks first the periphery? Again, why does hydrazine act so specifically upon the liver cells, even when subcutaneously injected? And why does it limit its action so specifically to the cell cytoplasm, leaving the nucleus practically uninjured? It seems probable that the use of this drug in studies of fatty metamorphosis might throw light upon some of the obscure phases of this puzzling subject. As to the significance of the effects of hydrazine for physiology I may quote from Underhill and Kleiner, as follows: "The most striking feature of the action of hydrazine upon the animal body is the absence of abnormal relationships in the principal urinary constituents. Yet according to histological examination the liver is profoundly altered in structure and a large proportion of the cells is apparently inactive. The only inference that can be drawn from such evidence is that through the persistence of a small number of normally functionating liver cells this organ is enabled to maintain its intermediary metabolic processes in approximate equilibrium. This is in harmony with the recently published observations of Jackson and Pearce upon the production of artificial liver necrosis by injections of hemotoxic sera, and as they have aptly pointed out, constitutes one of the best examples of the 'factors of safety' or protective adaptations in the animal body."

In conclusion, I take pleasure in expressing my indebtedness to Professor Underhill for the opportunity of making this study, and for much of the material that was used, as well as for his permission to publish this phase of the studies upon the action of hydrazine.
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Explanation of Plate XXVII.

Fig. 1. Showing the fat deposited about the central veins. Stained with Sudan III and hematoxylin, the fat appearing black in the photograph. Condition observed 24 to 48 hours after poisoning.

Fig. 2. Later stage than Fig. 1. About the central veins, shown black in the photograph, are only stroma and liver cell nuclei. The light areas represent corners of lobules; the fat (photographed black) occupies an intermediate zone in the liver lobule, as a band surrounding the relatively unaffected corners of the lobules.

Fig. 3. Same as Fig. 2, but higher magnification. The pale area in the center represents parts of adjacent lobules about a portal radical, which is separated by a dark colored band of fatty degeneration from the central parts of the lobules, where only a few large globules of fat remain.