HISTOCHEMICAL DATA CONCERNING SOME METABOLIC DISTURBANCES IN ACUTE RADIATION SICKNESS

A.E. Ivanov and N.N. Kurshakova

(Supervisor - Active Member AMN SSSR N.A. Kraevskii)
(Presented by Active Member of AMN SSSR N.A. Kraevskii)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50, No. 7, pp. 58-63, July, 1960
Original article submitted October 13, 1959

The metabolism of radiation sickness has been studied basically by biochemical methods, which naturally could not give a complete picture concerning the microlocalization and character of changes of the studied processes in separate structural elements. Histochemical observations conducted by certain authors touched mainly on protein metabolism and first of all on nucleoprotein metabolism [5, 7, 10, 15].

For this reason the authors have conducted histochemical investigations of some enzymes, lipids, and glycogen in the lungs and the liver of rabbits subjected to general action of filtered x-rays in a dose of 800 r (LD-80/30). The lungs and the liver were chosen as the subjects for the study because of their large role in the pathogenesis of acute radiation sickness, because of their special participation in carbohydrate and lipid metabolism and also because of the lack of similar data in the literature.

EXPERIMENTAL METHODS

Twenty-five rabbits were utilized in the experiments; three rabbits served as controls and 22 rabbits were killed 6 hours, 1, 3, 5, 7, 10, 15, and 20 days after irradiation. Besides staining the sections with eosin-hematoxylin and scarlet, fat was differentiated in the sections by means of Sudan black and Nile blue, glycogen by the method of Shabadash, succinic dehydrogenase activity by the method of Shneider and Shelton, cytochrome oxidase activity by the method of Moog, and alkaline glycerophosphatase by the method of Gomori.

EXPERIMENTAL RESULTS

The researchers have shown that under conditions of acute radiation sickness there took place a basic disturbance in succinic dehydrogenase and cytochrome oxidase activities. Both of these enzymes play an important role in the final stages of biological oxidation of carbohydrates, fatty acids, and proteins. Succinic dehydrogenase serves as a carrier of hydrogen protons and electrons from succinic acid into the cytochrome system, thus catalyzing the transfer of succinic acid into fumaric. Cytochrome oxidase, by oxidizing cytochromes, serves as an intermediate carrier of hydrogen toward oxygen with the consequent formation of water.

The lung and liver succinic dehydrogenase and cytochrome oxidase activities changed monotypically, in complete conformity with the periods of radiation sickness development in the whole organism. After a slight drop during the first 2-3 hours after irradiation there occurred a short-term increase in the oxidative enzymes' activity. This was evidenced by an increase in cellular protoplasmic granules and by intensification of their staining. The cells were almost completely stained with dark blue color during development of cytochrome oxidase or dark violet during development of succinic dehydrogenase. This was especially noticeable in macrophages and in the bronchial epithelium.

After a day the enzyme activities decreased sharply, reaching a very low level at the climax of the sickness. In this period the enzymes were almost undevelopable and only in some cells could there be observed a small number of faintly stained granules. Beginning with the 10th day, cytochrome oxidase and succinic dehydrogenase activities gradually increased, and at the 20th day the activity almost reached the normal level.

In this fashion, evidently, important links in the intracellular oxidative processes were disturbed during radiation sickness; this without doubt played a large role in the lowering of tissue oxygen utilization and in a number of local and general disturbances. Thus, for example, it was established that in parallel with the lowering in the oxidative enzyme activity, there was a lowering in the digestive function of the macrophages, and this in turn led to disturbance in the lung and liver barrier functions [3]. Besides this, a considerable lowering of cytochrome oxidase and succinic dehydrogenase activities could be one of the reasons for accumulation of toxic metabolites.

In the literature there are contradictory meanings concerning the action of irradiation on tissue respiration. Data of a number of authors, as well as of the present ones, attest to basic changes in activity of oxidative enzymes during ray injury [8, 9, 11, 14, 19]; other, mainly biochemical, investigations, on the contrary, deal

with preservation basic enzyme systems of tissue respiration [1, 2, 16, 17, 18, 20, 22, 24, 25]. However, the divergence of present data from literature data was evidently related to the fact that in the biochemical studies in which there were investigated blood, biological fluids, tissue sections and homogenates, there were determined the total oxidative enzyme activity; histochemical methods let us study the state of various substances in separate structural elements. It is well known, and this was confirmed by the authors' previous works [4], that the distribution of enzyme activity in different structural elements of the same tissue is unlike and it changes differently under pathological conditions.

In this fashion, the present data do not contradict literature data, but on the contrary they supplement them, letting one judge more correctly the character of oxidative enzyme changes in the tissues of an irradiated organism.

In contrast to the oxidative enzymes, the activity of alkaline glycerophosphatase during the first days after irradiation changed slightly, but subsequently it increased considerably, remaining at the high level even during the recuperative period—on the 15-20th days (Fig. 1). Analogous observations were made by other investigators [21].

Glycogen investigations have shown that during the first hours after the action, the glycogen content decreased sharply; it was evidenced only in the central portions of the hepatic lobes (Fig. 2) and in individual cells of the bronchial epithelium and the cartilage, whereas liver glycogen was evidenced not only in hepatic cell protoplasm, but also in the lumens of intralobular capillaries and central veins. Thus it can be thought that in the beginning of sickness there takes place intensification of splitting and washing out of glycogen from the liver. Data of a number of biochemical investigations discuss this; these authors have observed development of hyperglycemia during the beginning of radiation sickness [2, 6,13,23].

At the end of the first day the liver glycogen was rapidly restored, remaining,however, lower than normal during the whole duration of the sickness. There was also a change in the character of glycogen distribution. During this period there was not noticed any significant difference in the glycogen content of the periphery and the center of the hepatic lobes; only in their different spots could there be observed separate groups of cells almost devoid of glycogen.

It is necessary to note that in some animals killed during the 7-10th day, the organ glycogen content was insignificant. Probably, such animals were killed during the preagonal period, when the glycogen stores decrease sharply [2].

The lowering of liver cell glycogen during the first hours after irradiation could be possibly tied in with the shock-like reaction arising as the result of irradiation, which is generally characterized by the sharp lowering of liver glycogen. Thus, such a lowering of glycogen is evidently not characteristic for all cases of radiation injury, but is connected with sharp reflector disturbances, produced by single massive external irradiation. In the case of acute injury by incorporated radioactive substances a similar decrease in glycogen in liver and in other organs during the first hours of the sickness does not take place [11].

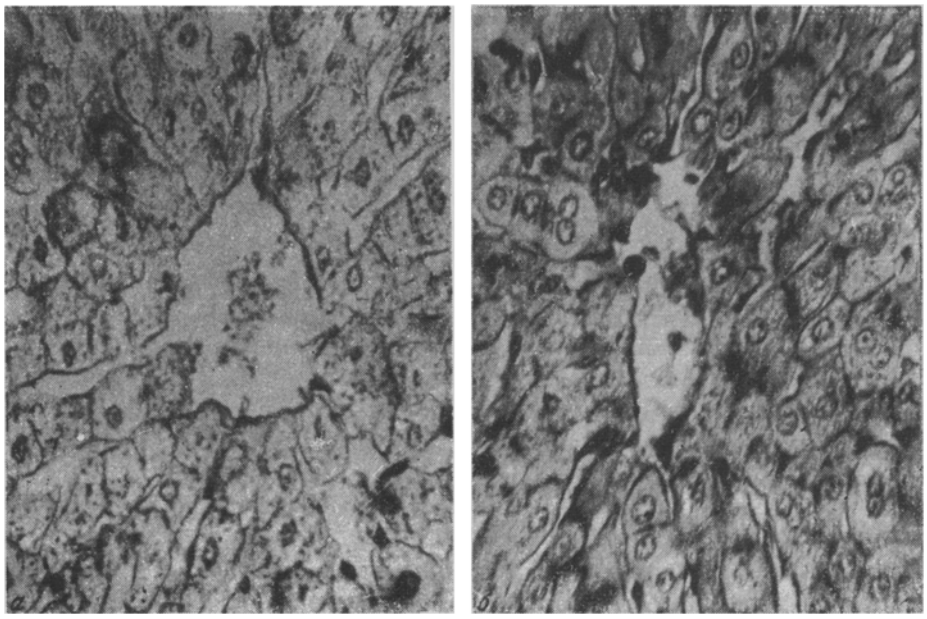

Fig. 1. Liver alkaline glycophosphate activity of normal (a) and on the 7th day after irradiation (b). Oc. 10×, ob.40×. Staining according to Gomori.

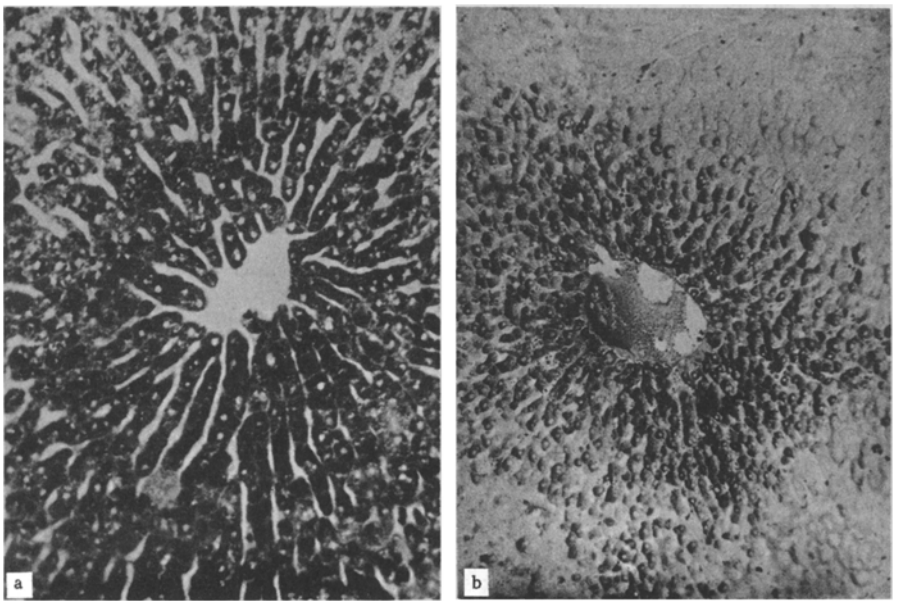

Fig. 2. Glycogen content in the liver of a normal rabbit (a) and at 6 hours after irradiation (b). Oc. 10 ×, ob. 8 ×. Staining according to Shabadash.

The disturbance of carbohydrate metabolism was not limited by the lowering of the stores of free liver glycogen. Absence of glycogen in considerable part of bronchial cartilage cells, and also increase in the obtaining intensity of fibrous structures and the interwoven substance attested to deeper changes in tissue carbohydrate metabolism of the irradiated organism. In the cells of the bronchial epithelium and in the macrophages there was a concurrent accumulation of large quantities of sugars, which were not removed from the tissues following treatment with preparations of salivary amylase. Such an increase in mucous cell and histocyte sugar could be related to the increased decomposition of tissue polysaccharides and disturbance of processes of their subsequent transformation in the cells.

Concurrently with glycogen changes there was a disturbance in lipid metabolism. Already observed at six hours after irradiation there was an increase in the content of liver neutral fat and an almost complete disappearance of phosphatides. In healthy animals phosphatides, appeared in liver cells in the form of separate globules, and after irradiation such globules disappeared; the protoplasm was stained diffusely in a gray-pink color. Analogous protoplasmic staining was observed during lipid development with Nile blue, scarlet, and also during the staining of the preparation with eosin- hematoxylin; it was most rapidl connected with changes of cellular lipo-and glycoproteins.

After a day the amount of neutral fat in hepatic cells increased greatly. This was indicated by a large number of pink droplets in the protoplasm of the majority of cells following staining with Nile blue. To an equal degree there was an increase in phosphatides, which also in a large number filled the protoplasm of hepatic cells. However, the increase in phosphatides was not prolonged and possibly was related to the tendency of the organism to restore the disturbed relationship between the processes of neutral fat hydrolysis and the synthesis of phospholipids. At the climax of the sickness the cellular neutral fat content remained high and the number of phosphatides fell below normal. Such a decrease in phosphatides could be partially dependent on the increased activity of alkaline glycerophosphatase during this period of the sickness.

If the animals survived the acute phase of radiation sickness (10 days), the phosphatide content of liver cells increased gradually; the neutral fat appeared as before in large quantity. During this the character of fat distribution changed. If during the first days and at the climax of the disease the fat appeared mainly in peripherally situated cells, then during the 15-20th days it was as a rule observed in the central portion of the lobes. Such a distribution of fat permitted speculation on various mechanisms of lipid metabolism disturbances in these or other periods of radiation sickness. Elevated fat content of lobular periphery in the beginning of the sickness indicated simple adiposity, which could be connected with the lowering of liver glycogen and disturbance in the processes of digestion of the surplus neutral fat entering the liver. Appearance in such a case of protoplasmic fat decomposition evidently did not play an important role.

Observation of lipids primarily in the central portion of the hepatic lobes was a sign of fatty dystrophy of the hepatic cells. Such a change in hepatic cells in latter

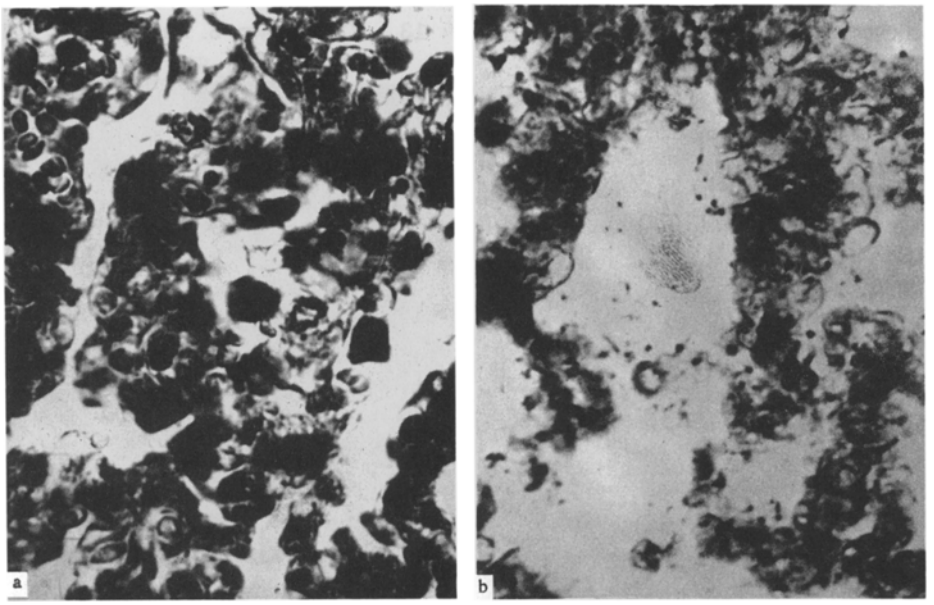

Fig. 3. Lung phospholipid content of a normal rabbit (a) and on the 7th day after irradiation (b) Oc. 10 ×, ob. 40 ×. Staining with Sudan black.

periods after irradiation developed most rapidly as the result of general metabolic disturbances during radiation sickness, while one of the primary reasons for this could be the appearance of exhaustion as the outcome of the sickness.

The disturbance of lipid metabolism in the lungs took place in a different manner. During the whole period of the sickness neutral fat did not appear in the cells of lung tissue, with the exception of cartilage cells; the content of phosphatides decreased sharply (Fig. 3). At the same time there was an appearance of fat—which stained blue with Nile blue—in septal cells, macrophages, and bronchial epithelium. Their appearance could be connected with the lowering of oxidative enzyme activity at the climax of the sickness; as the result there was an accumulation of incompleted oxidized products of fat cleavage, which were formed in the liver. With the increase in oxidative enzyme activity, the content of incompletely oxidized products of fat cleavage was decreased and there were observed essentially phosphatides.

In this way, the presented materials attest to the essential disturbances of some metabolic phases of lung tissue and liver cells. Evidently, the most highly expressed disturbances occurred in lipid metabolism, while the glycogen content during the course of the sickness remained at a level sufficient to maintain the activity of the irradiated organism. Only during the first hours after irradiation was there a sharp decrease in the liver glycogen content, which possibly could be one of the causes of the early lethal results of radiation sickness. This is supported by the acute destruction of liver in animals perishing in the first few hours after irradiation.

With regard to the changes in metabolism of separate lipid fractions, the observations show that under the influence of irradiation there was a many-fold increase in liver neutral fat content and in the incompletely oxidized by-products of fat cleavage in the lungs. At the same time the quantity of phospholipids was decreased significantly.

SUMMARY

Carbohydrate and lipid metabolism, as well as the activity of alkaline glycerophosphatase, cytochrome oxidase, succinic dehydrogenase, and succinic dehydrogenase, were studied histochemically in the liver and lungs of irradiated rabbits. The activity of succinic dehydrogenase and cytochrome oxidase drops considerably in animals with acute radiation sickness, whereas the activity of alkaline glycerophosphatase is markedly elevated. In addition, a considerable increase of the neutral fat content in the liver, with accumulation of underoxidized products of its splitting in the lungs. The amount of phospholipids is usually markedly reduced in both organs. The amount of glycogen in the liver changes but little; only during the first hours after irradiation and in the dead animals is a loss of the glycogen store in the liver noted.

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