COMPARATIVE HISTOCHEMICAL DATA ON GLYCOGEN CHANGES IN X-RAY AND STRONTIUM-90 RADIATION SICKNESS

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In our previous investigations [5] we demonstrated that, under conditions of acute radiation sickness induced via x-rays (800 r), a serious impairment takes place of the glycogen content in the liver, cardiac muscle, lungs, and other organs of the irradiated animals. Thus, within the first few hours after irradiation a marked reduction of the liver glycogen is observed and an increase of its content in the cells of cardiac muscles. During the latter stages, on the contrary, a certain restoration of the glycogen content of the liver and cardiac muscle is noted. However, during the entire acute period of the disease the glycogen content remains reduced. Only in animals who had recovered from the acute stage of the disease did glycogen content in the organs gradually reach its normal level.

These observations fully conform with the data of various clinical, pathologoanatomical and biochemical investigations during various periods of the acute radiation sickness.

It could be assumed that under the effect of incorporated radioactive substances the character of corresponding histochemical observations will differ from the previously described ones, since there is a definite pecularity in the clinical and pathologoanatomical pictures of this type of radiation injury.

On this basis, we carried out histochemical studies of glycogen and alkaline glycerophosphatase in the liver, cardiac muscle and lungs of rabbits during the early stages of chronic radiation sickness induced by the introduction of strontium-90.

METHOD

A total of 33 rabbits was used in the experiments. Of these, 21 received intravenously strontium-90 in a 0.5 microcuries/gm dose. Twelve healthy rabbits served as control. The rabbits were killed within 6 hours and 1,3,5,7,10,15 and 20 days from the moment of strontium-90 administration. Determination of glycogen was made according to the A. L. Shabadash method [14], and alkaline glycerophosphatase by the Gomori method. We also determined the amount of energy absorbed in the liver, cardiac muscle and lungs.

RESULTS

In all experimental animals the initial stage of the disease (up to 20 days) proceeded with marked phenomena of acute radiation sickness, though with a less expressed leucopenia than in the case of x-ray irradiation. The number of leucocytes per cubic mm of blood was reduced to 2000-3000.

Thus, we had sufficient reason to evaluate the character of glycogen changes in the investigated organs in acute radiation sickness caused by total x-ray irradiation and during the acute period of a chronic radiation sickness caused by the incorporation of strontium-90.

We took into account that strontium-90 (beta-emitter) is accumulated basically in the bones very soon after its incorporation. Therefore, all pathologoanatomical changes in the liver, cardiac muscle and lungs which take place following administration of strontium-90 should be regarded mainly as the result of general disturbances caused by the presence of a radioactive substance in the organism.

The detected morphological changes in the liver, cardiac muscle and lungs manifested themselves in phenomena of acute impairment of the hemodynamics, increased permeability of vascular walls, and to a greater or lesser extent in pronounced dystrophic changes in the parenchymatous cells and interstitial tissue. These changes have been sufficiently described in detail in the literature [3,8,11] and we shall therefore not dwell on them.

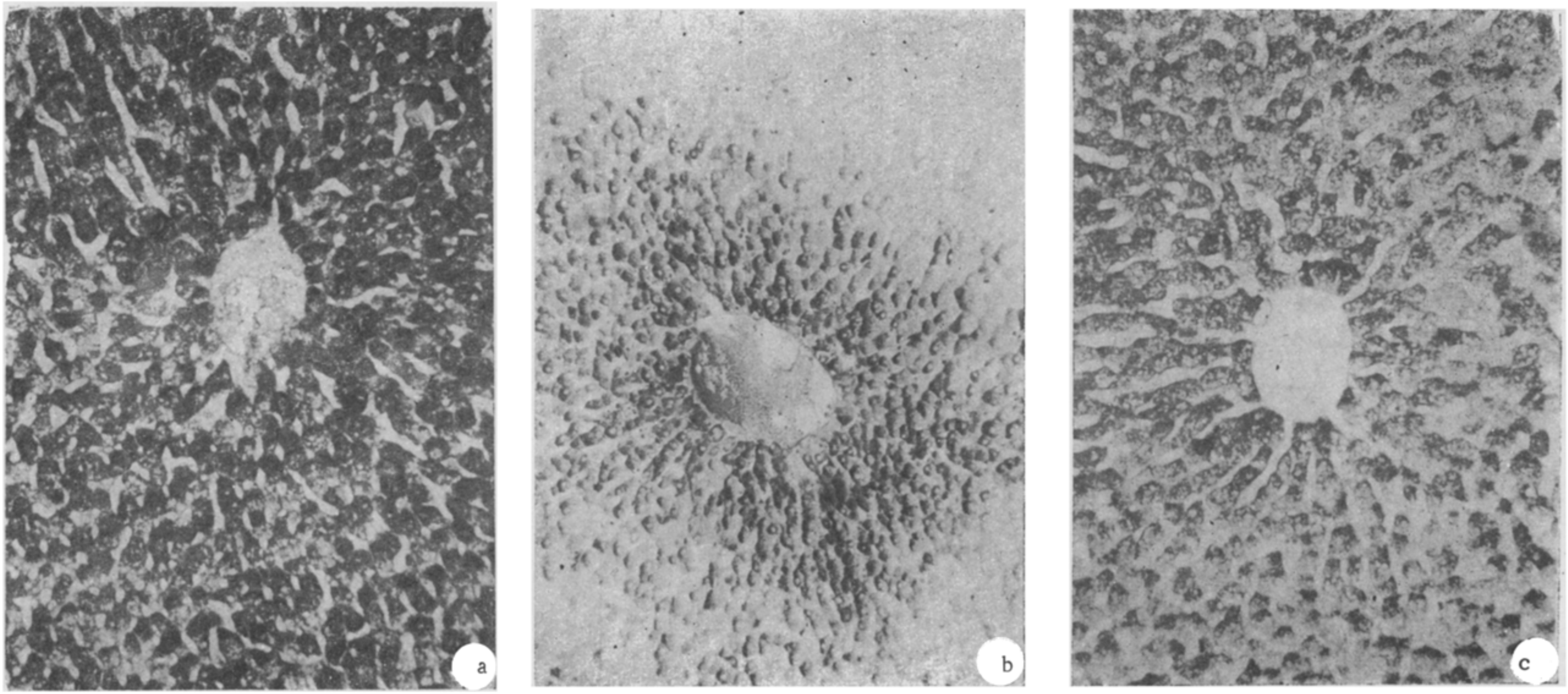

Fig. 1. Changes in the glycogen content in the liver of healthy and irradiated animals. a) Control (healthy rabbit); b) 6 hours after irradiation with x-rays in an 800 r dose; c) 6 hours after the introduction of strontium-90 in the amount of 0.5 microcuries/gm. Staining by Shabadash method. Magnification obj. 10 ×, ocular 10 ×.

As regards glycogen changes, the data of histochemical observations during the first few hours following the introduction of a radioactive substance proved to be non-uniform. Despite repeated experimental check-ups, some animals showed a reduced glycogen content, others its increase, and in some instances its content did not differ from control. In the healthy control animals no such fluctuations of the glycogen content were observed. Analogously, the activity of alkaline glycerophosphatase varied. In some cases, despite an increased activity of the enzyme, the glycogen content of the liver showed no changes. In the cardiac muscle and lungs of all animals, parallel with the reduced glycogen content an increased activity of alkaline glycerophosphatase was noted. All this indicates the different character of the initial reaction in response to the introduction of strontium-90. This fact is apparently not accidental, since it fully conforms with the observations of other investigators who have also noted the non-uniform character of the derangement of various organic functions during the early stages following the effect of radioactive substances [10,13].

The nature of distribution of glycogen during the first hours and days following strontium-90 incorporation differed from its distribution after x-ray irradiation. This was most clearly observed in the liver. Whereas following x-ray irradiation glycogen could be elicited only in the cells of the central part of lobules, a uniform distribution of glycogen over the entire lobule was observed following strontium-90 administration (Fig. 1). In the first case, the rapid decrease of glycogen is, possibly, connected basically with the reflex shifts in the carbohydrate metabolism; in the second case a gradual development of deeper disturbances of intracellular metabolism takes place. This is partially attested to by the increased activity of alkaline glycerophosphatase.

The above-stated equally refers to the changed content of glycogen also in other organs. Thus, we observed no initial increase of glycogen content in the cardiac muscle,which is generally observed under conditions of x-ray irradiation. In the lungs also a definite reduction of glycogen content was observed in the macrophages and hyaline cartilages of the bronchi.

An increased content of glycogen in the investigated organs in some animals can be connected during the early stages following the introduction of strontium-90 with its redistribution as well as with liberation of the so-called structural glycogen not elicited under ordinary conditions via our method. The latter explanation, in our opinion, seems to be more likely.

On the 2nd-3rd day and during the latter stages of the disease, the glycogen content of the liver, cardiac muscle and lungs was considerably lower than in control animals. The reduction was observed in all animals, and it was more marked as the disease progressed (Fig. 2). However, the glycogen distribution in the tissues now had a uniform character similar to the one in acute radiation sickness induced with x-ray irradiation. The only difference was that in the case of strontium-90 no restoration of glycogen content had taken place toward the end of the observation period (15th-20th day). Simultaneously, a considerable increase of alkaline glycorophosphatase activity was observed.

In summarizing the above data, it is important to point out the substantial difference in the character of changes of the glycogen content in acute radiation sickness induced by x-rays and in the acute period of the chronic radiation sickness induced by the internal administration of strontium-90. In the first instance, the microscopic picture of glycogen distribution has much in common with the picture observed during a state of shock. It can be assumed, therefore, that the decrease of glycogen during the early stages of acute radiation sickness is connected mainly with acute functional shifts originating under the effect of external irradiation. In the second instance, the glycogen change takes place in all parenchymatous cells and is characterized by a gradual reduction of its content as the disease progresses. This type of change in the distribution of glycogen corresponds to the nature of the effect of incorporated radioactive substances on tissues. Whereas upon a single external irradiation there is a one-stage absorption of a very large quantity of radiation energy, in the affection caused by incorporated substances there is a gradual accumulation of energy absorbed by the tissues (see table).

Analysis of the obtained data indicates that in some animals apparently the impairment of glycogen synthesis predominates,as a result of which we observed a uniform reduction of glycogen in all cells of the liver, cardiac muscle and lungs. In other animals, as a result of the predominance of phanerosis of the structural compounds which include glycogen, the latter is elicited in excessive amounts. Finally, at the early stages following the introduction of radioactive substances the glycogen content of the tissues may show no changes. This fact conforms with the data of a number of authors [1,2,19,21] who had also found a normal quantity or an increased glycogen content in the tissues in radiation sickness. In their opinion, this is due to the delay in the disintegration of glycogen and its synthesis from the products of increased disintegration of proteins and fats.

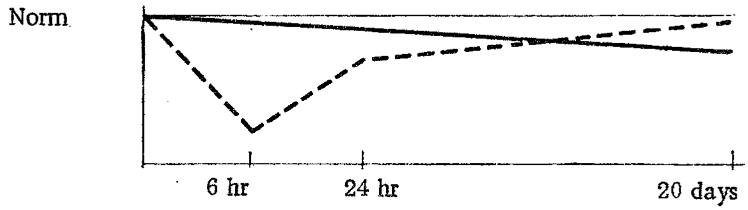

Fig. 2. Comparative diagram of the change in glycogen content in rabbits' liver under the effect of x-rays (----) and strontium-90 (——).

Quantity of Energy Absorbed(in rads)by the Tissues of the Liver, Lungs, and Cardiac Muscle under the Effect of Strontium-90 in a 0.5 microcurie/gm Dose

Organ	Time during which the energy absorption took place, days					
	1	2	3	5	10	20
Liver	0,015	0,023	0,026	0,034	0,064	0,11
Lungs	0,17	0,20	0,22	0,24	0,31	0,32
Cardiac muscle	0,02	0,05	0,053	0,071	0,10	0,15

One of the causes of the retarded splitting of glycogen may be the change in oxidation processes in the tissues of an irradiated organism [5,6,9]. As regards the reduction of the glycogen content at the height of the disease, one may assume that the mechanism of this phenomenon under conditions of external irradiation as well as after incorporation of radioactive substances is of an identical nature. Apparently, in both instances a profound disturbance of the metabolism takes place. However, in contrast to the opinion of a large number of authors [4,12,16,18,20,24,25] who think that the reduced glycogen content in the liver is connected with the insufficient absorption of carbohydrates from the intestines, there are data available at present which point to the leading role of inhibition of the glycogen-forming function of the liver in the impairment of glycogen metabolism [7].

Apparently, the cause of reduced glycogen content in tissues in radiation sickness is also the increased hydrolysis of the esters of phosphoric acid which are formed upon phosphorylation of monosaccharides in the process of their absorption from the intestinal canal and as a result of the phosphorolysis of glycogen. This is attested to in particular by the increased activity of alkaline glycerophosphatase. In this case an enhanced and premature disintegration takes place of compounds needed for the synthesis of glycogen and replenishment of the energetic and plastic reserves in the cells of an irradiated organism.

Thus, our data correspond to the observations of investigators who had observed a considerable reduction of glycogen in tissues following exposure to various sources of radiation [4,15,17,22,23]. Some divergence of our data from those of other authors can be, perhaps, explained on the one hand by the difference in the methods of investigation (histochemical and biochemical) and, on the other, by the non-uniform conditions of radiation action. As shown by our observations, the latter have a substantial effect on the investigation results.

SUMMARY

A histochemical study was made of the glycogen content in the liver, cardiac muscle and lungs of rabbits at the initial period of chronic radiation sickness provoked by the intravenous injection of strontium-90 in the dose of 0.5 μC per g of body weight. An essential difference was noted in the character of the initial changes of glycogen content in radiation sickness caused by the irradiation with x-rays and strontium-90. In the first case the changes in the glycogen content may be explained by the functional disturbances, whereas in the second—by more profound derangements in the carbohydrate metabolism. At the height of the disease the cause of reduced glycogen content is the same, and, according to the authors' data, is to a certain extent connected with the increased activity of alkaline glycerophosphatase.

LITERATURE CITED

1. D. A. Golubtsov, Theses of the Conference. Early Mechanisms of Radiation Sickness[in Russian] (Khar'kov, 1958) p. 27.
2. B. M. Graevskaya and R. Ya. Keilina, Vestnik Rentgenologii i Radiologii 4 (1955) p. 21.
3. A. E. Ivanov, Arkhiv Patologii 1 (1957) p. 31.
4. I. I. Ivanov, V. S. Balabukh, et al., in the book: Metabolism in Radiation Sickness [in Russian] (Moscow, 1956) p. 208.
5. A. E. Ivanov and N. N. Kurshakova, Theses of Reports of 3rd All-Union Congress of Pathologoanatom [in Russian] Khar'kov, July 6-11, 1959, p. 182.
6. A. E. Ivanov and N. N. Kurshakova, Arkhiv Patologii 22, 3 (1960) p. 34.
7. R. Ya. Keilina, Biokhimiya 24, 6 (1959) p. 966.
8. N. A. Kraevskii, Essays on the Pathological Anatomy of Radiation Sickness [in Russian] (Moscow, 1957).
9. P. F. Minaev, Zhurnal Obshchei Biologii 15 (1954) p. 401.
10. B. B. Moroz and S. P. Grozdov, Farmakologiya i Toksikologiya 22, 6 (1959) p. 544.
11. N. I. Mudretsov, Theses of the All-Union Conf. on Med. Radiology, Experimental Med. Radiology [in Russian]. (1957) p. 208.
12. S. R. Perepelkin, Theses of Reports of the 8th All-Union Congress of Physiologists, Biochemists and Pharmacologists, (Moscow, 1955) p. 473.
13. V. P. Fedotov, The State of the Carbohydrate Function of the Liver in Dogs Affected by Polonium [in Russian], Candidate's Dissertation (1958).
14. A. L. Shabadash, Glycogen Histochemistry of a Normal Nervous System [in Russian] (Moscow, 1949).
15. A. Ya. Shulyatikova, Transact. of the All-Union Conf. on Med. Radiology. Experim. Med. Radiology [in Russian], (Moscow, 1957) p. 111.
16. K. W. Buchwald, Experim. Med. 58 (1931) p. 827.
17. J. Denson and E. Grey, Proc. Soc. Experim. Biol. and Med. 82, 4 (1935) p. 707.
18. H. M. Dickson, Amer. J. Physiol. 182 (1955) p. 47.
19. P. Fischer, Arch. internat. Physiol. 42 (1954) p. 134.
20. T. J. Haley, Radiation Res. 2 (1956) p. 483.
21. R. E. Kay and C. Enteman, Proc. Soc. Experim. Biol. and Med. 91, 1 (1956).
22. P. Lelievre, C.R.Soc.Biol. 148 (1954) p. 927.
23. B. Levy and R. Rugh, Proc. Soc. Exp. Biol. and Med. 82, 2, 203 (1953).
24. M. Lourau and O. Lartique, Compt. Rend. Acad. Sci. 232 (1951) p. 1047.
25. L. F. Nims and E. Sutton, Am. J. Physiol. 177 (1954) p. 51.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.