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# ALKALINE PHOSPHATASE ACTIVITY CHANGES IN ORGANS OF RATS KILLED WITH ROENTGEN-RAYS*

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In our Institute the effect on living tissues of gamma rays originatig from the disintegration of radium and that of Roentgen rays has been subject of general studies. In the first place we have been investigating changes in the enzyme systems of irradiated animals and human tumors. In this paper we wish to report on the behavior of the alkaline phosphatase reaction performed on tissues of rats.

The phosphatases are dephosphorylising enzymes which split off $PO_4$ from phosphoric acid esthers by means of hydrolysis. Phosphatase can be found everywhere in vegetable and animal tissues. The phosphatases have been classified differently by different authors. In general 1. phosphomonoestherases, 2. phosphodiestherases, 3. pyrophosphatases and 4. apyrases are distinguished.

For phosphomonoestherases, based on how they react to alpha and beta glycerophosphate, as substrate, what reaction they give to Mg and how they are distributed in different organs, Folley and Kay have put forward the following classification: Type $A_1$ (alkaline phosphatase), optimal activity at pH 9. Type $A_2$ (acid phosphatase), optimal activity ranging from pH 4,5 to pH 5. Type $A_3$ (vegetable phosphatase), optimal activity at pH 6 and finally type $A_4$ (acid erythrocyte phosphatase), optimal activity at about pH 6.

Gömöri — and independently from him Takumatsu — were the first to succeed in demonstrating histochemically acid and alkaline phosphatase.

In this paper we wish to report on those alkaline phosphatase investigations which we performed — according to Gömöri's method — on organs of rats killed with lethal doses of rays. The reason why we selected rats for our first experiments was that the rat, contrary to other experimental animals, is capable of synthesising vitamin C. Irradiated animals namely lose a very great quantity of ascorbic acid through excretion, and the resulting vitamin C deficiency — especially in guinea pigs — leads to a very marked decrease of

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phosphatase acticity of tissues. For our experiments we used 20 couples of rats, mostly males. Their average weight was 150 g, and they were 8 to 10 months old. The rats were given a total irradiation of 2000 to 4000 *r*, in one lethal dose or in fractionated doses. For irradiation we used a Siemens Stabilivolt apparatus with Greinacher switch and with a Siemens Doglas inlay tube. 180 kV, 15 mA irradiations were given, from a distance of 50 cm., using a 0,5 *Cu* filter. To each of the irradiated animals belonged a control rat, similar in bodily condition and kept under the same circumstances. The rats kept on

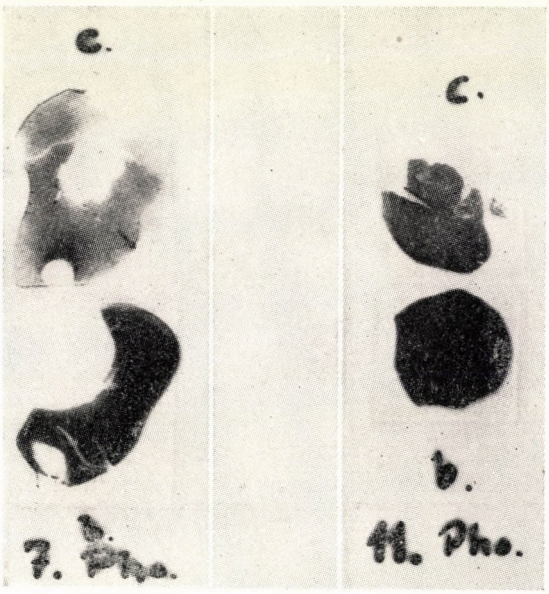


*Fig. 1.*
Macroscopic photographs of histological sections. c marks sections of the organ from controls, b, from experimental animals. Sections of the liver, after having performed the phosphatase reaction

standard diet died generally after a period of 3 to 4 days following the irradiation. At 24 to 36 hours following the irradiation there was a marked loss of appetite, but the animals still kept on eating. When the irradiated animal died spontaneously, the control animal was also killed by a blow to the nuchal region. Identical organs of both animals were embedded at the same time, the histological sections were mounted on the same slide and were treated with Gömöri's method. For fixation 85 per cent ice-cooled alcohol, for embedding increasing series of alcohol, benzol and paraffine were used. As a substrate sodium glycerophoshate, as buffer sodium diaethylbarbiturate were used. The precipitated $Ca_3(PO_4)_2$ was made visible by means off Kossa's reaction.

In the histological sections in this way prepared we observed in irradiated animals a very marked increase of phosphatase acticvity. *Most conspicuous was the greatly increased phosphatase activity of the liver*, which could be detected even macroscopically in the sections (Fig. 1.). The liver of the normal animal is — according to Gömöri's description and our observations made in the controls — negative as regards phosphatase activity ; in some cases there may be some activity limited to the biliary capillaries and to the walls of the vessels. (Fig. 2.) In irradiated animals we found very great phosphatase activity in the liver

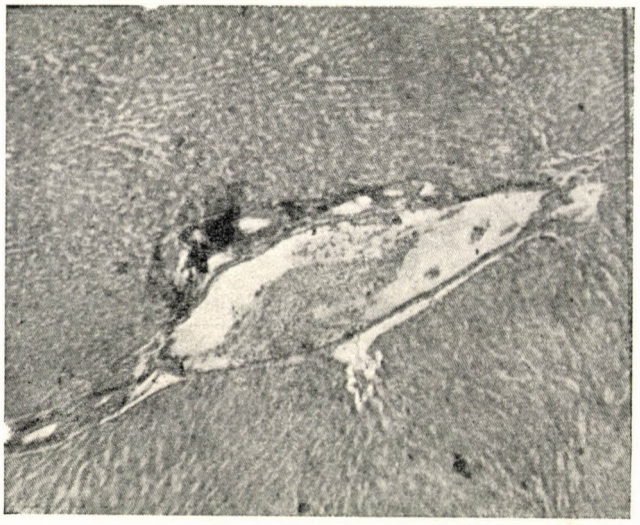

*Fig. 2.*
The liver of a control. The parenchyma of the liver is free of phosphatase activity ; phosphatase activity is seen in the wall of bile ducts and of greater vessels. (Zeiss microscope, objectiv 3, ocular 5)

tissue, showing zonular arrangement (Fig. 3.). It was in the potoplasm of the liver cells and in the bile ducts surrounded by these cells that high degree of enzyme activity could be observed (Fig. 4.). In the Kupffer cells, on the other hand, no phosphatase activity could be demonstrated. In other organs higher enzyme activity could be demonstrated in the membrana basalis of the testis, as well-as in the bronchial epithel, in the edematous alveolar fluid and in the desquamated alveolar cells of the lungs. In the kidneys the results were more difficult to evaluate, since normal kidneys also show high-degree phosphatase activity. In the intestines no evaluations could be made, due to the rapid intestinal autolysis occurring in irradiated animals. Unfortunately we could not investigate the bone marrow. This and the central nervous system will be studied separately.

Summing up we may state that *in the organs of rats killed with a lethal dose of Roentgen-rays there is an increase of the phosphatase activity. This increase is very marked in the liver.*

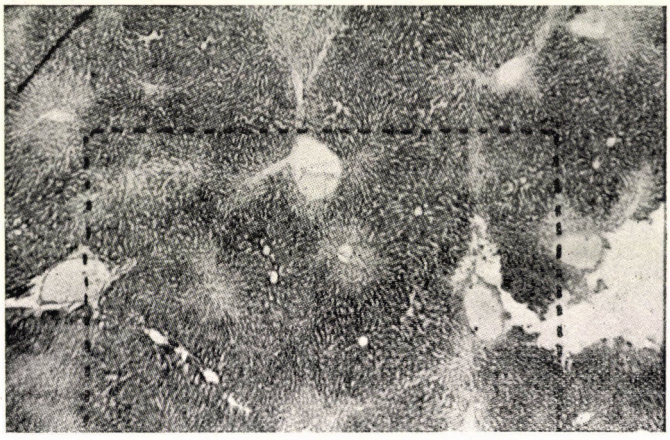

*Fig. 3*
General view of the liver of an irradiated animal. Zonal arrangement of phosphatase activity. The area in the square is shown in greater magnification in the next Fig. (Objectiv O, ocular 5)

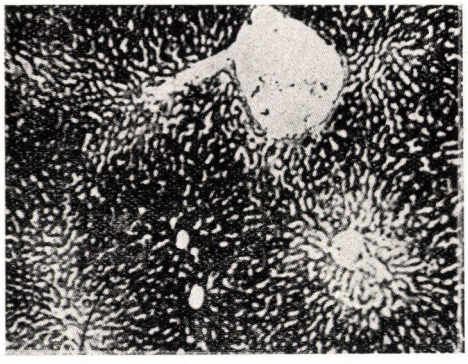

*Fig. 6*
Greater magnification from Fig. 3. (Objectiv 3, ocular 5 of a Zeiss microscope)

The occurrence of phosphatase activity in the liver may offer a partial explanation to the hypoglycemic conditions observed by Straus and Rother, Rother, and other authors, as well as to the disappearance of the liver glycogen following irradiation (Rother) in experimental animals.

The question arises: Why does the phosphatase activity of different organs — and mainly that of the liver — increase following irradiation? To the answer we must know the following data of literature.

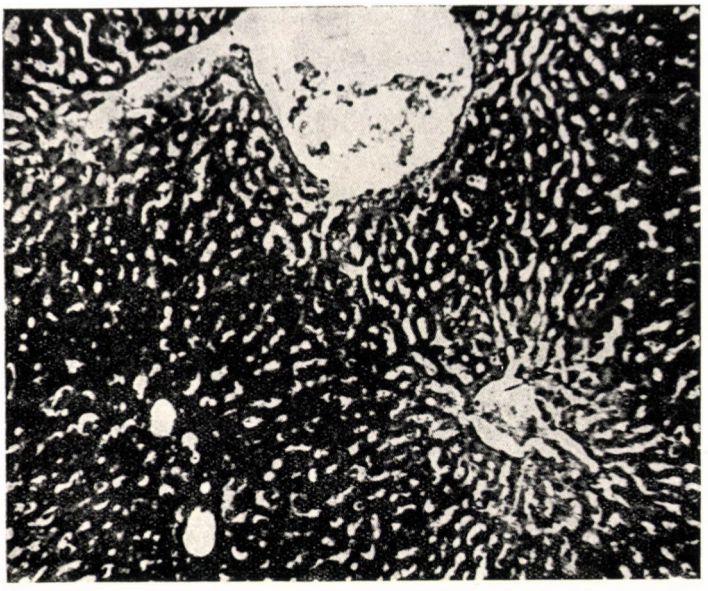

*Fig. 4*
Higher magnification (Zeiss optics, objectiv 9, ocular 5)

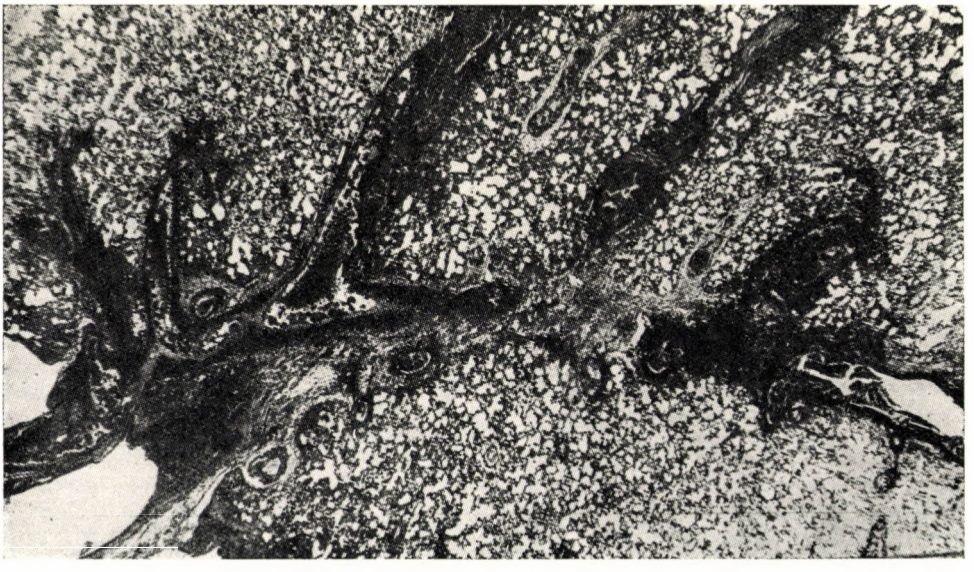

*Fig. 5*
Microscopic view of the lung of an irradiated animal

According to Errera's investigations, irradiation with Roentgen rays markedly reduces the otherwise very high viscositiy of thymonucleohiston; this is probably due to depolymerisation. In his opinion a similar process occurs in living cells on irradiation.

Holmes experimented with Jensen's rat sarcoma. He injected $P^{32}$ isotope into the animals and found that on irradiation the nuclein metabolism, i. e. the incorporation of $P^{32}$ into the thymonuclein fraction, was materially reduced. Thus he confirmed Hevesy's previous observations made on animals having two tumors. Isolated irradiation of one of these tumors similarly decreases $P^{32}$ uptake in the non-irradiated tumor.

Caspersson and Thorell have found that in the course of embryonal development the changes in phosphatase activity run parallel with the changes in concentration of nucleinic acids respectively of nucleoproteids. It appears very likely that this enzyme takes part in the synthesis and destruction of nucleinic acids.

Krugelis, Danielli and Catcheside have found in the cells of Drosophilia, in the chromosomes, phosphatase activity in such a trabecular distribution which more or less corresponds to the arrangement of the Feulgen-positive bundles.

Finally it should be mentioned that — in contrast to the former static theory — also in the enzymology it is the dynamic conception that prevails, i. e. we consider enzymes to be effects and not separate substances. The same substance that in a certain state of the cell plays the part of a metabolite, and is fuel supplying energy, under other circumstances may act as an enzyme. Our experiments and the quoted data of literature suggest that *the phosphatase activity occurring in the liver may be brought into connection with the depolymerisation of thymonucleohiston brought about by irradiation, which presents itself in the form of an enzyme effect.*

Further histochemical and other investigations are in progress with which we wish to obtain further evidence concerning the validity of this theory.

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*Summary*

In our Institute we investigate the effects of Roentgen and gamma rays on living tissues and on tumors. In the first place it was the changes of enzymes that were subjected to investigation by means of known histochemical reactions. In the present paper we report on the changes of alkaline phosphatase activity in tissues of rats killed with lethal doses of rays.

In our experiments rats were used, because these animals are capable of synthesising vitamin *C*: in other animals irradiation with Roentgen rays gives rise to excessive vitamin *C* excretion and this reduces the phosphatase activity of their organs. The rats were irradiated with 2000—4000 r, in one dose or in fractionated doses (180 kV. 18 mA, 0,5 Cu, 50 cm). To each irradiated animal there was a control one, being of the same bodily condition and kept under similar circumstances. When the irradiated animal died spontaneously, the control was also killed by a blow to the nuchal region. The organs of the two animals were prepared identically and at the same time. Identical organs were mounted on one slide. The sections then

were treated with Gömöri's method. In certain organs considerable increase of phosphatase activity was found. The most conspicuous increase in phosphatase activity could be observed in the liver, which is negative otherwise, i. e. activity is limited to the bile capillaries and to the walls of the vessels. In other organs greater enzyme activity could be demonstrated in the basal membrane of the testis, in spermiums, in the bronchial epithelium, in the alveolar edematous fluid and in desquamated alveolar cells of the lungs. Changes in activity were more difficult to evaluate in other organs (autolysis of the intestines, difficulties in decalcination in the bone marrow, great activity in the kidney of normal animals etc. :)

*Conlusions.* The increase in the phosphatase activity of the liver on irradiation seems to account for the hypoglykemic conditions observed in connection with irradiations and for the disappearance of liver glycogen in experimental animals. The increase in the phosphatase activity of the liver, on the other hand, may be brought in connection with the depolymerisation of thymonucleohiston due to irradiation, which presents itself in the form of an enzyme effect.

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# ИЗМЕНЕНИЕ АКТИВНОСТИ ЩЕЛОЧНОГО ФОЗФАГАЗА В ОРГАНАХ КРЫС УБИТЫХ РЕНТГЕНОВСКИМИ ЛУЧАМИ

Др. К. Рев, др. Л. Рев, др. В. Вартерес

Резюме

В наших опытах мы исползовали крыс, из-за их способности синтетизировать витамин с. Мы подвергли крыс действию рентгеновских лучей, количеством от **2000—4000 P**, поданных сразу или по частям. (180 к **V**, 18 мА, **0,5 Cu**, 50 см). На каждое подопытное животное мы исползовали по одному контрольному животному. В части органов мы нашли значительное повышение активности фозфагаза. Бросается в глаза прежде всего проявление активности фозфагаза в печеночной ткани, где она обычно отсутствует, или-же ограничивается на желчные капилляры и на стенку сосудов из вышеизложенных следует: повышение активности фозфагаза в почени под действием лучей по видимому объясняет, гипергликемические состояния, наблюдаемые под влиянием лучей и изчезнование гликогена в печени подопытных животных. Повышение активности фозфагаза в печени повидимому стоит в связи со деполимеризацией тимонуклеогистона, происходящей под влиянием лучей и являющейся следствием действия энзима.