HISTOCHEMICAL CHANGES IN RAT LIVER CELLS AFTER INTRODUCTION OF A CARCINOGEN (o-AMINOAZOTOLUENE)

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We showed in our previous paper [1] that a close correlation exists between alkaline phosphatase activity and content of basophilic substance in liver cells in the early stages of malignization. It was found that during the first 4-5 months of feeding a diet containing o-aminoazotoluene there was a lowering of alkaline phosphatase activity of the nucleus and nucleoli of liver cells, together with almost complete disappearance of basophilic substances from the cytoplasm and nucleoli of these cells.

The content of basophilic substances increases considerably in the later stages of malignization (6-8 months), and this is associated with a rise in alkaline phosphatase activity in the cytoplasm and nucleoli of the liver cells.

The present paper is devoted to a study of the alkaline phosphatase activity and content of basophilic substances in liver cells at late stages of malignization (10-22 months).

EXPERIMENTAL METHODS

The experiments were performed on albino rats of both sexes, weighing 150-200 g. The animals were given a synthetic diet [1], to which a daily addition of 0.001 ml of 6% o-aminoazotoluene in sunflower seed oil was given to 96 test rats, but not to 55 control rats.

The experiments were conducted for from 10 to 22 months, over which time a group of test rats was killed every month, together with a group of controls. Fragments of liver were fixed in cooled 80% alcohol and embedded in paraffin. Alkaline phosphatase was determined by the Gomori method, as modified by Bizel. Liver slices 6-7μ thick, taken for the controls, were incubated in a medium not containing glycerophosphate, or else the enzyme was first inactivated by boiling in distilled water for 3 minutes.

Ribonucleic acid was determined by Brachet's method. The sections were stained with methyl green and pyronine, as were also the control preparations previously treated with ribonuclease.

EXPERIMENTAL RESULTS

Examination of sections stained with methyl green and pyronine shows that the liver cells of control rats are rich in basophilic substances (Fig. 1, a), which are present in the nucleoli, where they stain diffusely with pyronine, and in the cytoplasm, as small aggregates and granules.

After treatment with ribonuclease the basophilic substances disappear from the cytoplasm and nucleoli of liver cells; it may hence be concluded that the basophilic properties of the cells are due to ribonucleic acid.

Alkaline phosphatase of the liver cells of the control rats was found basically in the nucleus and nucleoli (Fig. 2, a). The activity in the nuclei was inconsiderable, being localized chiefly in the nuclear membrane at

sites of chromatin distribution. The nucleoli exhibit stronger phosphatase activity, staining diffusely. Alkaline phosphatase is usually absent from the cytoplasm, or else there is only very little of it.

Histological study of liver cells at an advanced stage of malignization (10-22 months) showed that the material fell into two groups: one, of 81 rats, in which the liver cells were rich in basophilic substances, and the other in which they are practically absent (15 rats).

In the first group the liver was enlarged and of firm consistency, with an uneven surface covered with protuberances of pale rose color, about the size of a millet seed; discrete areas of calcification were evident. Microscopic examination of liver sections from animals of this group showed that after feeding with o-aminoazo toluene for 10-12 months the parenchyma cells had an increased content of basophilic substances, and this was particularly marked in the peripheral zone and in the perivascular cells. It was possible at this stage to recognize separate cells or groups of cells in process of breakdown, although no definite necrotic foci were as yet present. All the lobules contained cells rich in basophilic substances distributed as large aggregates throughout the cytoplasm, and also present in considerable amount in the nucleoli (Fig. 1, b). Atypical cells are plentiful; these may be large or very small, and their cytoplasm and enlarged nucleoli are rich in basophilic substances. Cells with two nuclei are numerous, and cells with three or more nuclei may be encountered.

The blood vessels and bile ducts of the hepatic parenchyma are dilated.

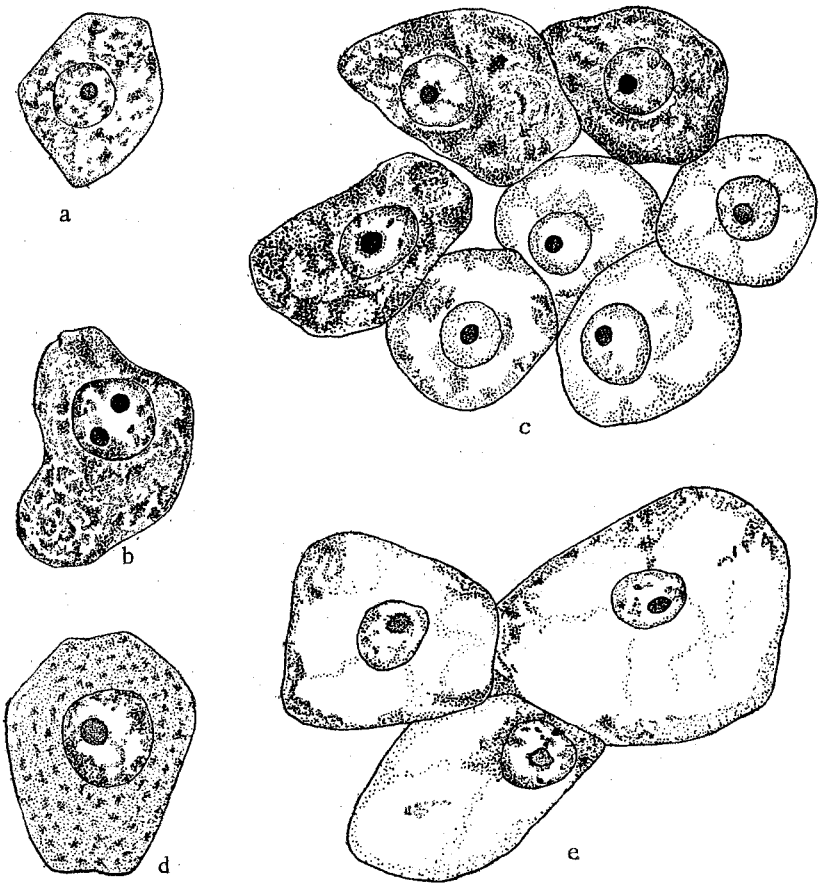

Fig. 1. L ver cells of rats fed with o-aminoazotoluene (basophile staining). a) normal liver cell, b) after 10 months, c) after 12 months, d) liver cells of rats dying after 22 months of feeding, e) after 10 months. Fixed in 80% alcohol. Stained by the Unna-Pappenheim procedure.

The phosphatase activity of the liver cells was found to be much higher than for control animals (Fig. 2, b). The nucleoli are particularly rich in phosphatase, which is uniformly distributed throughout the cytoplasm and the nucleoli. Alkaline phosphatase is found as aggregates in the nucleus, chiefly on the membrane and around the nucleoli. Cells very rich in alkaline phosphatase may be encountered near the blood vessels, although they are sometimes also seen at a distance from them. Liver cells not containing phosphatase may also be seen.

In the later stages of the experiment (12-22 months) clearly demarcated areas are to be seen, containing necrotic cells; these areas are interconnected by means of narrow protoplasmic isthmi. The cell nuclei are in various stages of breakdown, and are missing from many of the cells, and binuclear cells are seen more frequently than during the earlier stages of malignization. The blood vessels and bile ducts are considerably enlarged.

Peripheral zone cells and perivascular cells are very rich in basophilic substances. The nucleoli of such cells are enlarged, and have a high content of basophilic substances.

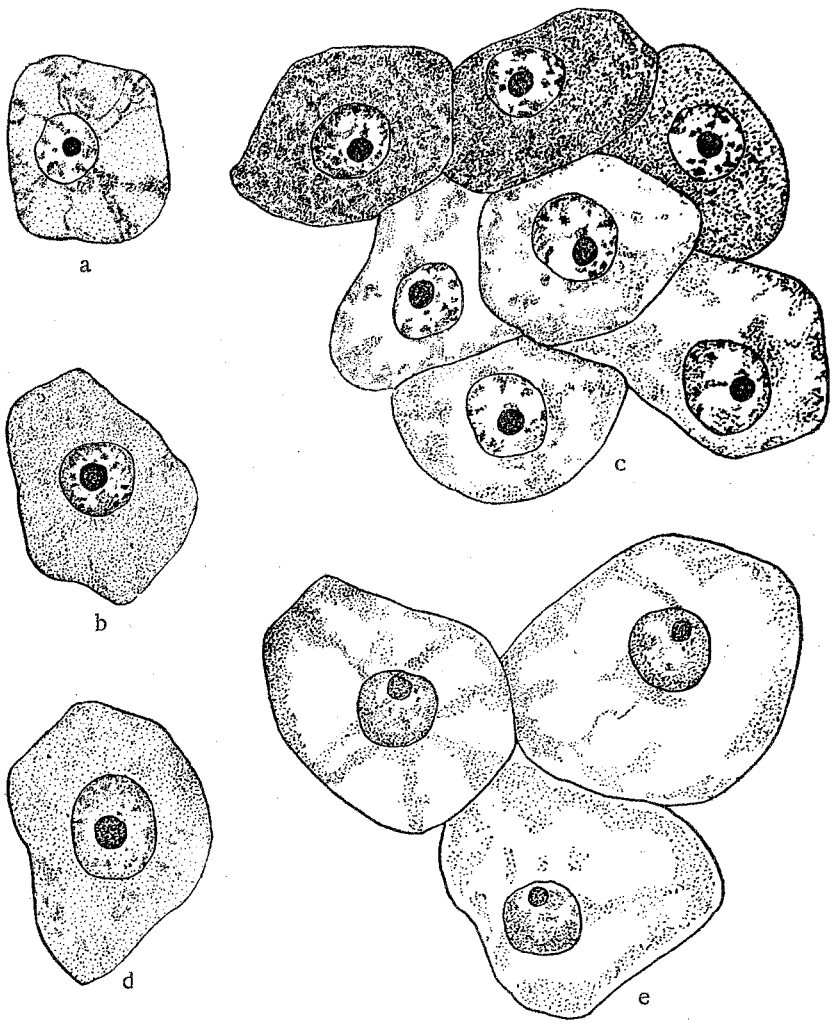

Fig. 2. Liver cells of rats fed with o-aminoazotoluene. Alkaline phosphatase reaction. a) normal liver cells, b) after 10 months, c) after 12 months, d) liver cells of rats dying after 22 months of feeding, e) after 10 months. Fixed in 80% alcohol. Stained by Gomori's method.

Basophilic substances are not seen in the cytoplasm and nucleoli of degenerate cells from malignant nodules. Cells in the earliest stages of degeneration still contain a certain amount of basophilic substances, distributed as large aggregates on the nuclear membrane and around the weakly basophilic nucleoli. Cells from the liver tissue surrounding the necrotic nodules contain considerable amounts of basophilic substances in the cytoplasm and the nucleoli (Fig. 1, c).

Groups of cells are to be found in all the hepatic lobes which are rich in basophilic substances, and these groups may alternate with fairly large groups of cells with a smaller content of basophilic substances in the cytoplasms and the nucleoli.

The cells of such areas frequently contain vacuoles, due probably to fatty degeneration processes. In contrast to the above described nodules, these areas do not contain conglomerates of cells, and are not sharply demarcated from the surrounding tissue; moreover, they contain individual cells with a high content of basophilic substances.

The cells of the necrotic nodules contain only a small amount of alkaline phosphatase, localized in the nucleoli and the nuclear membrane (Fig. 2, c).

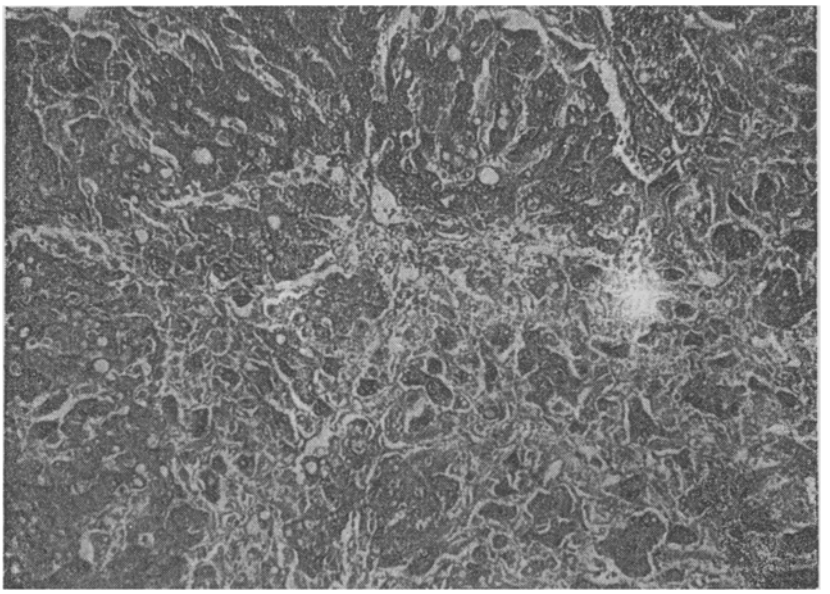

Fig. 3. Hepatic cirrhosis in rats fed with o-aminoazotoluene for 12 months. Photomicrograph: magnification, objective 40, ocular 10. Mallory stain.

The cells surrounding these nodules, and the perivascular cells, are rich in phosphatase, which is localized in the nuclear membrane, the chromatin, and the nucleoli. It is absent from foci of fatty degeneration, or present in only very small amount.

Some rats exhibit advanced hepatic cirrhosis after 12-22 months of feeding with o-aminoazotoluene. Connective tissue strands, containing numerous cellular elements, divide the liver tissue into separate sections (Fig. 3). The surviving liver cells of such sections usually contain a moderate amount of basophilic substances, but their alkaline phosphatase activity is inconsiderable. The distribution of the basophilic granules and of the alkaline phosphatase is similar to that described above.

The structure of the hepatic cords is highly disordered in rats dying during the experiment. The cell outlines are blurred, and the liver cells appear like a syncytium.

Marked cirrhotic changes are evident in many cases. The connective tissue septa and the spaces between liver cells are infiltrated with round cells and with blood elements. The blood vessels and bile ducts are dilated.

Basophilic substances in the cytoplasm appear as small droplets or aggregates, filling the whole cell. The nucleoli are weakly stained by pyronine (Fig. 1, d).

The phosphatase activity of the liver cells is small; the enzyme is diffusely distributed in the cytoplasm, nucleus, and nucleoli (Fig. 2, d).

The second group of rats included animals whose liver had a yellowish-red color, a smooth surface, and a soft consistency. Basophilic substances were practically absent over the whole period from 10 to 22 months. The small amount present is distributed near the cell membrane and at the nuclear membrane, in the form of large aggregates; a small amount can be found in the nucleoli, which stain faintly with pyronine (Fig. 1, e).

A considerable part of the liver cell cytoplasm is occupied by large vacuoles, joined to each other by narrow protoplasmic bridges.

Phosphatase activity is very small in such cells. The enzyme is found only on the nuclear membrane and in the vacuolized cells (Fig. 2, e).

It appears from our results that in the first group of animals increase in the content of basophilic substances, with simultaneous rise in alkaline phosphatase activity of the liver cells, is to be found only up to 10-12 months of feeding with o-aminoazotoluene, i.e., during the process of development of the hepatoma.

At more advanced stages of malignization (12-22 months) we were not able to establish any close correlation between the content of basophilic substances and phosphatase activity. This is probably a result of necrotic changes of the tumor tissue, such as presence of cells containing fluids and nuclear debris, infiltration of blood cells, and in some cases proliferation of connective tissue.

The considerable fall in content of basophilic substances observed in liver cells of the second group of animals is not, in our opinion, connected with development of the tumor; further research will, however, be required for the elucidation of this problem.

It may be concluded from our experimental results that liver ribonucleic acid and alkaline phosphatase participate in general metabolic processes connected with intensified synthesis of proteins in development of hepatomata.

LITERATURE CITED

[1] Z. A. Ryabinina, Bull. Exptl. Biol. Med., 1951, 11, 409-412.