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 5% 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 6-7 μ 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-aminooazotoluene 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](image-url)

Fig. 1. Liver cells of rats fed with o-aminooazotoluene (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.