EFFECT OF CARCINOGENIC AND NON-CARCINOGENIC AMINONITRO COMPOUNDS ON THE ULTRAVIOLET AND BLUE FLUORESCENCE OF TADPOLE LIVER CELLS

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From a theoretical and practical viewpoint, the morphologic and functional changes in cells which arise in the earliest period of action of carcinogens are extremely important. Such morphological changes in cells have not been detectable to the present time, but much indirect data indicate the possibility of their existence [8, 11, 18].

The method of ultraviolet luminescence microscopy is effective for investigation of early changes in cells acted upon by some aminoazo dyes [2, 5]. The high sensitivity of this method has been demonstrated in many articles: detection of characteristic changes in the intensity of UV fluorescence by liver cells in embryogenesis [9], cells of various tissue cultures during mitotic division [6], blood cells after irradiation injury [1, 3, 10].

Comparison of the course of changes in the intensity of UV and blue fluorescence of liver cells acted upon by the aforesaid 2 substances is of interest for the following reasons. There are data that carcinogenic substances act, in the first place, on mitochondria [16, 17, 19, 20]. On the other hand it has been established [14, 15, 21] that the intensity of blue fluorescence dependent on reduced diphosphopyridinucleotide (DPN-H) characterizes the degree of oxidation-reduction processes in the mitochondria. Mitochondria also possess a marked UV fluorescence, which in its turn permits the hypothesis of a correlation between this fluorescence and cellular processes of respiration. Therefore, the study of changes in UV and blue cellular fluorescence of cells treated with carcinogenic substances permits certain conclusions about the action of these substances on the cellular respiratory system in the process of carcinogenesis.

The aim of this article was to study the changes in UV fluorescence of tadpole liver cells treated with 2 related aminoazo compounds: orthoaminoazotoluol (OAAT) and diethylaminoazobenzol (DEAB); the first of these is a carcinogen and causes formation of liver tumors in mice and rats, the 2nd has no carcinogenic activity [8, 22].

METHODS

Tadpole liver cells were studied by 2 methods: 1) by obtaining microphotography of cell fluorescence in UV light and in transmitted UV light and 2) by photo-measurement of UV and blue fluorescence.

The experiments were performed on tadpoles of Rana temporaria at the 27-28th stage of developement [12]. The aminoazo compounds used were fed to the tadpoles once as an 0.1% mixture in egg yolk; the dye crystals were dissolved in a glass mortar in egg yolk until a completely homogeneous mass was obtained. The food containing the azo dye was given ad libitum to the tadpoles for 15 min to 5 h. After stopping contact with the test substances the tadpoles were killed, the liver removed and rapidly placed in 0.01 M versene solution in physiological solution. Then a suspension of liver cells was placed on the quartz objective glass. The photometry and photography techniques for such preparations for UV fluorescence have been described in detail elsewhere [1, 2, 4, 5]. A feature of the present work was the carrying out of parallel observations in UV light and blue light fluorescence on the very same cells by using apparatus equipped with changeable light filters. To stimulate UV fluorescence we used a gas light filter, which transmits the spectral regions 25-280 millimicrons in wave length, and before the photomultiplier,

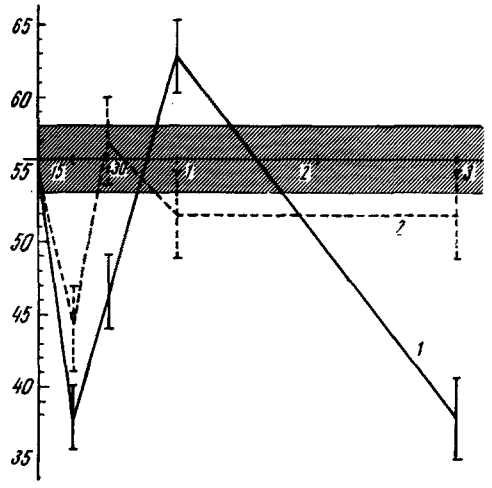

Fig. 1. Dynamics of change in ultraviolet fluorescence intensity. On abscissa – time of contact with aminoazodyes, on ordinate – intensity of fluorescence under unit conditions (galvanometer scale divisions). Curves are constructed from mean values, vertical lines signifying limits of confidence interval; area of confidence interval for normal liver cells (control) cross-hatched. 1) OAAT; 2) DEAB.

a filter for the region 320-380 millimicrons. In order to stimulate blue fluorescence a light filter was used which transmits wave length 350-370 millimicrons and in front of the photomultiplier, 420-450 millimicrons.

Tadpole liver cells may vary in size and structural detail; therefore, we chose the most typical ones for photometry, one which did not vary morphologically. The values for intensity of fluorescence obtained by cytophotometry, were subjected to statistical treatment.

RESULTS

Despite the constant variations in intensity of fluorescence of individual cells, the mean values for intensity of UV fluorescence obtained on different days for control tadpoles varied very little. This applied equally to blue fluorescence as well.

In Figs. 1 and 2 the dynamics of changes in UV and blue fluorescence of tadpole liver cells acted upon aminoazodyes are presented in graph form. In Fig. 3 are microphotographs of liver cells in the light of their UV fluorescence at different periods of the experiment. As seen from these figures, already after 15 min feeding of aminoazo dyes a sharp, statistically valid fall in the intensity of UV fluorescence of liver cells is observed with OAAT evoking more marked fall in fluorescence than DEAB. Change in the intensity of fluorescence clearly appears also in the microphotographs taken in the light of the UV cellular fluorescence (Fig. 3b). Marked changes in the structure of liver cells did not occur: only a slight decrease in the brightness of the fluorescence of the small dustlike and larger granules (evidently mitochondria in the cytoplasm) was observed.

After 30 min of feeding the azocompounds a rise in the intensity of UV fluorescence of the cells is observed, weaker with the action of OAAT than with DEAB, with which the fluorescence reaches a normal level. Marked changes in cellular structure were not noted in this time period (Fig. 3c).

After feeding azodyes for 1 h, the difference in action of OAAT and DEAB is preserved: with OAAT a sharp rise in the intensity of UV fluorescence which actually exceeds normal levels, is observed; DEAB essentially does not alter the liver cell fluorescence and it almost remains within the values characteristic for the control (see Fig. 1). At this time changes in the fine structure of the liver cells are revealed in the experiments with OAAT: against a background of small, dust-like fluorescent granules in the cytoplasm, swollen round, clearly shining granules of mitochondria are observed; they are decreased in number (Fig. 3d). DEAB also evokes some mitochondrial swelling but their number and fluorescence are unchanged.

Feeding DEAB for 3 h did not provoke changes in the intensity of UV fluorescence – it remained at a level close to control values. At the same time, 3 h after feeding OAAT a significant fall in the intensity of UV fluorescence was observed. Liver cells of tadpoles which had received OAAT were also clearly distinguished by morphology: their cytoplasm was somewhat swollen, the number of fluorescing granules in the cytoplasm was further diminished; mainly small, dustlike fluorescent structures were seen in the cytoplasm. In liver cells of tadpoles which had received DEAB only some swelling of the cytoplasm and mitochondria could be detected.

Thus, a marked, statistically valid difference in the effect upon UV fluorescence of tadpole liver cells from carcinogenic and non-carcinogenic animoazo compounds is observed; these differences are detectable in each of the periods studied but are particularly demonstrable upon comparison of the curves which express the total dynamics of changes in the intensity of fluorescence with time in the experiment.

If we represent the dynamics of changes in blue fluorescence intensity over time as affected by OAAT and DEAB (see Fig. 2), then the curves obtained appear extremely similar to those examined above for UV fluorescence (see Fig. 1).

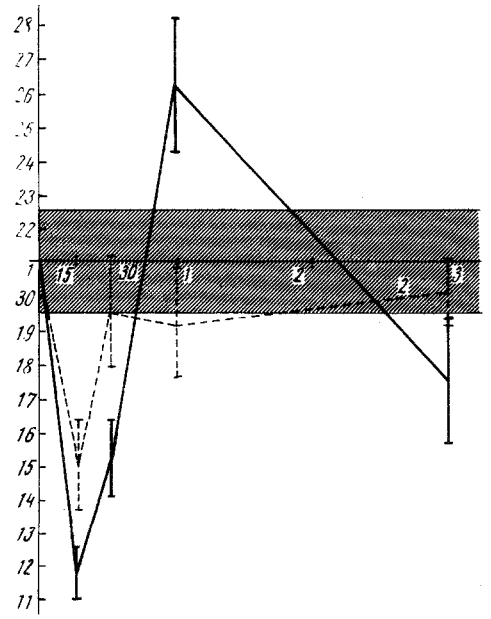

Fig. 2. Dynamics of changes in blue fluorescence intensity. Graph construction and designations same as in Fig. 1.

In conclusion, we observed still another feature of the action of aminoazo compounds on tadpole liver, which, although it is not related to cellular luminescence, represents, in our opinion, a certain interest. After feeding of aminoazodyes, the liver cells undergo division considerably more easily than in the control, the situation arising that OAAT more strongly than DEAB breaks intercellular bonds.

The data obtained indicate that already at the early period of action of aminoazo compounds, definite differences exist in the reaction of tadpole liver cells to OAAT and DEAB. On the basis of these experiments we cannot yet categorically state that our observed data are related directly to the carcinogenic activity of one of the substances used. We may only suggest that such a correlation is possible. For a conclusive resolution of this question it will be necessary to perform experiments with other pairs of related compounds, one of which is a carcinogen. We consider that further investigations by the method of UV fluorescence microscopy on our model and on mammalian cells may lead to clarification of this question.

Change in the intensity of blue fluorescence, as is known, is related to the intensity of cellular respiration or blockade of the respiratory enzyme chain [14, 15, 21]. Change in the intensity of UV fluorescence of the mitochondria, evidently, is also related to the state of several components of the respiratory enzyme chain [4, 7, 13]. Therefore, as a working hypothesis we consider that the difference in reaction of tadpole liver cells to the action of two tested aminoazocompounds is related to the detrimental action of the carcinogenic azodye on the mitochondria, and, consequently, in a broad sense, on cellular respiration.

SUMMARY

Model experiments with feeding tadpoles with carcinogenic and non-carcinogenic aminonitrocompounds revealed differences in the intensity of the ultraviolet and blue fluorescence of liver cells and in their fine morphology at early periods (15 min, 30 min, 1 h and 3 h) of contact with the tested substance. The authors suppose that these differences are due to the specific effect of a carcinogenic compound on mitochondria and cellular respiration.

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