# ELECTROPHORETIC ANALYSIS OF RAT SERUM PROTEINS IN THE EARLY STAGES OF EXPERIMENTAL CARCINOGENESIS

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Administration of dimethylaminoazobenzene to albino rats for 60-100 days causes an increase in the concentration of $\gamma$-globulins and a decrease in the concentration of albumins in the blood serum.

Carcinogenic substances are known to be bound by the proteins of the tissues in which a tumor arises [9]. The compounds formed by tissue proteins with carcinogens are abnormal antigens [1-5]. Antibody formation against abnormal antigens has not yet been investigated. Meanwhile, some investigators [6-8] consider that such antibodies play an important role in the process of tumor development.

It is possible that electrophoretic analysis of the serum proteins of animals in the early stages of carcinogenesis may reveal changes in the protein fractions characteristic of the process of antibody formation. The possibility is not ruled out that abnormal antigens formed in the tissues by the action of a carcinogen may enter the blood and that the changes in the serum protein fractions taking place as a result may also be detected by electrophoretic investigation of the serum proteins.

The object of the present investigation was to study the state of the serum protein fractions in the blood of rats during administration of the hepatotropic carcinogen dimethylaminoazobenzene, using the method of electrophoresis in agar-agar.

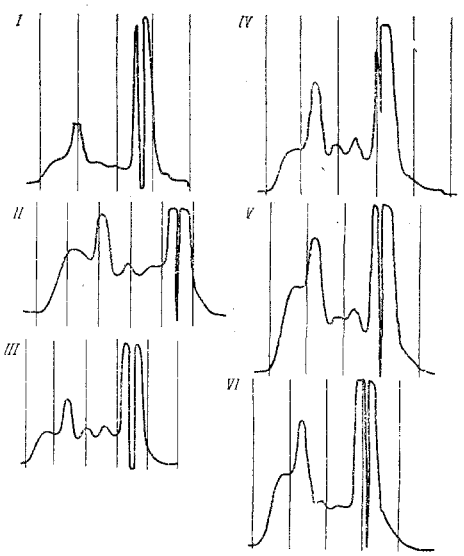


Fig. 1. Densitograms of serum proteins of normal rats (I) and rats receiving carcinogen for 4 (II), 15 (III), 30 (IV), 60 (V), and 100 (VI) days.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 120-140 g. The experimental animals received the carcinogen with the diet in a daily dose of 10 mg, while control animals received a normal diet without addition of the carcinogen. The rats were sacrificed by decapitation 4, 15, 30, 60, and 100 days after the beginning of administration of the carcinogen. Blood serum was obtained by the usual method and fractionated by electrophoresis. The sera of 10-15 rats at the same stage of the experiment were pooled and investigated as a single specimen of serum. For electrophoretic fractionation of the serum proteins, glass plates measuring 9 × 12 cm were covered with 1% agar solution in borate buffer, pH 8.6, to form a layer 2 mm thick. Gutters measuring 1 × 0.2 cm were cut out of this layer of agar in the center of the plate, one above the other. The test sera were introduced into the gutters in a volume of 0.01 ml. Electrophoresis was carried out in borate buffer, pH 8.6, with a falling voltage gradient of 8.3 V/cm and a current gradient of 3.3 mA/cm for 2.5-3 h. The plates were fixed in 2% acetic acid solution in 60° ethanol for 4-6 h. They were then placed in a solution in amido black 10B for 24 h. Dye not bound by proteins was removed by washing with 2% acetic acid. The plates were dried at room temperature. For quantitative estimation of the fractions, the plates were examined with the MF-4

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TABLE 1. Serum Protein Fractions of Normal Rats and Rats Receiving Carcinogen (in %)

| Stages of experiment | No. of experiments | Fraction moving toward anode | Fraction at starting line | Fractions moving toward cathode | | | | |
|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI |
| Normal | 9 | 12,1±0,86 | 31,7±0,87 | 9,8±1,58 | 8,0±1,12 | 19,4±0,83 | 19,8±1,19 |
| 4 days | 4 | 11,4±0,5 | 26,6±0,04 | 9,8±0,58 | 10,5±0,53 | 17,9±0,9 | 23,5±0,5 |
| | | $P=0,5—0,4$ | $P=0,05—0,02$ | — | $P=0,05$ | $P=0,3—0,2$ | $P=0,02—0,01$ |
| 15 days | 6 | 10,3±1,17 | 29,3±1,74 | 12,7±0,88 | 10,3±0,6 | 16,9±0,56 | 20,1±0,88 |
| | | $P=0,3—0,2$ | $P=0,3—0,2$ | $P=0,2—0,1$ | $P=0,1—0,05$ | $P=0,05—0,02$ | $P=0,8$ |
| 30 » | 12 | 10,9±1,03 | 29,6±2,44 | 8,8±0,74 | 8,3±0,59 | 19,1±0,61 | 20,4±1,07 |
| | | $P=0,4$ | $P=0,4$ | $P=0,5$ | $P=0,8$ | $P=0,8—0,7$ | $P=0,9$ |
| 60 » | 9 | 9,2±0,89 | 32,4±1,24 | 11,4±1,0 | 9,0±2,39 | 18,7±1,14 | 19,2±1,49 |
| | | $P=0,05—0,02$ | $P=0,7—0,6$ | $P=0,4$ | $P=0,7—0,6$ | $P=0,7—0,6$ | $P=0,8—0,7$ |
| 100 » | 9 | 8,16±0,84 | 32,5±1,54 | 8,7±1,09 | 8,2±0,61 | 17,7±0,92 | 23,28±0,9 |
| | | $P=0,01$ | $P=0,7—0,6$ | $P=0,6—0,5$ | $P=0,9—0,8$ | $P=0,2—0,1$ | $P=0,05—0,02$ |

microphotometer. The protein content in the fractions was expressed in percent. Significance of the changes discovered was assessed by the Fisher–Student method. Differences were regarded as significant for which P = 0.05.

## EXPERIMENTAL RESULTS

The experimental results are given in Fig. 1 and Table 1. The blood sera of the normal and experimental rats were separated into 6 protein fractions. One of the fractions moving toward the anode evidently consisted of albumins, and another remaining at the starting line, evidently of $\alpha$-globulins, while the 4 other fractions moving toward the cathode were evidently $\beta_1$-, $\beta_2$-, $\gamma_1$-, and $\gamma_2$-globulins. When expressed as percentages, the $\alpha$-globulin fractions gave the highest values and the $\beta$-globulins the lowest. With administration of the carcinogen, changes in the isolated protein fractions were observed. The albumin content of the serum fell during administration of the carcinogen for 60-100 days. The level of the $\alpha$-globulins also fell on the 4th day of the experiment. The content of $\beta_2$-globulins increased on the 4th day while that of the $\gamma_1$-globulins fell on the 15th day of the experiment, and the content of $\gamma_2$-globulins increased both on the 4th and the 100th days of the experiment. Statistical analysis showed that all these changes were significant. It may be that the observed changes in the serum $\gamma$-globulins of the experimental animals on the 4th and 100th days of the experiment are evidence of antibody formation at these periods of carcinogenesis. It is difficult to account for the other changes in the protein fractions.

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