# CYTOPLASMIC ANTIGENS OF THE LIVER

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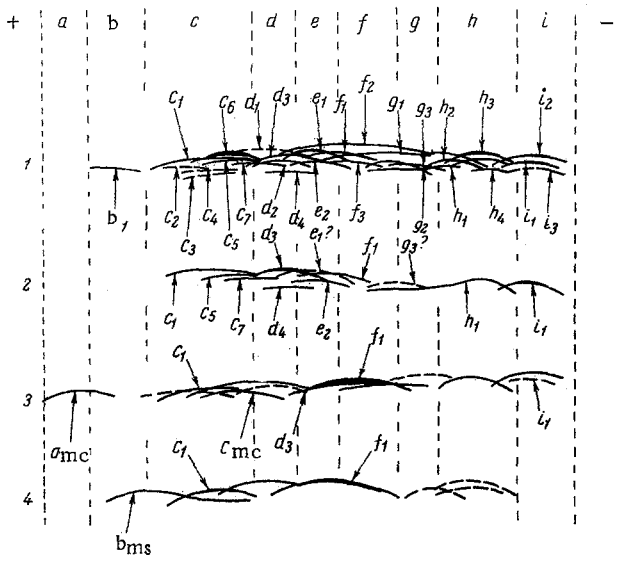
Information about the antigenic spectrum of the cells and tissues of the body is essential when studying the nature of tissue incompatibility, the mechanisms of formation of immunologic tolerance, the development of autoimmune conflicts, and so on. The characteristics of organ-specific antigens may also be very useful in the analysis of differentiation, morphogenisis, and malignant degeneration of cells taking place in the living body [2, 4, 7, 13, 15]. Despite many publications on these problems, it is only recently that the complexity of the antigenic spectrum and the multiplicity of organ-specific antigens of certain organs have been established immunochemically [1, 5, 6, 8, 12, 16].

The object of this investigation was to study the characteristics of the cytoplasmic proteins of the liver.

## EXPERIMENTAL METHOD

Male August rats weighing 165-180 g were fasted before the experiment for 24 h. Under Nembutal anesthesia the liver was washed free from blood by perfusion through the thoracic aorta with cold buffered physiological saline (0.035 M Tris buffer, pH 7.4-7.5; 0.25 M sucrose, 0.025 M KCl, 0.005 M $MgCl_2$). The liver was minced in the cold and homogenized with five volumes of the same buffer solution in a glass homogenizer with a Teflon pestle.

After separation of the nuclei, fragments, and whole cells (by centrifugation for 40 min at 1500 g) the fractions of mitochondria, microsomes, and cell juice were obtained by preparative centrifugation of the homogenate on a Spinco-L untracentrifuge under the following conditions respectively: 15 min at 15000 g and 90 min at 105000 g. After repeated washing of the fractions, the membrane proteins of the mitochondria and microsomes were extracted by treating the cytoplasmic granules with 0.5% sodium desoxycholate. The subfractions thus obtained were dialyzed against the same buffer solution and concentrated, and after determination of the protein content [10], they were used for immunization of 4-5 rabbits with each of the antigens. For this purpose the animals received three injections, at intervals of three weeks, of a mixture of 35 mg protein of each fraction together with Freund's complete adjuvant, and the same dose of protein was subsequently given in 5-6 intramuscular injections. The immune globulins in the antisera were freed from some of the ballast proteins and concentrated by




Immunoelectrophoresis of cytoplasmic antigens of rat liver. The antigens used were liver cell juice (1 and 2), membrane proteins of mitochondria (3) and microsomes of the liver (4). Precipitation lines developed by antiserum against liver cell juice exhausted by serum proteins (1) and proteins of other organs (2) and also by sera against mitochondria (3) and microsomes (4) exhausted by rat serum proteins. The arrows pointing to precipitation lines of proteins of cytoplasmic granules (3 and 4) denote only organ-specific antigens.

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Distribution and Some Properties of Antigens of Various Fractions of Liver Cytoplasm

| Protein fractions distinguished by electrophoresis | Relative electrophoretic mobility ($I_X/I_A$) | Antigens and their origin | Characteristics of antigens |
|---|---|---|---|
| a | 1.27–1.18 | Mitochondria, $a_{mc}$ | Fraction- and species-specific antigen detected by sera against mc of various organs |
| b | 1.18–1.02 | Cell juice, $b_1$ | Species-specific antigen present also in other organs |
| | | Microsomes, $b_{ms}$ | Organ- and fraction-specific (microsomes) antigen whose precipitation line continues like a tail into the zone of c-proteins, glycoprotein |
| c | 1.02–0.78 | Cell juice, $C_2$, $C_3$, $C_4$ | Species-specific antigens detected also in other organs |
| | | Cell juice, granules, $C_1$ | Organ-specific antigen present among proteins of granules and cell juice |
| | | Cell juice, $C_5$, $C_7$ | Organ- and fraction-specific (cell juice) antigens |
| | | Cell juice, $C_6$ | Species-specific, thermostable, alcohol-insoluble antigen isolated from a series of organs by Witebsky's method |
| | | Mitochondria, $C_{mc}$ | Organ- and fraction-specific (mitochondria) antigen |
| d | 0.78–0.65 | Microsomes, mitochondria, cell juice, $d_1$ | Species-specific antigen, readily detected among membrane proteins of mitochondria and microsomes of several organs but with difficulty in cell juice |
| | | Cell juice, $d_2$ | Species- and fraction-specific (cell juice) antigen |
| | | $d_4$ | Organ- and fraction-specific (cell juice) antigen |
| | | $d_3$ | Organ- and fraction-specific (cell juice) thermostable alcohol-insoluble antigen separated by Witebsky's method and present in mitochondria as traces |
| e | 0.65–0.55 | Cell juice, $e_1$, $e_2$ | Organ- and fraction-specific (cell juice) antigens |
| f | 0.55–0.42 | Microsomes, mitochondria, $f_1$ | Organ-specific antigen predominant among proteins of mc and ms but detected with difficulty in hyaloplasm of liver, glycoprotein |
| | | Cell juice, mc–$f_2$ | Species-specific antigen heterogeneous as regards electrophoretic mobility |
| | | $f_3$ | Species-specific antigen |
| g | 0.42–0.34 | Cell juice, $g_1$ | Species- and fraction-specific (cell juice) antigen, immunologically homogeneous, but heterogeneous as regards electrophoretic mobility |
| | | $g_2$ | Possesses catalase activity, readily detected among proteins of cell juice, less so in mitochondrial fractions |
| | | $g_3$ | Species- and fraction-specific (cell juice) lipoprotein |
| h | 0.34–0.14 | Cell juice, $h_1$ | Organ- and fraction-specific (cell juice) antigen whose electrophoretically fast tail migrates with f-proteins |
| | | $h_2$, $h_3$, $h_4$ | Species-specific antigens of cell juice also detected among proteins of cytoplasmic granules |
| i | 0.14– | Cell juice, $i_1$ | Organ- and fraction-specific (cell juice) antigen, glycoprotein (?) |
| | | Cell juice, mc, $i_2$, $i_3$ | Species-specific antigens well represented among proteins of cell juice and less so in mitochondria |

Legend: mc–fraction of mitochondria, ms–fraction of microsomes. $I_X$–electrophoretic mobility of test antigens in Difco agar gel, $I_A$–electrophoretic mobility of human serum albumin in the same gel.

salting out with ammonium sulfate. By addition of increasing doses of lyophilized protein preparations of blood serum, erythrocytes, kidney, spleen, and lungs and by removal of the precipitate thus formed, the immune sera were then exhausted until all antibodies against these organs had been removed, as verified by the ring-precipitation test and by Ouchterlony's gel-diffusion reaction, and they were then again concentrated by drying in a current of cold air or from the frozen state. The liver proteins were then subjected to electrophoresis and immunoelectrophoresis in 1% agar gel (Difco) along the lesser diameter of glass dishes measuring 13 × 18 cm, cooled during the experiment with tap water (using medinal buffer, pH 8.2, $\mu$ 0.05 as electrolyte, voltage gradient 9 V/cm). After electrophoresis the precipitation lines were developed by immune sera against the same and other cytoplasmic fractions of the liver. Fraction-specific antigens were determined by treating the agar gel with sera cross-exhausted with proteins of the other fractions and with serum proteins. The precipitation lines of some antigens were also identified by their enzymic activity (catalase, peroxidase) or by staining for glycoproteins and lipoproteins [19]. The "organ-specific BE (boiled ethanol insoluble) antigens" were separated from the heated liver homogenate by alcohol precipitation as described by Witebsky [11].

## EXPERIMENTAL RESULTS

During electrophoretic analysis the cytoplasmic proteins of the liver were separated into 9-10 fractions with different electrophoretic mobility (zone of a-i proteins). More prolonged separation showed that among the h-proteins of the hyaloplasm there were two further subfractions. In analogous experiments, the identical proteinograms of the water-soluble proteins of the mitochondria and microsomes were no less complex than the proteinograms of the cell juice proteins, although electrophoretically faster components were well represented in the former, and slow components in the latter.

Immunoelectrophoretic investigation of the cytoplasmic granules revealed more serum proteins (albumins, $\alpha_1$-, $\alpha_2$, and $\beta_1$-globulins) than in the cell juice, and this may perhaps account for predominance of electrophoretically fast subfractions among the proteins of the cytoplasmic granules. By means of rabbit anti-liver sera exhausted with blood proteins, the largest number of antigens (26-27) was found among the proteins of the mitochondria and microsomes. The results of the comparative immunochemical investigation of the proteins of these fractions are represented schematically in the figure; more detailed information of the individual antigens is given in the table.

All the antigens detected by immunophoresis by means of rabbit sera may be regarded as species-specific antigens characteristic of rats and absent in rabbits. The probability that some rat proteins are identical with intracellular rabbit antigens and that the latter possess autoantigenic properties was excluded by special experiments in which an immunoelectrophoretic analysis was made of the tissue proteins of an immune rabbit by means of autoserum.

As a result of these experiments, among the species-specific antigens of rat liver, proteins possessing organ- and fraction-specificity were detected by means of cross-exhausted sera. The relationships between them were highly intricate and complex: some fraction-specific antigens were not organ-specific liver antigens were not connected with any particular fraction and could be detected in the proteins of the cell juice and also in the cytoplasmic granules. Contrary to the view that a high content of organ-specific antigens is found in the fraction consisting of proteins of cytoplasmic granules [4, 14, 15, 19], in our experiments a great variety of organ-specific antigens was demonstrated in the cell juice of the rat liver. Among the membrane proteins of microsomes and mitochondria only one or two simultaneously organ- and fraction-specific antigens and only a few of the organ-specific antigens widely represented in the liver cell juice fraction could be detected (see figure).

These results may be explained by the high specialization of the cytoplasmic granules in cells performing similar functions in the cells of different organs and even of different species of animals. This is confirmed by the fact that in a cell-free system the species specificity of the serum albumin synthesized by the liver microsomes is determined by the origin of added messenger RNA, whereas microsomes synthesize albumins of immunologically different species specificity with equal success [17].

Although ribosomal proteins of different origins possess well marked heterogeneity and contain many components [18], in our experiments immunization of rabbits with rat microsomes caused the formation of antibodies predominantly against membrane proteins, thus demonstrating the absence of immunologic differences in the composition of the ribosomal proteins of different species of animals.

The experiments described above showed that a high content of both species-specific and organ-specific antigens is present in liver cell juice. This fraction of the cells may therefore be particularly interesting in connection with immunochemical investigations of certain pathological and physiological processes taking place in the body.

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