DISTRIBUTION OF AZOCARCINOGEN-BINDING PROTEIN IN THE ORGANS OF RATS AND MICE

G. A. Bannikov and T. A. Chipysheva

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Monospecific antibodies against a liver protein binding azocarcinogens in rats were obtained. An antigen identical with this protein was found in extracts of the liver, kidney, small intestine, testis, and ovary of rats and also in extracts of the liver and kidney of mice. The content of this antigen was sharply reduced in the liver and kidney of embryos and of rats in the early postnatal period. In the light of these findings the possible functional role of azocarcinogen-binding protein in the normal cell is discussed.

The study of a protein obtained from rat liver and capable of binding azocarcinogens (ABP) is interesting because of its possible role in hepatocarcinogenesis [10, 11].

In the investigation described below the presence of ABP was tested in the organs of rats and mice at various stages of development by the use of monospecific antibodies.

EXPERIMENTAL METHOD

ABP was obtained by Ketterer's method [8] with slight modifications from the liver of adult rats killed 48 h after receiving 50 mg 3'-methyl-4-dimethylaminoazobenzene (3'-MDAB). Extracts listed in Table 1 were used as antigens. All extracts were obtained by homogenization of tissue in three volumes of distilled water at pH 7.4-7.6. The homogenate was centrifuged for 15 min at 6000 g.

For immunization, the ABP preparation with a protein concentration of 2.5 mg/ml was mixed with an equal volume of Freund's complete adjuvant (Difco) and injected into rabbits in doses of 0.2 ml into the popliteal lymph glands of both hind limbs [7]; the same dose of antigen was injected intravenously into the animals 1 month later. Blood was taken on the 7th, 9th, 11th, and 14th days after the last injection. Two preparations of ABP obtained at different times were used for immunization. Altogether nine animals were immunized.

Monospecific antibodies were obtained as follows: 1) by elution of antibodies from immunosorbents obtained with the aid of glutaraldehyde from extracts of the spleen and kidney [4]; 2) by exhaustion of serum with spleen extract under the control of the precipitation test; γ globulins from the exhausted sera were concentrated by alcoholic precipitation [2] and purified on DEAE-cellulose (DE-52, Whatman) [12]; 3) by decomposition of precipitates formed during successive incubation of the serum with extracts of spleen and kidney, in glycine-HCl buffer (pH 2.4). The material obtained in this way was centrifuged and passed through a column of Sephadex G-200 (3 × 50 cm), equilibrated with the same buffer. During gel-filtration on this column the pure antibodies were separated from soluble antigen–antibody complexes and free antigen. The precipitation test was carried out in the micromodification of Gusev and Tsvetkov [1]. Standard test systems [3] were used to identify the various antigens. Semiquantitative analysis of the antigens was carried out by the method of Khramkova and Abelev [3].

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TABLE 1. Content of Antigen Identical with ABP in Various Tissues of Rats and Mice

Organ	Age of animal	Content of antigen	
		titer	%
Liver	Adult	512	100
	30 days	512	100
	10-12 days	4–64	0.75–12.5
	3 days	2	0.4
	Newborn	8	1.5
	Embryos of different ages	Original dilutation 16	0.2–3
Kidney	Adult	32–128	7.5–25
	30 days	128	25
	10-12 days	4–8	0.75–1.5
	3 days	Original dilutation 2	0.2-0.4
	Newborn	8	1.5
	Embryos of different ages	2–4	0.4–0.75
Small intestine	Adult	Original dilutation 32	0.2–7.5
Testis	Adult	Original dilutation 4	0.2–0.75
Ovary	Adult	Original dilutation 2	0.2–0.4
Liver of C3HA mice	Adult	4–32	0.75–7.5
Kidney of C3HA mice	Adult	4–32	0.75–7.5
Limit of sensitivity		–	0.2

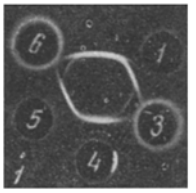
Note: No antigen identical with ABP was found in extracts of the spleen, lung, forestomach, glandular part of the stomach, pancrease, skin, heart muscle, striated muscles, brain, pituitary, thymus, parotid salivary gland, adrenal cortex and medulla, wall of the urinary bladder, thyroid gland and uterus, and also in the serum of the rats.

The carcinogen was determined in precipitates formed by incubation (for 30 min at 37°C) of pure monospecific antibodies with the semipurified preparation of ABP (to obtain this preparation the procedure of purification of ABP was stopped at the stage of filtration of the material through the DEAE-cellulose column at pH 8.0). Doses of antibodies neutralizing 1-2 mg of the semipurified ABP preparation were chosen as a first step in the precipitation test. The resulting preparation, white in color, was washed twice with physiological saline, pH 7.2-7.4. The presence of the carcinogen in the precipitate was determined by dissolving it in 85% formic acid. The appearance of a pink color indicated the presence of the azocarcinogen [13]. The precipitate obtained by incubation of antiserum against bovine serum albumen and a solution of this protein mixed with the semipurified ABP preparation was used for the control.

EXPERIMENTAL RESULTS

Three types of antibodies, not identical in the precipitation test, were found in the immune sera (Fig. 1). Antibodies of type I, which were present in all sera in the highest titer, reacted with the ABP preparation and with liver and kidney extracts to form one common precipitation band. Antibodies of type II were detected in the test with the ABP preparation and with liver and spleen extracts and they also formed a common precipitation line. The tier of antibodies of this type varied in the different sera. Antibodies of type III, which reacted only with liver extracts, were present in three of the nine sera. Two types of antibodies reacting with the ABP preparation were thus found in the sera. Monospecific antibodies of both types were isolated from the serum by different methods. Since the type I antibodies did not react in the precipitation test with spleen extract, the order of isolation was always the same: initially the type II antibodies were extracted from the serum, and the type I later. The highest yield of antibodies was obtained by the use of the method of hydrolysis of the precipitate in acid medium.

The presence of two types of antibodies in the serum reacting with the ABP preparation can be explained as follows: 1) the ABP preparation used for immunization contained two proteins binding the carcinogen, or 2) it contained impurities not binding the carcinogen. To solve this problem the carcino-



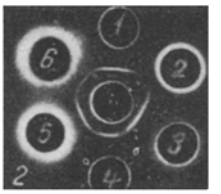


Fig. 1 Fig. 2

Fig. 1. Detection of different types of antibodies in antiserum against ABP. Top well contains ABP preparation; extracts of rat organs: 1,5) kidney; 3, 6) spleen; 4) liver. Central well contains native native ABP antiserum.

Fig. 2. Presence of antigen identical with ABP in extracts of various organs: 1) ABP preparation; extracts of rat organs: 2) spleen, 3) liver, 4) kidney, 5) small intestine, 6) testis. Central well contains monospecific type I antibodies.

gen was determined in precipitates obtained by incubation of whole serum and also of pure monospecific type I and II antibodies with the semipurified ABP preparation. The carcinogen was found only in precipitates formed by the whole serum and type I antibodies with the semipurified ABP preparation. Precipitates formed by type II antibodies and the control precipitates did not give a color with formic acid. Only the type I antibodies thus reacted specifically with the ABP. Antigen detected in the ABP preparation by type II antibodies is evidently an impurity not containing the carcinogen. The type III antibodies were probably explained by accidental impurities in the ABP preparation taken for immunization, for they were completely absent in most of the sera. The results of these investigations, indicating that antiserum against ABP contains antibodies of several types, are in agreement with those obtained by Baldwin et al. [5]. These workers did not obtain a monospecific serum on immunization with ABP isolated by Ketterer's method.

An antigen identical with ABP was discovered with the aid of type I monospecific antibodies in extracts of the liver, kidney, testis, and small intestine of adult rats and also in the liver and kidney of C3HA mice (Fig. 2). The results of semiquantitative analysis of this antigen in the extracts of the various organs are given in Table 1. They show that the content of antigen identical with ABP in extracts of the liver and kidneys of embryos and newborn animals, and also in young rats aged 3, 7, and 12 days is much lower than in extracts of the liver and kidneys of adult rats.

It can be postulated on the basis of these results that ABP is a product of the differentiated cell and that it participates in the postnatal activity of the organ. This gives interest to a report by Litwack et al. [9] that one of the proteins binding a cortisol metabolite is identical with ABP. It is a striking fact that intensive accumulation of labeled corticosteroids during their administration to animals takes place in organs which, as the present experiments show, contain an antigen identical with ABP [6].

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