FORMATION OF ANTIGENIC COMPOSITION OF THE CHICK MESO- AND METANEPHROS DURING ONTOGENESIS

N. G. Andreeva

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Two organ-specific and six inter-organ antigens were detected in the definitive chick kidney. During ontogenesis inter-organ antigens appear first in the kidney tissues, followed by organ-specific antigens.

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To discover the role of immunological factors in morphogenetic processes during tissue transplantation and in many other cases the consecutive study of antigenic differentiation of organs during development is an important subject for study [3]. In this respect the kidneys are a most convenient organ because during ontogenesis they pass through morphologically well-defined stages of pro- meso-, and metanephros, so that changes in antigenic composition of the developing organ can be correlated precisely with changes in its structure. Results of immunological study of kidney tissue differentiation published in the literature are few and fragmentary [5, 8].

We, therefore, decided to investigate the course of formation of the antigenic composition of the chick meso- and metanephros.

EXPERIMENTAL METHOD

Concentrated immune sera against the kidney tissues of adult chickens and obtained from 8 rabbits were used in the experiments. Each serum was concentrated by McErlean's method [7]. To obtain the sera various methods of immunization were used [1]. The most specific sera were those obtained by the method of "blocking secondary antigens." These sera contained only very small amounts of antibodies against one of the serum antigens, which were easily removed by Björklund's method of additional absorption [4], or were free from such antigens. Sera against adult chicken kidney tissues, obtained by this method, as a rule detected both organ-specific and inter-organ antigens [2, 9]. Extracts from the kidneys of 4-, 6-, 8-, 10-, 12-, 14-, 16-, 18-, and 20-day chick embryos were used as antigens (at these times of development of chick embryos the kidneys have the appearance of structures well-differentiated from adjacent tissue, which can easily be separated by ophthalmic forceps), from the kidneys of newborn chicks, and also from the tissues of the kidneys and other organs of fully grown chickens (liver, spleen, heart, lung). Extracts of these tissues were diluted 1 : 5 with Tris-buffer, pH 7.5 (this was the initial dilution used for preparation of all subsequent dilutions). The antigenic composition of the developing kidney was analyzed by the Ouchterlony agar-precipitation reaction [8].

EXPERIMENTAL RESULTS

Serum against the kidney of adult chickens (No. 34) reacted with the initial extract from chicken kidney tissues with the formation of 6 precipitation bands (Fig. 1A and B). The extracts formed up to 7 or 8 precipitation bands with the other sera (Nos. 1, 31, and 1048). In experiments in which the antisera were diluted 1 : 2 and 1 : 4, the number of precipitation bands remained unchanged. The antisera did not react in higher dilutions. When extracts of kidney tissue was titrated with the antisera, no additional precipitation bands were discovered. After absorption of the antisera by Björklund's method not only with normal serum, but also with liver of adult chickens, only 2 of the total number of antigens detected were found to

Laboratory of Immunology of Embryogenesis, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 3, pp. 83-86, March, 1968. Original article submitted December 15, 1966.

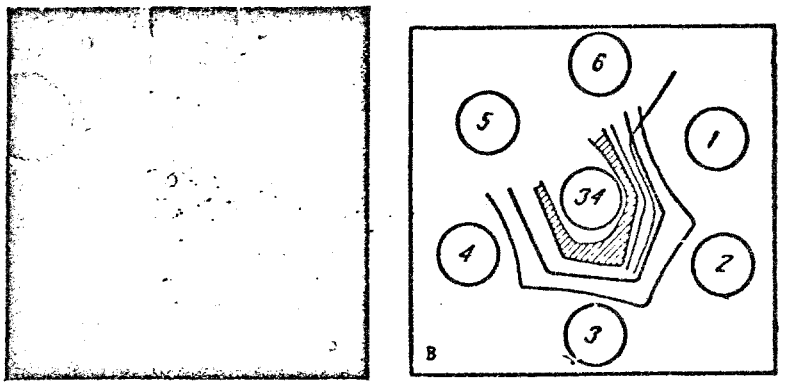

Fig. 1. Gel-precipitation reaction by Ouchterlony's method with antiserum against adult chicken kidney tissues. A) Central well contains antiserum, peripheral wells extracts from kidney (1, 2), and liver (3, 4) of adult chickens; control – normal adult chicken blood serum (5, 6). B) The same represented schematically.

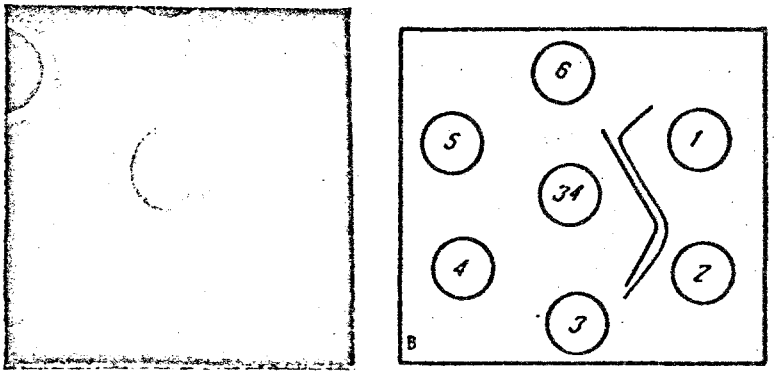

Fig. 2. Gel-precipitation reaction by Ouchterlony's method with antiserum against adult chicken kidney tissues absorbed by extract from adult chicken liver. A) Central well contains antiserum, peripheral wells extracts from kidney (1, 2), and liver (3, 4) of adult chicken; 3) the same represented schematically.

be specific for kidney (Fig. 2A and B). This was confirmed by reactions with other tissues. The sera mentioned above reacted with extracts of the heart and lungs to form 2 precipitation bands, and 3 and 4-5 precipitation bands, respectively, with extract of spleen and liver (Fig. 1A and B). In subsequent experiments the formation of the antigenic composition of kidney tissues was studied in ontogenesis. Typical results of this investigation are shown in Table 1 (they were obtained serum No. 31 and the original extracts equalized as regards protein concentration).

The following conclusion may be drawn from analysis of the results given in Table 1 and Figs. 1 and 2. Two organ-specific antigens and 6 inter-organ antigens, identical with the antigens of other chick organs, were found in the definitive chick kidney. During formation of the kidney in ontogenesis its antigenic composition changes continually. When the anlage of the organ first appears, inter-organ antigens are found initially. Later, when the mesonephros is completely formed as a morphological structure, organ-specific antigens appear (at the 8th-10th day of incubation). In the course of degeneration of the mesonephros at the 18th-20th day of incubation, the content of inter-organ and organ-specific antigens falls. Soon after appearance of the rudimentary metanephros, inter-organ antigens also appear initially (on the 10th day of incubation). During differentiation of the metanephros they increase in amount, and organ-specific antigens begin to appear in the metanephros of 14-16-day embryos. Formation of the antigenic composition of the definitive kidney is evidently basically complete by the 16th day of embryonic development, apart from 1 or 2 components which are formed in the metanephros of the chick 3 days after birth.

TABLE 1. Results of Precipitation Reaction in Agar Gel between Sera against Adult Chicken Kidney and Tissue Extracts from Chick Embryos and Adult Chickens

Tissues from which extracts obtained	Antigens*								Total No. of antigens
	1	2	3	4	5	6	7	8	
Tissues of 4-day chick embryo†	0	0	0	0	0	0	0	0	0
Mesonephros at 6 day of incubation	0	0	0	0	0	+	0	0	1
» 8 » »	0	0	0	0	+	+	0	+	2
» 10 » »	0	0	+	+	+	+	+	+	6
» 12 » »	0	0	+	+	+	+	+	+	6
» 14 » »	0	0	+	+	+	+	+	+	6
» 16 » »	0	0	+	+	+	+	+	+	6
» 18 » »	0	0	0	0	+	+	+	+	4
» 20 » »	0	0	0	0	+	+	0	0	2
Metanephros 10 » »	0	0	0	0	0	+	+	0	2
» 12 » »	0	0	0	0	0	+	+	0	2
» 14 » »	0	0	0	0	+	+	+	0	3
» 16 » »	0	+	+	+	+	+	+	+	7
» 18 » »	0	+	+	+	+	+	+	+	7
» 20 » »	0	+	+	+	+	+	+	+	7
» of 3-day chick									
Kidneys of adult chickens	+	+	+	+	+	+	+	+	8
Liver » »	+	+	+	+	+	+	+	+	8
Spleen » »	+	+	+	0	0	+	+	0	4—5
Lung » »	0	0	+	0	0	+	0	+	3
Heart » »	0	0	0	0	0	+	0	+	2

* Precipitation bands counted from peripheral wells towards centrals.

† The trunk portion of the embryos was used in the experiments.

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