ANTIGENS OF THE HUMAN EMBRYONIC METANEPHROS AND THEIR COMPARISON WITH THOSE OF THE ADULT

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It was shown by immunoelectrophoresis that specific kidney antigens detected in human embryos have the mobility of blood serum γ-globulin. An antigen identical for the kidney and liver was found in the metanephros of fetuses starting with the 14th week of development. This antigen has the mobility of serum β_2-globulin. Interorgan antigens of the kidney identical with antigens of many organs (liver, heart, lung, spleen) were found in the metanephros at the earliest stages of human development studied (starting with the 6th-7th week). Their relative electrophoretic mobility was identical with that of serum albumin and α_2- and β_2-globulins.

Information in the literature on the formation of the subcellular structure of the kidney during human embryonic development is fragmentary [3, 4]. The writer has previously [1, 2] described the results of a study of the formation of the antigenic structure of kidney tissues at different stages of human intrauterine development.

The object of this investigation was to study the relative electrophoretic mobility of kidney antigens in human embryos and the adult.

EXPERIMENTAL METHOD

Agar and tissue extracts were made up in veronal-medinal buffer, pH 8.6, ionic strength 0.025. The same buffer was used to fill the electrode compartments of the apparatus. The agar solution was poured in a layer 1.6 mm thick on a sheet of defatted glass measuring 9 × 12 cm. Wells for the extracts were cut out of the solidified agar plate. To determine the level of electro-osmosis, human albumin for normal blood serum was constantly added to one of the wells, and dextran, which under these experimental conditions has no electric charge, was added to another. The apparatus was connected through a rectifier to the grid and the voltage so regulated that the potential gradient was 4.5-5 V/cm. Under these conditions separation continued for 90 min. The current was 11-13 A. At the end of electrophoresis, gutters parallel to the axis of migration of the antigens were cut out of the agar and filled with the corresponding antisera. Antibodies reacting with blood serum proteins were removed from the antisera by absorption. For this purpose normal human blood serum was dropped preliminarily into the gutter. After 1-2 h the remains of the normal serum were removed by aspiration and the antiserum was added. The plate was placed in a humid chamber. The results were analyzed 24 h later. To determine the relative electrophoretic mobility (U_X/U_A) of the antigens the distance traveled by the reference protein (albumin, U_A) from the point of zero mobility (the location of dextran, detected by 96° alcohol) was first measured. The distance (U_X) traveled by the test protein from the point of zero mobility was then measured.

EXPERIMENTAL RESULTS

Eight antigens with different relative mobilities were identified by immunoelectrophoresis and also by means of the agar diffusion test in the human kidney extract: antigen 1 had a relative electrophoretic

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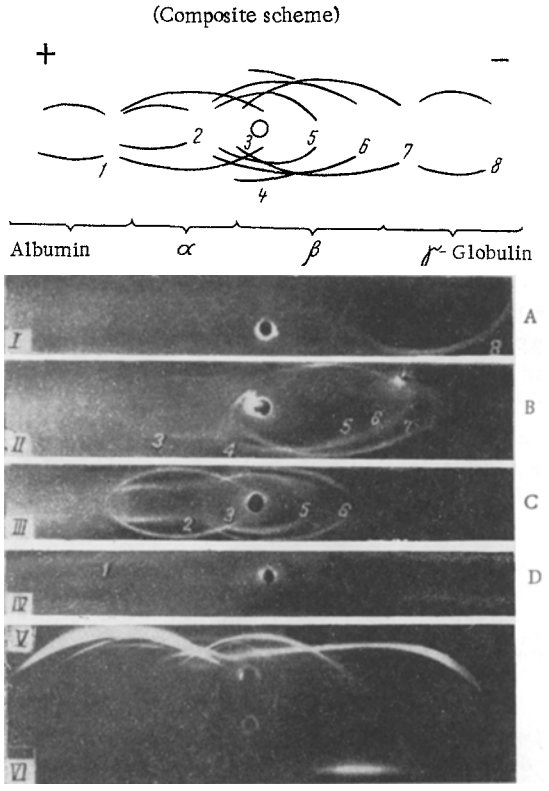

Fig. 1. Results of immunoelectrophoresis with sera against human kidney, liver, and heart and human kidney extract. Contents of wells: I-IV) human kidney extract; V) human blood serum; VI) dextran; contents of gutters: A) serum against human kidney tissues; B) serum against human liver tissues; C) serum against human heart tissues; D) serum against human blood serum. 1-8) Precipitation arcs.

mobility of 1.04 [zone I) albumins], antigen 2 a mobility of 0.85 [zone II) α-globulins], antigen 3 a mobility of 0.72 [zone III) α_2-globulins], antigen 4 a mobility of 0.52 [zone IV) β_1-globulins], antigens 5 and 6 had mobilities of 0.41 and 0.32 respectively [zone V) β_2-globulins], and antigens 7 and 8 had mobilities of 0.17 and 0.18 respectively [zone VI) γ-globulins]. The kidney antigens as a whole can be divided into 6 groups: those with the mobility of serum albumin and of α_1-, α_2-, β_1-, β_2-, and γ-globulins, (Fig. 1). Antigens 1 and 2 were found principally by sera against the kidney, liver, and heart not absorbed by serum proteins. However, antigen 1 was also detected by means of the absorbed antiheart serum in several different human organs: the kidney, liver, heart, diaphragm, adrenal, and stomach. This antigen was found to differ immunologically from serum albumin.

Antigen 3 was systematically detected in the human kidney, liver, and spleen by sera against kidney and spleen absorbed by serum proteins. Antigen 4, however, was present in the kidney only in a low concentration, for antibodies reacting predominantly with extract from the liver, but not from the kidney, could be obtained against it. This antigen was also detected easily in liver extract by the corresponding antiserum. Antigen 5 was regularly found only in the kidney extract and liver by sera against human kidney and liver, but not by serum against human heart. Antigen 5 was found to be specific for kidney and liver, but in the liver it was evidently not homogeneous because antiliver serum, absorbed by kidney extract, continued to detect this antigen in the liver although the precipitation arc in these cases was ill-defined. On the other hand, antikidney serum, absorbed by liver extract, did not detect this antigen in kidney extract.

Antigen 6 was found in human kidney extract by various sera (against kidney, liver, and heart). Identical antigens, with relative electrophoretic mobilities of 0.33, 0.35, 0.36, and 0.39 were represented in several other human organs (liver, heart, adrenal, stomach). It can evidently be concluded from these results that antigen 6 is of the interorgan type. Antigen 7 was found in human kidney extract predominantly by serum against human kidney, but in some cases by serum against human liver. However, the analogous antigen was not found in human liver extract. Antigen 8 was found only by means of serum against human kidney. These results suggest that antigens 7 and 8 are organ-specific for the kidney. The number of serum antigens in human kidney extract was not always strictly constant, evidently because of their insufficiently complete removal during washing of the organ.

The results of determination of the relative electrophoretic mobility of antigens of the developing human embryonic metanephros are given in Table 1.

As Table 1 shows, serum proteins (α-globulins) as well as interorgan albumin and an antigen with relative mobility in the zone of β_2-globulins (0.34, 0.31, and so on) were found in the metanephros in the earliest stage of development of human embryos (starting from the 6th-7th week of development). An antigen identical for the kidney and liver, with the relative mobility of β_2- globulin (0.47, 0.45), was found in the metanephros of fetuses starting with the 14th week of development. Kidney-specific antigen with an electrophoretic mobility of 0.16 was found in the metanephros of 18-week human fetuses. However, in the preceding stages (6-14 weeks of development) antigens not found in the adult kidney, and with mobility slightly different from that of adult kidney antigen (0.07 and 0.12), were found in the metanephros. These

TABLE 1. Mean Relative Electrophoretic Mobility of Antigens of the Human Embryonic Metanephros

Human kidney antigen	Zone of mobility	Age of fetuses (in weeks)				
		6—7	10	12	14	18
1	Albumin	1,04	1,05	1,04	1,09	1,05
2	α-Globulin	0,87	0,87	0,88	0,86	0,85
3	α_2-Globulin	0,77	0,74	0,76	0,74	0,72
4	β_1-Globulin	—	—	—	—	—
5	β_2-Globulin	—	—	—	0,47	0,45
6	β_2-Globulin	0,34	0,31	0,31	0,31	0,30
7	γ-Globulin	0,07	0,10	0,12	0,12	0,16
8	γ-Globulin	—0 22	—0,20	—0,19	—0,19	—0,18

Legend: — no antigen present.

results suggest that in the adult kidney these antigens are evidently present, but in such small quantities that although they can induce antibody formation, they are insufficient to produce a visible reaction with existing antibodies.

The results of immunoelectrophoresis thus added to those obtained by the agar-diffusion reaction and also provided some information on one of the properties of the antigens detected, viz. their relative electrophoretic mobility.

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