

EXPERIMENTAL BIOLOGY

ORGAN ANTIGENIC SPECIFICITY OF RENAL AND CARDIAC HUMAN EMBRYONIC TISSUE

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Until now the process of formation of tissue antigens during embryogenesis has been investigated chiefly in animals [1,7-10]. Only a few works concern organ-specific antigens in developing human embryonic tissue.

R. F. Averkina [2] was able to demonstrate organ-specific antigens in human embryonic cardiac tissue by means of a complement-fixation reaction. The problem of the time at which renal and cardiac organ-specific antigens are formed during human embryogenesis has not yet been decided, and our understanding of the mechanism of development of these two organs is thereby held back [4].

We have made a study of the antigenic properties of developing human renal and cardiac embryonic tissue. We were particularly concerned to investigate organ-specific antigens at various periods of embryogenesis (before placentation, after the placenta had been completed, and between the 35th and 40th week), and to make corresponding studies in the human adult.

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In our work we used guinea pigs and the anaphylactic reaction with desensitization [5,8]. The method has been described previously [6].

The experiments whose results are shown in tables 1 and 2 were carried out as follows. Twenty-four guinea pigs were sensitized with subcutaneous injections of 32 mg of a suspension of renal tissue from human embryos at various stages of development (6-9, 15-19, 35-40 weeks), and with a suspension of adult human renal tissue (Table 1).

In the I, II, and III sets of experiments the animals received renal tissue from human embryos 6-9, 15-19, and 35-40 weeks old respectively; in the fourth set of the experiments they received the tissues of adult human renal tissue.

In the second experiment, 24 guinea pigs were sensitized with 32 mg of a suspension of human embryonic or adult cardiac tissue (Table 2). In experiments I, II, and III a suspension of human cardiac tissue from embryos 6-9, 15-19, and 35-40 weeks old respectively was given, and in the fourth set a suspension of adult human cardiac tissue was administered.

On the twenty-first day after the sensitizing injection all animals were desensitized with respect to species-specific human antigens by an intraperitoneal injection of 1 ml of human serum diluted 1:5. After a test for completeness of desensitization (intravenous injection of 0.6 ml serum diluted 1:5) all the animals received 0.6 ml of an aqueous saline solution of adult renal and cardiac tissue, i.e. 75mg of protein* each intravenously.

*The amount of protein in the extracts was determined by Kjeldahl's micro-method.

TABLE 1. An Anaphylactic Reaction in Guinea Pigs Sensitized with Human Renal Embryonic Tissue. An Injection of Extract of Adult Renal Tissue.

| No. of guinea pigs | Sensitization (subcutaneous) | | Desensitization (intraperitoneal) | | | Test for completeness of desensitization (intravenous) | | | 24th injection (intravenous) | | |
|--------------------|------------------------------------------------------------------|--------------|-----------------------------------|--------------|----------|--------------------------------------------------------|--------------|----------|-------------------------------------|--------------|----------|
| | Antigen | Dose (in mg) | Antigen | Dose (in ml) | Reaction | Antigen | Dose (in ml) | Reaction | Antigen | Dose (in ml) | Reaction |
| 1 | Suspension of renal tissue from a human embryo (6-9 weeks old) | 32 | Adult human serum | 1.0 | + | Adult human serum | 0.6 | - | Extract of adult renal human tissue | 0.6 | + |
| 2 | The same | 32 | The same | 1.0 | + | The same | 0.6 | - | The same | 0.6 | ++ |
| 3 | " " | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 71 | " " | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 72 | " " | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 79 | " " | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 33 | Suspension of renal tissue from a human embryo (15-19 weeks old) | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 34 | The same | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 35 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 61 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 62 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 63 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 11 | Suspension of renal tissue from a human embryo (35-40 weeks old) | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 14 | The same | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 16 | " " | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 126 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 127 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 129 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 77 | Suspension of adult human renal tissue | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 78 | The same | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 80 | " " | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 94 | " " | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 97 | " " | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 99 | " " | 32 | " " | 1.0 | + | " " | 0.6 | - | " " | 0.6 | ++ |
| 111 | Unsensitized guinea pigs | . | . | . | . | . | . | . | Extract of adult renal human tissue | 0.6 | - |
| 112 | The same | . | . | . | . | . | . | . | The same | 0.6 | - |
| 113 | " " | . | . | . | . | . | . | . | " " | 0.6 | - |

Note: + indicates tremor, scratching of the nose and the ears, ruffling of the fur, breathlessness, and some fall in temperature; ++ indicates the same symptoms exhibited to a greater degree; guinea pig sneezes frequently; +++ indicates the same symptoms manifested still more strongly, spasmodic jumps in the air, coughing, animal lies on its side, but survives; - indicates no symptoms of anaphylactic shock; no injection given.

TABLE 2. Anaphylactic Reaction in Guinea Pigs Sensitized with Human Embryonic Cardiac Tissue. The Injection of an Extract of Adult Human Cardiac Tissue

| No. of guinea pigs | Sensitization (subcutaneous) | | Desensitization (intraperitoneal) | | | Test for completeness of desensitization (intravenous) | | | Critical injection (intravenous) | | |
|--------------------|-------------------------------------------------------------------|--------------|-----------------------------------|--------------|----------|--------------------------------------------------------|--------------|----------|--------------------------------------------|--------------|----------|
| | Antigen | Dose (in mg) | Antigen | Dose (in ml) | Reaction | Antigen | Dose (in ml) | Reaction | Antigen | Dose (in ml) | Reaction |
| 4 | Suspension of cardiac tissue from human embryo 6-9 weeks old | 32 | Serum from adult human | 1.0 | ++ | Serum from adult human | 0.6 | - | Extract from cardiac tissue of adult human | 0.6 | ++ |
| 5 | The same | 32 | The same | 1.0 | ++ | The same | 0.6 | - | The same | 0.6 | ++ |
| 6 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | ++ |
| 70 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | ++ |
| 74 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | ++ |
| 76 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | ++ |
| 30 | Suspension of cardiac tissues from human foetuses 15-19 weeks old | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | ++ |
| 31 | The same | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 32 | " " | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | ++ |
| 59 | " " | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 66 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | ++ |
| 68 | " " | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 7 | Suspension of cardiac tissue from human foetuses 35-40 weeks old | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 8 | The same | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 9 | " " | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 120 | " " | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 123 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 125 | " " | 32 | " " | 1.0 | +++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 37 | Suspension of cardiac tissue from adult human | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 44 | The same | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | ++ |
| 92 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 85 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 91 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 100 | " " | 32 | " " | 1.0 | ++ | " " | 0.6 | - | " " | 0.6 | +++ |
| 87 | Desensitized guinea pigs | . | . | . | . | . | . | . | Extract of tissue from adult human | 0.6 | - |
| 93 | The same | . | . | . | . | . | . | . | The same | 0.6 | - |
| 54 | " " | . | . | . | . | . | . | . | " " | 0.6 | - |

Indications as in table 1.

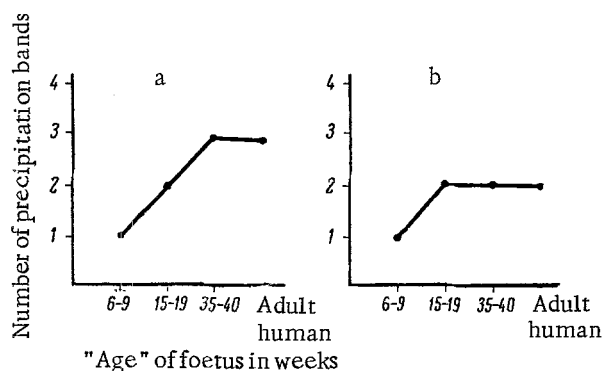


Fig. 1. Results of the reaction of precipitation in agar between anti-adult human serum and antigens against renal tissue of human foetuses of various ages; a) reaction on plates b) reaction in capillary tubes.

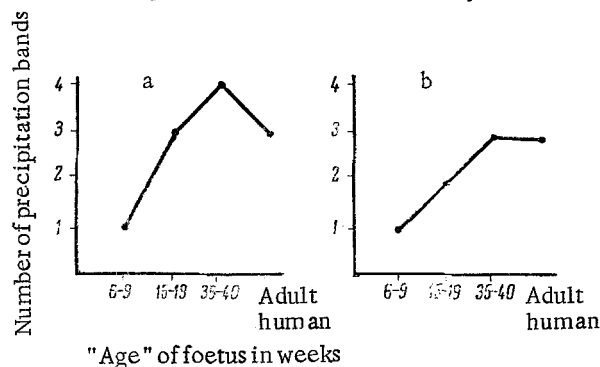


Fig. 2. Results of precipitation in agar between anti-adult-human serum and antigens of the heart of human foetuses of various ages. Indications as in Fig. 1.

In these experiments, as antigens we used aqueous saline extracts of human embryonic cardiac and renal tissue from embryos 6-9, 15-19, and 35-40 weeks and as a control we used corresponding extracts from adult human tissue. The extracts were prepared as in the previous experiment except they were diluted by 1:5 instead of 1:10.

The extracts were used as antigens for the reaction of precipitation in agar plates [11], and for micro-precipitation in agar capillary tubes [3].

We used sera obtained from rabbits immunized with cardiac and renal adult human tissue. Altogether we obtained 12 sets of sera with antibodies against organs of titers from 1:400 to 1:1600.

As can be seen from the results shown in Fig. 1 in the precipitation in agar reaction extracts from renal tissue of human embryos 6-9 weeks old formed a single precipitation band when tested against anti-adult human serum; extracts of renal tissue from human embryos 15-19 weeks old gave two precipitation bands with the same serum; extracts of renal tissue from foetuses 35-40 weeks old or from adult human subjects gave three precipitation bands.

The results obtained in precipitation or micro-precipitation in agar plates or in micro-precipitation in capillary tubes were similar.

They showed that renal tissue of human embryos at all stages of development contains antigens resembling those of the adult human kidney. However, the extent of the resemblance varied; as the human embryo develops so also does the number of antigens which resemble those of the adult.

We obtained similar results in a study of the differentiation of human embryonic cardiac tissue (Fig. 2).

In the reaction of precipitation in agar with cardiac tissue from human embryos 6-9 weeks, 15-19 weeks and 35-40 weeks old we obtained 1 band, 3 bands and 4-5 bands respectively; with extracts from adult cardiac tissue we obtained 3 precipitation bands.

Extracts for the injection were prepared as follows. The cardiac and renal tissue, washed free from blood, was rapidly cut up into small pieces with scissors in a sterile porcelain mortar, and then ground with sand as physiological saline was gradually added (1 g of tissue to 9 ml. of saline). The resulting suspension was left in a refrigerator for twenty-four hours and then centrifuged for twenty minutes at 3,000 revs/minute. For the experiments we used the supernatant fluid.

As can be seen from Tables 1 and 2, in all the animals there was a positive anaphylactic reaction. Because all the guinea pigs were desensitized with respect to species-specific antigens, this reaction could be caused only by a residual sensitivity with respect to organ-specific antigens.

The results of these experiments show that at all periods of development renal and cardiac tissue had a marked organ-specific antigenicity. It can also be seen that the intensity of the reaction in guinea pigs sensitized by suspensions of renal or cardiac tissue at later stages of development (mainly + + +) is somewhat greater than in animals sensitized with suspensions made from younger cardiac or renal tissue (reaction mainly + +). These results indicate that possibly the organ-specificity of the kidney and heart increases somewhat during development.

In order further to analyze the development of organ-specific antigenicity in human embryonic cardiac and renal tissue we used the reaction of precipitation in agar.

Thus human embryonic cardiac tissue at all stages of development contains antigens resembling those of the adult. The extent of the resemblance varies; as the embryo develops the number of antigens resembling those of the adult also increases.

The experiments have shown that renal and cardiac tissue from human embryos and fetuses aged 6-40 weeks contain corresponding organ-specific antigens. During development the number of such antigens gradually increases. The maximum number of organ-specific antigens is formed by the 35-40th week of intrauterine development.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.