AN ANALOG OF HUMAN EMBRYONIC PREALBUMIN-1 IN ANIMALS

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The discovery of analogs of human embryonic proteins in the blood of animals provides fresh opportunities for the study of these proteins both in embryogenesis and in carcinogenesis on experimental models. One such embryonic protein is embryonic prealbumin-1 (EPA-1), which is a glycoprotein [5]. EPA-1 was first found in amniotic fluid and in the blood serum of human fetuses [2, 3, 6], in tissue extracts of various tumors, and also in cultures of embryonic and adult fibroblasts [1, 2, 6, 7].

A detailed study of antigenic determinants of the EPA-1 molecule showed that this molecule (or molecules) contains two groups of determinants which differ in immunochemical specificity. On these grounds it was suggested that there are two forms of embryonic prealbumin: EPA-1 and EPA-1S [4].

The object of the present investigation was to look for analogs of human EPA-1 (AEPA-1) in certain animals.

EXPERIMENTAL METHOD

Antisera against EPA-1 and EPA-1S were obtained in rabbits, using semipurified preparations of EPA-1, obtained from human amniotic fluid, and also from desmoid tissue extracts, for immunization [3, 4]. Antiserum against AEPA-1 was obtained by immunizing rabbits with a preparation isolated from bovine amniotic fluid.

This preparation was isolated in the same way as human EPA-1.

Immunodiffusion analysis was carried out with the aid of a standard test system against EPA-1, EPA-1S, and AEPA-1, by the method of Khramkova and Abelev [8], and also by crossed immunoelectrophoresis in agarose gel [9]. To study the analogs amniotic fluid and fetal blood serum were taken in the first half of embryonic development, and adult human and animal blood serum also was used.

To prove that the AEPA-1 thus found is an analog of human EPA-1, a preparation of bovine AEPA-1 was obtained. To do this, bovine AEPA-1 from amniotic fluid was precipitated by means of a monospecific antiserum against this protein. The precipitate was carefully washed with physiological saline to remove protein impurities and dissociated with 0.05 M glycine-

TABLE 1. Results of Immunochemical Determination of EPA-1 and EPA-1S Analogs in Animals

Species of animals	Presence of EPA-1 and EPA-1S		
	maternal blood serum	fetal blood serum	amniotic fluid
Mouse	—	—	—
Rat	—	—	—
Guinea pig	—	—	—
Rabbit	—	—	—
Sheep	—	+	+
Pig	—	+	+
Cow	—	+	+

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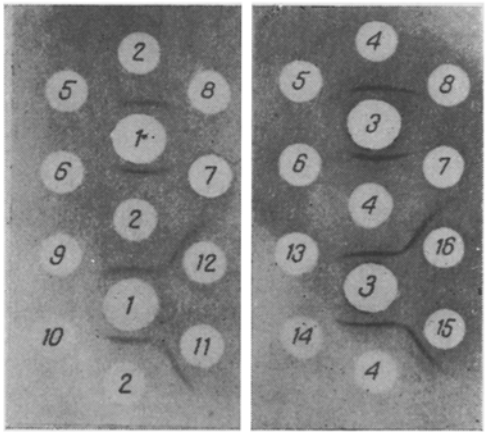

Fig. 1

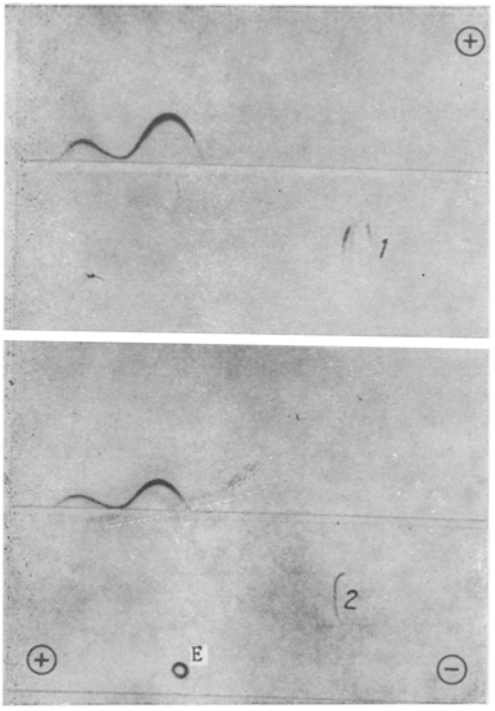

Fig. 2

Fig. 1. Immunochemical analysis of EPA-1 and EPA-1S in a cow. 1) Antiserum against EPA-1, 2) standard EPA-1 preparation, 3) antiserum against EPA-1S, 4) standard EPA-1S preparation, 5) amniotic fluid of calf fetus, 6) calf fetal blood serum, 7) maternal cow's blood serum, 8) physiological saline, 9) mixture of antiserum against EPA-1 with amniotic fluid of calf fetus, 10) mixture of antiserum against EPA-1 with calf fetal blood serum, 11) mixture of antiserum against EPA-1 with blood serum of maternal cow, 12) mixture of antiserum with physiological saline, 13) mixture of antiserum against EPA-1S with amniotic fluid of calf fetus, 14) mixture of antiserum against EPA-1S with calf fetal blood serum, 15) mixture of antiserum against EPA-1S with blood serum of maternal cow, 16) mixture of antiserum against EPA-1S with physiological saline.

Fig. 2. Immunoelectrophoretic analysis of human EPA-1 and cow AEPA-1. 1) Amniotic fluid of human fetus, developed by antiserum against EPA-1; 2) amniotic fluid of calf fetus developed by antiserum against AEPA-1. E) Zone of albumin stained by Evans' blue.

HCl buffer, pH 2.2. Sulfosalicylic acid was added to the dissolved precipitate to a final concentration of 0.6 M, after which the antibodies thrown down in the residue were removed by centrifugation at 6000 rpm. The AEPA-1 preparation was left in the supernatant. It was dialyzed against distilled water for 4 days at 4°C and used in the subsequent experiments.

EXPERIMENTAL RESULTS

The results of analysis of analogs of immunochemical determinants of the human EPA-1 molecule in various animals are given in Table 1. No reactions of complete immunochemical identity were observed between EPA-1 and AEPA-1. However, amniotic fluid and blood serum of calf, pig, and sheep fetuses gave an effect of inhibition of the precipitation arc (Fig. 1). This was not observed in other animals.

On the addition of samples giving an inhibition effect to antisera against EPA-1 and EPA-1S, they were exhausted (Fig. 1). It can thus be definitely concluded that immunochemical analogs of EPA-1 and EPA-1S determinants are present in cows, sheep, and pigs.

Antisera obtained by immunization of rabbits with a semipurified AEPA-1 preparation from calf amniotic fluid, after exhaustion with adult cow plasma, revealed an antigen in the amniotic fluid which resembled human EPA-1 in its electrophoretic mobility (Fig. 2). The physicochemical properties of AEPA-1 also were the same as those of human EPA-1. It was also

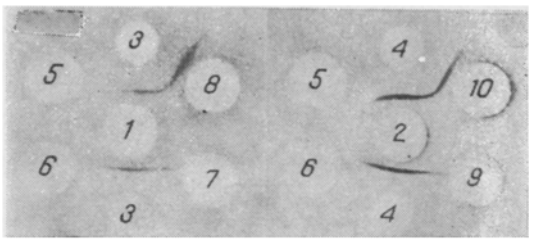

Fig. 3

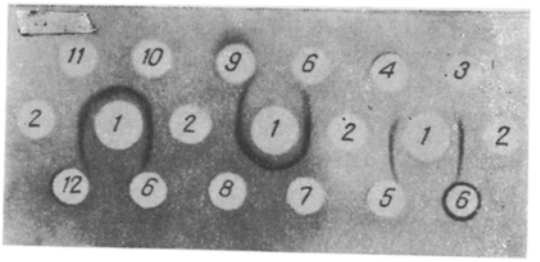

Fig. 4

Fig. 3. Immunodiffusion analysis of human, sheep, and pig AEPA-1. 1) Antiserum against AEPA-1, 2) standard AEPA-1 preparation, 3) amniotic fluid of human fetus, 4) human fetal blood serum, 5) blood serum of mother of human fetus, 6) physiological saline, 7) amniotic fluid of pig fetus, 8) pig fetal blood serum, 9) blood serum of mother pig, 10) amniotic fluid of sheep fetus, 11) sheep fetal blood serum, 12) blood serum of mother sheep.

Fig. 4. Immunochemical analysis of EPA-1 and EPA-1S in AEPA-1 preparation isolated from precipitate. 1) Antiserum against EPA-1, 2) antiserum against EPA-1S, 3) standard EPA-1 preparation, 4) standard EPA-1S preparation, 5) AEPA-1 preparation, 6) physiological saline, 7) mixture of antiserum against EPA-1 with AEPA-1 preparation, 8) mixture of antiserum against EPA-1 with physiological saline, 9) mixture of antiserum against EPA-1S with AEPA-1 preparation, 10) mixture of antiserum against EPA-1S with physiological saline.

found in amniotic fluid and fetal blood serum but was absent in the blood serum of adult animals. By means of a standard test system against cow AEPA-1 it was shown that similar proteins were present in the amniotic fluid and blood serum of sheep and pig fetuses and that they gave a reaction of complete immunochemical identity. No AEPA-1 was found in other animals. Human amniotic fluid and fetal blood serum gave an inhibition reaction of the AEPA-1 precipitation arc, just as in the case of the reaction between cow, sheep, and pig AEPA-1 with a test system of human EPA-1 and EPA-1S (Fig. 3).

To obtain complete proof of the identity of human EPA-1 and cow AEPA-1, immunochemical determinants of human EPA-1 and EPA-1S molecules were determined in a protein preparation obtained from the precipitate of monospecific antiserum against cow AEPA-1 and calf amniotic fluid. It was found that the cow AEPA-1 preparation exhausts antiserum against human EPA-1 and EPA-1S (Fig. 4).

It can thus be concluded from the results of these experiments that immunochemical analogs of the EPA-1 and EPA-1S determinants are present in cows, sheep, and pigs. They were not found in the other animals studied.

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