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ANALOGS OF HUMAN EMBRYONIC PREALBUMIN-2 IN ANIMALS

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UDC 612.124:547.962.3]:612.646-019

KEY WORDS: embryonic prealbumin-2; collagen; analogs of embryonic proteins.

The presence of immunochemical analogs of human embryonic proteins in animals is of great importance because their discovery has opened the way to the experimental study of these proteins.

The object of this investigation was to make an immunochemical study of analogs of embryonic prealbumin-2 (EPA-2) in animals. EPA-2 is a protein found in the blood serum and amniotic fluid of human fetuses. This protein has not been found in the blood serum of blood donors or pregnant women [1].

EXPERIMENTAL METHOD

Antisera against EPA-2 were obtained in rabbits, using the glycoprotein fraction of human amniotic fluid for immunization [1]. Immunochemical identification of EPA-2 analogs was carried out with the aid of a standard test system for this protein by the method of Khramkova and Abelev [5].

The cellular localization of EPA-2 in tissues of calf fetuses and adult animals was studied by the indirect immunofluorescence analysis method. Tissue sections 4-8 μ thick were fixed with alcohol and acetic acid [7] and embedded in paraffin wax [8]. The sections were labeled by means of antibodies against EPA-2 obtained with the aid of an immunosorbent. EPA-2 immobilized on ACA-34 ultrogel by means of glutaraldehyde [6] was used as the immunosorbent. The technique of immunofluorescence analysis was described by the writers previously [2]. To study analogs, amniotic fluid, tissue, and blood serum from fetuses in the first half of embryonic development, and tissue and blood serum from adult animals were chosen. The tissue extracts were prepared in Tris-glycine buffer, pH 8.3, with the addition of detergents [4].

EXPERIMENTAL RESULTS

Immunochemical analysis of EPA-2 showed the presence of analogs of this protein in the blood serum and amniotic fluid of calf, pig, and sheep fetuses (Table 1). EPA-2 was not found in the blood serum of adult animals. Analogs of EPA-2 in animals gave a reaction of complete immunochemical identity with human EPA-2 (Fig. 1) and they also had similar physicochemical properties and electrophoretic mobility to those of prealbumin (Fig. 2).

The results of the study of EPA-2 in biological fluids, tissue extracts, and tissue sections from various organs of calf fetuses and cows are given in Table 2. The highest content of EPA-2 in animals, just as in man, was found to be in the amniotic fluid of the fetuses. The EPA-2 level in tissue extracts of fetal organs varied from 0.5 μg/ml in extracts of the spleen, liver, lung, and brain to 16-32 μg/ml in extracts of skin and of the umbilical cord.

Department of Biochemistry and Problem Laboratory for Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 62-64, July, 1981. Original article submitted January 9, 1981.

0007-4888/81/9207-0928$07.50

TABLE 1. Results of Immunochemical Determination of EPA-2 Analogs in Animals

Species of animals	Content of EPA-2		
	maternal blood serum	fetal blood serum	amniotic fluid
Mouse	—	—	—
Rat	—	—	—
Guinea pig	—	—	—
Rabbit	—	—	—
Sheep	—	+++	+++
Pig	—	++	++
Cow	—	+	+

TABLE 2. Results of Immunochemical Determination of EPA-2 in Tissue Extracts, Biological Fluids, and Sections of Cow's Organs and Tissues

Material	Calf fetus		Cow	
	concn. of EPA-2, μg/ml	EPA-2 on sections	concentrations of EPA-2, μg/ml	EPA-2 on sections
Blood serum	16	Not determined	0	Not determined
Amniotic fluid	64	Not determined	Not determined	Not determined
Tissue extracts of organs:				
Stomach	2	++	0,5	+
Small intestine	8	+++	8	+++
L. intestine	4	+++	4	+++
Skin	32	+++	2	+++
Kidney	8	++	2	++
Spleen	0,5	+	0	+
Heart	0	—	0	—
Liver	0,5	+	0	+
Muscle	8	+++	0	++
Lung	0,5	+	0	+
Aorta	0	—	0	—
Brain	0,5	—	0	—
Umbilical cord	16	+++	Not determined	Not determined

Legend. Reaction of immunofluorescence analysis expressed as: +++ strong, ++ average, + weak, — negative.

Analysis of EPA-2 in tissue extracts of organs of adult animals revealed the presence of this protein in the large and small intestines, stomach, skin, and kidney of cows.

The content of EPA-2 in tissue extracts from organs of adult animals was lower than in fetuses, except in the case of the large and small intestine.

Immunofluorescence analysis of EPA-2 on sections of fetal organs showed that this protein was localized in connective tissue fibers, except in the kidney. In sections through the fetal kidney, fluorescence was observed in the cytoplasm of epithelial cells of the tubules.

The absence of fluorescence on sections through the aorta indicates that EPA-2 is mainly found in the structure of collagen and reticular fibers. The discovery of EPA-2 in the cytoplasm of epithelial cells of the renal tubules is perhaps connected with the secretion and reabsorption of this protein in various parts of the kidney.

The study of tissue sections from organs of adult animals gave similar results but fluorescence in the cytoplasm of epithelial cells of the renal tubules was weaker than in the fetus. The results do not agree with those of immunochemical determination of EPA-2 in tissue extracts of the corresponding organs. For instance, no EPA-2 could be found in tissue extracts of the spleen, liver, muscle, and lung whereas specific fluorescence of structures containing EPA-2 was found in sections from these organs.

It can thus be concluded from these results that EPA-2 is a protein which is closely bound with the structure of collagen fibers. However, its role in the collagen fiber is not yet clear. It can only be suggested that EPA is a protein component either of procollagen (neutral salt-soluble collagen) or of the noncollagen structures of collagen fibers. This suggestion is in agreement with data on formation of collagen fibers in relation to age [3]. With age, the relative proportion of neutral salt-soluble collagen falls sharply, and this coincides with the discovery of EPA-2 in the amniotic fluid and fetal blood serum during

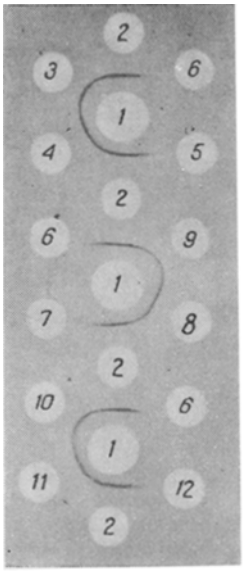

Fig. 1

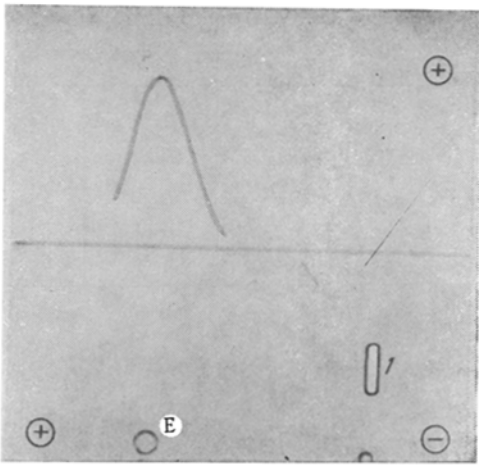

Fig. 2

Fig. 1. Immunochemical study of EPA-2 in amniotic fluid and blood serum of cows, sheep, and pigs. 1) Antiserum against human EPA-2, 2) standard preparation of human EPA-2, 3) blood serum of calf fetus, 4) amniotic fluid of calf fetus, 5) cow's blood serum, 6) physiological saline, 7) pig's blood serum, 8) amniotic fluid of pig fetus, 9) blood serum of pig fetus, 10) blood serum of sheep fetus, 11) amniotic fluid of sheep fetus, 12) lamb's blood serum.

Fig. 2. Immunoelectrophoretic analysis of EPA-2 from cows. 1) Amniotic fluid of calf fetus, developed by antiserum against human EPA-2. E) Zone of albumin stained by Evans' blue.

embryonic development and in adult human blood serum [1]. This may account for the presence of large quantities of EPA-2 in fetal blood serum and tissues and the fall in the content of this protein in the tissues and its complete absence in the blood serum of the adult animal.

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