

IMMUNOCHEMICAL INVESTIGATIONS OF TISSUE
 α_2 -GLOBULIN IN NORMAL AND TUMOR TISSUES
OF THE HUMAN KIDNEY

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UDC 616.36 + 616.61)-006-008.039.
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An α_2 -globulin with molecular weight of $473,000 \pm 6000$ was identified immunochemically in the tissues of the internal organs. The content of this protein in normal kidney tissues and in the tissue of a kidney tumor (hypernephroid carcinoma) was determined semiquantitatively (by titration). The content of the α_2 -globulin in the kidney tumor tissue was found to be almost 10 times higher than in normal kidney tissue. No α_2 -globulin was found in the blood serum of healthy persons during life, but it could be detected by the usual method of immunodiffusion analysis in the blood serum of some patients with hepatocellular carcinoma.

In recent years considerable interest has been aroused in the study of the antigenic composition of normal and tumor tissues and, in particular, of the so-called specific antigens of human and animal tumors. Research in this direction has led to the discovery of α_2 -fetoprotein during the development of a chemically induced hepatoma in mice and rats [1] and of hepatocellular carcinoma in man [2], the discovery of an embryonic carcinoma antigen in a tumor of the large intestine [5], and of specific α -globulin in a tumor of the kidney [4].

This paper describes the results of an immunochemical study of a tissue α_2 -globulin whose content is appreciably increased in kidney tumor tissue.

EXPERIMENTAL METHOD

Tissue extracts were prepared in Tris-glycine buffer with Triton X-100 by grinding in a mortar with powdered glass and subsequently freezing and thawing the homogenate and centrifuging at 9000 rpm.

Rabbits were immunized by repeated subcutaneous injections of extracts of a kidney tumor (hypernephroid carcinoma) which was mixed before the injections with an equal volume of 2% solution of potassium alum. The immune sera were exhausted with dry plasma and donors' blood serum. Two batches of nonspecific antisera against the tissue α_2 -globulin were used in the work.

High-voltage immunoelectrophoresis was carried out under the following conditions: 1% Difco agar solution, veronal-medinal buffer, pH 8.6, ionic strength 0.05. Immunodiffusion analysis was carried out by Ouchterlony's method [6] in the modification of Khramkova and Abelev [3]. The sensitivity of the method was about 0.5 mg% protein.

The kidneys removed for malignant disease were studied. Kidney extracts were prepared from two different areas: from the tumor tissue itself and from tissue of the same kidney not visually affected by the malignant growth. Extracts of the kidneys and of other internal organs (liver, spleen, stomach, intestine,

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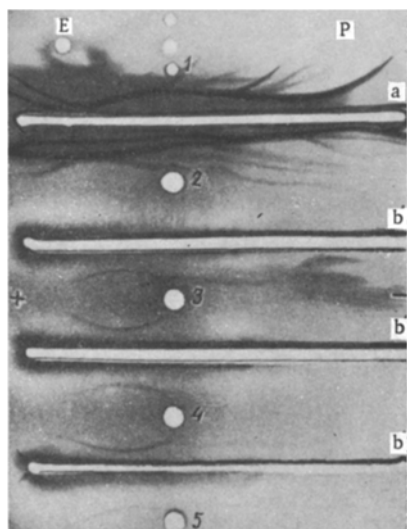


Fig. 1. Immunoelectrophoretic analysis of tissue α_2 -globulin: 1) blood serum of donor; 2) of patient with hypernephroid carcinoma of the kidney; 3) tissue extract of kidney tumor; 4) of liver tumor; 5) normal kidney; antiserum against extract of kidney tumor before (a) and after (b) its exhaustion with dry plasma and donor's serum; reference substances: P) pyronine; E) Evans' blue dye.

The results of semiquantitative determination of α_2 -globulin in the kidney tumor tissue and in the visually healthy area of the same kidney showed (Table 1) a marked difference in the content of this protein. Compared with the visually healthy kidney tissue and the tissue of a kidney removed at autopsy (control), the kidney tumor tissue (hypernephroma, hypernephroid carcinoma) contained about ten times as much of this

TABLE 1. Content of Tissue α_2 -Globulin in Extracts of Normal Kidney and Kidney Tumor Tissue

Material tested	No. of samples tested	Result of determination of α_2 -globulin	
		no. of positive samples	result of mean titer
Hypernephroid carcinoma	8	1:32—1:256	1:92
Hypernephroma changing into carcinoma	1	1:64	
Tubular-solid carcinoma	1	1:64	
Visually healthy tissue of a kidney affected by carcinoma	8	1:4—1:16	1:8
Normal kidney removed at autopsy (control)	18	1:2—1:16	1:6

pancreas, lungs, adrenal) from adult persons dying from diseases other than cancer were used for comparison. Blood sera of fetuses (caesarian section), newborn infants (umbilical blood), adults (donors), and some cancer patients also were tested by immunodiffusion analysis.

EXPERIMENTAL RESULTS

Immunoelectrophoretic analysis (Fig. 1) showed that the protein under investigation migrated in the zone of the α_2 -globulin, the precipitation arc of which began in the zone of the β_1 -globulins and ended in the zone of the α_1 -globulins. The electrophoretic mobility of the α_2 -globulin relative to albumin was 0.74. According to the results of gel filtration on Sephadex G-200, the molecular weight of the α_2 -globulin was $473,000 \pm 6000$.

TABLE 2. Content of Tissue α_2 -Globulin in Tissue Extracts of Internal Organs and in Blood Serum

Materials tested	No. of samples tested	No. of positive samples	Result of titration of α_2 -globulin
Tissue extracts:			
liver	6	6	1:2—1:32
spleen	8	8	1:2—1:8
stomach	7	6	1:2—1:4
small intestine	7	7	1:2—1:4
large intestine	7	7	1:2—1:4
pancreas	5	5	1:2—1:4
lungs	6	4	Weakly positive reaction
adrenal	5	4	The same
Blood serum of patients:			
with hypernephroma, hypernephroid carcinoma of the kidney	8	0	—
with hepatocellular carcinoma	50	9	Weakly positive reaction
Of fetuses:			
autopsy	32	30	1:8—1:64
caesarian section	6	0	—
Of adults:			
donors	24	0	—
autopsy	9	8	1:22—1:8
Of newborn infants (umbilical blood)	20	0	—

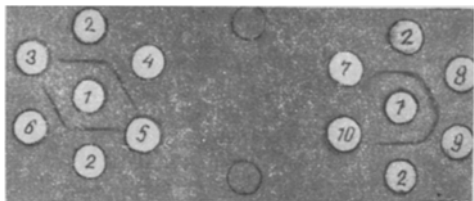


Fig. 2. Immunodiffusion analysis with a standard test system (antiserum against tissue α_2 -globulin obtained after exhaustion (1) and standard antigen (2)). 3) Serum from donor's blood; 4) from cadaver blood; 5) blood serum from patient with hypernephroid carcinoma; 6) tissue extract from normal kidney; 7) lungs; 8) from liver tumor; 9) blood serum from patient with hepatocellular carcinoma; 10) from fetus (caesarian section).

protein. Meanwhile the visually healthy tissue of a kidney removed for cancer and the tissue of a normal kidney taken at autopsy showed no significant difference in their content of the α_2 -globulin.

It must be emphasized that the tissue α_2 -globulin studied is not an organ-specific kidney antigen. This protein was also found in various amounts in normal tissue (autopsy) of certain internal organs (Table 2). The tissue α_2 -globulin was not found in the blood serum of fetuses, newborn infants, and healthy adults. Meanwhile, this protein was found fairly frequently in cadaver blood (autopsy) (Table 2). It is a clinically interesting fact that liberation of the tissue α_2 -globulin into the blood stream was found in certain patients with carcinoma of the liver during life (Fig. 2). During the period of tumor breakdown tissue α_2 -globulin can evidently be found in the patient's blood stream.

The results of these experiments show that malignant degeneration of the kidney may be accompanied by the accumulation of normal tissue α_2 -globulin in the tumor tissue. It would

be interesting to study the dynamics of the tissue α_2 -globulin in the internal organs during the development of a malignant tumor and in the blood serum of patients with cancer.

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