IMMUNOELECTROPHORETIC INVESTIGATION OF URINARY PROTEINS

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Immunoelectrophoretic analysis of the urine of intact male August rats revealed certain immunochemically unchanged serum proteins and also uroproteins specific for urine, identical to some proteins of kidney cells. The results may be useful for estimation of the state of glomerular filtration and activity of excretory processes in the kidneys.

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Proteinuria is observed in a wide range of pathological conditions, especially in kidney disease. Comparison of the spectrum of urinary proteins in health and disease, and, in particular, identification of the origin and properties of individual urinary protein components are therefore of the greatest importance [1]. Because of the great practical importance of this problem many investigations have been made of the uroproteins, comparing them with blood proteins and kidney homogenate [2, 6, 7, 11, 13, 14, 18]. However, no complete description of the spectrum of the urinary proteins of healthy individuals have yet been given, and the sources of individual protein components and the way in which they reach the urine have not yet been explained. For the comparative investigation of complex protein systems the method of immunoelectrophoretic analysis has been found particularly useful, for it combines adequate preliminary electrophoretic fractionation of the proteins with high specificity and sensitivity of immunologic reactions for the indentification of individual components of the fractions.

The object of this immunochemical investigation of the urinary proteins of intact experimental animals using immune sera against proteins of the blood serum and of the hyaloplasm, mitochondria, and microsomes of kidney cells was to determine the origin, identity, and properties of the individual protein components of the urine.

EXPERIMENTAL METHOD

Male August rats weighing 160-180 g were kept on a standard diet during the experiments, with water ad lib., in cages with facilities for collecting the urine and solid excreta separately. Each cell contained 5 or 6 animals. Animals of 15 groups were investigated at different times. The total urine excreted by the animals of each group in the 24 h was dialized against physiological saline and water and then concentrated 20-25 times by drying in a current of cold air or from the frozen state. Proliferation of microorganisms was prevented by carrying out all these procedures at a temperature of 4°, and by addition of merthiolate to the urine. After the urine samples had been concentrated in this way, the protein content was determined [15], and electrophoretic fractionation was then carried out for 120 min in 1% agar gel using a voltage of 9 V/cm under the conditions described previously [3]. Electrophoresis of 9-11 parallel samples, followed by staining for protein, glucoproteins, and lipoproteins, were carried out simultaneously [17]. The relative and absolute contents of individual protein fractions in the urine were determined by densitometry of the stained preparations after electrophoresis in the Eri-10 extinctiometer. The relative electrophoretic mobility of the fractions was found from the formula:

$$a = U_x/U_a,$$

where a represents relative electrophoretic mobility, U_X mobility of the test fraction, and U_a mobility of human serum albumin determined in the same experiment. Development of the preparations after electrophoresis was carried out with rabbit sera obtained by immunizing rabbits with serum and urine proteins,

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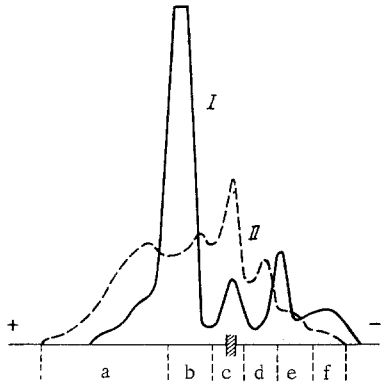

Fig. 1. Distribution of proteins during electrophoresis of blood serum (I) and urine (II) of rats.

proteins of mitochondria and microsomes, and proteins of kidney cell juice. Cytoplasmic granules and hyaloplasm of the kidney cells for immunization of the rabbits were isolated by fractional centrifugation of rat kidney homogenate [4]. The schemes of immunization of the rabbits, and methods of treatment and cross exhaustion of the sera have been published previously [3, 4]. After electrophoresis and immunodevelopment using exhausted and unexhausted sera, precipitation lines of individual proteins were identified by comparison with kidney, serum, and ovarian proteins by Ossermann's method [16] and by special staining for glucoproteins, lipoproteins, ceruloplasmin, haptoglobin, and proteins [17]. The precipitation line belonging to serum transferrin obtained by immunoelectrophoresis of urinary and serum proteins of intact rats was detected by means of $Fe^{59}Cl_3$ in radioimmunoelectrophoresis experiments by the method of Baker and co-workers [9].

EXPERIMENTAL RESULTS

The quantity of proteins excreted by August rats in the 24-h period showed very slight variation, and in repeated determinations at different times of year it was 12.5 ±0.2 mg protein per animal.

Urinary proteins were separated by electrophoresis into 6 fractions with relative electrophoresis mobilities of: a-proteins 1, b 0.79, c 0.66, d 0.51, e 0.4, and f-proteins 0.28. By their electrophoretic mobility only the a- and e- uroproteins corresponded to the serum α_1 and β- globulins, and the other uroprotein fractions differed from the serum proteins in this characteristic (Fig. 1).

The content of the various uroprotein fractions in the urine fell parallel to the decrease in their electrophoretic mobility. The "faster" a-, b-, and c- proteins, for instance, accounted for up to 80% of the total urinary proteins (36.2 ± 0.8, 24.0 ±0.15, and 21.5 ±0.75%), whereas the content of d-, e-, and f-proteins in the urine was 12.5 ±0.26, 4.3 ±0.3, and 1.5 ±0.08% (statistical analysis after Bailey [10]).

By immunochemical tests with anti-urine serum, 11-12 antigens were detected in the urine. Comparison by Ossermann's method with blood proteins and immunodevelopment of the peaks by serum against blood proteins identified serum albumin, γ-globulin, and one component each of the serum proteins with mobility of α_1, α_2, and β_1-globulins (Fig. 2). Haptoglobin, transferrin, ceruloplasmin, and serum lipoproteins and macroglobulins were not found in any sample of urine tested. The serum albumin excreted in the urine possessed higher electrophoretic mobility and increased physicochemical heterogeneity of its molecules, so that treatment with serum against rat blood proteins after electrophoretic fractionation of the uroproteins led to its precipitation over a wide zone instead of the clearly defined precipitation line observed during analysis of blood serum proteins.

Serum γ-globulin was present in the urine as two subfractions with different electrophoretic mobilities, so that the precipitation line of the "fast" subfraction of this protein during immunoelectrophoresis of the urinary proteins continued as a second wave far ahead into the zone of the a-uroproteins. The "fast" γ-globulins of the urine differed to some extent from the main fraction in their antigenic properties, so that this subfraction of the urinary proteins could be clearly detected only with the aid of anti-urine serum and could not be detected by treatment with immune serum against rat blood proteins. The reasons for this may lie both in qualitative differences in the antibody content in this subfraction in the two sera or in liberation of "latent" antigenic determinants during formation of low-molecular weight fragments of γ-globulin. Low-molecular weight fragments of antibodies and of serum γ-globulin have frequently been detected in the biocolloids of human and animal urine [6, 11, 18, 19], and according to the findings of Webb and co-workers [18, 19], the low-molecular weight fragment of α_2-globulin in healthy human urine differed to some extent in its antigenic properties, just as in our experiments on rats, from the unchanged protein molecules. The high physicochemical heterogeneity of serum proteins found in the urine may be due both to degradation of these proteins to low-molecular weight fragments in the urinary tract and also to elimination of their low-molecular weight fragments from the body along with a proportion of unchanged molecules circulating in the blood stream.

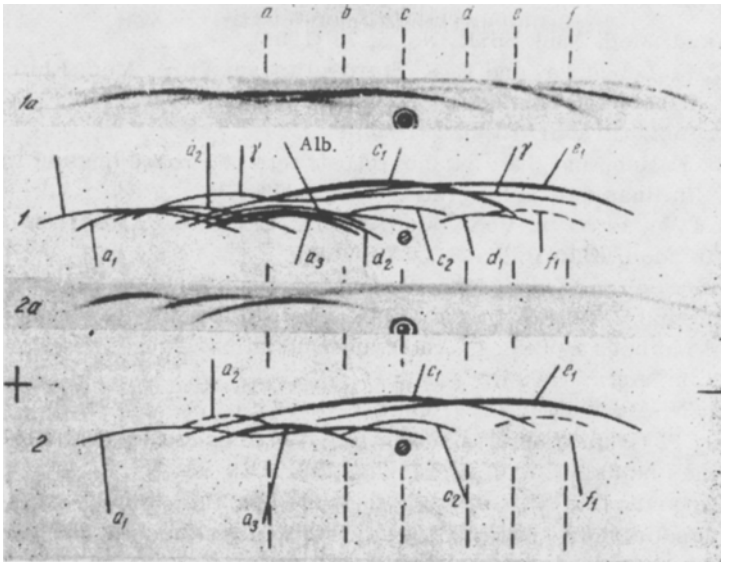

Fig. 2. Immunoelectrophoresis of rat urinary proteins. 1, 2) Drawings; 1a, 2a) photographs. Developed with rabbit antisera against rat urinary proteins, unexhausted (1 and 1a) and exhausted with rat blood serum proteins and erythrocytes (2 and 2a).

Anti-urine serum, when completely exhausted with rat blood serum protein and erythrocytes, revealed a further 7 antigens by electrophoresis of the uroproteins, among which the dominant member was c_1-antigen. The precipitation line of this antigen started in the zone of the d-uroproteins and continued into the zone of a-proteins, staining well for glucoproteins. Electrophoretically "fast" and "slow" subcomponents were also found in the a_1-uroprotein, the precipitation line of which continued as a "tail" into the c-uroprotein zone. In the a-protein zone, besides a large number of serum proteins, a glucoprotein which we called a_3-antigen was also clearly detected. On comparison with proteins of various kidney subcellular structures, the a_1- and a_3-uroproteins were identical to antigens of the microsomes, whereas the slow e_1-uroprotein was detected only among the cell juice proteins of the testis. The principal c_1-uroprotein was detected both among the hyaloplasm proteins and the kidney microsmes, while c_2-antigen, not identical to any of the water-soluble antigens of the kidney cytoplasm or nuclei or testicular cell juice apparently entered the urine from epithelial cells of the rat urinary tract.

The experiments thus showed that certain immunochemically unchanged serum proteins as well as certain uroproteins specific for urine, identical to certain proteins of the kidney cells, are present in the urine of intact animals. Proteins may enter the urine both during filtration of plasma through the glomeruli and as a result of secretory and excretory processes in the renal tubules [8, 12]. As was shown previously [5], up to 20 different proteins can be distinguished immunochemically among the cytoplasmic proteins of the kidneys, of which 3 microsomal antigens (a_1, a_2, c_1) and one protein of the kidney cell juice fraction (c_1-antigen) are also found among the uroproteins of intact rats. The technique of isolation of cytoplasmic granules from kidney homogenate ensured freedom from contamination of the granules with proteins of the cell juice, urine, and blood serum of the rats, so that the accumulation of certain kidney proteins of mainly microsomal origin in the urine indicated their elimination from the body as a result of active tubular excretion, and not of glomerular filtration or death of the epithelial cells of the renal tubules.

Immunochemical detection of blood serum proteins among the uroproteins can thus shed light on the state of glomerular filtration, whereas the discovery of certain proteins specific for urine in the urine, when compared with the results of cytological investigations using anti-urine sera, provides an index of the activity of excretory processes in the kidneys.

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