

**Experimental Histuria and Histemia**  
**Tissue Macromolecules in Blood and Urine Due to Hepatic Necrosis**  
**in Rabbits<sup>1</sup> (34365)**

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Specific antitissue sera differentiate those urinary macromolecules which are antigenically related to tissue constituents from those which are plasmalike. We have proposed the term "histuria" to denote a significantly increased output of tissuelike macromolecules in urine (1, 2). We have demonstrated this phenomenon to be frequent in the course of some pathological conditions of the kidney and also possible in some extrarenal diseases.

When the pathological damage is located outside of the kidney, the tissue molecules may be present in the blood before being excreted in urine. To confirm this mechanism, we produced experimental hepatic necrosis in rabbits by ligating their common bile duct, and thereafter looked for tissue macromolecules both in their plasma and urine. We propose the term "histemia" to denote the abnormal presence of tissuelike macromolecules in the plasma.

*Methods.* Six wild Burgundy rabbits (males and females), weighing about 3 kg, were operated on and four were simultaneously used as controls.

*Technique used to isolate urine macromolecules.* Rabbits lived in individual cages. Urine collection was continuous into a large

funnel adapted to the cage bottom.

The urine was filtered through a nylon sieve on the top of the funnel and immediately collected with a plastic hose into a refrigerated bottle. To ensure exactly a 24-hr urine collection, the bladder was forcibly voided at the beginning and the end of each period. Cells and debris were eliminated by centrifugation at 2000g for 10 min and thereafter the temperature of urine samples never rose above 6°. The processing of the urine consisted in essence of two sequential filtrations on a cellulose nitrate membrane<sup>2</sup> and a subsequent lyophilization. All technical details of the whole process are given in Ref. (2).

*Measurement of urine macromolecule outflow.* The mean urinary macromolecule outflow per minute was computed from the total weight of the macromolecules of the whole urine volume collected during each 24-hr period. Normal samples were collected sequentially from controls and from rabbits before operation.

*Plasma.* Plasma samples were collected from citrated blood drawn in the middle of each 24-hr period of urine collection, both in control and experimental animals.

*Common bile duct ligation.* Anesthesia was performed by the electronarcosis method (4). Surgery was performed under aseptic conditions. The common bile duct was dissected just above the proximal part of the

<sup>1</sup> This work was done in the "Centre de Recherches sur la Pathologie Rénale" of "Association Claude-Bernard" and "I.N.S.E.R.M." We would like to acknowledge J.-P. Benhamou and J.-N. Maillard for their advice on the surgical technique and for performance of hepatic tests in their laboratory, and P. Dorrington-Ward for his help in translation.

<sup>2</sup> Ultrafilters Lsg 60, Membranfilter, Göttingen, West Germany.

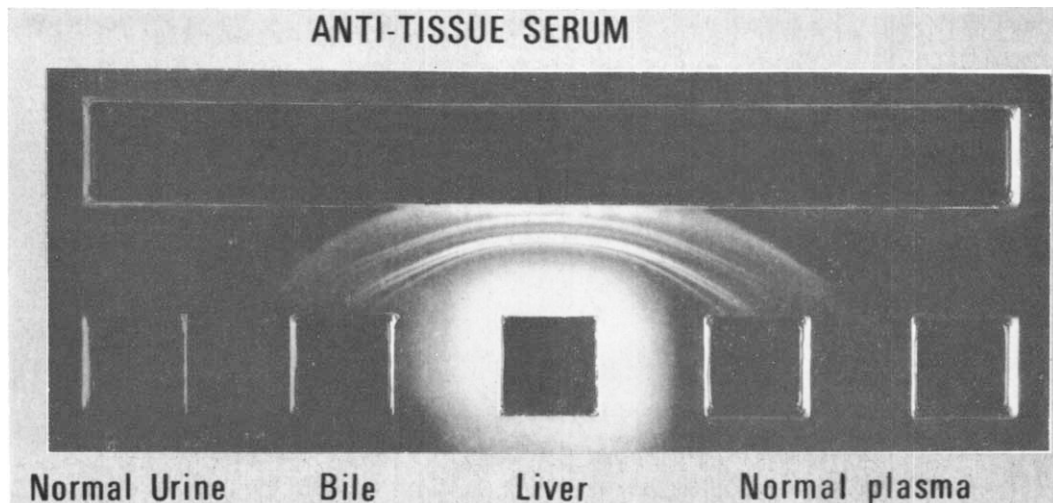


FIG. 1. Checking the specificity of the antitissue serum: (trough), duck antirabbit kidney serum (after absorption with plasma), 0.1 ml, conc  $10\times$ . (wells; from the left to the right), normal rabbit urine, 2 min macromolecule output (0.1 mg); rabbit bile, 0.01 ml, diluted  $\frac{1}{4}$ ; lyophilized watersoluble rabbit liver extract, 0.03 mg of dry powder; normal rabbit plasma, 0.01 ml; normal rabbit plasma, 0.01 ml, conc.  $5\times$ .

duodenum, then tied with a silk braid.<sup>3</sup> The cystic duct was also ligated with a flax thread at the neck of the gallbladder, to avoid its bursting. Some animals died spontaneously during the first day and those which survived were in a very poor condition at the end of the second day, and were sacrificed at this time. A "mock operation" was performed on two rabbits as technical controls, consisting of the same surgical procedure but in these the knots were not tightened.

*Immunoanalysis of urine and plasma.* Antitissue serum was obtained from a duck immunized with rabbit kidney by 26 weekly intraperitoneal injections [each of 180 mg of dry powder of kidney soluble fraction prepared as we have previously described (5)].

This antiserum was chosen from many others prepared in the same manner, because it fortuitously had a low titer of antibodies against whole blood antigens and a high titer of antibodies against tissue constituents. Thus it was easy to remove the undesirable antibodies by absorption with sequential volumes of rabbit plasma.

The antibodies of the absorbed serum precipitated multiple categories of water-soluble tissue antigens, most of them being present both in the kidney and liver extracts (5). No antibodies vs. plasma and bile were disclosed by immunoanalysis (Fig. 1).

Duck antirabbit plasma serum was prepared using the same technique of immunization. Liver water-soluble extracts, used as a control of the antiserum activity, were prepared as we have previously described (5).

Immunoanalyses (Ouchterlony method and immunoelectrophoresis) were performed in an acrylic gel, under standardized conditions (6). Reading of the results, performed by two observers, has been expressed both as the number of precipitates and also as the intensity of the precipitation ( $\pm$ , +, ++).

The definition of aliquots submitted to analyses was as follows: for urine, the quantity of urine macromolecules corresponding to a 2-min outflow, and for plasma a constant volume of 0.01 ml and a concentration of 5 times.

*Other techniques.* The SGPT, serum alkaline phosphatase, serum and urine bilirubin concentrations were measured by

<sup>3</sup> No. 5 (decimal gauge No. 9). Les Laboratoires Bruneau (Paris).

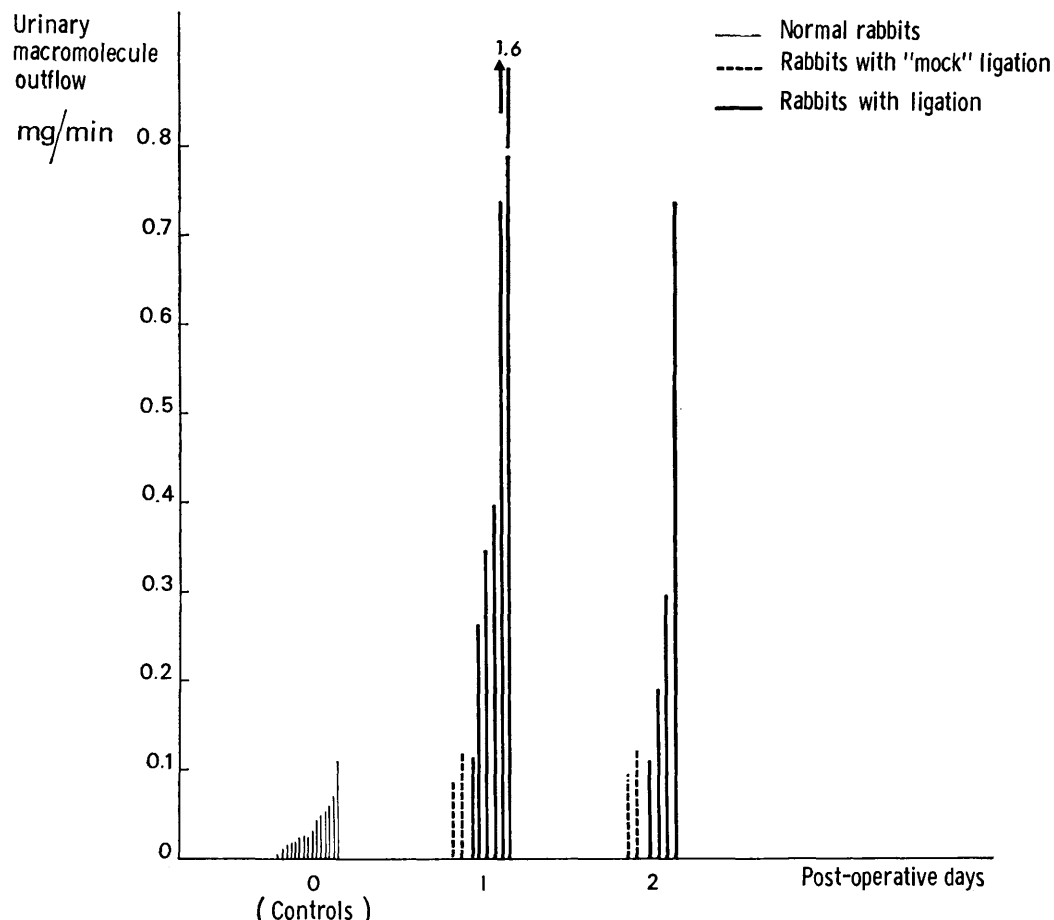


FIG. 2. Urinary outflow of total macromolecules after common bile duct ligation. The mean value of 15 controls is 0.04 mg/min; 0.11 mg/min for "mock ligations"; 0.58 mg/min for the first day after ligation and 0.34 mg/min for the second day. Individual values are shown.

the quoted techniques (7, 8). Fragments from histological examination have been fixed with formaldehyde (10%) and embedded in paraffin. Sections were stained with hematoxylin-eosin and Masson trichrome.

**Results. Total urinary macromolecule output** (Fig. 2). The mean value of total urinary macromolecule output in 15 normal rabbits samples was 0.04 mg/min, with 66% of the results between 0.01 and 0.05 mg/min. This value compares with 0.11 mg/min on the first and second postoperative days in animals submitted to a "mock operation" as controls.

Animals with the common bile duct ligation had significantly increased output of urine macromolecules: 0.58 mg/min on the first

operative day and 0.34 mg/min for the surviving animals on the second day (mean values).

**Urinary output of plasma proteins.** In comparison with the normal and control rabbits, the output of diverse plasma proteins increased slightly in the urines from rabbits with the common bile duct ligated.

**Serum enzymes and bilirubin.** In normal rabbits, SGPT mean level was 35 Frankel U, serum alkaline phosphatase mean level was 2.8 Bessy U, bilirubin plasma level was below 0.5 mg/10 ml and no bilirubin was detectable in urine.

In rabbits submitted to "mock operation," all these values were in normal range, except

for SGPT which was increased to a mean value of 140 Frankel U on the first postoperative day and of 122 on the second day. In rabbits with common bile duct ligation, all the above substances were increased, as shown in Table I.

TABLE I.

Day:	Postoperative		Units
	1	2	
SGTP	162	115.6	(Frankel U)
Serum alkaline phosphatase	16.3	10	(Bessey U)
Serum bilirubin	5.6	11.2	(mg/100 ml)
Urine bilirubin	23.3	29.8	(mg/100 ml)

*Histology of the liver and the kidney.* In the control animals ("mock ligation") the liver and kidneys were normal. In the animals with the common bile duct ligated, their livers contained necrotic areas disseminated around portal venules. Necrosis was variable among the different animals in its intensity and extension. In the center of the necrotic areas, hepatocytes appeared "empty" with only the cell membranes persisting, while in the periphery they were hyperacidophilic. In the kidneys of the two rabbits, which died spontaneously, some tubular expansion with casts and a few fibrinoid thrombi in the glomerular tufts were noted.

*Histuria after common bile duct ligation* (Fig. 3). In 15 normal samples, the technique we used has detected no tissue-like antigens except for 2 faint positive results consisting of one precipitate. After "mock operation," a faint histuria was observed in one of the two rabbits. After common bile duct

ligation, histuria was always significant both in intensity and number of the precipitates observed, as is shown in Table II.

Immunoelectrophoretic analysis of the positive samples showed that the tissue-like macromolecules were spread from the cathodic to the anodic zone (Fig. 4, upper).

*Histemia after common bile duct ligation.* In the plasma of the control rabbits, no tissue-like macromolecules were detectable. After common bile duct ligation, plasma of the rabbits always contained tissuelike macromolecules in variable amount (Fig. 5). The number of precipitation lines observed in the

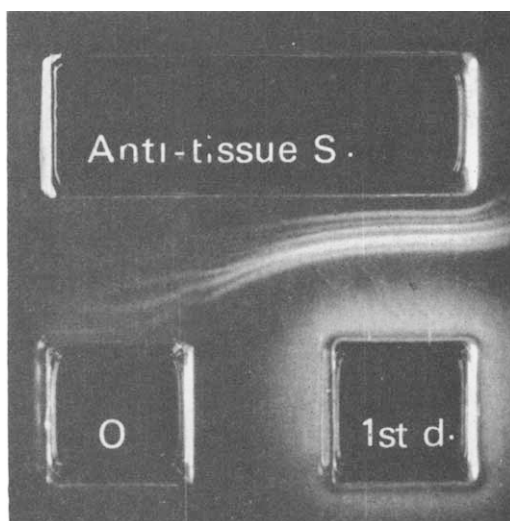


FIG. 3. Immunoanalysis (Ouchterlony type) of the urinary macromolecules from a rabbit after ligation of the common bile duct: (trough), duck antirabbit kidney serum (0.03 ml, conc 10X). (wells), 2-min urinary macromolecule outflow from the day before the operation on the left hand, and from the first postoperative day on the right hand.

TABLE II.

	Before operation		After operation				
			day:				
			1		2		
Controls							
Normal	0	0	0	0	0	1±	1±
"Mock operation"	0	0	0	0		1±	2±
Ligation	0	0	3++ <sup>a</sup>	7++ <sup>a</sup>		1+	2+
	0	0	3+	2+		1+	2+
	0	0	5+	3+		3+	2+

<sup>a</sup> These two rabbits survived only 1 day after the operation.

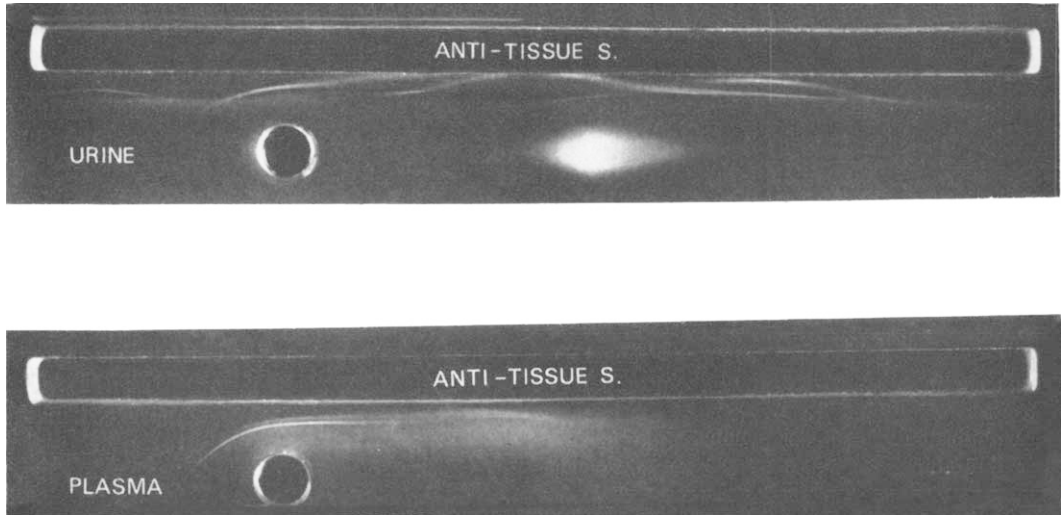


FIG. 4. Comparative immunoelectrophoretic analyses of urine and plasma of the same rabbit, on the first day after common bile duct ligation: (trough), duck antirabbit tissue serum (0.2 ml, conc 10 $\times$ ). (upper well), 2-min urinary macromolecule outflow; (lower well), 0.02 ml of plasma, conc 3 $\times$ .

plasma was from 2 to 6 by Ouchterlony technique, and their intensity was often strong, as is shown in Table III.

Immunoelectrophoretic analyses of these tissue-like antigens in the plasma showed that

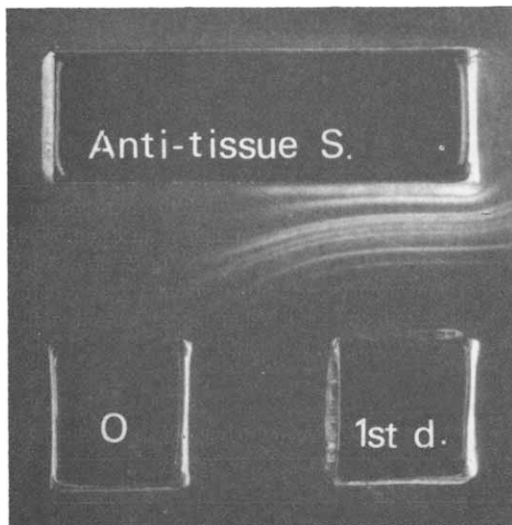


FIG. 5. Immunoanalysis (Ouchterlony type) of the plasma macromolecules from a rabbit after ligation of its common bile duct: (trough), duck antirabbit tissue serum (0.03 ml, conc 10 $\times$ ). (wells), rabbit plasma (0.01 ml, conc 5 $\times$ ) from the day before the operation on the left hand, from the first postoperative day on the right hand.

their electrophoretic mobility was in the  $\beta$  zone (Fig. 4, lower).

*Correlations. Correlations between histuria and total urinary macromolecule output.* There is a correlation between the total urinary macromolecule output and the importance of histuria (intensity and number of the precipitates). When histuria was significant, the macromolecule outflow was nearly always greater than 0.1 mg/min, and when histuria was very faint or nil, the macromolecule outflow was nearly always less than 0.1 mg/min.

*Correlations between hepatic necrosis and histuria and histemia.* A consistent correlation exists between the intensity of hepatic necrosis and that of simultaneous histuria (Fig. 6) both for intensity and number of the precipitates. The same correlation exists for histemia.

*Correlations between histemia and serum enzymes.* There was no correlation between the SGPT level and the histemia intensity when all the data are plotted together, but a correlation exists when the "mock operation" rabbits are excluded because histemia was absent and SGPT was high in this last condition. When serum alkaline phosphatase level was abnormal ( $> 6$  Bessey U), a significant

TABLE III.

	day:	After operation							
		1				2			
Controls, normal	0	0	0	0	0	0	0	0	0
“Mock operation”	0			1±		0			1±
Ligation		5+++ <sup>a</sup>		5+++ <sup>a</sup>					
		3+++		2+++		2+++		2+	
		2+++		2+		2+++		3+	

<sup>a</sup> These two rabbits survived only 1 day after the operation.

histemia was regularly observed, but there was no constant distribution between the two phenomena. The same distribution was observed for bilirubin plasma level as for alkaline phosphatase.

*Correlation between histuria and urine bilirubin level.* No correlation exists here.

*Correlation between postoperative survival time and histuria and histemia.* There is a significant correlation between intensity of histemia or histuria during the first postoperative day and the spontaneous survival time, as shown in Table IV. As a control, no correlation exists between the survival time and the total urinary protein outflow.

*Correlation between histuria and histemia.*

There is a significant correlation between the general intensity of the two phenomena ( $p < 0.01$ ). The antigenic patterns of the tissue-like macromolecules simultaneously present in urine and plasma have been compared by the two types of immunoprecipitation used in this work. Some of these tissue macromolecules appeared antigenically similar, but for the most part they were different antigenically (Fig. 7) and in their electrophoretic mobility (Fig. 4).

*Discussion.* The present data show tissue-like macromolecules excreted in urine of animals with necrotic lesions in the liver and with normal kidneys. This is an experimental approach to the phenomenon we have de-

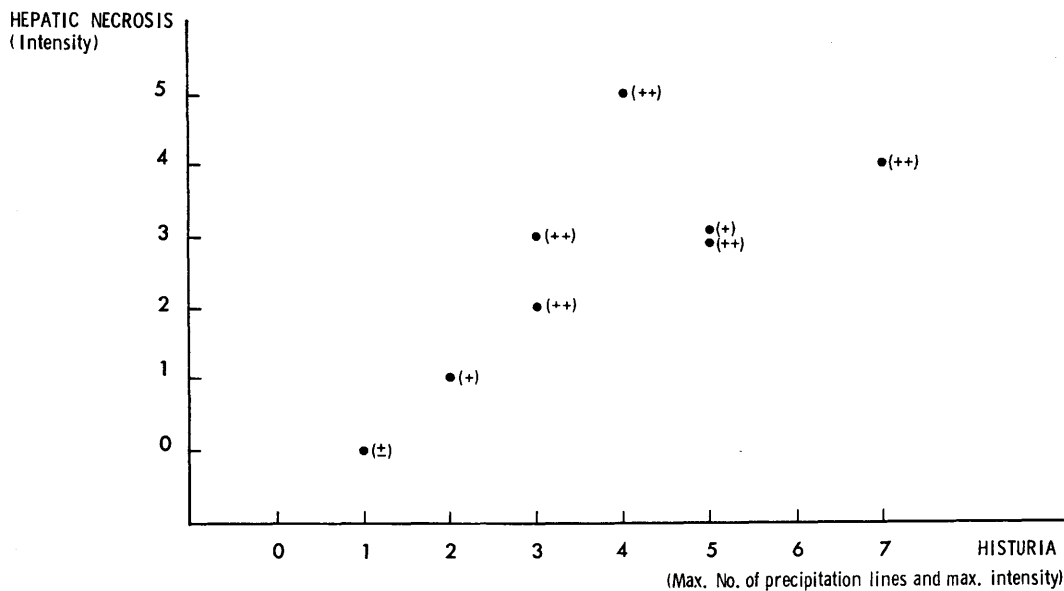


FIG. 6. Correlation between the intensity of histuria and the importance of hepatic necrosis. The importance of hepatic necrosis has been scored in five different grades according to the extension and the number of the necrotic areas observed.

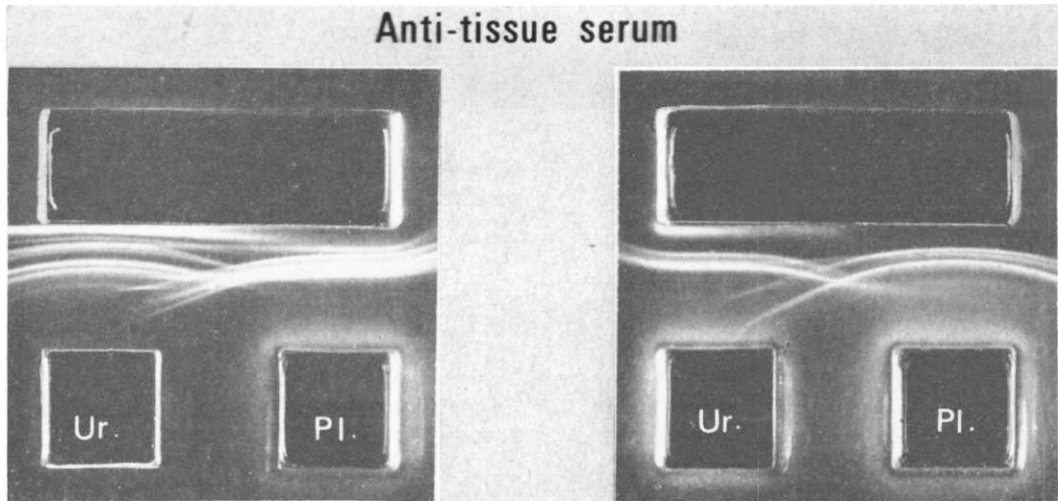


FIG. 7. Comparative immunoanalysis of simultaneous histemia and histuria in juxtaposition. The two sets of photographs correspond to two different rabbits on the first day after common bile duct ligation. (trough), duck antirabbit tissue serum: 0.03 ml, conc 10 $\times$ . (wells, right wells of each set), rabbit plasma:0.01 ml, conc 5 $\times$ ; (left wells of each set), 2-min urinary macromolecules. (left set), 2 antigens are common between plasma and urine; for the others, 3 exist only in plasma and 5 only in urine; (right set), 1 antigen is common between plasma and urine; for the others, 3 exist only in plasma and 3 only in urine.

scribed as "histuria" (1, 2). The data also show tissue-like macromolecules circulating in the blood stream, a phenomenon for which we propose the term "histemia."

The data demonstrate that the actual mechanism of histuria may be via histemia when the organic lesion is outside of the kidney and urinary tract. We had already anticipated histemia as one of the two possible mechanisms for histuria (1), but it had not yet been demonstrated except in a very

few instances during renal transplantation with jaundice (3).

However, the presence of tissue macromolecules circulating in the blood stream and originating from organic lesions has been widely known for a long time. For example, it is routinely shown by enzymatic techniques in pathological lesions of the liver or the myocardium, but it has been rarely studied by an immunological approach (9, 10). This abnormal circulation of organic macromolecules has been suspected as a possible origin of auto-immunity (11).

We have already emphasized the great similarity which exists between the nonblood tissue antigens of the liver and those of the kidney (5). This is why we used as antitissue reagent for the analyses, an immune serum induced by rabbit kidney injections. Many of the macromolecules antigenically determined to be of tissue origin probably have an enzyme activity. This is the case for alkaline phosphatase whose blood level increase was simultaneous with the histemia, but the SGPT was not involved in the precipitated antigens since there was no correlation in

TABLE IV.<sup>a</sup>

	Animals which survived		
	24 hr	At least 48 hr	
Histemia	5++ 5++		2+
			2++
			2++
			3++
Histuria	3++ 7++		2+
			3+
			3+
			5+

<sup>a</sup> The distribution of the product (number  $\times$  intensity of precipitates) has a  $p < 0.05$ .

intensity between its abnormal level and the concomitant histemia.

In this experiment, the close correlation between histemia and histuria is in agreement with the assumption that histemia is the mechanism which may precede histuria when the originating lesion is outside of the kidney. A more precise analysis of the antigenic and electrophoretic properties of the tissue-like molecules, simultaneously observed in plasma and urine, shows that only a few of them are similar. It appears that the tissue molecules in the blood stream are those of large size which cannot pass the glomerular membrane, and the tissue molecules in the urine are those of a smaller size which might have been rapidly excreted through the glomerular wall. This hypothesis is in agreement with the electrophoretic mobilities observed for the two categories of the tissue-like macromolecules: only the  $\beta$  zone for those in plasma contrasting with the large electrophoretic spread of those present in urine. These smaller molecules may be intact molecular constituents from the necrotic liver, or molecular moieties from the same origin. The partial antigenic community observed between the tissuelike molecules from plasma and urine is in good agreement with this last possibility.

The faint concomitant outflow of urinary plasma proteins with the large outflow of tissuelike macromolecules probably does not incriminate a kidney lesion. It may be simply explained by tubular reabsorption competition (12), a theory which assumes that any abnormal protein excretion in the urine compels a simultaneous plasma protein excretion.

Thus it appears that an early intense histuria or histemia gives a poor prognosis in terms of survival time. This relationship is probably the reflexion of increased hepatic necrosis.

*Summary.* Patchy necrotic lesions of the

liver were produced in rabbits by common bile duct ligation. This surgical procedure does not result in any detectable alteration in the kidney. Urinary outflow of tissuelike macromolecules was detected by immunoprecipitation techniques using a specific duck antirabbit tissue serum. The presence of a significant amount of tissue-like macromolecules was demonstrated in the blood stream at the same time. Comparative immunoanalyses of these abnormal plasma and urine proteins showed that a few of them were identical, but that the majority had differing antigenic and electrophoretic patterns. It is probable that the smallest of the molecules (or moieties) originating from the liver had passed through the glomerular membrane, and that the biggest ones had been retained in the blood stream at least for a longer time. This experiment demonstrates that histemia occurs before the histuria resulting from an organic extrarenal lesion.

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Received April 18, 1969. P.S.E.B.M., 1969, Vol. 132.