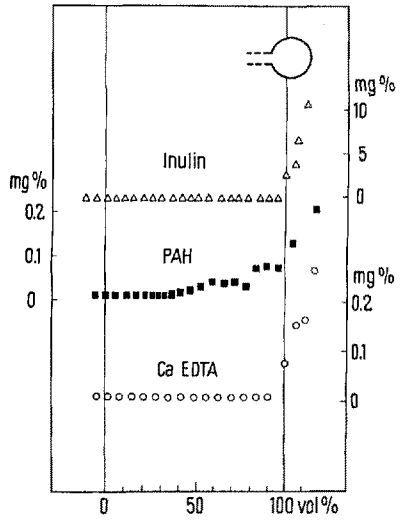
The identical results of these two series of experiments which are, surprisingly, indicative of non-participation of tubular mechanisms in the excretion of EDTA by the avian kidney, were confirmed in further experiments comparing the rate of cumulative EDTA excretion with the rate of inulin excretion in the chicken. In experiments in which urine was collected from both kidneys together, however, and in which $CaNa_2EDTA$ and inulin (150 mg/kg and 25 mg/kg respectively) were injected simultaneously and intravenously, no difference was found in the rate of excretion of these two substances, thus indicating that they were excreted by the same renal mechanism, i.e. by simple glomerular filtration.



Excretion patterns of inulin, sodium *p*-aminohippurate and $CaNa_2$-EDTA in individual drops of urine collected from the ipsilateral ureter after instantaneous injection in the renal portal system in the chicken. Different time intervals of individual drops are substituted in the Figure by volume % of the urine volume contained in the kidney at the moment of injection.

The following conclusions may be drawn from these experiments: (1) in rats, the EDTA anion is excreted by tubular secretion as well as by glomerular filtration; (2) this tubular secretion is not dependent on the pH value of the urine; (3) it is not inhibited by the administration of sodium *p*-aminohippurate, diodrast, probenecid or quinine; (4) in the chicken EDTA anion is excreted by glomerular filtration only.

The only explanation of these results appears to be that EDTA is not excreted by either of the known tubular secretory mechanisms (i.e. neither by the hippurate system nor the organic base system), but by some other mechanism specific for mammals and not existing in birds.

Zusammenfassung. Die Ausscheidungsgeschwindigkeit nach intravenöser Injektion von $CaNa_2$ÄDTA bei Ratten war höher als die des Inulins und änderte sich weder mit dem pH-Wert des Harnes noch mit der Belastung durch hohe Dosen von PAH, Diodrast, Probenecid oder Chinin. Bei den Hühnern hingegen wurde kein wesentlicher Unterschied zwischen der Ausscheidungsgeschwindigkeit von $CaNa_2$ÄDTA und Inulin gefunden und eine tubuläre Sekretion der ÄDTA konnte sogar bei der Anwendung der Methode von SPERBER nicht bestätigt werden.

J. HELLER and J. VOSTAL

Institute of Industrial Hygiene and Occupational Diseases, Prague (Czechoslovakia), November 7, 1963.

Inhibition of Dog Fibrinolytic System in Experimental Tubular Necrosis of Kidney

Urokinase, a plasminogen activator excreted in urine, may be a product of kidney, as already suggested by some authors[1,2]. PAINTER and CHARLES[3] demonstrated an accumulation of soluble plasminogen activator during the growth of cultures of monkey and dog kidney cells in serum free media. A great fibrinolytic activity in venous renal blood has been found by BULUK et al.[4–6]. According to these authors, about 94% of urokinase is secreted by the kidney into the general circulation, and 6% only into urine.

It is well known that mercury chloride produces necrosis of kidney tubular cells, particularly of those in the Henle loops[7].

The purpose of this paper is to investigate the influence of mercury chloride intoxication upon the fibrinolytic system in dog.

Experiments were performed on 23 mongrel dogs. 14 dogs were injected with mercury chloride, subcutaneously, in a daily dose of 3 mg per 1 kg of weight during 5 days. Then blood was drawn from tibial and renal veins of those dogs under a general anaesthesia. Control dogs were treated in a similar way.

The following determinations were performed on dog plasma: prothrombin time[8], fibrinogen level[8], Factor V[9], Factor VII[10], Factor VIII[11], plasminogen and plasminogen proactivator[12], and antiplasmin[13]. Euglobulin fibrinolysis was measured using both test tube[14] and fibrin

[1] T. ASTRUP and I. STERNDORFF, Proc. Soc. exp. Biol. Med. *81*, 675 (1952).
[2] K. N. KAULLA and N. RIGGENBACK, Thromb. Diath. Haem. *8*, 162 (1960).
[3] R. H. PAINTER and A. F. CHARLES, Amer. J. Physiol. *202*, 1128 (1962).
[4] K. BULUK and M. FURMAN, Exper. *18*, 146 (1962).
[5] K. BULUK and M. MALOFIEJEW, Acta phys. Polonica, *14*, 371 (1963).
[6] M. FURMAN, Doctor Dissertation, Bialystok (1963).
[7] J. OLIVER, M. MACDOWELL, and A. TRACY, J. clin. Investig. *30*, 1305 (1951).
[8] A. J. QUICK, *The Physiology and Pathology of Haemostasis* (Kimpton, London 1951).
[9] P. WOLF, J. clin. Path. *6*, 34 (1953).
[10] F. KOLLER, A. LOELIGER, and F. DUCKERT, Acta Haemat. *6*, 1 (1951).
[11] J. P. SOULIER and M. J. LARRIEU, J. lab. clin. Med. *41*, 849 (1953).
[12] S. NIEWIAROWSKI, Path. Biol. (Paris) *7*, 2557 (1959).
[13] M. NIEWIAROWSKA and Z. WEGRZYNOWICZ, Thromb. Diath. Haem. *3*, 279 (1959).
[14] E. KOWALSKI, M. KOPEĆ, and S. NIEWIAROWSKI, J. clin. Path. *12*, 215 (1959).

plate methods[15]. A correlation was found between the results of both methods. Fibrinolytic activity was usually expressed as the fibrinolytic index $f = 1000/t$ min (t = clot lysis time).

Figure 1 shows a rapid decrease of the fibrinolytic activity after mercury chloride injections. This phenomenon is followed by a gradual increase of the fibrinogen level. Fibrinolytic activity of the renal venous blood of the normal dog is several times higher than that of its peripheral blood. After intoxication, the fibrinolytic activity decreases in the peripheral blood 8 times, and in renal blood 12 times (Figure 2). It can be seen from Figure 3 that the fibrinogen level is considerably higher in peripheral than in renal blood. In the case of kidney damage, the fibrinogen level in venous peripheral blood increased approximately 3 times and in venous renal blood approximately 4 times. Figure 4 shows an increase of plasminogen, plasminogen proactivator and Factor VIII activities under the above-mentioned condition. The prothrombin time, the level of Factor V and that of Factor VII did not show any changes, antiplasmin activity being reduced to 30% of its initial value.

Our experiments revealed a slight elevation of the urea level and transaminase activity in the serum of intoxicated dogs. Necrosis of tubular cells was ascertained by means of microscopic investigations. The glomeruli were only slightly affected (Dr. L. REJNIAK, Department of Pathological Anatomy).

The hypothesis of kidney secreting urokinase into the general circulation is sustained by the results given below: (1) Fibrinolytic activity of renal blood is considerably higher than that of peripheral blood. (2) Significant depletion of the fibrinolytic activity occurs in tubular necrosis of kidney. It may be suggested that the fibrinolytic activity of renal blood is related to the secretion rate of urokinase.

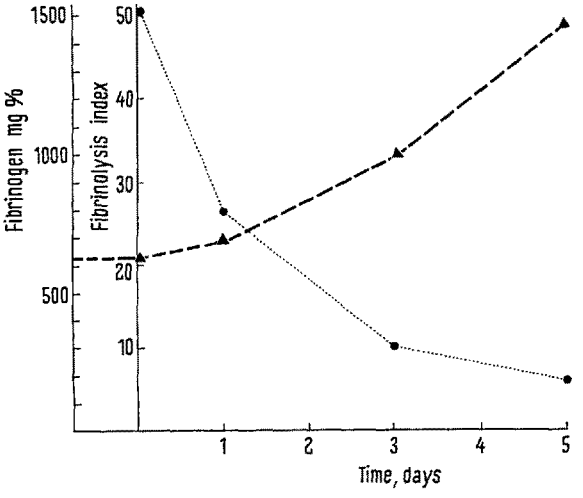


Fig. 1. Euglobulin fibrinolysis and fibrinogen level in dog during progressive kidney damage. Broken line: fibrinogen level. Dotted line: fibrinolysis index. Mean values obtained from 4 experiments.

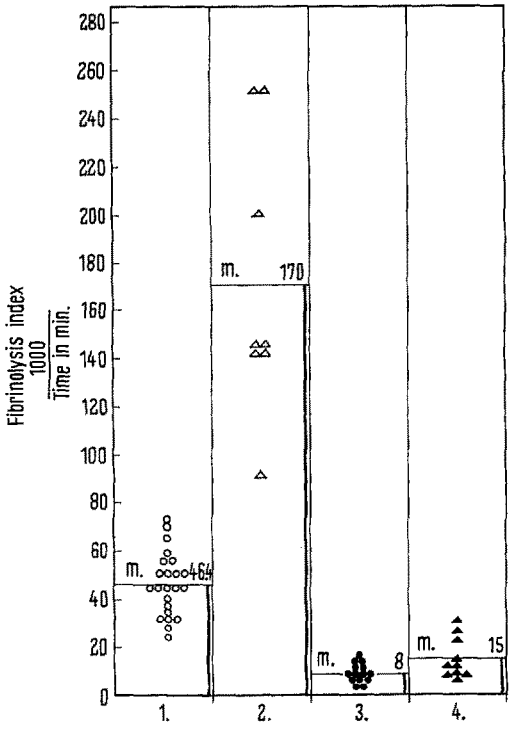


Fig. 2. Euglobulin fibrinolytic activity: 1. Venous peripheral blood of normal dogs. 2. Venous renal blood of normal dogs. 3. Venous peripheral blood of intoxicated dogs. 4. Venous renal blood of intoxicated dogs.

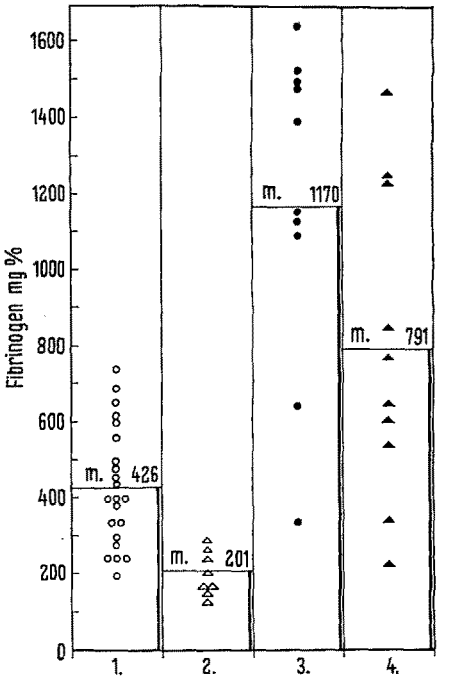


Fig. 3. Fibrinogen level of peripheral and renal blood of normal and intoxicated dogs. Explanation given in Figure 2.

[15] T. ASTRUP and M. ALKJAERSIG, Arch. Biochem. *37*, 99 (1952).

GUEST and CELANDER[16] observed an increase of plasma fibrinolytic activity and a decrease of fibrinogen and plasminogen level after an injection of purified urokinase preparation.

According to our observations, the level of plasminogen and fibrinogen in venous renal blood was lower than in peripheral blood. We suggest that an excess of plasminogen, plasminogen proactivator, fibrinogen and Factor VIII in blood plasma of intoxicated dogs is the result of the depletion of urokinase secretion rate caused by kidney damage.

An important role of urokinase secretion may be suggested for the maintenance of fibrinolytic balance in the organism. This enzyme, supplied by the kidney to the general circulation, would continuously activate plasminogen into plasmin. Subsequently, plasmin would digest fibrinogen and other factors sensitive to its action, e.g. A.H.G. A rapid inactivation of urokinase and plasmin in the blood stream might also occur.

It is not excluded that the decrease of antiplasmin activity in experimental kidney damage is a compensatory phenomenon.

It may be assumed that hypercoagulability and hyperfibrinogenemia, found in various renal diseases by several authors[17–20], are related to the depletion of urokinase secretion rate.

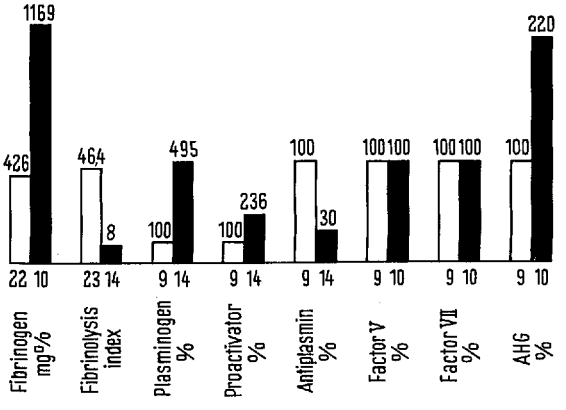


Fig. 4. Influence of $HgCl_2$ intoxication upon some clotting and fibrinolytic factors of peripheral blood in dogs. White columns: values before experiments. Black columns: values after experiments. Values under columns correspond to the number of determinations.

Résumé. Au cours de la necrose expérimentale des tubules rénales du chien, l'activité fibrinolytique du sang veineux rénal et périphérique se diminue progressivement à mesure qu'augmente le taux du fibrinogène, du plasminogène et du Facteur VIII.

S. NIEWIAROWSKI, J. PROKOPOWICZ, A. POPŁAWSKI, and K. WOROWSKI

Department of Physiological Chemistry, Medical School, Bialystok (Poland), August 5, 1963.

[16] M. M. GUEST and D. R. CELANDER, Texas Rep. Biol. Med. *19*, 89 (1961).
[17] F. H. SCHULTZ, *Das Fibrinogen* (VEB Georg Thieme, Leipzig 1953).
[18] J. H. LEWIS, M. B. ZUCKER, and J. H. FERGUSON, Blood *11*, 1073 (1956).
[19] R. GROSS, H. NIETH, and E. MAMMEN, Klin. Wschr. *36*, 107 (1958).
[20] O. EGEBERG, Scand. J. clin. lab. Investig. *14*, 2, 163 (1962).

PRO EXPERIMENTIS

Separation of Catalase and Other Red Cell Enzymes from Hemoglobin by Gel Filtration

Recent work in this laboratory has concentrated on the isolation of catalase from small amounts of human blood. It was found that gel filtration through dextran gel (Sephadex G-100) provides an excellent means to accomplish in one step the separation of catalase from the bulk of the proteins present in a concentrated hemolysate of human erythrocytes. Since it was observed that in addition to catalase also other enzymes can easily be separated, it seems that this procedure is of particular value as an initial step in the preparation of certain red cell enzymes.

As starting material, samples of highly concentrated hemolysate were prepared as follows: Blood containing 10% v/v A.C.D.-solution[1] was centrifuged, the sediment resuspended and washed 3 times in an equal volume of 0.95% NaCl-solution. The packed red cells were then lysed in the same volume of distilled water. In order to complete hemolysis, the material was frozen and thawed three times. Removal of any particulate matter (stroma) was accomplished by filtration in the following manner: A layer (1 cm) of dry 'Hyflo-supel-cel' powder[2] was placed on a G-3 sintered glass funnel ($d = 3.5$ cm). After a small amount of distilled water had been sucked through, the hemolysate (20 ml) was added on top of the layer. Slight vacuum was applied until the water had passed the funnel and hemolysate was then collected separately by efficient suction with an oil pump. Usually 12–15 ml portions of hemolysate could be obtained before the filter was blocked.

For the gel filtration of this material a column, 2.0 cm in diameter and 52 cm in height, was used. The column was packed at room temperature with Sephadex G-100 according to FLODIN[3] and equilibrated in the cold-room with the salt solution to be used. The sample was applied by slowly pipetting the desired volume (3–15 ml) onto the top of the column. Elution was accomplished by a 0.1%

[1] Solutio Anticoagulans; U.S.P. 14th Revision (1950), p. 550.
[2] A filter aid product from diatomaceous sediment, of Johns-Manville Corp., New York.
[3] P. FLODIN, J. Chromatography *5*, 103 (1961).