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.

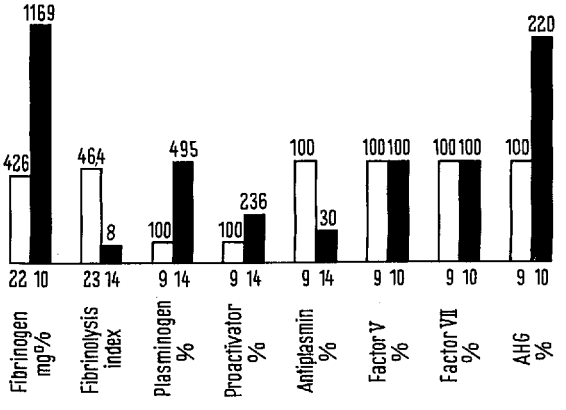

Fig. 4. Influence of $HgCl_2$ intoxication upon some clotting and fibrinolytic factors of peripheral blood in dogs. White columns: values before experiments. Black columns: values after experiments. Values under columns correspond to the number of determinations.

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.

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

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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.
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NaCl solution containing 0.75% chloroform from a reservoir connected to the column by flexible tubing such that the level of the stock solution was a few centimeters above the top of the gel column. The flow rate was approximately 40 ml per h. In the effluent fractions catalase activity[4], hemoglobin[5] and protein by the Folin-Lowry colour[6] were determined. In some experiments lactate dehydrogenase[7], aldolase[8] and pyruvate kinase[9] activity were measured in addition. Figure 1 demonstrates an elution pattern obtained from 3 ml of hemolysate; Figure 2 shows a pattern obtained from 15 ml of hemolysate on the same column. It is evident that catalase and the other enzymes measured can be separated to a high degree from hemoglobin and other proteins by gel filtration on Sephadex G-100. In our hands, this procedure also proved to be useful in the isolation of catalase active material from blood of 'acatalatic' individuals, as well as in the preparation of hemoglobin solutions virtually free of catalase[10].

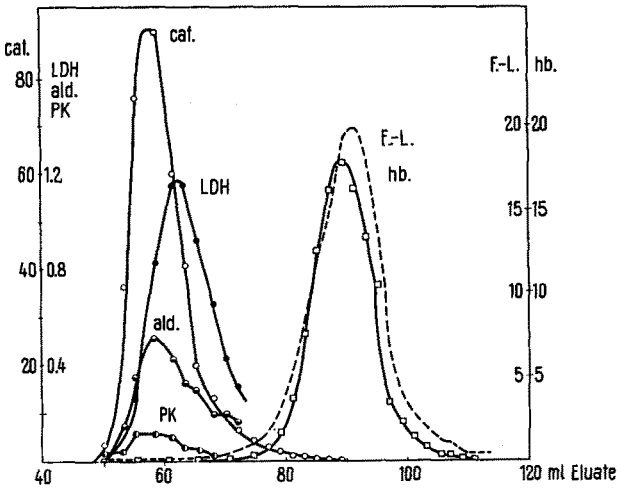

Fig. 1. Gel filtration of 3 ml of hemolysate on Sephadex G-100 (see text); o—o catalase (cat.) as milli-equivalents of perborate used in 5 min per ml; •—• lactate dehydrogenase (LDH) as international enzyme units per ml; ⊖—⊖ aldolase (ald.) as international enzyme units per 10 ml; ⊙—⊙ pyruvate kinase (PK) as international enzyme units per 10 ml; □—□ hemoglobin (hb.) as mg per ml; ---- Folin-Lowry colour (F.-L.) as optical density at 750 nm ($l = 1$ cm) in the eluate.

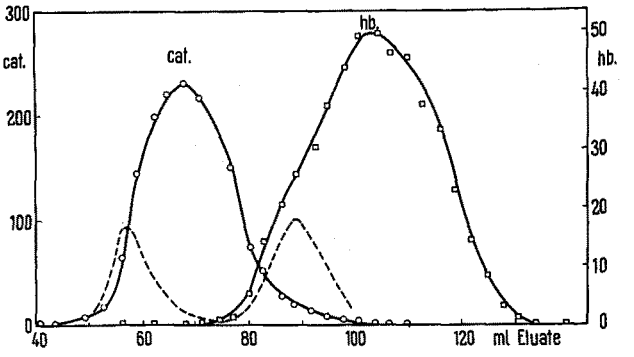

Fig. 2. Gel filtration of 15 ml of hemolysate on Sephadex G-100 (see text) o—o catalase (cat.) as milli-equivalents of perborate used in 5 min per ml; hemoglobin (hb.) as mg per ml. Broken lines indicate the catalase and hemoglobin peaks as they were found in the experiment represented in Figure 1. It is to be noted that the positions of the leading edges of the catalase peaks and of the hemoglobin peaks respectively are nearly the same in the two experiments.

Zusammenfassung. Es wird über ein einfaches Verfahren zur Abtrennung von Katalase und anderer Enzyme aus konzentriertem Haemolysat mit Hilfe der Gel-Filtration unter Verwendung von Sephadex G-100 berichtet.

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Automatisches Auftragen von Papierchromatogrammen

Substanzgemische, deren Komponenten sich in ihren Rf-Werten wenig voneinander unterscheiden, lassen sich papierchromatographisch nur dann sauber trennen, wenn es gelingt, den Startfleck klein zu halten. Ist man gezwungen, grössere Lösungsmengen aufzutragen, so kann dieser Arbeitsgang äusserst langwierig sein, insbesondere dann, wenn die zu fraktionierenden Verbindungen keine Erwärmung vertragen. Dies wiederum kann beim Arbeiten mit radioaktiven Präparaten besondere Vorsichtsmassnahmen notwendig machen, die die Vorbereitung der Chromatogramme zusätzlich erschweren.

Wir sind seit einiger Zeit dazu übergegangen, alle Papierchromatogramme automatisch aufzutragen. Dazu bedienen wir uns eines Dauerinfusionsgerätes (Typ «Unita I» der Firma Apparatebau B. Braun, Melsungen, Deutschland), dessen Halterahmen sechs Injektionsspritzen aufzunehmen vermag (vgl. Figur). Bei diesem Apparat lässt sich die Vorschubgeschwindigkeit der Spritzenkolben in 24 Stufen so variieren, dass bei Verwendung von 5 cm^3-Pipetten stündlich Mengen zwischen 7,5 mm^3 und 30 cm^3 abgegeben werden.

Auf den zur Befestigung der Kanülen angesetzten Konus wird jeweils ein PVC-Schlauch aufgeschoben, der die Verbindung zur eigentlichen Auftragepipette, einem zu einer feinen Spitze ausgezogenen Glasröhrchen (Fassungsvermögen etwa 250 mm^3) herstellt. Auf diese Weise ist es möglich, durch Wahl verschieden langer Schläuche sechs waagerecht ausgelegte Papierchromatogramme gleichzeitig zu versorgen. Damit die Chromatographiebögen nicht durch Kapillarkräfte unkontrollierbare Flüssigkeitsmengen aus den Pipetten heraussaugen können, erwies es sich als erforderlich, die Spritzen einschliesslich der Verbindungsschläuche und der Kapillaren luft-