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CLOTTING CHANGES INDUCED BY METAL IONS IN VITRO

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Addition of UO_2^{++}, Sr^{++} and Pb^{++} to plasma in concentrations from 12,5–62,5 μg/ml does not influence the prothrombin activity. Mercury ions cause a prolongation of the plasma prothrombin time resulting in a reduction of the prothrombin index values to about 55%. This effect of mercury depends on the concentration of mercury ions and the exposure time. The highest reduction in prothrombin index values was observed at concentrations from 25,0–37,5 μg/ml after 3 hours of exposure. At higher or lower concentrations the effect was less pronounced.

Several metal ions when applied either in vivo or in vitro have an activating or inhibitory action on the blood coagulation process.

Inorganic compounds of mercury cause hypercoagulability with increased prothrombin and fibrinogen levels in dogs and rabbits (1). Intramuscular injections of mercurial diuretics diminish the clotting time in hemophiliacs (2). On the other hand bleeding time of rabbits is increased if wounds are washed with $HgCl_2$ or other sulfhydryl group reagents (3).

Oral feeding of uranyl chloride causes an increase in the prothrombin time, clotting time and clot retraction time in rabbits (4). The trombin fibrinogen system was found to be more sensitive to uranyl acetate than most of the other enzyme systems, which might explain the lengthening of the clotting time in animals heavily poisoned by uranyl acetate (5). In some inhalation experiments, however, exposure to uranyl ions caused no definite responses for plasma fibrinogen or prothrombin clotting time in dogs and rabbits (6).

Strontium salts accelerate the conversion of the fibrinogen to fibrin by thrombin (7) and activate the human brain thromboplastin (8). Cal-

cium has, however, a greater specificity for plasma coagulation than strontium (9, 10). In higher concentrations than optimum, calcium and related ions (strontium, magnesium and barium) have an inhibitory action on coagulation. It is assumed that the inhibitory action is due to the stabilising effect on fibrinogen (10).

In lead poisoning, especially during lead colics, decreased prothrombin activity was observed in many cases. At the same time a reduction of the factor VII was almost constant (11).

In our experiments the effects of strontium, mercury, lead and uranyl ions on the prothrombin activity in vitro were compared.

METHODS

In experiments with the rat's blood, the blood of female rats weighing from 200–250 g was used. Blood samples were taken in light ether anaesthesia. 8 millilitres of blood were drawn by cardiac puncture into siliconed syringes containing 0.3 ml of sodium oxalate (1.34% solution). The blood was immediately transferred to siliconed conical centrifuge tubes and spun for 10 minutes at 3000 rpm. The plasma was drawn off by suction and divided into two test tubes. 0.5 ml of saline was added to the first test tube which served as control. Metal ions were added in a volume of 0.5 ml to the second test tube. Both test tubes were kept in a water bath at 37° C.

The one stage prothrombin time was determined by Quick's (12) method 8, 30, 60, 90, 120, 150 and 180 minutes after the addition of metal ions. All the values were obtained in triplicate. The results are expressed as prothrombin index values.

In some experiments metal ions were added to human blood. Blood samples were taken by venous puncture, the further procedures being the same as described for the rat's blood. Human blood was used only in experiments in which the effect of mercury ions was investigated.

Metal ions were added in concentrations of 12.5–62.5 μg of Pb^{++}, Sr^{++} and UO_2^{++} per millilitre of the plasma and mercury ions in concentrations from 1.7–87.5 μg per millilitre.

RESULTS

Addition of lead, uranyl and strontium ions in concentrations from 12.5—62.5 μg/ml does not cause any changes in the prothrombin index values.

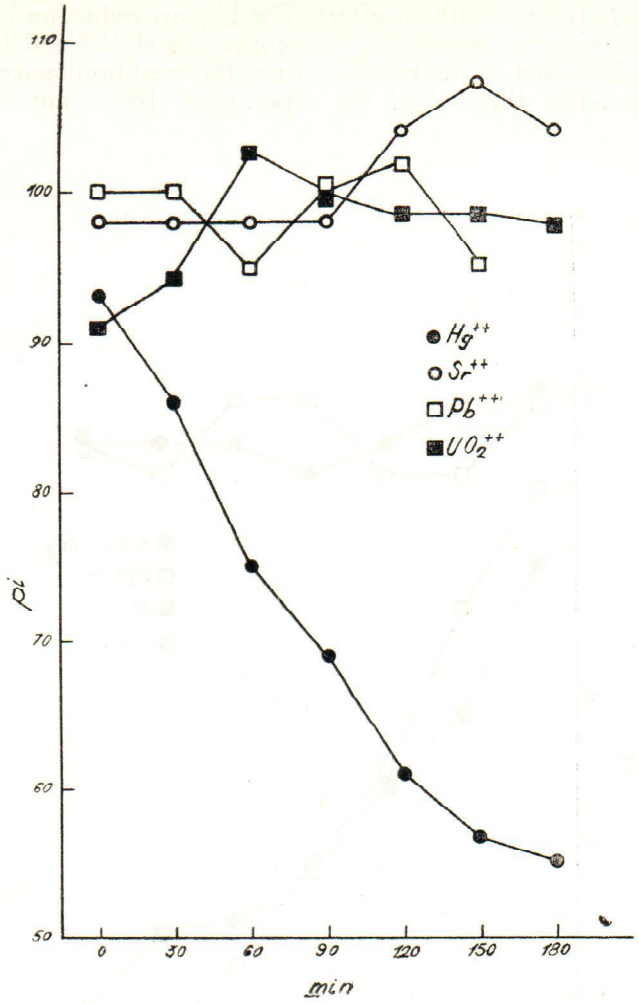

Fig. 1. The effect of the addition of Hg^{++}, Sr^{++}, Pb^{++} and UO_2^{++} to the rat's plasma (25 ug/ml) on prothrombin index values at various intervals.

Fig. 1 shows the effects of 25 μg Hg^{++}, UO_2^{++}, Pb^{++} and Sr^{++} on prothrombin index values at different times after exposure to metal ions. Mercury ions, however, cause a lengthening of the clotting time. By increasing the exposure time, the effect of mercury becomes more pronounced producing prothrombin index values of about 55% after 180 minutes. This effect of mercury depends on the concentration of

the metal ions (Fig. 2). Low and high concentrations of mercury (12.5 and 62.5 μg/ml) were without effect. The highest reduction in the prothrombin activity was observed at concentrations of 25.0 and 37.5 μg/ml. The effect depended on the exposure time, the most pronounced changes being observed at the end of the experiment (180 minutes after exposure).

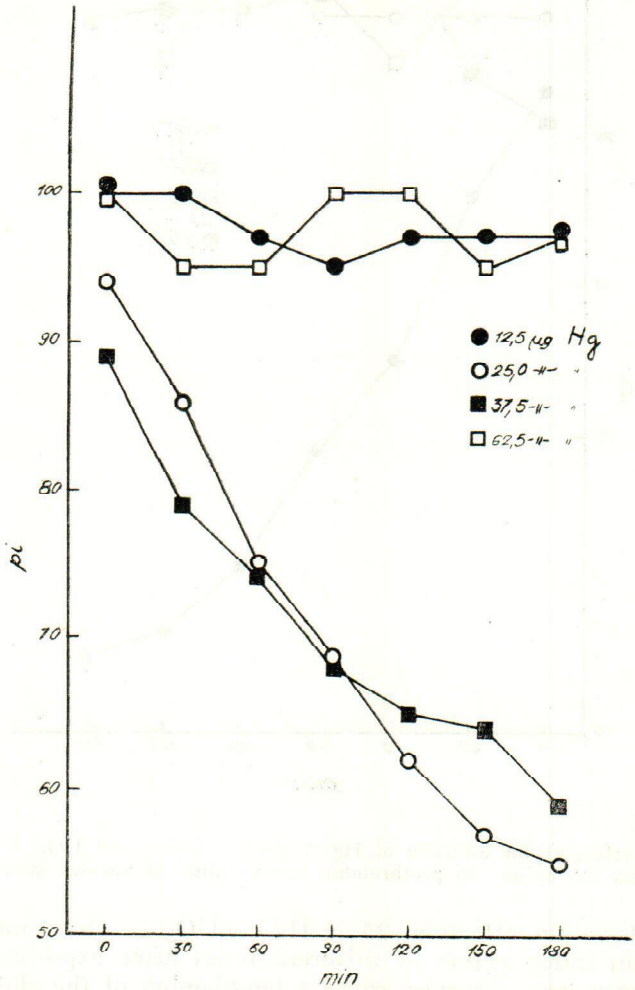

Fig. 2. The effect of different concentrations of Hg ions on prothrombin index values. The effect of the exposure time.

In experiments in which the effect of mercury ions on human plasma was studied concentrations from 1.7–75.0 μg/ml of plasma were used. Lower concentrations were used in order to find threshold values at

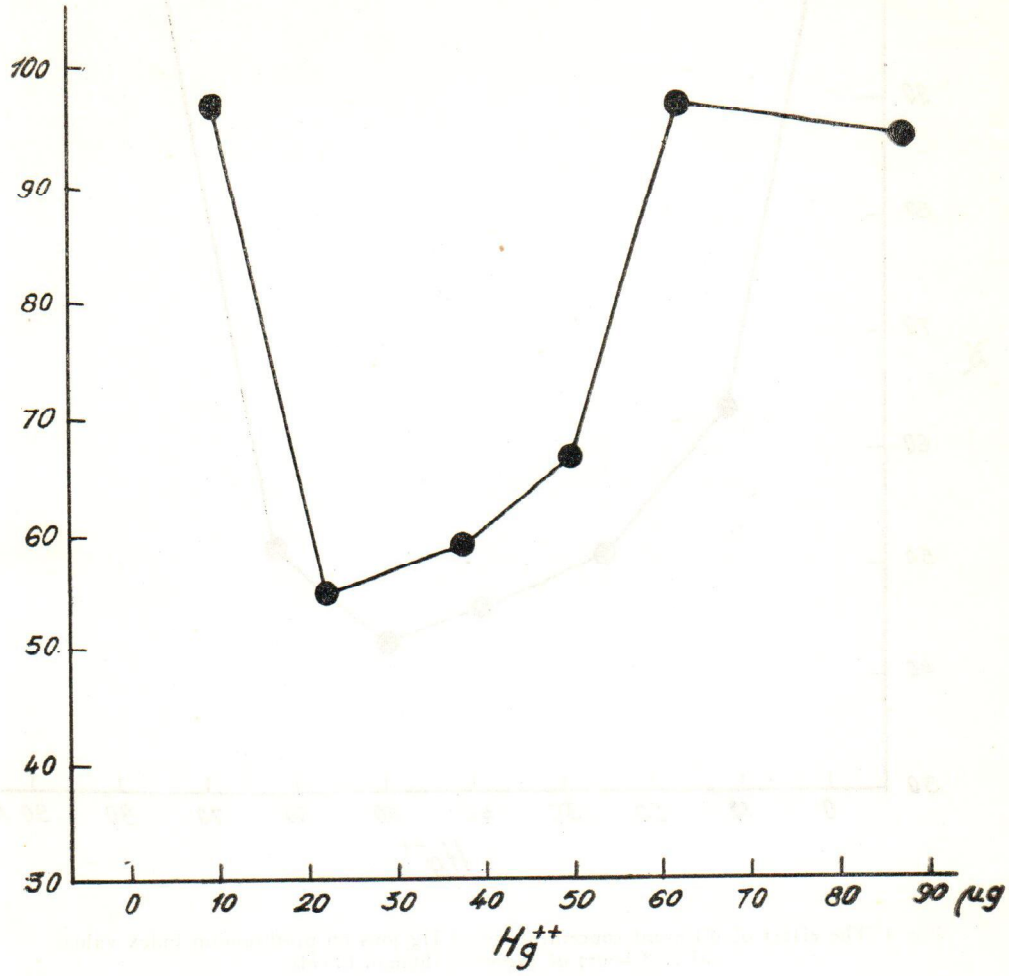

Fig. 8. The effect of different concentrations of Hg ions on prothrombin index values after 3 hours of exposure (rat's blood).

which no effect was observed, since 12.5 μg/ml still caused marked effects on prothrombin index values of human plasma. The dependence on the exposure time was the same as in rat's plasma, but prothrombin

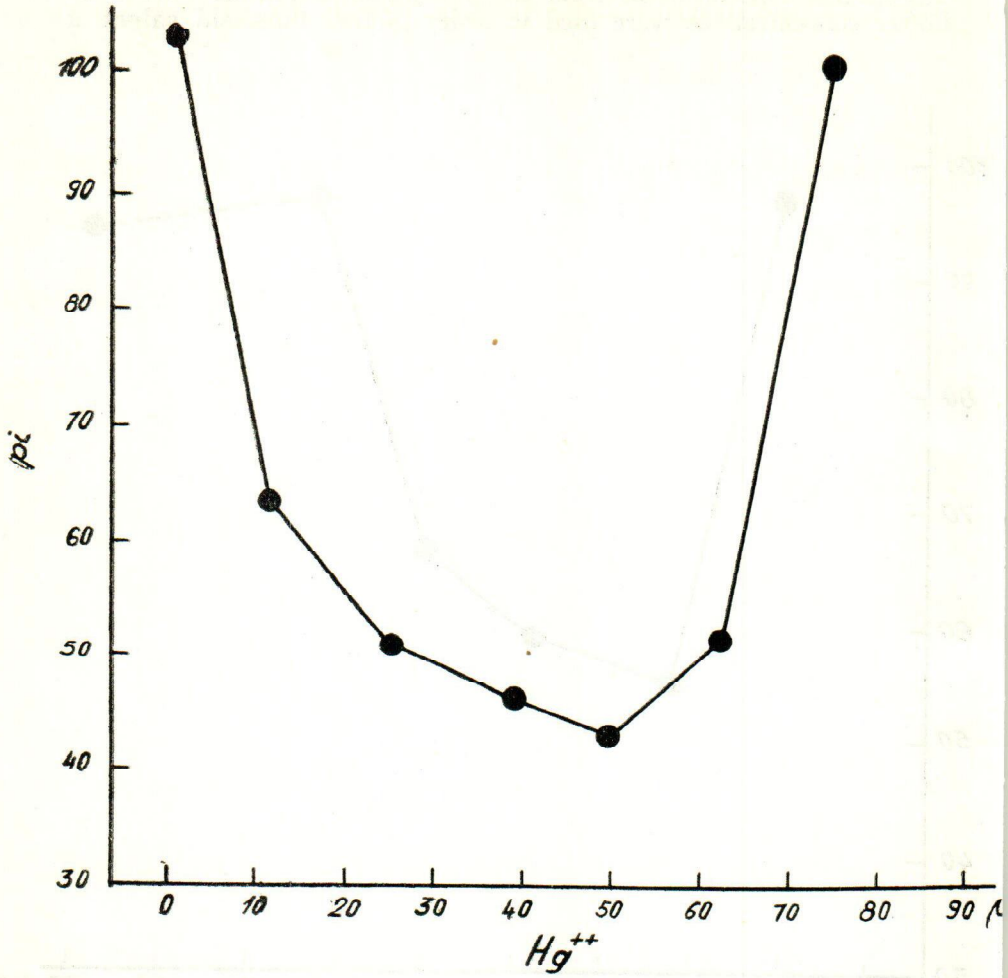

Fig. 4. The effect of different concentrations of Hg ions on prothrombin index values after 3 hours of exposure (human blood).

index values, obtained after 180 minutes of exposure were lower (between 40–50%). Fig. 3 and 4 show the effect of various concentrations of mercury ions added to the rat's or human plasma regarding the influence of these ions on prothrombin index values after 180 min. of exposure.

DISCUSSION

In our experiments Sr^{++}, UO_2^{++} and Pb^{++} when added to plasma in vitro did not cause any changes in prothrombin index values. The fact that Sr ions had no effect in our experiments might be explained by the presence of an excess of Ca ions which have a greater specificity for plasma coagulation. The presence of Sr^{++} in concentrations higher than optimum did not exert any inhibitory action on plasma clotting.

Uranyl ions inhibit clotting partially or completely depending on whether the uranyl acetate is added to plasma or to tromboplastin (5). This related to the concentrations approximately 10 times higher than used in our experiments, which might explain the difference in results.

Although lead ions caused no changes in the plasma coagulation process in vitro, the decrease in the prothrombin activity observed in some cases of lead poisoning might be due to hepatic damage brought about by lead ions (11).

The effect of mercury ions might be explained by the interaction of Hg ions with sulfhydryl groups of plasma proteins. In some experiments, dogs with an experimental depletion of plasma proteins, survived the doses of $HgCl_2$ fatal to normal dogs and excreted Hg in the urine much more rapidly than was the case in controls, indicating an interaction of mercury ions with plasma proteins (13). The fact that mercury ions affect plasma clotting only within a limited range of concentrations might be explained by the finding that other bivalent cations like Sr, Mg and Ba accelerate or retard clotting on the basis of their concentration, so that coagulation and prothrombin times are a resultant of a positive i. e. activating and negative i. e. inhibitory action of these ions (10).

Different results obtained with rat's blood might indicate slight differences in the number of SH groups responsible for plasma clotting in the rat's and the human blood.

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Sadržaj

UTJECAJ IONA METALA NA ZGRUŠAVANJE KRVI

Koncentracije UO_2^{++}, Sr^{++} i Pb^{++}, koje se kreću od 12,5–62,5 μg/ml plazme, ne utječu na protrombinsku aktivnost.

Živini ioni produžuju protrombinsko vrijeme plazme. To se odražava na protrombinskom indeksu padom do otprilike 55%. Učinak žive ovisi o koncentraciji živinih iona i o vremenu ekspozicije. Protrombinski se indeks najjače smanjio kod koncentracije živinih iona od 25,0–37,5 μg/ml plazme nakon trosatne ekspozicije. Kod viših i nižih koncentracija učinak je bio manji.

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