platelet aggregation ($p > 0.05$) while it strongly delays the beginning and inhibits the speed of the disaggregation ($p < 0.05$). Therefore the sensitivity of the disaggregation to manganese is much more marked than that of the aggregation.

The initial platelet aggregation speed was calculated by the tangent to the initial part of the O.D. curve. Figure 2 shows that, by adding $MnCl_2$, there is a dose-dependent inhibition of the ADP-induced initial platelet aggregation speed, which resulted in a statistically significant value until a final concentration higher than 10^{-3} M).

Discussion. Present results provide evidence that manganese (added to PRP contemporaneously with ADP) could impair platelet aggregation and inhibit disaggregation. As Mn^{2+} inhibits also clot retraction[1], our results support the hypothesis, previously formulated by DAY-HOLMSEN[2], White[3] and de GAETANO et al.[4] that the platelet adhesion-aggregation reaction is mediated by an activation of the same platelet contractile system which is responsible for the clot retraction.

BÜLBRING and TOMITA[6] and BRADING et al.[7] demonstrated the competition of Mn^{2+} with the internal calcium in the smooth muscle cells; the effect of Mn^{2+} on clot retraction previously described[1] and that on platelet aggregation here reported could be due to the competition of Mn^{2+} with the platelet calcium.

Certainly our results proved that both phenomena, aggregation and disaggregation are Mn^{2+}-sensitive, even if the mechanisms involved in the coming-out of platelets from aggreagte are more sensitive to Mn^{2+} than those involved in their going into it.

Riassunto. È stata studiata, con l'aggregometro, la variazione di responsività all'ADP delle piastrine di ratto all'aggiunta di $MnCl_2$. $MnCl_2$ inibisce sia l'aggregazione, indotta da ADP, che la disaggregazione. Questo effetto si presume sia dovuto alla competizione di Mn^{2+} con il calcio piastrinico.

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The Effect of Antidiuretic Hormone on the Extravascular Protein in the Renal Medulla

There is now a great deal of evidence that the renal medulla contains a relatively large pool of exchangeable albumen, much of it extravascular[1]. In previous experiments[2] we were able to show that, in adult rats, the amount of dye-labelled protein which passed out of the medullary vessels during the two minutes after the injection of the dye was significantly greater in animals undergoing a water diuresis than in those in which the diuresis had been checked by antidiuretic hormone (ADH), suggesting that the hormone diminishes the permeability of the medullary vessels. The present experiments were planned to study this phenomenon in a different way by first loading the medulla of diuretic rats with labelled protein and then studying the removal of this from the interstitial tissue after the injection of ADH. In the previous experiments, a prolonged saline washout was used to eliminate all intravascular protein but, since this sometimes gave somewhat erratic results, in the present work no washout was attempted but the total protein space in the medulla was calculated from estimations of the medullary and plasma dye levels.

Materials and methods. 12 male Wistar rats weighing between 230 and 300 g were used. Each was given 2 i.p. injections of warm distilled water at 1 h intervals and the resulting diuresis confirmed 1 h after the last injection by examining the urine osmolality. Each rat was then given an i.v. injection of 0.5 ml/100 G of an Evans Blue solution, prepared as described previously[2]. 10 min later, 0.5 ml of blood was removed for analysis and a further sample of urine taken. The rats were then given a s.c. injection of either 50 mU of Pitressin Tannate (Parke-Davis) in 0.1 ml oil or a control injection of a similar volume of oil only. After a lapse of 30 min, further blood and urine samples were taken and the left kidney was excised, and allowed to drain. It was then dried, the capsule stripped off, and the whole inner medulla removed, taking care to avoid contamination with blood from the rest of the kidney. The inner medulla was weighed, homogenized and its Evans Blue content estimated by the technique previously described[2]. The blood samples were centrifuged and the dye levels in the plasma estimated by the same method.

Results and discussion. The level of Evans Blue in the plasma fell during the 30 min which elapsed between taking the 2 blood samples but there was no significant difference in the rate of fall between the diuretic and the antidiuretic animals (Figure 1). As can be seen from Figure 2, a significantly smaller Evans Blue space was found in the medulla in the rats which had been given ADH than in the diuretic rats. One of the ADH-treated rats had an Evans Blue space of 0.261, which placed it among the diuretic results but in this animal the urine osmolality rose to only 830 mOsm which was considerably below that of the other ADH-treated animals and is, in fact, below that usually found in normal rats.

There is good evidence that, in the doses which we used, injected Evans Blue binds firmly and completely to plasma proteins[2] although WILDE et al.[3] have recently presented evidence to show that during the first 2 min after the injection, some unattached dye passes into the interstitial tissue. WILDE, however, used very much larger doses of Evans Blue (almost 4 times as large as ours) and it is not surprising that this amount, injected into hamsters, did not mix fully with plasma until some unbound dye had leaked into the medullary tissues.

The use of an estimate of the Evans Blue space as an indication of the amount of extravascular albumen depends, of course, on the constancy of the volume of the intravascular space since the diminution of the space found after ADH injection could be due to vasoconstriction. When ADH is given in physiological doses (i.e. sufficient to cause an antidiuretic effect without pressor activity) it seems, however, to produce no change

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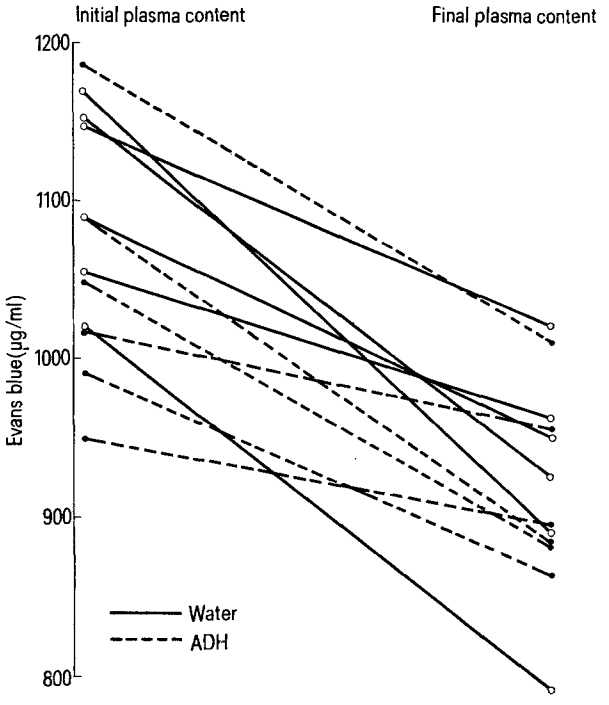

Fig. 1. Plasma Evans Blue values before ADH or control injection and at end of experiment. There is no significant difference in the fall in plasma levels between the diuretic and the antidiuretic animals.

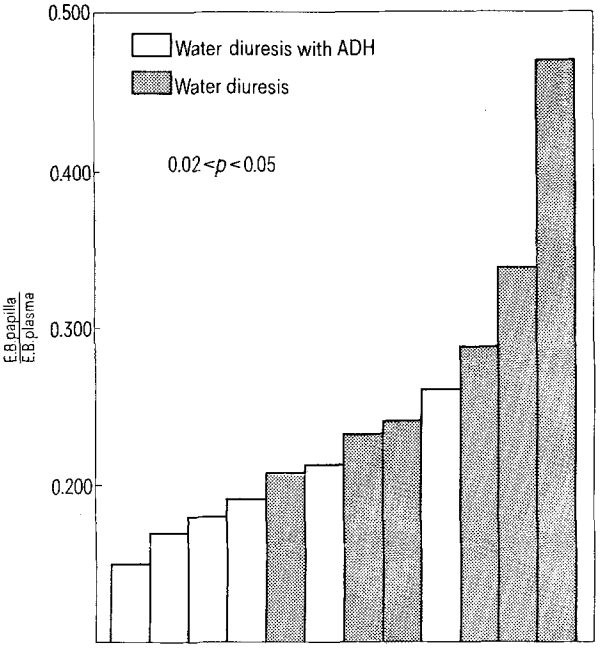

Fig. 2. Papillary Evans Blue space in 6 diuretic and 6 antidiuretic rats 30 min after ADH or control injections. The ADH treated animals had significantly lower values. The ADH treated rat with a space of 0.261 had a final urine osmolality of only 830 mOsm (see text).

in vascular volume in the inner medulla or in the total kidney volume[4–7]. TISHER et al.[8], however, did find a reduction in size of the medullary vessels after giving ADH to rats with diabetes insipidus but the dose of the hormone in these experiments was very large (1 unit s.c.) and the kidneys were fixed by intravascular perfusion before being sectioned and the vessels measured. Taking into consideration the results of our previous experiments, it seems likely, therefore, that the reduction in Evans Blue space indicates a true reduction in the amount of extravascular protein although it is not possible to tell whether this is due to an increased removal of protein from the tissue or to a diminished rate of replenishment from the vessels. It was not due to any change in the plasma content of the dye-protein complex (Figure 1). It is interesting that MORRISON and SCHNEEBURGER-KEELEY[9] found previously injected ferritin in some of the inner medullary interstitial cells of diuretic rats but not in control animals. Since the cells apparently picked up the ferritin from the interstitial tissue this may be a further indication of the permeability change which is reported in the present paper.

The function of the extravscular protein is uncertain. We believe that under conditions of antidiuresis, the oncotic pressure of the protein within the vessels – already raised as a result of glomerular filtration and the removal of water from the descending vasa recta – is responsible for the uptake of water from the interstitium by the ascending vasa recta, so that the medullary osmotic gradient is maintained. In diuresis, the vessels become more permeable and the oncotic pressure gradient across the endothelium is reduced or abolished altogether so that less water is taken up by the ascending vasa recta and the osmotic gradient is diminished. It is possible that a similar reduction of the gradient may explain the loss of concentrating ability which occurs in potassium deficiency since SHIMAMURA and MORRISON[10] have presented evidence that the permeability of the vessels to ferritin and horseradish peroxidase is increased in this condition. PANNER and RIFKIN[11] also reported visual evidence of an increased amount of previously injected horseradish peroxidase in the papillae of potassium depleted rats but were unable to show this quantitatively until 4 h after the injection.

Résumé. Douze rats diurétiques ont reçu une injection de Evans Blue et, après 10 min, une injection de 50 mU ADH ou d'huile inerte. Chez les rats qui avaient reçu l'ADH «l'espace Evans Blue» fut plus petit que chez les rats diurétiques.

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