17–32 of the human molecule or having electrical charges and conformational properties quite similar to this fragment[5].

Résumé. Les cellules C de la thyroide de rat ont été localisées par un anticorps anticalcitonine humaine (immunofluorescence indirecte). L'inhibition de la réaction par la calcitonine humaine et ses fragments a été étudié.

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[5] Fragments 1–10, 11–32, 17–32, 24–32 were kindly supplied by Merck, Sharp and Dohme and synthetic human calcitonin was a generous gift of Ciba Basel.

Effect of Manganese on the ADP-Induced Platelet Aggregation

In a previous work it was found that Mn^{2+} inhibits clot retraction and that it induces a very quick and complete relaxation of a fully contracted clot, as well as preventing clot retraction[1]. As substances known to influence the clot retraction could also modify the adhesion-aggregation reaction [2–4], it seemed interesting to see whether the aggregation of platelets by ADP is dependent on some similar connections to comparably Mn^{2+}-sensitive mechanisms. The present paper shows that the responsiveness to ADP of the rat platelets is effectively modified by adding $MnCl_2$.

Methods and materials. Venous blood was collected by plastic syringe from the right ventricle in open-chest animals (Albino Wistar Rats, body weight 300–400 g) anaesthetized with ether.

Platelet – rich plasma (PRP), Platelet – poor plasma (PPP) and platelet count were performed as previously described[5]. The ADP-induced platelet aggregation was tested in PRP with a standard platelet concentration (700.000/μl) by an aggregometer (169 Platelet Aggregation Meter, Evans Electroselenium Ltd) and recorded by Speedomax XL 690 Series Recorder (Lees and Northrup, North Wales and Philadelphia) as usual[5].

Chemicals used are: Adenosine-5′-diphosphate (ADP) trisodium salt (Boeringher, Mannheim, Germany) dissolved in veronal buffer, pH 7.4, at a concentration of 10^{-3} *M* and stored at −20 °C until use; manganese: $MnCl_2$, crystals, (Mallinckrodt Chemical Works, St. Louis –New York–Montreal).

Statistical analysis: the data obtained were elaborated for their statistical significance by the 2 sample *t*-test, for 0.05 probability.

Results. Figure 1 shows the average O.D. changes of PRP tested in 42 experiments as follows: a) 12 experiments: PRP+ADP (final concentration 10^{-4} *M*); b) 8 experiments: PRP+ADP (as above) + $MnCl_2$ (final concentration 10^{-1} *M*); c) 14 experiments: PRP+ADP (as above) + $MnCl_2$ (final concentration 10^{-2} *M*); d) 8 experiments: PRP+ADP (as above) + $MnCl_2$ (final concentration 10^{-3} *M*).

There is evidence that Mn^{2+}, inhibits both ADP induced platelet aggregation and disaggregation. The inhibitory effect on the aggregation is very marked at higher Mn^{2+} concentrations (until 10^{-2} *M*) at which also the disaggregation is prevented. If the final concentration is less, (10^{-3} *M*) manganese fails to influence the maximal

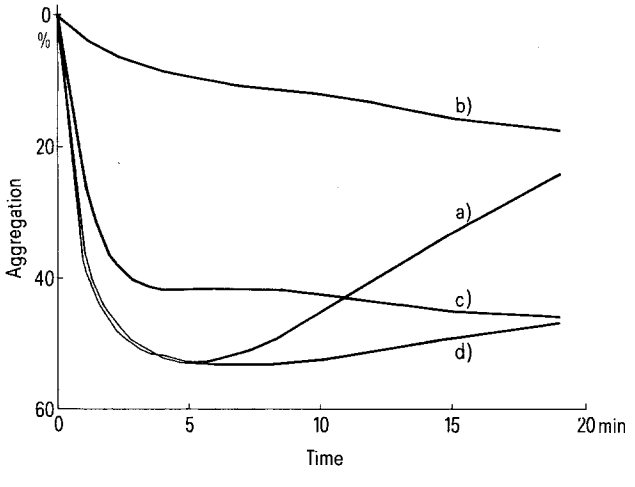

Fig. 1. Average O.D. changes observed in: a) PRP+ADP; b) c) d) PRP+ADP+$MnCl_2$. The final concentrations of ADP was always 10^{-4} *M*; that of $MnCl_2$ was: b) 10^{-1} *M*, c) 10^{-2} *M*, d) 10^{-3} *M*. Number of platelets: 700.000/μl. By the two sample *t*-test computed for the maximal amplitude of the curve, it was found that in respect to a) for b) and c) $p \leq 0.05$ and for d) $p > 0.05$. For the curve d) the values obtained were statistically significant in respect to a) for the beginning and the speed of the disaggregation (disaggregation 10 min after the maximal aggregation).

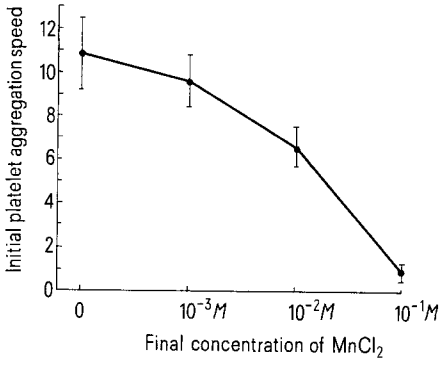

Fig. 2. Influence of manganese on the initial ADP-induced platelet aggregation speed. The speed was calculated through the tangent to the initial part of the curve conventionally expressed in cm. By the two sample *t*-test we found that the inhibition is statistically significant for a final concentration of Mn^{2+} 10^{-1} *M* and 10^{-2} *M*.

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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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