In anaesthetized guinea-pigs the human AT preparation specifically described evoked severe bronchospasm at doses of 2 mg/kg. The spasm was preceded by a short period of respiratory stimulation and was accompanied by a biphasic blood pressure response (fall followed by a rise). The same symptoms have been observed previously after injection of purified hog AT[6]. Doses of human and hog AT which were equally effective in the isolated guinea-pig ileum preparation were also about equivalent in producing the in vivo effects. After desensitization of a guinea-pig to the bronchial effect of human AT, the animal was also insensitive to hog AT.

Anaphylatoxin also formed when human serum was incubated with the AT-forming enzyme of cobra venom. Again, in order to recognize the activity it was necessary to purify the active principle and to concentrate it. Traces of AT were found occasionally after fractionation of non-incubated serum. When portions of these sera were activated by contact or with the cobra enzyme, they developed additional AT activity.

These results demonstrate that it is possible to generate AT activity in whole human serum by classical methods. In its biological properties the human AT is identical with other ATs, and it apparently acts on the same receptors, thus producing the phenomenon of cross-tachyphylaxis. Recently DIAS DA SILVA and LEPOW[7,8] detected a smooth-muscle-contracting principle in incubates of human complement factors C'1, 4, 2 and 3; the substance was a cleavage product of C'3 and was described as AT. It differed, however, from classical ATs in various biological properties and in that it did not form or was inactivated in whole human serum. Whereas these differences could have been partly explained by assuming species differences, the present experiments show that such differences do not exist. Rather AT appears to be a unique substance which does not show gross functional differences in different species, as regards formation and actions. The human C'3 cleavage product is not AT, as it has been investigated and described since its discovery by FRIEDBERGER. Smooth-muscle contraction with tachyphylaxis is not sufficient to characterize AT; this action is common to several peptides of venoms, phospholipase A, serum kininogenases, trypsin, etc.; some of these substances also release histamine from tissues.

A specific criterium for AT is the phenomenon of cross-tachyphylaxis with a classical AT preparation.

Zusammenfassung. Es ist möglich, auch in menschlichem Serum eine Anaphylatoxinbildung (AT) durch Kontaktaktivierung oder Kobragift zu induzieren. Wegen der geringen Mengen, die entstehen, muss das wirksame Prinzip vor dem biologischen Nachweis angereichert werden. Menschliches AT verhält sich in allen untersuchten Eigenschaften wie AT aus anderen Plasmaarten. Es unterscheidet sich von dem darmkontrahierenden Spaltprodukt aus der menschlichen Komplementkomponente C'3, das mithin nicht als AT angesprochen werden kann.

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The Induction of Blood Platelet Aggregation by Divalent Cations

The view that divalent cations are important in platelet cohesion is supported by the observation that platelet aggregates are disrupted and platelet-to-platelet adhesion is prevented by chelating agents such as ethylenediaminetetraacetate (EDTA) or citrate[1]. The adhesive property of the platelets is regained when the concentration of the divalent cations in the medium is normalized[2]. The clumping of platelets can be induced by aggregating agents such as adenosine diphosphate (ADP)[3], 5-hydroxytryptamine, the catecholamines and thrombin[4] in the presence of an appropriate concentration of calcium[5]. In addition, calcium and magnesium themselves are capable of inducing aggregation when added to the platelet suspension[6]. This paper deals with the effect of calcium, magnesium, strontium, barium, manganese, zinc and nickel on platelet aggregation in stirred platelet-rich plasma (PRP).

Materials and methods. The nephelometric method used to assess platelet aggregation was similar to that originally used by BORN[3] and has been described fully elsewhere[7]. Citrated PRP from sheep was used in these experiments which were carried out at 37 °C. The metal salt solutions (analytical reagent quality) were prepared in barbitone buffered saline and the volumes used did not exceed 0.1 ml per 3 ml samples of PRP. Other drugs used were: ADP, adenosine and 5-HT (SIGMA), bromolysergic acid diethylamide (BOL-148, Sandoz) and 2-chloroadenosine (prepared by Dr. M. H. MAGUIRE of this institute).

Results and discussion. All the divalent cations tested, with the exception of barium, caused platelets to clump. The platelet response differed qualitatively, dividing the cations into 2 groups: calcium, magnesium and strontium (the alkaline earth metals) required a lag period of not less than 2 minutes before the onset of aggregation; however, the transition elements nickel, zinc and manganese, all caused an immediate clumping response. The initial rate of aggregation brought about by magnesium, calcium and strontium was slow, followed by a rapid clumping stage which tapered off when the maximal degree of aggregation was achieved. This might indicate that a release reaction took place and that it proceeded in stages. Furthermore, the aggregation caused by all the metal ions, unlike that induced by ADP or 5-HT, was in all cases irreversible. The plots of the initial rates of platelet clumping in response to nickel and zinc were

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almost parallel to those representing aggregation induced by ADP (Figure 1). In the case of manganese, however, the rates were slower and the resultant aggregation reached only about 50% of the maximal response attainable by the other cations. The order of aggregating effectiveness of the divalent cations was obtained by comparing the time intervals needed for the clumping induced by the cation to reach a value of 50% of the maximal change in optical density of the PRP. Data in the Table show that the transition metal ions were more potent initiators of aggregation than the alkaline earths. Nickel was the most active cation. In subthreshold concentrations, all the metal ions with the exception of barium, potentiated the clumping of platelets induced by ADP or 5-HT. The relative aggregating ability of the divalent cations parallels the order in which they are able to form complexes with chelating agents[8,9]. This observation would appear to support the suggestion that under physiological conditions ADP complexes with calcium to form an active aggregator[10].

In the presence of adenosine or 2-chloroadenosine, which are specific and competitive inhibitors of ADP-induced platelet aggregation[11,12], the onset of clumping caused by the divalent cations was delayed. With adenosine which becomes rapidly deaminated in sheep platelet-rich plasma[13] the delay was short. The inhibition by 2-chloroadenosine which is more resistant to deamination than adenosine[14] persisted much longer (Figure 2). The antiserotonin agent BOL-148 specifically inhibits 5-HT-induced platelet clumping without affecting aggregation caused by ADP[7,15]. In the clumping induced by divalent cations BOL-148 showed no appreciable effect on the onset and the initial rate of aggregation, however, after platelets began to aggregate the clumping proceeded with greatly reduced velocity. The results obtained by using the specific ADP and 5-HT inhibitors indicate the possibility of the involvement of ADP and 5-HT in the divalent cation-induced platelet aggregation. It appears likely that the platelet response and the ultimate platelet cohesion is a result of the liberation from the platelets of nucleotides – especially ADP – which initiate the aggregation, and pharmacologically active amines, particularly serotonin, which reinforce and accelerate the clumping response.

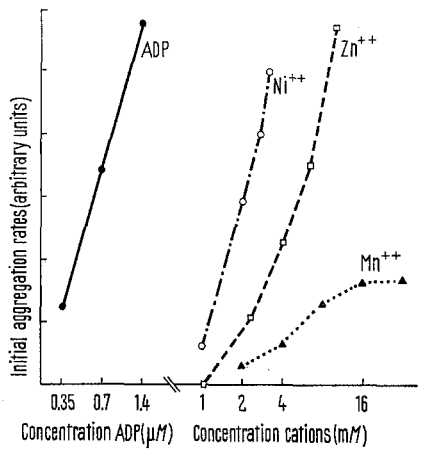

Fig. 1. Log dose-response curves for the aggregation of platelets in platelet-rich sheep plasma by ADP (●—●); Nickel (○-·-·-○); Zinc (□---□); and Manganese (▲·····▲). The aggregation rates were obtained from the initial slopes of the clumping curves and expressed in arbitrary units. Each point represents a mean of not less than 3 separate measurements.

The relative effectiveness of divalent cations as aggregators of platelets in PRP

	Cation[a]	Relative aggregating activity	Log K (formation constant of metal EDTA complex)[8]
Transition metals	Ni	1.00	18.62
	Zn	0.67	16.61
	Mn	0.16	14.04
Alkaline earths	Ca	0.13	10.96
	Mg	0.08	8.69
	Sr	0.05	8.63
	Ba	< 0.05	7.76

[a] Final concentration of 8.8 mM in PRP.

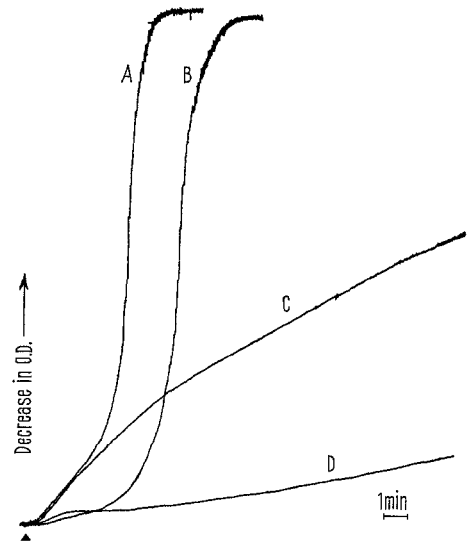

Fig. 2. The aggregation curves for platelet clumping induced by nickel (420 μM) in the absence of inhibitors (A); and in the presence of 5 μM Adenosine (B), 8 μM BOL-148 (C), and 1 μM 2-Chloroadenosine (D). The inhibitors were pre-incubated in the plasma for 3 min prior to the addition of nickel (at ▲).

Zusammenfassung. Im blutplättchenhaltigen Plasma wurden die Thrombozyten durch Nickel, Zink, Mangan, Kalzium, Magnesium und Strontium aggregiert, wobei Nickel die stärkste Wirkung hatte. Adenosin-Analoga und Antiserotonin unterdrückten den Effekt, was durch die Freisetzung von ADP und Serotonin aus den Thrombozyten bedingt zu sein scheint.

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