responsible for the appearance of nucleoli described as having regions with differing light transmission properties (see Figures 3, 4a and 4e of reference [1]) and probably corresponds to the phenomenon described by REISSENWEBER and CARDOSA[3] concerning the pars amorpha and nucleolonema. In many nucleoli of the explants on complete medium this linear structure was highly refractile and consisted of thin-appearing continuous connections between knobs or swellings occurring at apparently regular intervals (Figures 3a, b, 4a, b). The non-refractile and refractile conditions of this linear structure no doubt reflected different stages of growth activity of the explants. The linear nature of this refractile nucleolar component was also evident from the manner in which it lined the inside periphery of the nucleoli and that it frequently appeared as loose helices or looped structures (Figures 3a, b, 4a, b). Filamentous structures combined with dense looking droplets or knobs have also been observed in stained nucleoli of chicken fibroblasts[4]. Indications are that the linear nucleolar component described here is the same as the filamentous nucleolar component known as the nucleolonema[5–9].

Zusammenfassung. Es wird gezeigt, dass ein gradliniger Nukleolusbestandteil der Zitrusfrucht offenbar in vitro wachsen kann. Dies macht deutlich, dass es sich bei diesem Nukleolusbestandteil um das Nukleolonema handelt.

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Formation of Anaphylatoxin in Human Serum

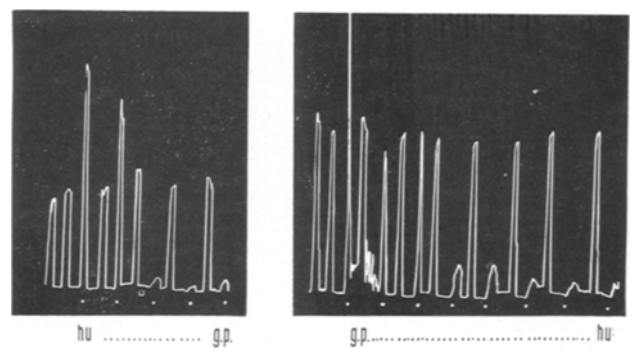
The term 'anaphylatoxin' (AT) was coined by FRIEDBERGER about 60 years ago and has been used since to designate the toxic principle which develops in guinea-pig serum on incubation with antigen-antibody precipitates. Once formed AT remains active. It is recognized by the shock produced after injection into normal guinea-pigs, or by the contraction followed by tachyphylaxis of isolated strips of guinea-pig ileum (for reviews see GIERTZ and HAHN[1] and VOGT[2]). The actions are the same in activated whole serum or plasma and in highly purified AT preparations from rat and hog serum[3,4].

The sera of only a few species are suitable for AT formation, by immune precipitates or other contact agents. Probably many investigators have tried without success to induce AT formation in human serum. According to SCHWOERER, BRANDT and VOGT[5] the failure is due to the lack of sufficient precursor – anaphylatoxinogen – to yield amounts of AT directly detectable in the serum. We have now succeeded in demonstrating the formation of AT in whole human serum after concentration of the active principle by procedures which have been used before in the purification of hog serum AT[4].

Methods. In a representative experiment, 880 ml fresh human serum were stirred for 60 min at 37 °C with 16 g baker's yeast as contact activator. After centrifuging the supernatant was diluted with 880 ml distilled water, cooled to about 4 °C, adjusted to pH 4 with HCl and adsorbed twice batchwise with CM cellulose (2.4 g each time). The adsorbent was washed with 0.1 *M* ammonium formate buffer pH 4 followed by 0.5 *M* acetic acid in a column. AT was then eluted with 0.1 *M* ammonium formate buffer pH 7.0. The protein-containing fractions (220 ml) were adsorbed on 10 g Amberlite XAD-2. The adsorbent was washed with water and eluted with a mixture of glacial acetic acid, methanol and water (2:1:1). The residue of this eluate was further purified by gel chromatography on Sephadex G-100 and Sephadex G-25. After lyophilization 8.3 mg AT were obtained, which were dissolved in 0.02 *N* acetic acid giving a stock solution which contained 1 mg/ml.

Results and discussion. The preparation described above produced maximal contractions of the isolated guinea-pig ileum at concentrations of 1.5 µg/ml bath. Another preparation was 4 times as active; the weight of other preparations was unknown. The preparations were much less active and pure than purified hog AT.

The contractions produced were blocked by the antihistaminic tripelennamine, and tachyphylaxis was evident. Cross-tachyphylaxis occurred with all other AT preparations tested (Figure): ATs from rat and hog sera, produced by incubation with the AT-forming enzyme of cobra venom; and ATs from serum or plasma of rat, guinea-pig and hog, produced by contact activation with Sephadex, zymosan or yeast.



Isolated guinea-pig ileum. Cross-tachyphylaxis between human (hu) and guinea-pig (g.p.) AT, both obtained by contact activation with yeast. Injections marked by dots are made with the desensitizing AT preparation, except for the last one which is the challenging injection of the other AT preparation. Contractions not marked by dots are due to acetylcholine (4×10^{-9} g/l).

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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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