preferably demonstrates non-adrenergic fibres. Both fluorescent and methylene-blue-stained fibres appear simultaneously in all heart valves of the rat except 1 aortic cusp (No. II in Figure 2). The 2 types of fibres run close together in the terminal vegetative ground plexus. It seems most reasonable to suppose that the 2 contiguous fibres are adrenergic and cholinergic, respectively.

It has been shown previously[2] that the plexa of the valves are a direct continuation of an endocardial nerve plexus, and the type of nerve supply found in the valves can be expected also in this endocardial plexus. Electron-microscopical studies[9] have confirmed this, and have shown that the arrangement with the 2 types of contiguous nerves can also be found among the nerves of the myocardium proper[10].

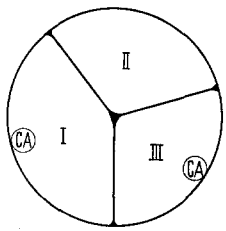

Fig. 2. Schematic representation of the aortic valve (see text). CA: Coronary artery.

Résumé. Toutes les valvules du rat, sauf une valvule de l'aorte, contiennent des nerfs adrénergique et cholinergique. Très souvent, les deux types d'axones courent très près l'un de l'autre comme ceux de l'iris du rat.

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University of Lund, Institute of Anatomy and Histology, Lund (Sweden), 24 February 1969.

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[10] This work was supported by grants from the Royal Physiographic Society at Lund, the Swedish Medical Research Council No. B69-14X-2321-02 and was carried out within a research organization sponsored by the Swedish Medical Research Council (Projects No. B69-14X-56-05C and B69-14X-712-04C).

Optical Evidence of a Linear Component in Nucleoli of Lemon Fruit Explants (*Citrus limon* L.)

Certain stages of nucleolar morphology of lemon fruit explants have been separated from each other by altering the nutrient environment[1]. These morphological stages included nucleolar enlargement with and without changes in light transmission properties as well as the formation of highly refractile nucleolar inclusions. Evidence is presented here that nucleoli 'showing regions with differing light transmission properties' as well as the formation of refractile nucleolar material observed in lemon fruit explants[1] are primarily associated with a linear component of the nucleolus.

Vesicle stalks from mature lemons (*Citrus limon* L.) were inoculated aseptically onto distilled water and mineral-sucrose solution[1] and placed in the dark at 25 °C. After 2 or 3 days in vitro the stalks were fixed in Randolph's CRAF solution and washed well in distilled water. The tissue was squashed between 2 dry uncoated microscope slides and dried over anhydrous $CaCl_2$ after separating the slides. The squashed material was covered with several drops of xylol for 15–20 min before mounting unstained in 'Sira' mountant or 'Permount'. Unstained squash preparations of freshly excised stalks fixed in CRAF solution served as controls.

Nucleoli of the control tissue were homogeneous in appearance as observed previously (Figure 1)[1,2]. Enlarged nucleoli were evident in explants maintained on distilled water some of which contained a relatively dense-appearing non-refractile material arranged in a linear fashion along the inside periphery of the nucleoli (Figure 2). This linear arrangement of non-refractile material was also evident in nucleoli of explants maintained on complete nutrient medium. It is this non-refractile stage of the linear nucleolar component that was most likely

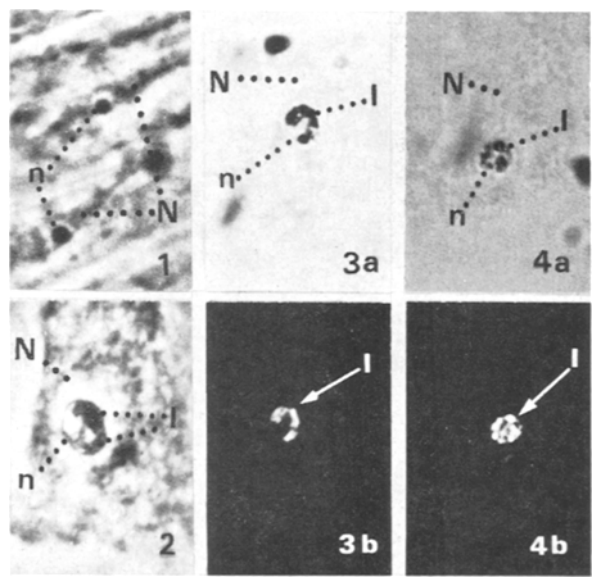

Fig. 1. Nuclei and homogeneous-appearing nucleoli of control tissue. Bright field. × 1250.

Fig. 2. Nucleus and nucleolus of distilled water explant showing linear arrangement of non-refractile material at right side of nucleolus. Phase contrast. × 1250.

Fig. 3a. Nucleus and nucleolus of explant on complete medium showing marked linearity of nucleolar component at right side of nucleolus. Note the looped appearance. Bright field. × 1250.

Fig. 3b. Phase contrast microscopy of nucleolar component in Figure 3a. × 1250.

Fig. 4a. Nucleus and nucleolus of explant on complete medium showing linearity of nucleolar component lining the entire periphery of the nucleolus. Bright field. × 1250.

Fig. 4b. Phase contrast microscopy of nucleolar component in Figure 4a. × 1250. N, nucleus; n, nucleolus; l, linear component.

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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

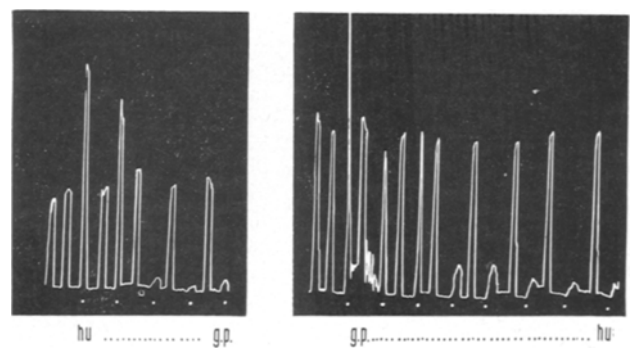
The term 'anaphylatoxin' (AT) was coined by FRIEDBERGER about 60 years ago and has been used since to designate the toxic principle which develops in guinea-pig serum on incubation with antigen-antibody precipitates. Once formed AT remains active. It is recognized by the shock produced after injection 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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