Adrenergic and Non-Adrenergic Valvular Nerves of the Heart

Adrenergic nerves have recently been demonstrated in significant numbers in the heart valves[1–3]. They most probably supply smooth muscles of the valves, although this point has not so far been finally proved. In a study of other smooth muscles, such as the iris dilator, adrenergic and cholinergic terminal fibres have been found to run concomitantly, forming a terminal ground plexus of tightly intertwined varicose axons[4–6]. This type of fibre arrangement seems to provide possibilities for mutual influence between adrenergic and cholinergic terminal axons. The heart has been widely investigated physiologically and pharmacologically, and it is of obvious interest to find out whether this principle of distribution of vegetative nerve fibres applies also to the heart or to any part of it.

Heart valves were dissected out from adult albino rats (Anticimex, Sweden) and prepared as whole-mounts, either directly or after incubation (10 min) in a methylene blue solution (150 µg/ml). Irides from the same animal stock were incubated in the same way as the heart valves, but also at different pH's ranging from 5.0–8.0, and then spread on microscope slides. For the pH ranges 5.0–6.5 a citrate buffer and for 6.0–8.0 a phosphate buffer were used. The whole-mounts were prepared for fluorescence microscopy according to the method of Falck and Hillarp. The combining of the histofluorescence technique and the methylene blue staining has recently been shown to demonstrate simultaneously adrenergic and non-adrenergic fibres[6,7].

The heart valves were found to contain adrenergic terminals and preterminals, although in varying numbers. All the cusps of the mitral and tricuspidal valves contained adrenergic and methylene-blue-stained fibres. Often the 2 fibre types ran close together, with several fibres in each strand (Figure 1). The fibre plexus was normally most wide-meshed near the free edge of the valves. Adrenergic fibres could often be seen in the chordae tendineae. The number of both types of fibres was highest in the mitral valve. In the aortic valve, there was a marked difference of the number of accompanying fibres between different cusps. Cusp I and III (Figure 2) had a fairly dense plexus, but cusp II had no fibres. It is of interest to note that the cusps with adrenergic fibres are where the 2 coronary arteries issue from. As in the mitral and tricuspidal valves, axons stainable with methylene blue were seen contiguous to the adrenergic fibres of the aortic and pulmonary valves. However, the strong affinity the valvular stroma has for methylene-blue often resulted in a partial disguise of the nerve fibres that made it difficult to assess fully the appearance of the plexus.

In the irides, the combined demonstration of adrenergic and non-adrenergic fibres showed the normal fibre pattern previously described, i.e. a well-developed meshwork of concomitant adrenergic and methylene-blue-stained fibres[6]. In electronmicroscopical studies, Richardson[7] corroborated that methylene-blue preferentially stains cholinergic fibres. At pH 6.0 and 6.5 both citrate and phosphate buffers were used with identical results, showing that the buffer type had no influence on the staining. At pH's lower than 6.5, vascular nerves were at times stained with methylene-blue. This occurred also in some few irides incubated at pH 7.5 and 8.0. Vascular fibres are purely sympathetic in the rat iris[8], and thus, in these cases, adrenergic fibres were stained with methylene-blue. However, it was invariably noted that, when such staining occurred, adrenergic fluorescent fibres were no longer demonstrable. No staining of vascular (i.e. adrenergic) fibres could be seen in places where the fluorescence was good. From these experiments it thus appears feasible that as long as fluorescent adrenergic fibres are still visible, the methylene-blue stain

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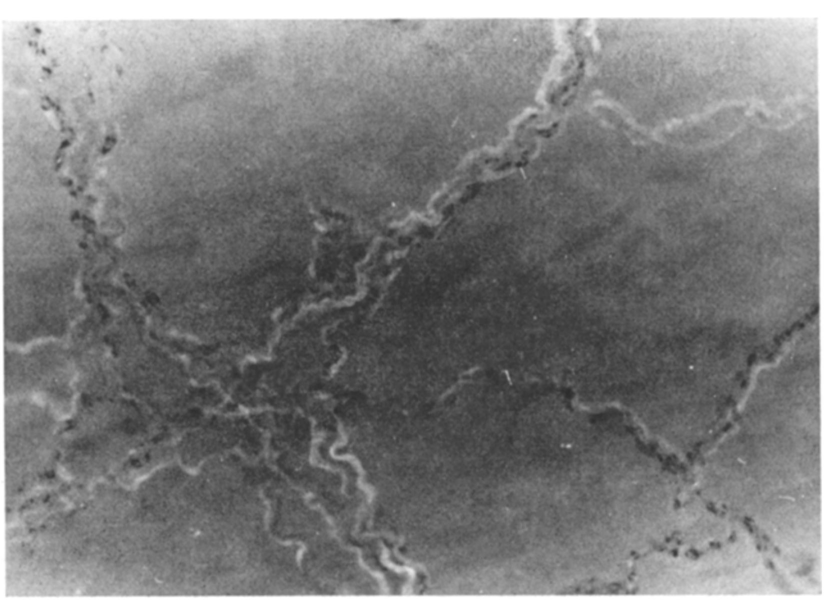

Fig. 1. Mitral valve, whole mount, rat. Fluorescent (adrenergic) and methylene-blue-stained (cholinergic) nerve fibres run close together. × 1200.

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. Electronmicroscopical 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].

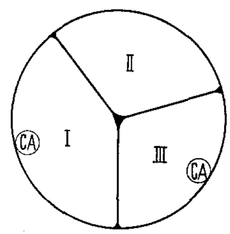

Fig. 2. Schematic representation of the aortic valve (see text). CA: Coronary artery.

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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[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

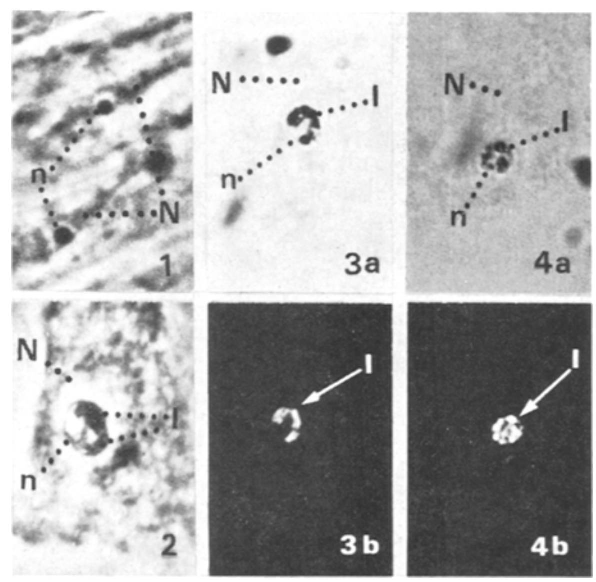

Fig. 1. Nuclei and homogeneous-appearing nucleoli of control tissue. Bright field. × 1250.

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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