Fluorescing Valvular Nerves in the Guinea-Pig Heart

A varicose nerve plexus has been demonstrated in ureteric mucosa by workers in this department using fluorescence techniques[1]. Although such plexuses are usually found at terminal effector sites, these workers point out that no such site is apparent in this situation. They further suggest that it is possible these nerves may fulfill an afferent function. Additional evidence for a possible sensory function of adrenergic nerves is provided by findings in the guinea-pig heart.

Materials and methods. Guinea-pigs were killed by either blows to the head or i.p. injection of Nembutal. The heart was rapidly removed and the atrioventricular junctional region dissected and placed on a cryostat chuck. Following quenching in isopentane cooled in liquid nitrogen the block was serially sectioned at 10–20 μm thickness. Adjacent sections were processed for routine histology, and to demonstrate cholinesterase and catecholamine activity. The Masson trichrome technique was used to show routine histology. Cholinesterase activity was visualized using GOMORI's technique[2] following fixation for 5 min in a phosphate buffered sucrose-formalin mixture at pH 7.4. Although previous experiments had shown guinea-pig nerves to contain only acetylcholinesterase[3] all sections were preincubated in $10^{-5}M$ Tipa (an inhibitor of non-specific cholinesterase) and incubated for 4–16 h using acetylthiocholine iodide as substrate. Adrenergic nerves were demonstrated using the fluorescence technique of SPRIGG's[4] using paraformaldehyde stored at 70.4% relative humidity. Sections were viewed and photographed using a Zeiss photomicroscope, excitor filter BG12/4 mm and barrier filter GG9/1 mm.

Results. Fluorescing nerves were present forming plexuses in both the bases and free edges of atrioventricular valves. At the valve base the adrenergic plexus was associated with muscle continued into the valve from the atrial wall. However, in the connective tissue of the valve an adrenergic plexus was present unassociated with either muscle tissue or blood vessels. The muscle at the valve base bore many resemblances to specialized cardiac tissue, and was associated with a richer plexus of acetylcholinesterase-containing nerves than adjacent atrial muscle (Figure 1). Some of the acetylcholinesterase-containing nerves were continued into the free edge of the valve. In this situation many more adrenergic than cholinergic nerves were present (Figure 2).

A rich fluorescing plexus was also present in the pulmonary valve cusps (Figure 3). Histology revealed that these cusps were composed entirely of fibrous and connective tissue. Sections processed to demonstrate acetylcholinesterase revealed no activity whatsoever in these cusps.

Discussion. Adrenergic nerves have been described in the valves by EHINGER et al.[5]. They suggest that the nerves are associated with valvular smooth muscle. In the mitral valve of the rat[6] they provide evidence that

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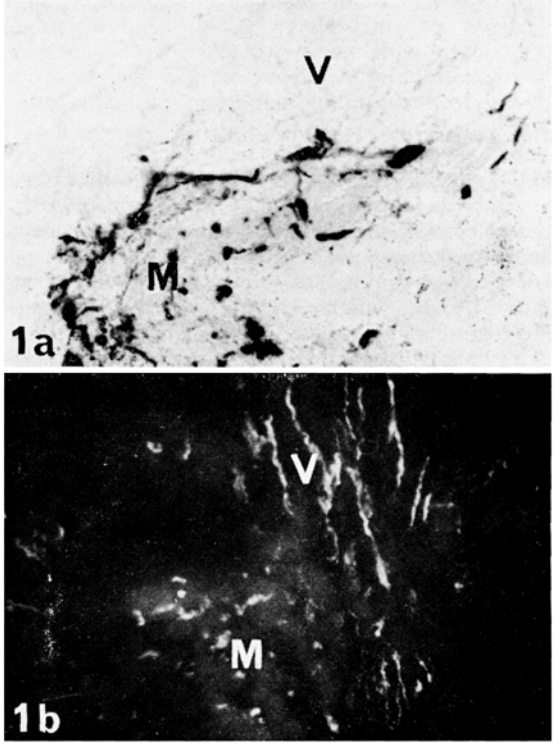

Fig. 1. Base of tricuspid valve processed to demonstrate a) acetylcholinesterase and b) catecholamines. The AChE-containing nerves are restricted to the muscle (M), whilst adrenergic nerves are also present within the valve (V).

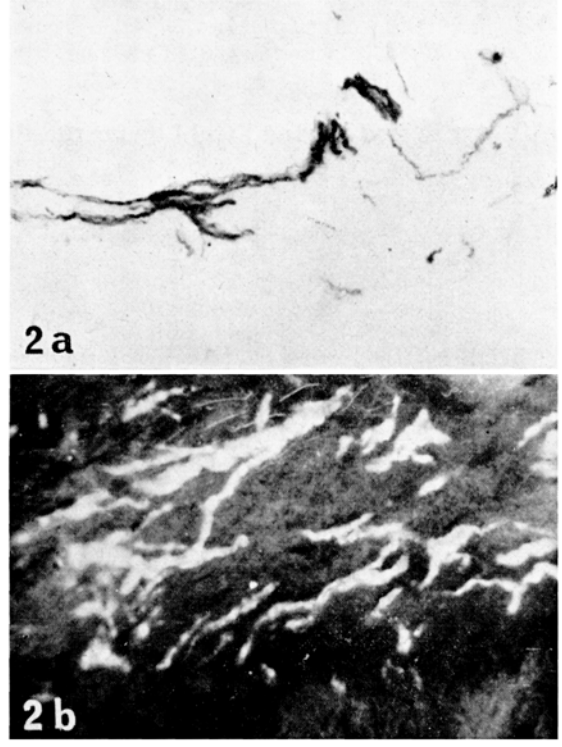

Fig. 2. Free portion of tricuspid valve processed to demonstrate a) AChE activity and b) catecholamines on adjacent sections. The adrenergic nerves outnumber the nerves containing AChE.

axo-axonal synapses may exist between adrenergic and cholinergic nerves. Whilst muscle is present at the bases of the guinea-pig atrioventricular valves, histology shows that the more distal portions contain only fibrous and connective tissue, yet still possess both adrenergic and cholinergic nerves. It is possible the function of the adrenergic nerves is to monitor the cholinergic nerves, but they far outnumber these nerves in this site. A further possibility is that they are en route to the ventricles, but this is unlikely since their morphology is typical of terminal axons. In the case of the pulmonary valve, no cholinergic nerves are present and using the light microscope the valve appears to consist solely of fibrous and connective tissue. A rich adrenergic plexus is also present in this valve. Since no obvious effector site has been demonstrated it is possible these adrenergic nerves may be serving an afferent function. Alternately they may be influencing nerves not demonstrated by cholinesterase techniques. As GOSLING and DIXON[1] point out, further evidence on this problem can come only ultrastructural investigations, and it is hoped to proceed with these in the near future.

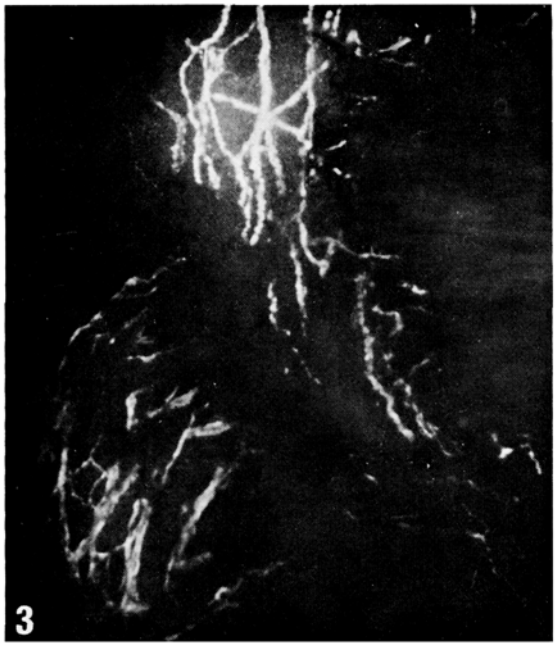

Fig. 3. Pulmonary valve cusps processed to demonstrate catecholamine activity. The adjacent section showed no AChE activity in similar sites.

Résumé. Des plexus fluorescents de nerfs dans les valvules du cœur du Cobaye sont décrits. Bien que du tissu musculaire et des nerfs cholinergiques fussent présents dans les valvules atrioventriculaires, les nerfs adrénergiques de la valvule pulmonaire ne se rapportaient qu'au tissu connectif fibreux. Puisque dans cette situation il n'y a pas d'emplacement effecteur évident ou que ces nerfs influencement des nerfs dont la présence n'est pas révélée par des techniques de cholinesterase ou qu'ils sont de caractère afférent.

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The Target Period During Fetal Life for the Production of Tellurium Hydrocephalus

The morphological anomalies in present congenital malformations are determined by the type and amount of the in-jurious agent, and by the particular moment (target pe-riod) of injury to the embryo and fetus[1,2].

Injuries to the conceptus during embryonic life – the organogenetic period – result in malformed organs (i.e., the nondivision of the telencephalon resulting in holoprosencephaly). Injuries during fetal life, when the formed organs are increasing their bulk, result in anomalies of growth such as absence or small or large gyri, porencephaly. Small doses of tellurium fed to gestating rats during their pregnancy, will result in the birth of hydrocephalic offspring in 50 to 100% of pregnancies[3–5]. The present report concerns the time of the target period for the production of tellurium-induced hydrocephalus in rats.

The diet given to the rats in the present experiments, consisted of 2.5 g of metallic tellurium (Fisher Company) added to 1 kg of normal rat food (Purina diet). The amount of this food given to and usually eaten per rat per day was 20 g (therefore containing 50 mg of tellurium). One group of 20 animals, was fed the tellurium diet every day of gestation (21 days) and 12 of them gave birth to litters containing an average of 8 animals, 6 of which were hydrocephalic. The experiments were divided into 2 types, the 'period insult' and the 'single insult'. The purpose of these experiments was to detect and define the particular period – the target period-during intrauterine development when the fetal rats were more susceptible to tellurium and consequently more likely to be hydrocephalic at birth. For the purposes of these studies, the 21-day-period of gestation was divided into 3 periods, the 1st period was from day 1 to day 9 inclusive, that is until the appearance of the neural plate, the 2nd period was from day 10 to day 15, the organogenetic period, and the 3rd period was from day 16 to 21.

The first experiment was named the 'period insult' because the tellurium diet was fed to the pregnant rats every day of 1 of the 3 gestative periods. Therefore 1 group of 20 pregnant rats received the tellurium diet every day of the 1st period, another group of 20 received it during the 2nd period and a 3rd lot received it during the last

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