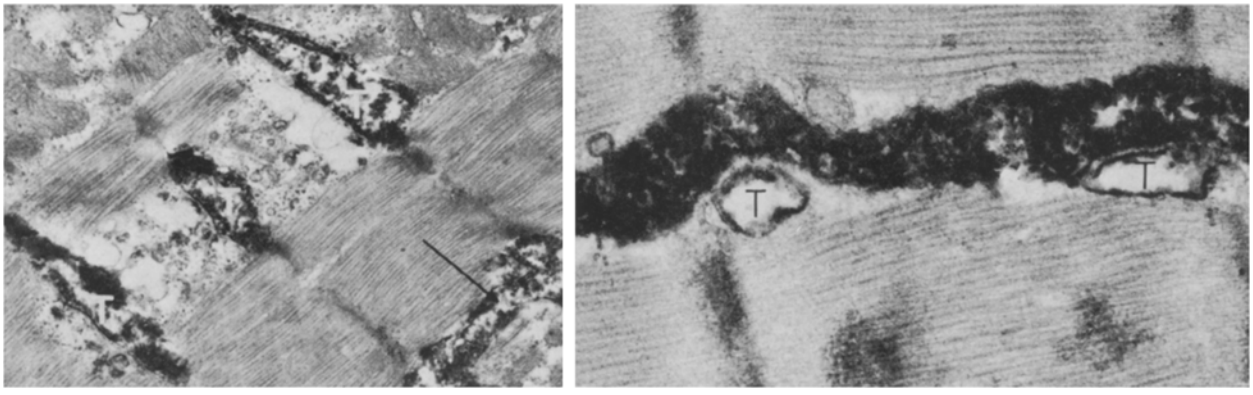

Fig. 2. a) and b) HRP-treated frog myofibres. Product of the enzymatic reaction is seen in the extracellular space (↑) and in the T-tubules (T), which run perpendicularly to the long axis of the myofibres at the level of successive Z-lines. In a) the T-tubules are cut longitudinally (×20,000); in b) the apertures of the TT (T) are cut transversely (×40,000).

In the mammalian cardiac muscle, couplings are regarded as a functional binding of the sarcolemma and the sarcoplasmic reticulum. Depolarization of the surface membrane, whether it is the wall of a transverse tubule or the peripheral envelope of the muscle fibre, leads to the release of calcium-ions stored in the SR. In the case of the ventricle of the frog, physiological experiments indicate the presence of an intra-cellular calcium store[5]. Hence, it would appear that, since the difficulty caused by the supposed absence of couplings has been eliminated, it is not necessary to consider a different mechanism of excitation-contraction coupling in the frog heart from that of the mammalian cardiac muscle.

Zusammenfassung. Auf Grund des grundsätzlich identischen Feinbaus (Vorhandensein von T-System und Koppelungen) des Myocards bei Frosch und Mammalia ist anzunehmen, dass in beiden Fällen auch die elektromechanischen Koppelungen identisch sind.

D. W. SCHEUERMANN

Institut für Biophysik und Elektronenmikroskopie der Universität, Moorenstrasse 5, D-4 Düsseldorf (BR Deutschland), 7 November 1973.

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Development of the Adrenergic Innervation in the Ureter and Vas Deferens in Rabbits

The peripheral sympathetic nervous system has been extensively studied with the fluorescence method of FALCK and HILLARP[1]. This highly specific histochemical approach has thrown new light on the morphological and functional aspects of the adrenergic transmitter. However, most investigations have been concerned with the completely developed adrenergic nervous system. At the present time only a few histochemical studies on the ontogenesis of the peripheral sympathetic nervous system have been reported[2-6]. In these investigations, various peripheral organs were studied but the development of the sympathetic innervation of the ureter was never considered.

We have investigated the development of the adrenergic innervation in the rabbit ureter and vas deferens, this latter organ being considered as a reference for fluorescence neurohistochemical studies. The appearance, distribution and subsequent development of catecholamine-containing nerves in these effector organs are reported.

Material and methods. 34 young male rabbits were studied at age 1, 2, 3, 4, 6, 8, 10, 14, 18, 21, 30 and 45 days. 12 embryos of the last week of gestation period were obtained by hysterotomy under nembutal anesthesia. Specimens consists of the distal parts of the ureter and vas deferens. All preparations were examined for the demonstration of fluorescent catecholamines according to the method of FALCK and HILLARP[1]. The sections were examined under a Zeiss fluorescent photomicroscope using a Philips high-pressure mercury lamp, a BG 12 excitation filter and a 470 u barrier filter.

All sections were studied at the same magnification and photomicrographs taken with the same exposure time. The number of fluorescent nerves were counted on these pictures.

Some tissues were incubated at 37 °C for 30 min in a modified Krebs-Ringer bicarbonate buffer containing norepinephrine in a concentration of 10^{-5} M[7]. The specificity of the fluorescence reaction was checked by omission of the paraformaldehyde reaction and supported by the fact that no fluorescence was observed after pretreatment of some animals with reserpine and α-methyl-metatyrosine[8].

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Results and discussion. Before birth, the ureter and vas deferens musculature are devoid of fluorescent nerve fibres. At birth, both organs were still devoid of fluorescent varicose nerve fibres suggestive of a functional neurotransmission mechanism. However, large fluorescent non-terminal nerve bundles were observed in the surrounding connective tissue issuing from the anterior pelvic ganglia (also called hypogastric ganglia)[9,10] which lay between the vas deferens, seminal vesicle and bladder base. This ganglia is part of the pelvic plexus[9–11] and is connected by the hypogastric nerve which appears as large fluorescent non-varicose nerve bundles. The ganglia give rise to a large number of fluorescent nerve bundles running towards the vas deferens, seminal vesicle, bladder base and lower ureter. At 3–4 days, rare fluorescent fibres started to appear in the musculature of both organs. These delicate fibres exhibited a low fluorescence intensity and the varicosities were irregularly distributed along the nerve fibres. The fluorescent fibres began to grow into the smooth muscle layer and during the 2nd week the entire musculature was invaded by a network of fluorescent varicose nerve fibres. This early network of adrenergic nerves was grossly similar to the adult smooth musculature pattern of innervation, although the nerve terminals were less numerous and had not yet their final appearance with regularly distributed and highly fluorescent varicosities. During the following 3–5 weeks, the density of the adrenergic network increased rapidly and the final adult pattern of innervation of the effector organ was reached about 4 to 6 weeks after birth. Incubation of

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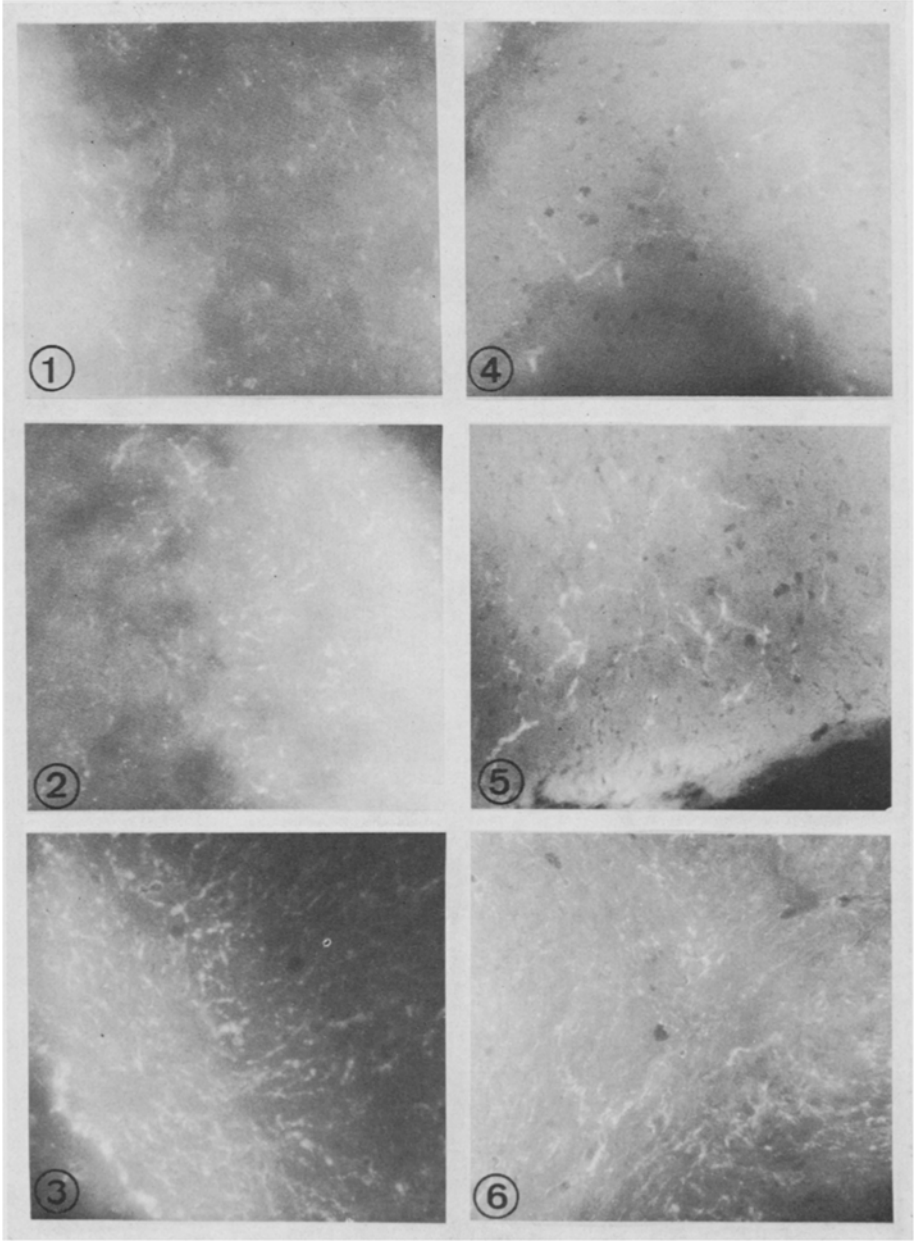

Fig. 1. Vas deferens (3 days). Rare fluorescent fibres start to appear in the musculature. ×300.

Fig. 2. Vas deferens (9 days). The entire musculature is invaded by a network of adrenergic nerves. ×300.

Fig. 3. Vas deferens (21 days). The density of the fluorescent varicosities has rapidly increased to reach the final adult pattern of innervation. ×300.

Fig. 4. Ureter (4 days). Discrete fluorescent varicosities appear at the periphery of the muscular layer. ×300.

Fig. 5. Ureter (14 days). The early network of adrenergic nerves is grossly similar to the adult pattern of innervation. ×300.

Fig. 6. Ureter (45 days). Definitive adult network of adrenergic nerves. ×300.

tissues in norepinephrine increased the intensity of the fluorescence but did not increase the amount of fluorescent nerve.

This suggests that the formaldehyde-induced fluorescence of catecholamines had detected most of the developing adrenergic nerves, a similar suggestion was made after administration of noradrenaline or inhibition of the metabolism[2,3].

The development of adrenergic nerves of both organs proceeded almost identically; however, the vas deferens seemed to develop its adrenergic innervation somewhat earlier than the ureter. Difference between both organs was also quantitatively significant: likewise in adult animals, the density of adrenergic nerve terminals is much lower in the ureter compared to the rich sympathetic innervation of the vas deferens[12].

The histochemical study demonstrated that before reaching the terminal part of the adrenergic nervous network, the outgrowing non-varicose nerve fibres had a larger diameter and a higher fluorescence intensity than the adult preterminal axons. The fluorescence of these non-terminal fibres decreased concomitantly with a progressive increase in the fluorescent varicosities of the developing terminal nerve fibres. Similar observations were made in other organs and species[3,4]. This suggests that during early development the adrenergic terminal nerve fibres move into, rather than form within, the effector organ to form the autonomic ground plexus.

At birth, the kidney is not fully differentiated[13,14], and it has been shown recently that full differentiation of the ureter is also not achieved at birth[15,16]. During development there appears to be a correlation between the amount of muscle and the presence of function. LEESON and LEESON[15] have observed that the rat ureter is composed of mature smooth muscle cells only by the 5th post-natal day and a fully developed lamina propria and muscularis are acquired over a period of 7 days. The rapid acquisition of a fully developed ureteral musculature coincides with the loss of the placenta as the principal excretory organ[15]. The fact that the ureter is devoid of functional adrenergic nerves at birth correlates well with the above-mentioned observations. The subsequent development of the adrenergic terminal innervation appears to be related to the underlying maturation of the smooth musculature.

Résumé. A la naissance, la musculature de l'uretère et du canal déférent du lapin est dépourvue d'une innervation adrénergique fonctionnelle. Les fibres nerveuses terminales apparaissent vers le troisième et quatrième jour et l'innervation augmente rapidement pour atteindre l'état adulte entre la quatrième et la sixième semaine. Le développement de l'innervation adrénergique périphérique est lié à la maturation sous-jacente de la musculature lisse.

C.C. SCHULMAN[17]

Department of Urology, Brugmann University Hospital, University of Brussels, 4, Place Van Gehuchten, B–1020 Bruxelles (Belgium), 21 January 1974.

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Stimulation of Cell Aggregation by Theophylline in the Asexual Reproduction of Fresh-Water Sponges (*Ephydatia fluviatilis*)

The gemmules of fresh-water sponges arise through the local aggregation in the mesohyle of the sponge of several types of amoeboïd cells. The first to aggregate are the archaeocytes, which will eventually become the vitellus-stuffed embryonic cells and the trophocytes at the expense of which the vitellus is build up by phagocytosis[1]. We have previously studied the physiology of gemmulation on populations of sponges of specified strain, age and size, grown in Petri dishes under various experimental conditions[2–5]. It has also been possible to observe the formation of a gemmule in very thin sponges[6], grown between 2 glass slides[7].

These experiments and observations strongly suggest that 1. the aggregation of cells during the building of a gemmule is oriented by a chemical signal and that 2. several of the physiological variables that modulate the frequency of gemmulation in a sponge population bear a striking resemblance to those implied in the aggregation of cellular slime-molds[8]. The identification of acrasin as cyclic 3′, 5′ adenosine monophosphate and of acrasinase as a phosphodiesterase[9–14] suggested that we should investigate a possible effect of either cyclic AMP or an inhibitor of phosphodiesterase on gemmulation.

Material and methods. We used fresh-water sponges of the species *Ephydatia fluviatilis*; strains α, β, γ and δ[15]. The sponges were cultivated from gemmules that had been gathered from open-air cultures in a pond near Brussels. Culture methods have been described in detail previously[2–4]. Briefly, the gemmules are incubated in a mineral medium, at 20 °C in the dark, except for some experiments bearing on the influence of light.

By grouping these gemmules in clusters before they hatch, we can control the individual size of the sponges as

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