ethanol series, cleared in xylol, and mounted in Permount.

Results. Of the 94 Con A-treated embryos, 86 had a malformed brain, which was shortened and distorted by irregular foldings. The neural tube showed varying degrees of openness. Somites, although less numerous than controls, were almost normal in appearance. Other structures were usually unaffected. Electron microscopic observations on the Con A-treated neuroepithelium revealed that cells were more rounded than those of controls, were not closely apposed, and showed loss of lateral cytoplasmic extensions (Figures 1 and 2). The apical surface was smoother than in the untreated cells and had no apical folds. Con A, at the concentration used, had no apparent effect on the integrity of microtubules (Figure 2) and microfilaments (Figure 3) except in severely affected neuroepithelium. Radioautographic studies showed that labeled materials were found mostly on the cell surface (Figure 4).

Discussion. The present study showed that 1. cellular processes associated with closure of the neural tube were more sensitive to Con A than were those essential to the formation of microfilaments and microtubules; 2. the initial site of Con A effect on neuroepithelium was the cell surface. These findings along with the known biological property of Con A[6,7] indicated that the observed neural tube defects were consequences of alterations in the cell surface of developing neuroepithelium. Indeed, the presence of glycoproteins and glycosyltransferases on the surface of cells which are in contact with a lumen has been implicated in cell adhesion and recognition during embryonic development[10–15]. Further experiments are presently underway in our laboratory to investigate the possible role of cell surface coat material in neurulation of chick embryos.

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Extravesicular Noradrenaline in Developing Peripheral Adrenergic Nerves

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Summary. Using a fluorescence technique numerous developing noradrenergic nerve terminals were observed in the muscle coat of the rat ductus deferens between 2 and 6 days postpartum. In the electron microscope similar developing nerve terminals possessed an extensive system of tubular endoplasmic reticulum but did not contain the small dense cored vesicles characteristic of mature noradrenergic nerve terminals. Thus the tubular reticulum is proposed as an alternative storage site for noradrenaline in developing adrenergic nerves.

Noradrenaline (NA) is believed to occupy membrane bound stores within peripheral adrenergic (sympathetic) nerve terminals and both pharmacological and electron microscopic studies[1–3] have shown that intra-axonal dense cored vesicles – both small (50 nm diameter) and large (90 nm diameter) – are the structures concerned with this amine storage. However, these and many other studies apply to adult material and relatively little information is available on NA in immature peripheral adrenergic nerves. Consequently, the distribution of NA in developing axons was examined using a microfluorescence technique and these results have been correlated with the fine structure of similar immature adrenergic nerves. The rat ductus deferens was chosen for examination since this tissue has been used extensively in studies on adult autonomic innervation and it is known to possess a rich supply of NA containing nerves[4,5].

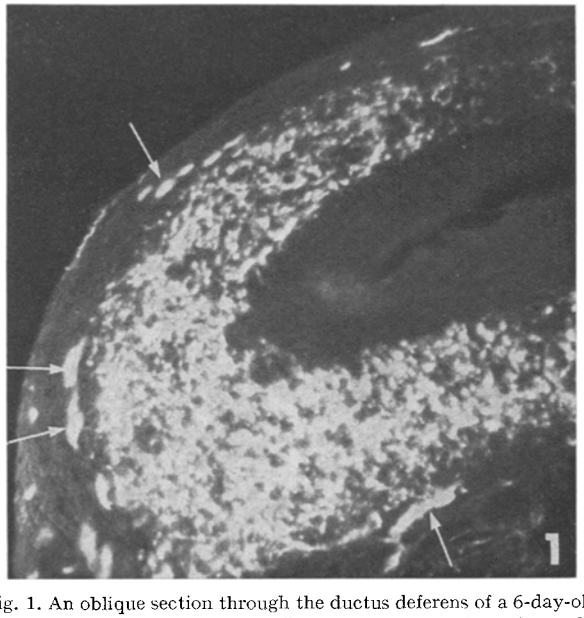

Fig. 1. An oblique section through the ductus deferens of a 6-day-old rat showing a dense plexus of fluorescent nerves throughout the muscle coat. Note the large fluorescent adventitial nerves (arrows). × 200.

Materials and methods. Male Sprague Dawley rats were killed at birth and on alternate days thereafter to provide a series of animals in age from 0–24 days. For each group, a minimum of 2 animals was examined and from every rat one ductus was processed histochemically for tissue NA using the technique described by FALCK and OWMAN[6]. The other ductus deferens from each animal was examined electron microscopically following fixation in either buffered potassium permanganate[7] or osmium tetroxide. Particular attention was focused on the urethral end of

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the ductus since this portion is known to be supplied mainly with NA containing terminal regions[8].

Results and discussion. Using the histochemical technique on new-born animals, the characteristic fluorescence associated with NA was found in nerves confined to the external aspect of the muscle coat. These nerves were relatively large and NA fluorophores were uniformly distributed along their length; finely beaded nerve fibres were absent from these preparations. At 2–4 days, fine branches extended into the muscularis from the adventitial nerves and, by 6 days postpartum, the complete thickness of the muscle wall was associated with NA positive nerves (Figure 1). At this stage some fibres possessed small varicosities not unlike those seen in the adult.

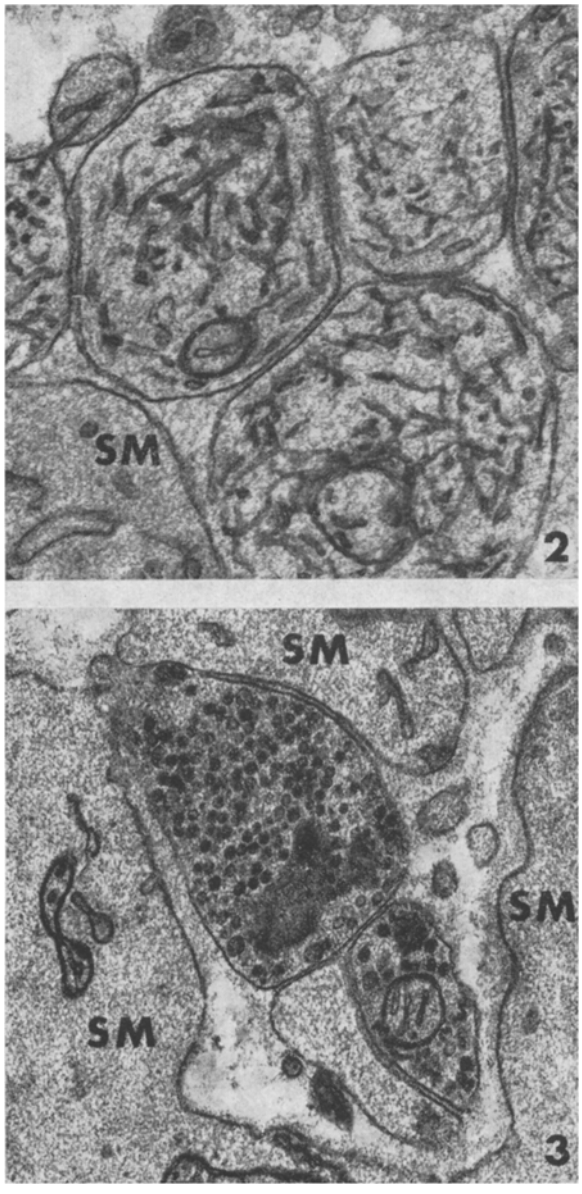

Fig. 2. A group of axons adjacent to a smooth muscle cell (SM) in the ductus deferens of a 6-day-old specimen. Each axon contains an extensive system of TR but is devoid of 50 nm diameter dense cored vesicles. Permanganate fixation. × 40,000.

Fig. 3. Two axons in the adult ductus are seen to contain numerous small granulated vesicles typical of noradrenergic effector regions. Endoplasmic reticulum is absent from these profiles (compare with Figure 2). SM, smooth muscle. Permanganate fixation. × 40,000.

8–10 days after birth the muscle coat was well innervated and a particularly rich plexus of brightly fluorescent finely beaded nerves was evident along the inner aspect of the muscle. This general arrangement was seen in older animals although the fluorescence associated with adventitial nerves continued to decrease so that 24 days after birth large nerves in this situation could not be visualized. Electron microscopy of tissue from new-born animals revealed large bundles of axons on the external aspect of the immature muscle layer. These axons contained an extensive system of tubular smooth endoplasmic reticulum (TR) together with neurotubules and neurofilaments. A similar fine structure was seen in occasional single axons laying in close association with myoblasts in the muscle coat of 2-day-old specimens. At 4 days, small groups of axons, the majority of which contained tubular membranous cisternae were observed amongst the myoblasts. By 6 days postpartum the coat was increased in thickness and the frequency of associated axons was also markedly raised. However, in all the axons examined up to this stage, small (50 nm diameter) dense cored vesicles similar to those found in the adult were never observed (Figure 2).

8 days after birth, in addition to quantities of TR, some axon profiles now contained occasional small and/or large dense cored vesicles. In later stages there was a gradual increase in the occurrence of small dense cored vesicles and this was accompanied by a concomitant decrease in TR within the axoplasm. Whilst such changes occurred gradually, the majority of axon profiles within the smooth muscle coat at 24 days were indistinguishable from those observed in adult preparations (Figure 3).

Our histochemical results support those of Owman et al.[9] and confirm the presence of NA in developing autonomic nerves of the rat ductus deferens. Similar findings have also been described recently in the rabbit ductus deferens by Schulman[10]. The characteristic fluorophores of NA were found to be present at birth and to gradually increase throughout the early postnatal period. In the adult a proportion of NA in peripheral terminals is stored within small dense cored vesicles readily identified after fixation for electron microscopy in potassium permanganate[7]. The present study however, has shown that intra-axonal NA occurs in the rat ductus deferens in the absence of theses structures in early postnatal life. An alternative site for NA storage must therefore exist in immature peripheral nerves and one possible candidate is the TR found in these nerves. Indeed, both Eränkö[11] and more recently Richards and Tranzer[12] have suggested that TR in the cytoplasm of adult adrenergic neurons may represent the storage site for extravesicular NA in these cell bodies. The latter workers have also proposed that the small dense-cored vesicles may be derived from the TR with which they are often associated. This process may also occur in developing adrenergic nerve terminals and our results indicate that further detailed study of 6–8-day-old specimens (during which time small dense cored vesicles are first recognized) may provide direct evidence for this process.

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