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Reinnervation of the rat adrenal medulla transplanted in the anterior eye chamber

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INTRODUCTION

In relation to the idea originally conceived by Langley (1892, 1895, 1897) that the reinnervation of mammalian autonomic neurons is specific, several studies have been attempted on the reinnervation of mammalian adrenomedullary chromaffin cells (Piezzi & Cavicchia, 1973; Carruba, Ceccarelli, Clementi & Mantegazza, 1974). In the report by Carruba *et al.* no reinnervation occurred when the cat adrenal medulla and superior cervical ganglion were transplanted together under the kidney capsule. They stated that the adrenal medulla, which is originally innervated solely by preganglionic cholinergic fibres, cannot be reinnervated by transplanted postganglionic neurons ('foreign' nerves), and concluded that the reinnervation process follows the original type of innervation. No nerve fibres were reported to be in contact with chromaffin cells of the rat adrenal medulla transplanted into the anterior eye chamber (Piezzi & Cavicchia, 1973), where various transplanted target tissues for autonomic postganglionic neurons are known to be reinnervated by nerve fibres derived from the host iris (Olson & Malmfors, 1970).

On the other hand, it has recently been shown physiologically as well as ultrastructurally that reinnervation and functional recovery can be obtained by regeneration of fibres derived not only from the original nerve, but also from 'foreign' ones, in studies of the autonomic ganglion cells (Ceccarelli, Clementi & Mantegazza, 1971; McLachlan, 1974; Raisman, Field, Ostberg, Iversen & Zigmond, 1974; Purves, 1975, 1976). In addition, the presence of nerve fibres was noted recently in the transplanted adrenal medulla, although a detailed description of the reinnervation was not given (Manuelidis & Manuelidis, 1975; Unsicker & Chamley, 1977; Unsicker, Zwarg & Habura, 1977).

In the present study an attempt has been made to examine the possibility of reinnervation of the adrenal medulla by 'foreign' nerves after transplantation into the anterior eye chamber.

MATERIALS AND METHODS

Thirteen adult albino rats from the same litter and weighing approximately 150 g were used for this study. A donor rat was anaesthetized with ether and the adrenal medulla was dissected out aseptically under a dissection microscope. The dissected adrenal medulla was then inserted into the anterior eye chamber of recipient rats according to the technique described by Olson & Malmfors (1970). Half the recipient rats were subjected to ipsilateral sympathectomy of the superior cervical ganglion at the time of transplantation. Two and six months after surgery the recipient rats were anaesthetized with ether and the eyeballs were removed. Half the rats were

pre-treated with 5-hydroxydopamine (5-OHDA) (50 mg/kg body weight) intraperitoneally in order to enhance the appearance of adrenergic nerve fibres.

The transplant attached to the iris was removed and immersed for 2 hours in 3 % glutaraldehyde solution buffered with sodium cacodylate (pH 7·4). The tissue was post-fixed in 1 % osmium tetroxide (using the same buffer) for 2 hours, followed by *en bloc* staining with aqueous 1 % uranyl acetate. Following dehydration in a graded series of ethanols the blocks were embedded in Epon 812 (Luft, 1961). Ultrathin sections were mounted on formvar film using grids with a single rectangular hole (1 × 2 mm) and were stained with uranyl acetate and lead citrate. They were examined with a JEM 200A electron microscope having an accelerating voltage of 100 kV. A large number of random sections as well as some series of 50–200 serial sections were examined. In the serial section study processes of adrenomedullary chromaffin cells and neuronal elements enveloped by satellite cells were reconstructed according to the method previously reported (Kondo, 1976, 1977*a*).

RESULTS

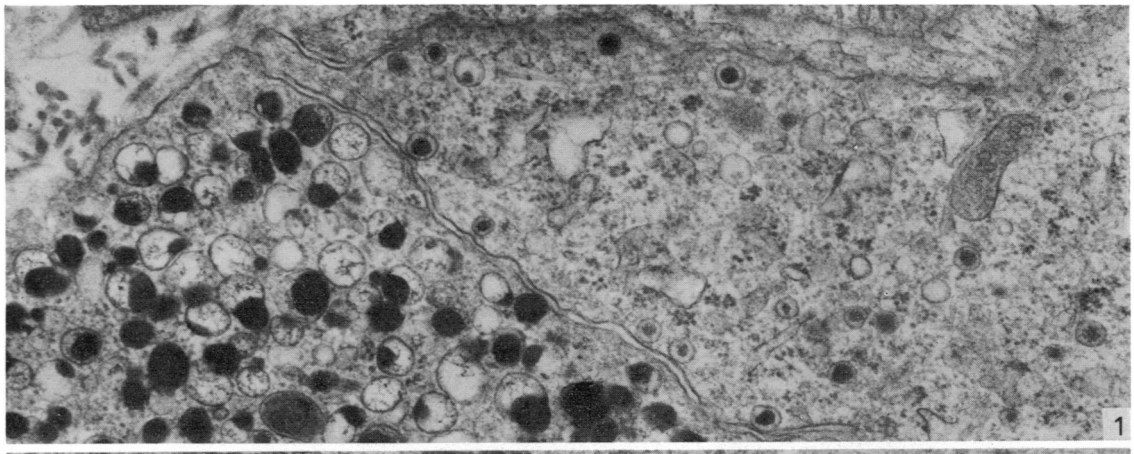
The results after different periods of transplantation were essentially identical and are therefore not described separately.

The transplants, consisting mainly of chromaffin cells, were attached to the iris and surrounded by the iridial stroma: they were richly vascularized with fenestrated capillaries. Chromaffin cells were arranged in small clusters. Desmosomal contacts were seen between adjacent cells. Some chromaffin cells were in direct apposition to the epithelial cells on the posterior surface of the iris, with intercellular spaces of approximately 15 nm, the basement laminae of both cells fusing to form a single continuous layer (Fig. 2). No membrane thickenings were found at the apposition sites. Three types of chromaffin cells were identified on the basis of the size and density of characteristic granular vesicles; namely noradrenaline cells (Fig. 1), adrenaline cells (Fig. 6), and cells containing small granular vesicles, 100 nm in mean diameter (Fig. 1). The first two types were predominant, and basically resembled their *in vivo* counterparts (Coupland, 1965). The diameter of vesicles characteristic of these two types was slightly decreased (from 240 to 180 nm in mean diameter) as compared with those in intact counterparts. The adrenaline cells were more numerous than the noradrenaline cells in spite of the fact that the adrenocortical tissue was markedly diminished in the transplant. The presence of the third type of cell has been noted in the intact adrenal medulla by Diner (1965), Hervonen (1971), Unsicker (1973, 1976) and Kobayashi & Coupland (1977). This type was more frequently encountered in random sections of the transplant than in the intact adrenal medulla, although its absolute number was still small. The small granular vesicles had a round dense core with a halo, which was sometimes located eccentrically in the vesicle. The density of the core was almost as great as that of a noradrenaline granule.

Fig. 1. Noradrenaline cell and small granule-containing cell in the transplant. Note the difference in size of vesicles in these two cells. Six months after transplantation. × 30000.

Fig. 2. Direct apposition between chromaffin cell (*C*) and epithelial cell (*E*) on the posterior surface of the iris. Note the confluent basement laminae (arrows). Arrowheads indicate tight junctions between adjacent epithelial cells. Six months after transplantation. × 30000.

Figs. 3–5. Serial sections of a cytoplasmic process of a chromaffin cell (arrows) enclosed together with a nerve fibre (*N*) by a common Schwann cell. Neither chromaffin granules nor ribosomes are seen in an extremely thin portion of the process. Six months after transplantation. × 30 000.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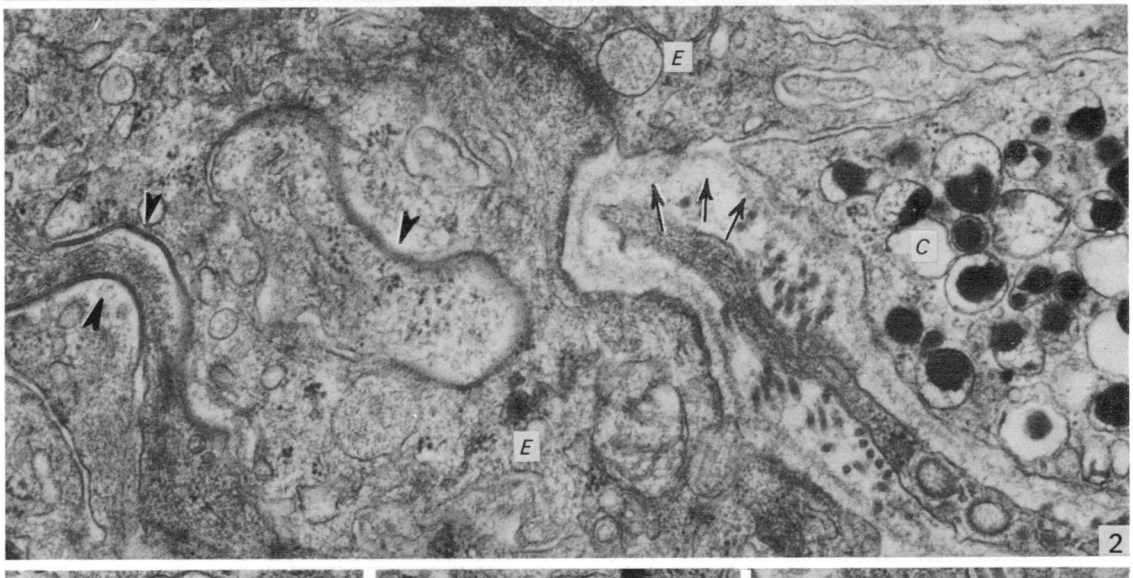
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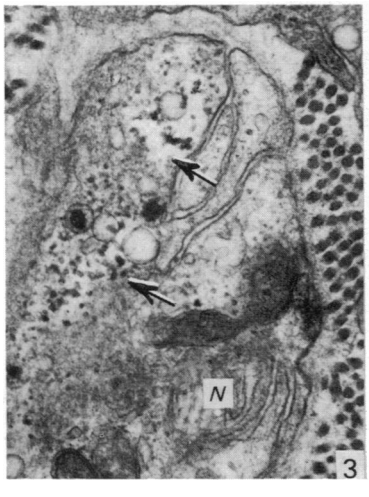
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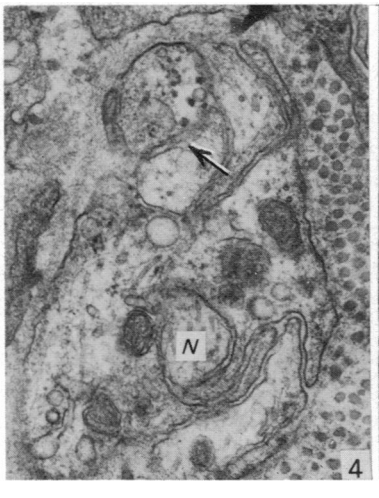
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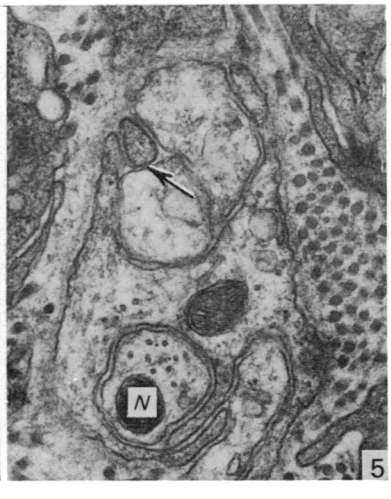
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Chromaffin cells developed cytoplasmic processes which sometimes projected into the stromal connective tissue. Process formation seemed to be a little more marked in ipsilaterally sympathectomized rats than in intact animals. Serial sections revealed that processes repeatedly changed diameter along their length (ranging from 0·1–1·2 μm). Granular vesicles characteristic of the chromaffin cells, and ribosomes, were seen throughout the processes, except where they were very thin (Figs. 3–5). Small clear 50 nm vesicles were not found in the processes.

A few ganglion cells were seen in the transplants. They had large oval nuclei with poor chromatin and prominent nucleoli and in general showed the ultrastructural features typical of autonomic ganglion cells (Elfvin, 1963; Yamamoto, 1963; Grillo, 1966). Two types of ganglion cells were identified as a result of administering 5-OHDA before death, one with larger granular vesicles, 100 nm in diameter, sparingly distributed throughout the perikarya (Fig. 6), the other, with several small granular vesicles, 50 nm in diameter, located in clusters at the periphery of the perikarya (Fig. 7). The former are presumably cholinergic, the latter adrenergic (Yokota & Yamauchi, 1974). Adrenergic ganglion cells were rarely encountered. The perikarya of ganglion cells were occasionally enclosed, together with adjacent ganglion or chromaffin cells, by common satellite cells, direct apposition of adjacent cells being seen.

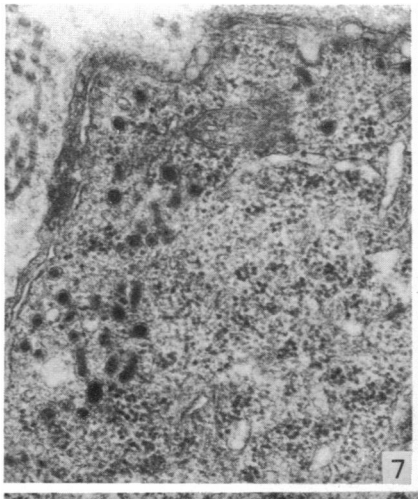
Nerve fibres were seen within satellite cells enclosing chromaffin and ganglion cells, although the number of the fibres was small as compared with that in the intact adrenal medulla. Cells located at the periphery of the transplant were associated with more numerous nerve fibres than those in the interior of the transplant. It was revealed in serial sections that the nerve fibres ran along the outer surfaces of cell clusters and sent only a few branches into the clusters. During their courses they changed diameter repeatedly (from 0·1 to 0·8 μm). Thickened portions of nerve fibres contained numerous vesicles, but tenuous portions contained very few.

Two types of nerve fibre were identified by their vesicles: cholinergic fibres contained small clear vesicles, 50 nm in diameter, mixed with large granular vesicles, 100 nm in diameter (Figs. 6, 11, 12), while adrenergic fibres had small granular vesicles, 50 nm in diameter (Figs. 8–10). The former were numerous, the latter rare. Although ipsilateral sympathectomy seemed to result in a decrease in the number, or even the complete disappearance, of the adrenergic fibres, the change following the operation was not striking because of the small number of the fibres present even before sympathectomy. Direct apposition between chromaffin or ganglion cells and two types of nerve fibres occurred, though infrequently. At such apposition sites a few efferent synapses were formed, a cluster of vesicles being concentrated at the membrane thickening on the nerve fibre side. The number of such synapses remained small even six months after transplantation.

Fig. 6. Adrenaline cell (*A*) and cholinergic ganglion cell (*G*) enclosed together with cholinergic nerve fibre (*Ch*) by common satellite cells. Following ante-mortem administration of 5-OHDA. Two months after transplantation. × 30000.

Fig. 7. Perikarya of adrenergic ganglion cell. Note small granular vesicles, 50 nm in diameter, in cluster following administration of 5-OHDA. Two months after transplantation. × 36000.

Fig. 8. Adrenergic nerve fibre (*Ad*) forming synapses on a cholinergic ganglion cell (*G*). Note small granular vesicles in the nerve fibre. 5-OHDA. Six months after transplantation. × 30000.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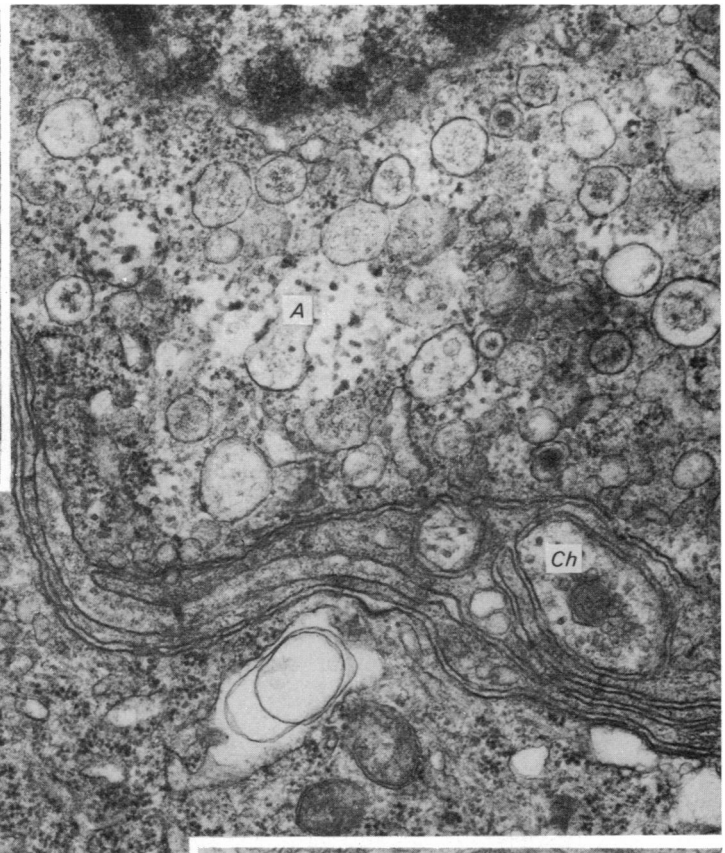
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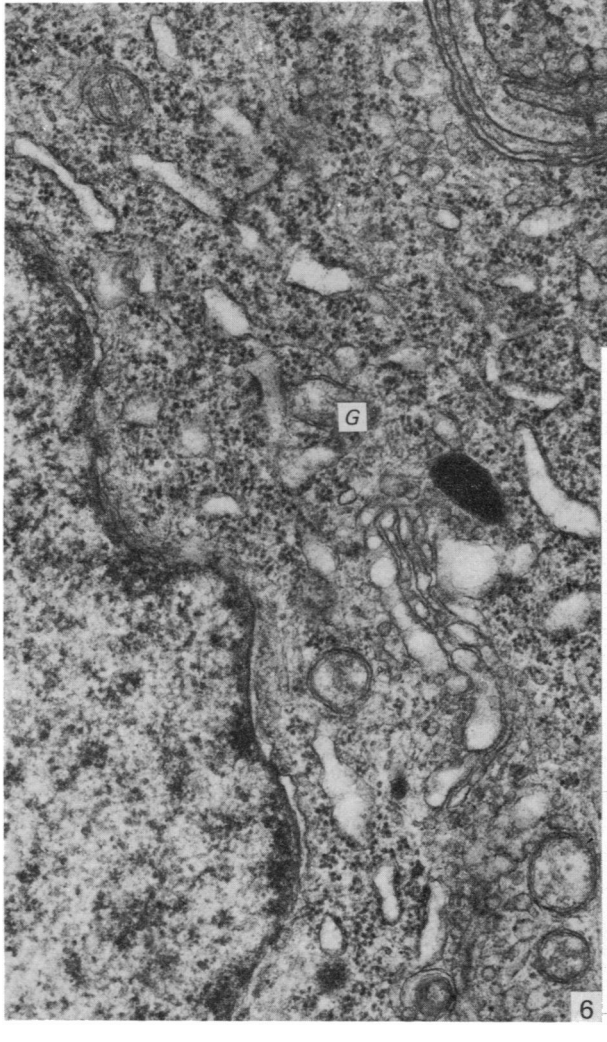
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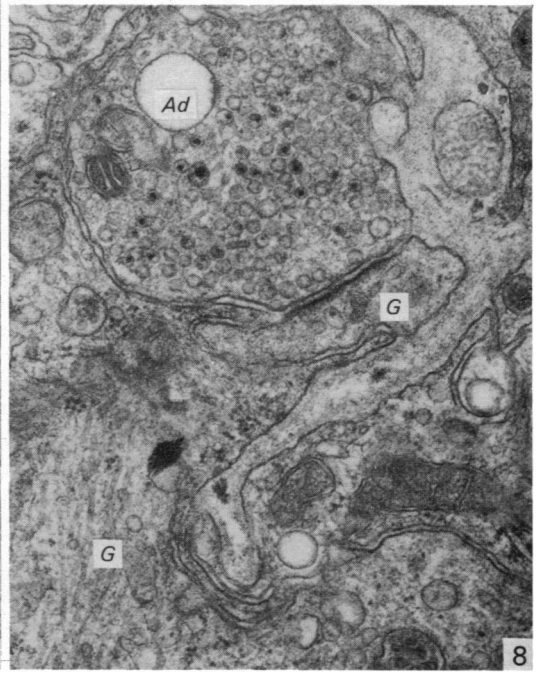
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G
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DISCUSSION

The development of long cytoplasmic processes in the adrenal medulla transplanted in the anterior eye chamber has been demonstrated by means of fluorescent microscopy (Olson, 1970). The present work verified that these long fluorescent processes were derived from the chromaffin cell itself. Similar process formation has recently been reported as a result of electron microscopic studies of adrenal chromaffin cells cultured *in vitro* (Manuelidis & Manuelidis, 1975; Unsicker & Chamley, 1977).

In contrast to Piezzi & Cavicchia (1973) and Carruba *et al.* (1974), the present study revealed many nerve fibres within satellite cells enveloping chromaffin cells. The satellite cells covered only small areas of the surface of chromaffin cells and so nerve fibres were only occasionally seen within satellite cells. This fact, and the uneven distribution of nerve fibres in the transplant, may explain why such nerve fibres within satellite cells escaped the notice of previous authors.

Several possibilities need to be considered with regard to the origin of the adrenergic and cholinergic nerve fibres in the transplanted adrenal medulla: they could be (1) extrinsic, from nerve fibres originally innervating the iridial musculature of the recipient rats, (2) intrinsic, pre-existing or newly formed, from ganglion cells which are sparsely distributed in the transplants, and (3) intrinsic, from chromaffin cells forming long cytoplasmic processes. This last possibility is most unlikely because neither granular vesicles characteristic of chromaffin cells, nor ribosomes, were present in the nerve fibres.

It is generally accepted that the ganglion cells in the intact adrenal medulla are cholinergic and give strongly positive histochemical reactions for cholinesterase activity (Lewis & Shute, 1968). The present study, however, revealed not only cholinergic, but also adrenergic, ganglion cells, when 5-OHDA was administered before death. The nerve fibres in the transplants probably arose from these two kinds of ganglion cell, although the number of ganglion cells is small compared to the number of nerve fibres. The presence of nerve fibres in adrenal medulla cultured *in vitro* (Manuelidis & Manuelidis, 1975; Unsicker & Chamley, 1977) supports this possibility. On the other hand, the fact that chromaffin cells at the periphery of the transplant were more frequently associated with nerve fibres than those in the interior suggests an extrinsic origin. The small number of adrenergic nerve fibres in normal rat adrenals makes it difficult to clarify the problem of the origin of the fibres by undertaking ipsilateral sympathectomy. Therefore it is not possible at present to state with any certainty which of the possible sources for nerve fibres is the correct one.

With regard to the small number of adrenergic nerve fibres in the transplanted adrenal medulla, the findings on the transplanted carotid body should be noted

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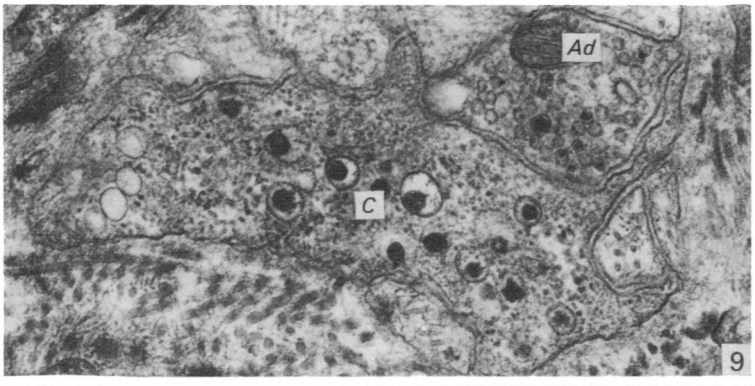
Fig. 9. Adrenergic nerve fibre (*Ad*) directly apposed to chromaffin cell (*C*). Note small granular vesicles in the nerve fibre. 5-OHDA. Two months after transplantation. × 30000.

Fig. 10. Different section of the same area shown in Fig. 9. Note synaptic membrane specialization (arrows). × 30000.

Fig. 11. Cholinergic nerve fibre (*Ch*) within satellite cell of transplanted adrenal medulla. Arrows indicate synapses on a chromaffin cell (*C*). 5-OHDA. Two months after transplantation. × 28000.

Fig. 12. Higher magnification of cholinergic synapses (arrows) on transplanted chromaffin cell (*C*). 5-OHDA. Two months after transplantation. × 50000.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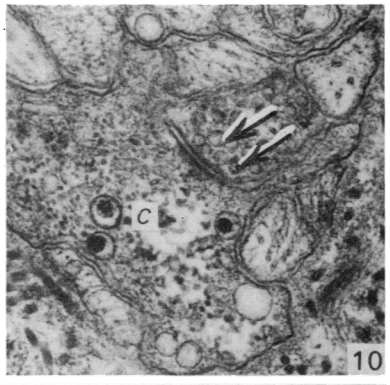
C
9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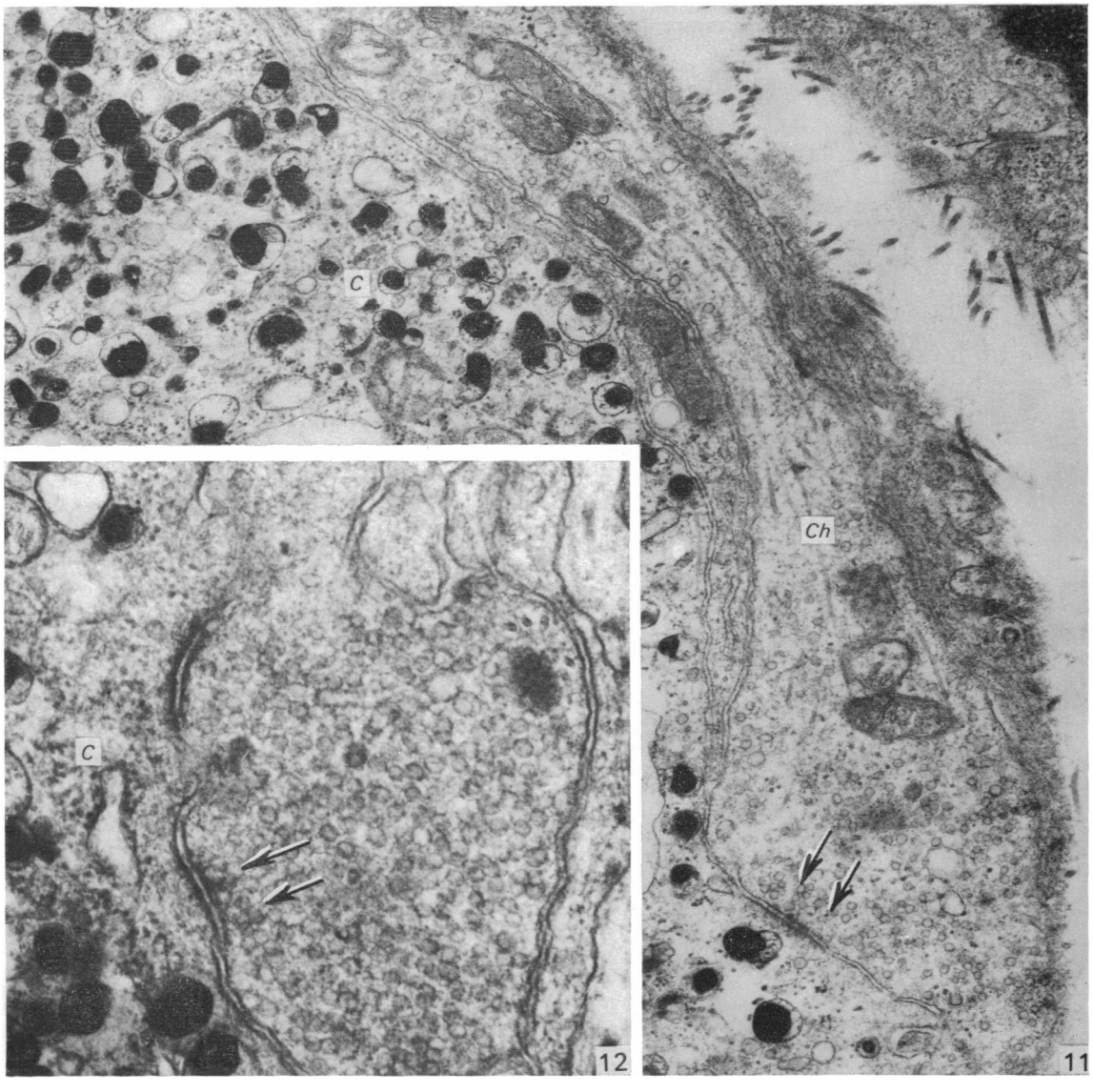
C
Ch
11

C
12

(Kondo, 1978). A distinct number of adrenergic nerve fibres were seen within sustentacular cells of the carotid body transplanted in the eye chamber of a recipient rat which had not been sympathectomized. It is known that the sustentacular cells of the intact carotid body enclose a distinct number of adrenergic nerve fibres (McDonald & Mitchell, 1975; Kondo, 1977*b*), but no such fibres are seen within the satellite cells of the intact adrenal medulla (Prentice & Wood, 1975). This difference in the distribution of adrenergic nerve fibres in these two organs under both normal and experimental conditions suggests that sustentacular and satellite cells may have different and specific affinities for adrenergic fibres.

SUMMARY

Adrenal medulla transplanted to the anterior chamber of the eye was studied by electron microscopy. Transplanted chromaffin cells consisted of noradrenaline cells, adrenaline cells and small granule-containing cells, and they formed long cytoplasmic processes. Some chromaffin cells were directly apposed to the iridial epithelial cells. A few adrenergic and cholinergic ganglion cells were seen in the transplant. Nerve fibres, mostly cholinergic, but a few adrenergic, were seen within satellite cells enclosing chromaffin and ganglion cells. A few synapses were formed on the chromaffin and ganglion cells, though the number of such synapses remained small even 6 months after transplantation. The possible origin of these nerve fibres, and the specificity of reinnervation are discussed.

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