The Innervation of the Adrenal Glands of Mammals; a Contribution to the Study of Nerve-endings.

By

Dorothy M. Willard,

Department of Zoology and Comparative Anatomy, University Museum, Oxford.

With Plate 24.

CONTENTS.

INTRODUCTION.

THE nature of the mechanism by which nerve impulses in one cell stimulate other cells to activity still remains a matter of considerable controversy, and the present study is an attempt to further our knowledge of the subject by an accurate study of the innervation of the adrenal glands.

Certain points in the general anatomical arrangement are almost unanimously accepted, namely, that the nerve-fibres from the sympathetic chain form a plexus on the capsule. From this plexus several nerve-bundles pass in through the cortex to the medulla, where the nerve-fibres branch profusely among the chromophil cells.

There is considerable doubt as to whether any of the nerve-fibres actually end in the cortex. Collateral branches are given off as the bundles pass through the cortex, and Dogiel (1894) and Fusari (1891) in adult animals, and Brauer (1932) in early embryonic forms of the chicken, describe nerves branching around cortical cells. Alpert (1931) saw the fibres ending within the cortical cell. On the other hand, Giacomini (1897) found very few nerves in the cortex of birds, and, in Selachians, Young (1933) could find no nerves in the interrenal except those to the blood-vessels.

As regards the innervation of the medullary tissue, the first question is whether the fibres are preganglionic as suggested by Elliott (1913). Young (1933) showed that in Selachians some at least of the motor fibres to the chromophil cells came from postganglionic nerve-cells lying in or near the suprarenal tissue, and that therefore in this case Elliott's hypothesis could not be altogether correct. It was hoped that further study of the intramedullary nerve-cells in mammals would throw more light on this question which is of some considerable interest in view of the different methods of action of pre- and postganglionic synapses (see Feldberg et al., 1934).

Perhaps the most interesting problem of all is the relation of the nerve-fibres in the medulla to the secretory cells. Fusari (1891) described a nerve net around groups of chromophil cells. He saw terminations ending in plaques or discoidal knobs, but contended that these were artifacts. Similar nerve nets around individual cells were found by Dogiel (1894).

On the other hand, Alpert (1931) describes the nerve-fibres to both cortical and medullary cells as penetrating within the cell 'either by a short straight twig or a delicately curving fibril which tapers down to end near the nucleus'.

There has been a similar difference of opinion as regards the innervation of other glands. Intercellular endings have been found by Cajal (1891) in the pancreas and salivary glands; by Arnstein (1895) in several glands; by von Greving (1934) in the pancreas; and by Stormont (1928) in the salivary and thyroid glands. Intracellular endings are reported by Kolmer (1905) in the dermal and lacrimal glands of *Triton cristatus*; by Tricomini-Allegro (1903–4) in the mammary gland; by Puglisi-Allegro (1904) in the lacrimal gland; and by Kubo (1933) in the liver. The general conclusion that the end organs lie within the cytoplasm is in line with the work of Boeke (1932) on nerve-endings in muscles, whereas the opposite conclusions have been reached among others by Hoff (1932), who believes that the terminal boutons seen in the spinal cord are merely in contact with the dendrites which they stimulate, and that there is no neurofibrillar continuity such as has been suggested by so many workers (see Tiegs, 1931).

There is, however, another possibility, namely, that the nerve-fibres passing close to the secretory cells can stimulate them without ending either within them or on their surface. That such stimulation does in fact occur is suggested by the whirls of fibres found particularly around sympathetic neurons (see Young, 1933).

Statement of Problems.

There are therefore five problems which have been dealt with during the present work.

1. To discover whether the nerve-fibres in the cortex innervate the secretory cells or merely pass through with the blood-vessels.
2. To determine whether there is any postganglionic innervation of the chromophil cells from intra-medullary neurons.
3. To discover whether the finest branches of the nerves in the medulla penetrate inside the cells which they innervate.
4. To find out whether the secretory cells are always innervated by terminal branches of the nerve-fibres, or whether the latter may deliver a stimulus as they pass among the cells.

Material and Technique.

For the principal study of the adrenal gland, the guinea-pig was used as representative of mammals. The male sex was uniformly chosen in the case of adults. For purposes of general comparison several embryonic stages (about 28 and 50 days) and a series of young animals of both sexes (1 and 2 hours, 2½, 4, 7, 9, 12, 14, 16, 18, and 21 days) were examined; also suprarenal glands from other mammals, rabbit and mouse. All the animals were killed by a blow on the head and dissected immediately afterwards.

Greatest success in the fixing and staining of the nerve-fibres was obtained with Cajal's chloral hydrate method.

(1) Fix for 24 hours in:

Chloral hydrate	2·5 gm.
95 per cent. alcohol	40 c.c.
Distilled water	40 c.c.
Pyridine	20 c.c.

(2) Wash in distilled water until little or no smell of pyridine remains and transfer to 97 per cent. alcohol for 24 hours.

(3) Wash in distilled water.

(4) Place in 2·5 per cent. silver nitrate at 37° C. 9–12 days was found to be the most satisfactory length of time. The longer times being better for nerve-cells.

(5) Short wash in distilled water (1 minute sufficient).

(6) Reduction 12–24 hours in

Hydroquinone or pyrogallic acid	1 gm.
Neutral formol	10 c.c.
Distilled water	90 c.c.

(Hydroquinone gave clearer results.)

(7) Dehydrate rapidly, embed in paraffin wax, and section 15–30 μ thick.

On some occasions the nuclei of these sections were stained with toluidin blue. This, however, was found to be unnecessary. The above method gave clearly fixed cells and nuclei, whereas the stain rendered indistinct the contrast between the black nerve-fibres and the yellow cytoplasmic background.

Innervation of the Adrenal Cortex.

The general arrangement of the nerves to the adrenal were found to be as described by other workers, namely, that nerve-bundles pass off from the plexuses in the capsule, through the cortex to the medulla (fig. 1, Pl. 24). Smaller branches are given off from the bundle as they pass through the cortex and some of these seem to pass into the cortical tissue itself and to end around the cells of the latter. In the cavy no nerve-endings were distinguished in this region, but only swellings on the course of the fibres as they pass the cell (*boutons de passage*). However, in the mouse both types of *boutons* were found. These *boutons* always seemed to lie on the surface of the cortical cells, never within them (fig. 2, Pl. 24).

There is, therefore, some evidence that the cells of the cortex may be directly innervated. However, it must be stressed that the cortex, in general, is exceedingly poor in nerve-fibres in comparison with the medulla. Even if the boutons described above do represent motor endings, yet it is not suggested that the majority or even a large proportion of the cortical cells are controlled in this way.

No nerve-cells were found in the cortex of young or adult guinea-pigs or mice, though Kubo (1934) has recently demonstrated their existence in man.

Occurrence of Nerve-cells in the Adrenal Medulla.

It has not been possible during the present investigation to settle definitely the question of the pre- or postganglionic origin of the nerve-fibres in the medulla. However, some evidence on this point is provided by the finding in the medulla of the younger forms of many nerve-cells, often as many as 20–25 in one section, 25 μ thick. Very few, however, are seen in the adult forms, in which they stain very indistinctly. This great difficulty in staining may account for their apparent rarity.

The author endeavoured to discover whether there is direct innervation of the chromophil cell from the intramedullary nerve-cell in the guinea-pig, but unfortunately either the nerve-fibres were cut too short by sectioning or the nerve-fibres from the nerve-cell were too complex to differentiate the course of separate nerves. However, Mr. Young kindly let me examine his sections of suprarenal tissue in Selachians, and several examples were found (fig. 3, Pl. 24). Whereas it cannot be concluded from this that a similar situation exists in the two animals, yet the observation suggests that at least some of the nerves in the medulla are postganglionic.

Innervation of the Adrenal Medulla.

The bundles of fibres which enter the medulla break up to form plexuses around the lobules into which the chromophil tissue is divided. From these plexuses smaller fibres run in and out among the cells of the lobule, forming a very complete network, such that in the adult every chromophil cell is in contact at least along one side with a nerve-fibre (fig. 4, Pl. 24). At intervals along the fibres there are swellings, boutons de passage, whose significance is discussed below, and some of the fibres come to an end as a terminal bouton (fig. 5, Pl. 24). A very careful examination has been made to discover whether either the fibres or the boutons penetrate into the cytoplasm of the chromophil tissue. This question is by no means easy to decide, but study of the preparations has led me to conclude that no such penetration occurs and that the nerve-fibres and boutons always lie on the surface of the chromophil cell, never within it.

Boutons de passage and boutons terminaux were present in the medulla of all adult animals examined, and in the later embryonic and young stages of guinea-pigs. However, even among adults of the same species there is no uniformity of distribution, for in many forms the boutons are concentrated in certain limited areas, whereas in other individuals they are scattered throughout the gland.

The boutons also vary in size, especially the boutons de passage (fig. 6, Pl. 24). Their exact structure is difficult to judge because of the many variations. Often the endings appear as hollow loops (fig. 6, Pl. 24) or oval bulbs either faintly (fig. 5, Pl. 24) or definitely (fig. 5, Pl. 24) fibrillar. Often, too, the nerve-fibres end in a slightly open fibrillar structure resembling a paint-brush (fig. 6, Pl. 24). Boutons de passage have an even more varied appearance: a hollow oval loop (fig. 6, Pl. 24); a loop with the nerve-fibre continuing through it (fig. 7, Pl. 24); or swellings composed of straight distinct fibrils (fig. 7, Pl. 24) or seemingly knotted and unevenly arranged fibrils (fig. 5, Pl. 24).

Considering the appearance of the structure of these boutons and the difficulty of staining them without altering their true nature, the author is unable to decide whether such neurofibrils in the boutons de passage and terminaux represent truly the living condition.

Previous investigators, who have been mentioned above, have stated that the boutons de passage and boutons terminaux act as synapses. This may be true, but there is another point to consider, namely, whether such boutons represent the chief or only method of excitation, and what may be their importance in the innervation of the gland relative to the contacts made by the nerve-fibres which run so close to the surface of the secretory cells. An examination of a series of equal areas of medullary tissue was therefore made in 1 and 2 hour, $2\frac{1}{2}$ and 4 days, and adult forms of the guinea-pig, both kinds of boutons and the medullary cells being counted, so as to enable the number of boutons per fifty medullary cells to be computed (see Table I). It is difficult to draw statistically exact

TABLE I

Summary Table of Number of Boutons.

Age of animal.	*No. of fields of 50 μ sq. examined.*	*No. of* boutons de passage *per 50 chromophil cells.*			*No. of* boutons terminaux *per 50 chromophil cells.*			*Total average.*
		Max.	*Min.*	*Aver.*	*Max.*	*Min.*	*Aver.*	
1 hour	20	10·6	0	3·7	2·7	0	0·9	2·3
2 hours	20	5·0	0	2·1	1·4	0	0·4	1·2
$2\frac{1}{2}$ days	20	6·2	0	2·2	2·5	0	1·2	1·7
4 days	5	5·6	2	3·6	1·4	0	0·6	2·1
Average of above 4 animals	65	10·6	0	2·9	2·7	0	0·9	1·8
Adult	20	3·5	0	2·6	1·8	0	0·8	1·4

conclusions from such an investigation for there is always the possibility that the groups of microscopic fields may not have been in equal degree random selections from the sections, and that these selections may not have been representative of all the variations in an entire medulla. However, the arithmetical means of a series of twenty counts gives the best estimate obtainable in practice.

The data are not adequate to show statistically the relative number of boutons in animals of different ages, but in general there seem to be rather fewer boutons in the older animals. This is interesting when compared with Windle's (1930) investigation of the spinal grey matter of kittens. He found no boutons appearing until the kitten was 21 days old, and then only boutons de passage, the number increasing with age. In the adrenal gland both types are found fairly early in embryonic life.

In comparing the average number of boutons de passage with that of boutons terminaux the results show the former to be 3½ times more numerous than the latter. This would suggest that the boutons de passage may be the more important. However, it is in considering the total number of boutons per fifty medullary cells that the results are most interesting.

There was considerable variation in the figures between different areas, the number of terminal boutons varying in the young animals from 0 to 2·7, in the adult from 0 to 1·8, and of the boutons de passage in the young from 0 to 10·6, and in the adult from 0 to 3·5. The average total number of boutons per fifty cells in the young forms was 1·8, and in the adult 1·4. In view of the smallness of these figures it seems certain that the boutons are not the main agent for stimulating the secretory cells, and in view of this it does not seem profitable to give any more detailed estimates of the variation between areas.

Mr. P. Heusner has been kind enough to let me mention, in this connexion, the work which he is now carrying out at Oxford on the superior cervical ganglion of cats. He has concluded that the boutons terminaux, if they exist, are too few in number to be of much significance, and that the close proximity of the finest preganglionic elements with the dendrites and the perikaryon of the postganglionic elements constitute the chief site of functional communication.

One can conclude from the above numbers that some other structure must aid in innervating these medullary cells. As has already been suggested it is quite possible that the nerve-fibres which form complex nerve nets throughout the medulla perform that function by stimulating the cell through contact with its surface. Such a method of stimulation has already been suggested for sympathetic ganglia (see Young, 1933), and would be in accordance with what we know of the general characteristics of secretory response, namely, the synchronous discharge of large numbers of cells. Such a type of innervation would clearly be less suitable for a tissue such as striated muscle in which the necessary correlation requires the division of tissue into separate functional units. Even in a gland such as the adrenal, one would expect that gradation in activity would be brought about by activation of larger or smaller parts of the gland, and it is most unlikely that the terminal plexuses among the chromophil cells constitute a continuous network in the sense understood by Stöhr (1928) and others, which, on the current theories of nervous activity, would imply that all parts of the medulla would necessarily be activated together. In fact the occurrence of terminal *boutons*, even if they are rare, shows that sooner or later the fibres do come to an end.

Summary.

1. The cells of the adrenal cortex of the cavy and mouse are sometimes innervated directly by nerve-fibres passing over and around the cells, but such nerve-fibres are very scarce in the cortex, the majority of whose cells are probably not under nervous influence.

2. There is a complex plexus of nerve-fibres among the cells of the adrenal medulla, the fibres of the plexus coming into close contact with the surface of the chromophil cells, but never penetrating inside them.

3. Formations comparable with the *boutons de passage* and the *boutons terminaux* of the central nervous system occur in this plexus.

4. However, the average number of *boutons* of both sorts per fifty medullary cells was found to be only 1·8 in young guinea-pigs, and 1·4 in adults. It is, therefore, concluded that they do not represent the main agent by which the secretory cells are activated, but that this function is performed by the nervous impulse passing in the network of fibres around the cell.

I am very grateful to Professor E. S. Goodrich and Mr. J. Z. Young for the suggestion of this problem, and their advice and assistance throughout the year. The work has been carried on in the Department of Zoology and Comparative Anatomy at the University Museum, Oxford.

EXPLANATION OF PLATE 24

All figures are from preparations made with Cajal's method as described. Drawings were made with Zeiss camera lucida, 4 compensating ocular, and oil-immersion objective.

Key to Lettering.

bp., bouton de passage; *bpn.*, bouton de passage with nerve-fibre passing through; *bt.*, bouton terminal; *c.*, cortex; *cap.*, capsule; *cbp.*, clearly fibrillar bouton de passage; *cbt.*, clearly fibrillar bouton terminal; *fbp.*, faintly fibrillar bouton de passage; *fbt.*, faintly fibrillar bouton terminal; *hbp.*, hollow loop bouton de passage; *hpt.*, hollow bouton terminal; *l.*, lobule; *m.*, medulla; *n.*, nerve; *nb.*, nerve-bundle; *nc.*, nerve-cell; *nf.*, nerve-fibrils; *pbt.*, paint-brush bouton terminal.

Fig. 1.—Photograph of section of adrenal gland in young guinea-pig showing general arrangement of nerves.

Fig. 2.—Section of the cortex next to capsule in a fourteen day old guinea-pig, showing nerve-fibres, several with boutons de passage, innervating the cortical cells.

Fig. 3.—Innervation of medullary cells in Selachians.

Fig. 4.—Detail of one medullary lobule in adult guinea-pig showing complex nervous plexus.

Fig. 5.—Drawing from a two and a half day guinea-pig showing large boutons de passage and smaller boutons terminaux.

Fig. 6.—Group of medullary cells in about a fifty day guinea-pig embryo showing different size boutons de passage and boutons terminaux. Also one ending resembling a paint-brush.

Fig. 7.—Group of medullary cells in an adult guinea-pig showing three different types of boutons de passage.

List of References

Alpert, L. K. (1931).—"Innervation of Suprarenal Gland", 'Anat. Rec.', **50.**

Arnstein (1895).—"Morphologie der Secretorischen Nervenendapparate", 'Anat. Anz.', **10.**

Boeke, J. (1932).—'Cytology and Cellular Pathology of the Nervous System', W. Penfield, New York, **1.**

Brauer (1932).—"Topographical and Cytological Study of Sympathetic Nervous Components of Suprarenals in Chick Embryo", 'Journ. of Morphol.', **53.**

Cajal, R. (1891).—'Terminación de los nervios y tubos glandulares del pancreas de los vertebrados'. Barcelona.

Dogiel, A. (1894).—"Nervenendigungen in den Nebennieren der Säugethiere", 'Archiv Anat. und Physiol.'

Elliott, T. R. (1913).—"Innervation of Adrenal Gland", 'Journ. of Physiol.', **46.**

Feldberg, W., Minz B., Tsudzimura, H. (1934).—'Mechanism of Nervous Discharge of Adrenaline', ibid., **80.**

Fusari (1891).—"De la terminaison des fibres nerveuses dans les capsules surrénales des mammifères", 'Arch. ital. de Biol.', **16.**

Giacomini (1897).—'Atti Accad. Fisiocr. Siena.'

von Greving (1924).—"Innervation der Leber", Müller, 'Lebensnerven'. Berlin.

Hoff, E. C. (1932).—"Central Nervous Terminals in Mammalian Spinal Cord and their Examination by Experimental Degeneration", 'Proc. Roy. Soc.', B, **111, 175.**

Kolmer (1905).—"Ueber das Verhalten der Neurofibrillen in der Peripherie", 'Anat. Anz.', **26.**

Kubo, Masaya (1933).—"Morphologische Studien ü. d. Endigung der Nierennerven", 'Mitt. Med. Akad. Kioto', **9.** Japanisch.

—— (1934).—'Innervation der Nebennierenrinde des Menschen', ibid., **11.**

Puglisi-Allegra (1904).—"Studio della glandula lacrimale", 'Arch. ital. di anat. e di embriol.', **3.**

Renner, O. (1914).—"Innervation der Nebenniere", 'Deutsches Archiv f. Klin. Med.', **114.**

Stöhr, P. jun. (1928).—"Das peripherische Nervensystem", 'Möllendorff's Handb.', **4.** (1) Berlin.

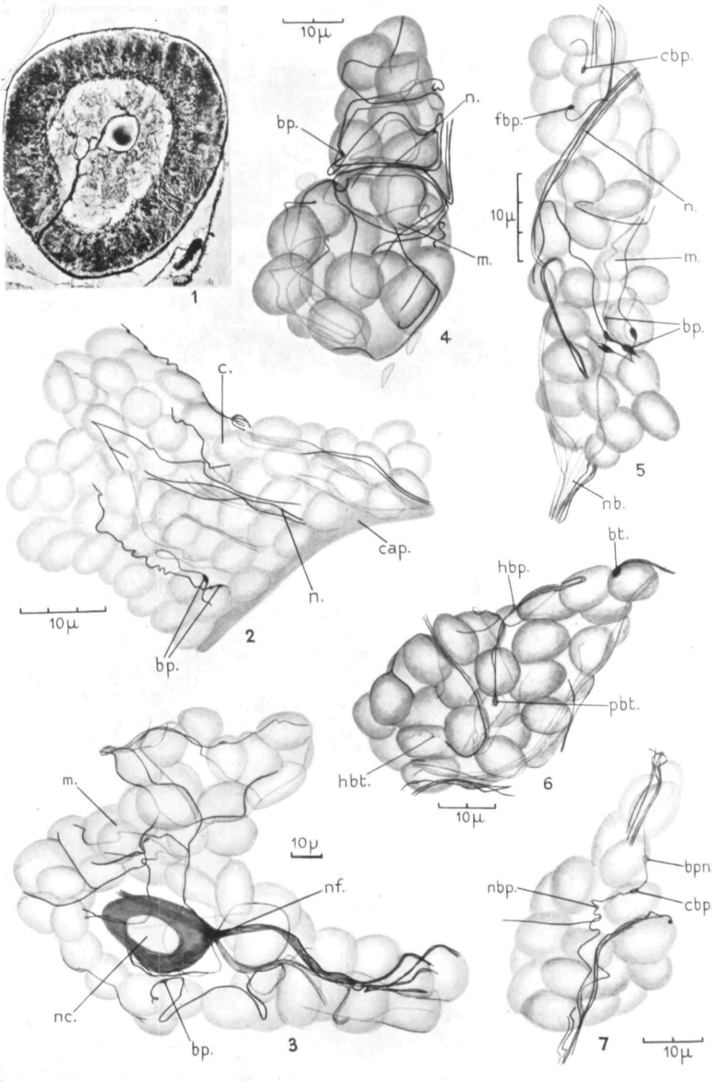
Stormont, D. L. (1928).—'Special Cytology', **1.**

Tiegs, O. W. (1931).—"Study of Neurofibril Structure of Nerve-cell", 'Journ. Comp. Anat.', **52.**

Tricomi-Allegra, G. (1903–4).—"Terminazione Nervose nella ghíandola mammaria", 'Anat. Anz.', **23.**

Windle, W. F. (1930).—"Normal Behavioral Reactions of Kittens, &c.", 'Journ. Comp. Neur.', **50.**

Young, J. Z. (1933).—"Autonomic System of Selachians", 'Quart. Journ. Micr. Sci.', **75.**



D. M. Willard, del.