J. Anat. (1971), **110**, 2, pp. 173–179
With 1 *figure*
Printed in Great Britain

Electron microscope observations upon the conus medullaris and filum terminale of human fetuses

H. J. GAMBLE

*Department of Anatomy, St Thomas's Hospital Medical School, London, S.E.*1

(*Accepted* 29 *July* 1971)

INTRODUCTION

Degenerative changes in cells of the developing nervous system have long been known to occur, and have recently been quantified by Hughes (1961) and Prestige (1965) in the anterior horns of the spinal grey matter and the dorsal root ganglia, respectively, of *Xenopus laevis*. It is obvious that their very small diameter would permit little to be seen by light microscopy of any degenerative changes which might occur in the unmyelinated axons arising from these degenerating cells, but some apparent axonal degeneration has been reported in peripheral nerves from human fetuses (Gamble, 1966), and it is implicit in the reports cited that degenerative changes are associated with the differentiation of the developing nervous system.

In a recent electron microscopic study of the spinal cord Bodian (1966*b*) considered that degenerative changes in fetal and newborn monkeys were more widespread, more prolonged, and more random than could readily be attributed to processes of differentiation alone. At the same time Bodian felt himself able to exclude hypoxia, malnutrition and genetic factors as causative agents of the degenerative changes, so that no comprehensive explanation was attempted.

The filum terminale and conus medullaris of the spinal cord derive from an otherwise nearly cylindrical structure, apparently by the combined effects of a dedifferentiation in its hindmost segments and an elongation of the skeleton greater than that of the spinal cord itself (e.g. Streeter, 1919). 'Normal' degenerative processes associated with differentiation within the central nervous system might then be expected to be most concentrated and most regularly found in these situations in fetuses at suitable stages of development.

MATERIALS AND METHODS

Eighteen human fetuses ranging in size from 6·5 to 14·0 cm C.R. length were obtained from hysterotomies performed in a neighbouring hospital. Some 20–30 min later the spinal cord was exposed from the lumbar segments distally, and immersed in chilled 5 % glutaraldehyde in phosphate buffer at pH 7·3. Slices of tissue were removed some 10 minutes later for further immersion in the glutaraldehyde solution and left for 2 h or more. After several rinses in a 10 % solution of sucrose, similarly buffered, the tissues were further fixed in a buffered 1 % solution of osmium tetroxide for 1 h.

After dehydration and embedding in Araldite, thick sections (about 1 μm) were stained with a mixture of Azure II and methylene blue (Richardson, Jarett & Fink, 1960) and examined by light microscopy. Thin sections were stained with uranyl acetate after mounting on uncoated grids and were examined with a Metropolitan Vickers EM6 electron microscope.

Where possible, three slices of tissue were taken for examination from each specimen: filum terminale internus, conus medullaris and a segment of upper sacral or lower lumbar spinal cord. However, in specimens of 6·5 or 7·5 cm C.R. length (HR 62, 63) the conus medullaris was itself the distal extremity of the cord, no filum having been formed. In some of the larger specimens, of 9·5 and 10·0 cm C.R. length (HR 68, 73, 80), tissue believed to be filum terminale was processed, but on subsequent

Table 1

Specimen	C.R. length (cm)	Estimated age (weeks)	Filum	Conus	Cord
HR 62	6·5	11–12	Not present	—	—
HR 63	7·5	11–12	Not present	—	—
HR 81	9·0	12–13	—	Not well preserved	—
HR 80	9·5	13–14	Not preserved	—	—
HR 68	9·5	13–14	Not preserved	—	—
HR 73	10·0	13–14	Not preserved	—	—
HR 83	10·0	13–14	—	Not well preserved	Not well preserved
HR 71	13·0	15	—	—	—
HR 72	13·0	15	—	—	—
HR 82	14·0	16	—	—	—

examination proved to be no more than a strand of connective tissue with blood vessels and nerve roots embedded in it. Perhaps, because of unavoidable delays between separation of fetus from mother and commencement of fixation procedures, really good fixation was seldom achieved and many specimens were discarded. The tissues finally available for study were as shown in Table 1.

RESULTS

The findings in the specimens of sacral or lumbar spinal cord were in general accord with those previously described (Gamble, 1969). Myelin was present in the marginal layers of the cords obtained from fetuses of C.R. length 10·0 cm or more, and no evidence of degeneration of cells or their processes was found. A continuous basement membrane covered the smooth outer surface of the cord, separating the cytoplasmic processes of marginal glia from the collagen fibrils and connective tissue cells of the outer pia mater.

In each conus medullaris examined, and in each filum terminale, the outer surface was contorted by indentation or deep cavitation; the surface of the cavity was often itself irregular (Fig. 1). The basement membrane faithfully followed the irregular surface formed by the marginal glial processes except that sometimes it was interrupted over a short interval, which was never seen to exceed 2 μm in breadth.

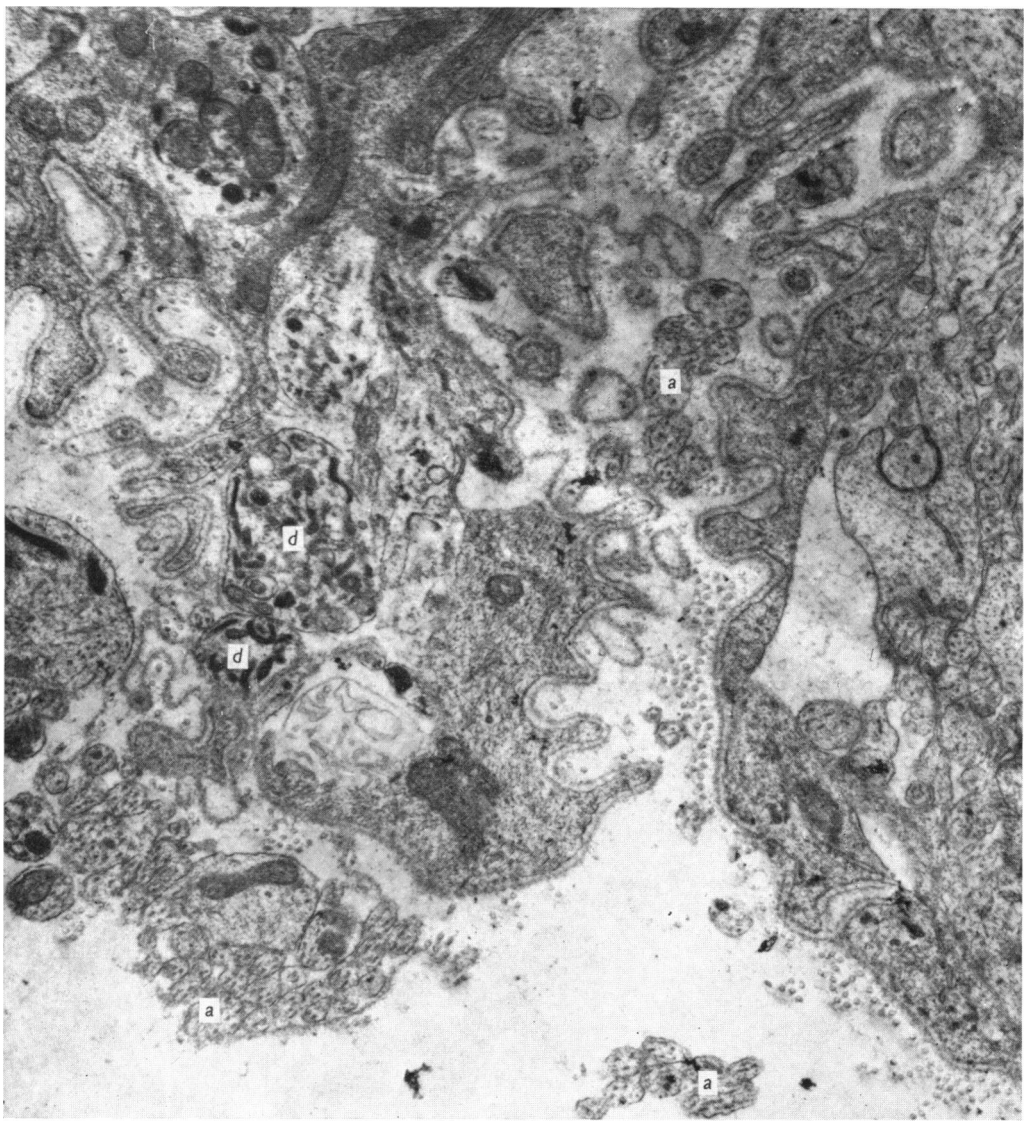

Fig. 1. Filum terminale of HR 71 (13·0 cm C.R. length) showing deep cavitation of outer surface with basement membrane largely following the wall. Collagen extends into the lumen of the cavity. Cytoplasmic processes with and without basement membrane investment lie in the cavity and outside in the pia mater: some resemble unmyelinated axons (*a*). Debris-filled cytoplasmic processes (*d*) occur in the marginal glia. (×17000.)

Occasionally the basement membrane was duplicated over a short distance so as to appear to close off a cavity. Some indentations were associated with blood vessels entering or leaving but the examination of near-serial sections showed that many indentations and cavitations had no direct relationship with such vessels.

In one specimen of the filum terminale (HR 82) and in two of the conus (HR 62, 68) indentation of the surface occurred in relation to glial processes of very markedly increased electron density where, on occasion, electron-dense intercellular material, regularly arranged, was conspicuous. In other specimens the marginal glial processes were rich in glycogen, but otherwise of normal electron density.

In several specimens (filum of HR 83, 71, 72; conus of HR 80, 72) cytoplasmic processes forming or lying in the boundary between neural ectoderm and the mesodermal tissues of the outer pia mater contained large numbers of dense bodies, resembling debris in appearance (Fig. 1) and assumed to be derived from degenerative changes in the parent or neighbouring cells. Cytoplasmic processes of similar appearance were also found lying free among the connective tissues of the outer pia mater, while their occurrence in the ectoderm/mesoderm junction sometimes coincided with a gap in the limiting basement membrane. None of the debris-filled cytoplasmic processes was seen to be nucleated and their true sizes are not known. Similar electron-dense inclusions were sometimes seen in cytoplasmic processes lying in perivascular spaces, or forming the boundary of the space. More debris occurred in an apparently random fashion; some of it showed a lamellar form, and appeared to be derived from myelin breakdown, and some occurred in fat droplets within cells which were presumably phagocytic.

The debris of degenerative processes was quite widespread within the filum and conus but none was seen involving the nucleated part of any cell. Within the central canal more debris was seen, similar to that described previously (Gamble, 1969); its source was obscure since the ependymal cells did not show degenerative changes. No proliferative changes were seen in the mantle or marginal layers of any specimen of conus or filum.

Where debris-filled cytoplasmic processes were found lying free in the interstitial spaces of the connective tissues of the pia mater there were frequently present, in addition, small (0·1–0·3 μm) rounded profiles, of moderate electron density, and containing microtubules (Fig. 1). They had the appearance of developing unmyelinated axons. Although some were apparently associated with satellite cell processes, or with a length of basement membrane, they were never seen wholly wrapped around by either of these. In specimen HR 71 coccygeal nerve roots were found in the connective tissues of the pia mater, and consisted of axon/Schwann cell complexes identical with those described in developing fetal ulnar nerve (Gamble & Breathnach, 1965; Gamble, 1966).

DISCUSSION

The results described suggest that the transformation of the caudal part of the human fetal spinal cord into conus medullaris and filum terminale is accompanied by a number of degenerative processes occurring simultaneously. The marginal glia of the fetal spinal cord ordinarily presents a smooth unbroken surface to the pial

connective tissues (Gamble, 1969) except where it is perforated by blood vessels entering or leaving (Malinsky, 1968). A mosaic of glial processes forms the surface and is in turn covered by an uninterrupted basement membrane. This condition has also been shown in chick embryo spinal cord by Wechsler (1966) and in the fetal spinal cord of the monkey by Bodian (1966*a*) and Bodian, Melby & Taylor (1968). In the present material the surfaces of conus medullaris and filum terminale were frequently grossly contorted in regions known to bear no close relationship to perforating blood vessels. Deep cavitations of the surface were followed faithfully by the investing basement membrane as were minor outward projections of marginal glia into the cavities. Sometimes the processes of marginal glia were extremely electron-dense although they lay alongside other seemingly well-preserved cytoplasmic processes. It seems probable that, being surplus to the new requirements of this part of the central nervous system, they were dying off. The process of cavitation of the surface, associated as it was with an apparent sloughing of axons and of glial processes (to which further reference will be made) probably reflects a reformation of the surface upon a reduced perimeter.

Debris derived from degeneration of axons and/or myelin degeneration may occur anywhere in the conus medullaris and filum terminale, and usually takes the form of dense bodies contained within cytoplasmic processes otherwise hardly distinguishable from those of astrocytes. Few such processes resemble the vesicle-filled structures figured by Bodian (1966*a*) and identified by him as 'gitter' cells of microglial origin. Debris-filled processes have been seen frequently in the marginal glia, sometimes coinciding with a break in the otherwise continuous basement membrane; they have also been found, again free of basement membrane, in the connective tissue spaces of the outer pia mater. It seems likely that they have been sloughed from the marginal glia. Their frequent association, in the outer pia mater, with unmyelinated axons lacking glial and basement membrane investment suggests that axonal sloughing also occurs. It is difficult to believe that sloughed axons could survive without glial support, and it must be assumed that the axons lying free in the outer pia mater would subsequently degenerate. The identification of unmyelinated axons in this rather surprising situation depends upon their marked similarity to undoubted axons in tracts of the marginal layer of the spinal cord and upon their content of microtubules, which are lacking in the cytoplasmic processes of the marginal glia. It seems probable that some of the reduction in bulk involved in the transformation of spinal cord through conus medullaris into filum terminale involves a redeployment of the astrocyte processes which form the marginal mosaic at the pial surface. As these processes are withdrawn to reform upon a reduced perimeter, so pieces of the cells break off and some of the more superficial axons (once the charges of these glial processes) are shed into the adjacent connective tissue spaces.

Other debris derived from the degeneration of axons and possibly of other cells is apparently removed by more conventional means; debris-filled cytoplasmic processes align themselves about blood vessels, at first in the glia which surrounds and delimits the perivascular spaces. Later they are shed into the perivascular space for further removal through the blood vessel itself. It is worth commenting that no debris-containing cytoplasmic process was ever seen to contain a nucleus. Consequently it seems probable that these processes represent pieces broken from surviving

cellular elements. In these circumstances it is improbable that they could survive for long, and there is no reason to link them with the degenerated structures found by Miller (1968) in the filum terminale and associated connective tissues of adult cats and squirrel monkeys.

All the evidence of degeneration obtained from the human fetal material has been of axonal and myelin (where present) degeneration with some involvement of phagocytic cells. Rather surprisingly, no degenerating neuroblast or neuron cell body was seen in any of the levels examined, nor were glial cell bodies affected in any recognizable way. The same appears to be true of the rather more widespread degenerative changes described by Bodian (1966*b*) in fetal and newborn monkey spinal cord, and of the conus medullaris and filum terminale of adult cats and squirrel monkeys described by Miller (1968). Possibly axonal collaterals, not affecting the cells of origin, have been largely involved, but the absence of changes in cell bodies remains surprising.

Despite the quite extensive degenerative changes which occur in the conversion of the caudal segments of the human fetal spinal cord into conus and filum, some axons persist in the adult filum and, indeed, become and remain myelinated (Testut, 1897; Gamble, 1967). From Miller's observation of the filum terminale in adult cats and squirrel monkeys, where normal nerve cell bodies, axons, dendrites and synapses exist alongside degenerated or degenerating neurites, one must suppose that there is an anatomical basis for nerve impulse conduction and transmission. How this might function is quite obscure.

One remaining feature of the human fetal conus medullaris and filum terminale requires comment, namely, the presence of cellular debris of unknown origin within the central canal. It has already been seen at higher levels of the cord (Gamble, 1969), but it is rather surprising that it should persist, or be renewed, into adult stages of development, as found by Miller (1968) in adult cat and squirrel monkey. Its fate is as obscure as its source.

SUMMARY

1. Filum terminale, conus medullaris and lumbosacral spinal cord from human fetuses of 6·5–14·0 cm C.R. length have been examined by light and electron microscopy.

2. Evidence of degenerative change has been found in axons (and in myelin, where present) and in phagocytic cytoplasmic processes thought to derive from astrocytes.

3. Some debris resulting from degenerative changes is probably removed through phagocytic cell processes entering into local blood vessels: other phagocytic cell processes enter and perhaps remain in the pial connective tissues.

4. Some axons of the shrinking central nervous system (filum and conus) are apparently shed into the surrounding pial connective tissue, by retraction of processes of the marginal glia which thereafter reforms a continuous surface. The further fate of these axons is assumed to be their degeneration *in situ*, since they lack any covering, whether of satellite cell or of basement membrane.

5. Some cellular debris is found within the central canal of both conus and filum but neither its origin nor its fate is known.

I thank Mr G. Maxwell for providing the sections studied in this work and Mr J. S. Fenton for the photography. I am indebted to Miss B. A. E. Coles for secretarial assistance, to Professor P. Rhodes for providing the specimens, and to the National Fund for Research into Crippling Diseases, by whom this work has been supported.

REFERENCES

BODIAN, D. (1966*a*). Development of fine structure of spinal cord in monkey fetuses. I. The motoneuron neuropil at the time of onset of reflex activity. *Bulletin of the Johns Hopkins Hospital* **119**, 129–149.

BODIAN, D. (1966*b*). Spontaneous degeneration in the spinal cord of monkey fetuses. *Bulletin of the Johns Hopkins Hospital* **119**, 212–234.

BODIAN, D., MELBY, E. C. & TAYLOR, N. (1968). Development of fine structure of spinal cord in monkey fetuses. II. Pre-reflex period to period of long intersegmental reflexes. *Journal of Comparative Neurology* **133**, 113–166.

GAMBLE, H. J. (1966). Further electron microscope studies of human fetal peripheral nerves. *Journal of Anatomy* **100**, 487–502.

GAMBLE, H. J. (1967). Observations upon the human filum terminale. *Journal of Anatomy* **101**, 631–632.

GAMBLE, H. J. (1969). Electron microscope observations on the human fetal and embryonic spinal cord. *Journal of Anatomy* **104**, 435–453.

GAMBLE, H. J. & BREATHNACH, A. S. (1965). An electron microscope study of human fetal peripheral nerves. *Journal of Anatomy* **99**, 573–584.

HUGHES, A. (1961). Cell degeneration in the larval ventral horn of *Xenopus laevis* (Daudin). *Journal of Embryology and Experimental Morphology* **9**, 269–284.

MALINSKY, J. (1968). The fine structure of spinal cord in human embryos. II. Marginal layers. *Acta Universitatis Palackianae Olomucensis-Facultatis Medicae.* **51**, 101–112.

MILLER, C. (1968). The ultrastructure of the conus medullaris and filum terminale. *Journal of Comparative Neurology* **132**, 547–566.

PRESTIGE, M. C. (1965). Cell turnover in the spinal ganglia of *Xenopus laevis* tadpoles. *Journal of Embryology and Experimental Morphology* **13**, 63–72.

RICHARDSON, K. C., JARETT, L. & FINK, E. H. (1960). Embedding in epoxy resin for ultrathin sectioning in electron microscopy. *Stain Technology* **35**, 313–323.

STREETER, G. L. (1919). Factors involved in the formation of the filum terminale. *American Journal of Anatomy.* **25**, 1–11.

TESTUT, L. (1897). *Traité d'Anatomie Humaine* Tome 2, Paris: Octave Doin et Cie.

WECHSLER, W. (1966) Die Feinstruktur des Neuralrohres und der Neuroektodermalen Matrixzellen am Zentralnervensystem von Hühnerembryonen. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **70**, 240–268.