THE DEVELOPMENT OF NERVE ENDINGS IN THE HUMAN FOETUS

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The development of nerve endings in the various tissues of the body is so closely bound up with the histogenesis of those tissues that any investigation of the former must include the latter, more particularly as the developing nervous elements are known to be influenced by their surroundings. Thus Detwiler (1) showed the attraction exerted by the limb buds for the growing motor nerves: Tello (2) speaks of the neurotropic attraction of developing muscle fibres for sensory nerves, and within the nervous system itself the directional growth of nerve fibres and final position of nerve cell groups appears to be determined largely by metabolic activity both local and peripheral, the process being known as neurobiotaxis (Kappers (3)).

The histogenesis of motor and sensory nerve endings in the chick has been described in great detail by Tello (2), who, in addition, gives an account of the differentiation of striped muscle cells: this account includes some few references to nerve endings in the human foetus of 6 months and at full term. Langworthy (4) refers to the presence of spindles in practically all muscles from the fourth month, although they were not demonstrated in the diaphragm nor in most of the intrinsic tongue muscles. The development of the muscles and their nerve fibres in the human foetus is referred to by Frazer (5), who states that during the fifth week muscles are indicated in the form of pre-muscle masses in the mesenchyme, and that at or before the 10 mm. stage the main nerves of the limbs are found within it: he says, further, that by the middle of the second month the sympathetic fibres have reached all the important rudimentary organs of the trunk, and that taste buds appear during the third month.

Other references to the *development* of the nerve endings in the human foetus are very scanty, although the fully differentiated endings are fully described in the current text-books (e.g. Maximow (6)). Recently Boeke (7, 8, 9) has described autonomic nervous networks and endings in connective tissue and various organs in Man.

CLASSIFICATION OF TYPES OF NERVE ENDINGS

Nerve endings are perhaps best considered in relation to the organs innervated. The following summary includes the more important (6).

A. *In muscle and tendon*

(1) *Striated muscle.*
- (*a*) Motor. Hypolemmal. End-plates.
- (*b*) Sensory.
  - (i) In muscle
    - (α) Interstitial.
      - Simple branchings in connective tissue between muscle fibres.
      - Encapsulated Golgi bodies.
    - (β) Epilemmal.
      - Simple nerve net enveloping muscle fibres.
      - Complex spindles.
  - (ii) In tendon
    - (α) Interstitial.
      - Simple branchings.
      - Encapsulated Golgi bodies and Pacinian corpuscles.
    - (β) Epitenal.
      - Complex Golgi organs.
- (*c*) Sympathetic. Rich plexus in relation with the blood vessels, and in the interstitial connective tissue (7).

(2) *Smooth muscle.*
- (*a*) Network with branching fibres ending in bead-like thickenings possibly within the cells.
- (*b*) Various sensory endings in connective tissue between muscle bundles.

(3) *Cardiac muscle.*
- Probably similar to smooth muscle.

B. *In epithelial tissue*

(1) *In glands.*
- Network outside the basement membrane with branches that make a second network on the inner surface: thin, branching varicose threads ending with thickenings between the cells.

(2) *In epithelial layers.*
- Several superimposed networks, with varicose threads ending between the cells. Also special tactile cells.

C. *In connective tissue*

(1) *Free endings.*
- (*a*) Nerve nets.
- (*b*) Arborised terminal branchings.
- (*c*) Non-encapsulated glomeruli.

(2) *Encapsulated endings.*
  (*a*) Encapsulated glomeruli.
  (*b*) Meissner's tactile corpuscles.
  (*c*) Krause's end bulbs, and Pacinian corpuscles.
(3) (?) *Sympathetic plexus.*
  Throughout all tissues of the body (7).

In the present investigation only a few of these various endings have been identified, namely: in striped muscle motor end-plates, interstitial Golgi bodies, epilemmal spindles; in smooth muscle and cardiac muscle the branching network with varicose fibres; in epithelial tissue the branching fibres from the network with their varicosities in both glands and epithelial coverings; and in connective tissue the free endings and Pacinian corpuscles and the (?) sympathetic plexus. In all probability the other kinds of endings can also be found in their various stages of development, and as suitable material comes to hand it is hoped to extend the present rather fragmentary observations.

The minute structure of the various types of fully formed nerve endings has been described in detail for various animals and in some cases for the human (see Maximow (6), Tello (2)): the present investigation confirms these descriptions for the human nerve endings and traces out their development.

PRESENT INVESTIGATION

A. Material and methods

The material used in this investigation consists of human foetuses of the following approximate ages:

*6 weeks (16 mm.)	1 specimen	12 (or 12+) weeks	4 specimens
7 weeks	1 specimen	14 weeks	1 specimen
*7½ weeks (25 mm.)	1 specimen	20 weeks	1 specimen
8 weeks	1 specimen	22 weeks	1 specimen
*8+ weeks	1 specimen	26–28 weeks	2 specimens
10 weeks	2 specimens	F.T.	2 specimens

Only material showing no post-mortem changes has been used; this is difficult to obtain, accounting for the gaps in the series. Most of the specimens were fixed in 7 per cent. formalin, and then treated by a silver-pyridine method (10). Serial sections, cut at 8 or 10 μ, were mounted in Gurr's neutral mounting medium. It is important to have serial sections when looking for nerve endings as most misleading appearances can be produced by oblique sections of nerve trunks when these are altering their plane of direction. The three specimens marked * were fixed in formalin as usual, imbedded, cut serially at 10 μ, and stained with Scott's haematoxylin and Biebrich scarlet or with Mallory's aniline blue connective tissue stain: these showed the muscle and nerve trunks but not the nerve endings. In addition, muscle from many of the specimens was fixed in formalin or formo-acetic Müller, and the sections stained with haematoxylin and Biebrich scarlet, with Mallory's connective tissue stain, and with Weigert's resorcin-fuchsin stain. The ear of one specimen was treated by de Castro's (11) method.

B. Results

The results obtained are most conveniently considered under the following headings:

(1) Differentiation of striped muscle and tendon, and of their sensory and motor nerve endings.

(2) Differentiation of nerve endings in

- (*a*) Smooth muscle.
- (*b*) Cardiac muscle.
- (*c*) Epithelial tissue.
- (*d*) Connective tissue.

(1) *Differentiation of striped muscle and tendon, and of their nerve endings*

6 *weeks* (16 mm.). The muscle cells are not yet differentiated.

7 *weeks*. At this age the muscle masses of the limbs consist of fusiform cells elongated for the most part in the longitudinal direction of the future muscle: these are the myoblasts of Tello in which no fibrils can be seen. The extrinsic eye muscles are only just beginning to differentiate, but in the tongue there are already fine muscle fibres with peripheral fibrils showing very fine cross-striations and several central nuclei: these are Tello's myocells. No nerve fibres were seen in the distal parts of the limbs. In the tongue, however, there is already a rich supply of nerve fibres, many with terminal swellings, particularly in relation to the finely striated developing muscle fibres.

7½ *weeks* (25 mm.). The limb muscle cells still possess neither fibrils nor striations, but many of the elongated cells contain several nuclei in a row.

8 *weeks*. The limb muscles are now beginning to possess fine muscle fibres showing fibrils and cross-striations, these being particularly well seen in the muscles of the hand and of the foot and in the sole. The number of nuclei within the cells is very marked; in addition the connective tissue nuclei between the muscle cells are increasing in number.

The nerve fibres in these muscles have branching and bulbous endings associated with nuclei, and lying in between and on the surface of the fine muscle fibres. It is common to see a bifurcation at the tip of the growing nerve fibre with a nucleus lying in the cleft. In addition, a very fine plexus of fibres can be made out between some of the muscle fibres: this is probably nervous and belongs to the connective tissue; it is apparently not derived from the clearly defined nerve fibre groups that are supplying the muscles (see below).

8½ *weeks*. Some of the muscle fibres have begun to grow considerably in girth.

10 *weeks*. At this stage there appears to be a very active evolution of the muscle fibres. The connective tissue between them increases very greatly,

enlarging the bulk of the whole muscle: at the same time the muscle fibres grow greatly in size, appearing as rather isolated, very bulky, fibres. These are the primitive myotubes, with well-marked peripheral fibrils and cross-striations, and a central row of nuclei; they are separated from one another by the proliferating connective tissue (see Plate I, fig. 1). The muscles of the fore-limb and hand are further advanced than those of the hind-limb and foot.

At the same time the tendons are differentiating by an increase of connective tissue elements, and loss of myotubes at these points: definite tendon fibres are not yet seen, but the elongated cells produce laminae.

At this stage of active growth and differentiation of the myotubes a definite neurotropism can be made out: the exploratory nerve fibres with their enlarged endings are related to the large muscle cells. In addition, the junctions between muscle and tendon are comparatively richly supplied with nerve fibres.

12 *weeks and* 12 + *weeks*. The very large muscle fibres are no longer apparent as the primitive myotubes are dividing longitudinally: these are now seen in groups which are separated from one another by the connective tissue, the arrangement being seen best in transverse sections. At this stage the appearance is that of very great capillary development and proliferative activity on the part of the connective tissue in addition to the multiplication of the myotubes. In those regions where development is most advanced (as in the tongue muscle) the individual fibres are becoming separated from one another by the insinuation of the connective tissue between them. At the same time the tendinous fibres are becoming differentiated with cells in rows between them.

No definite nerve endings can be made out, but the exploring fibres with knob-like terminals are very numerous, and extend to the extremities of the fingers and toes.

20 *weeks*. The division of the myotubes is now probably completed: the muscle fibres are slender, and possess well-marked fibrils and striations, but the nuclei are still central.

At this stage comparatively complex sensory nerve endings are present in the muscles of the arm and the leg both of the epilemmal spindle type and of the interstitial Golgi body type (see Plate I, fig. 2). None were seen in the tongue muscles which are more fully differentiated than those of the limbs, but on the other hand there is in the tongue a beginning of differentiation of rudimentary motor endings associated with nuclei on the surface of the muscle fibres.

22 *weeks*. The nuclei in some of the muscle fibres are now in a peripheral position, due either to an active migration or to an increase in fibril material.

The developing sensory organs are very numerous in the muscles of the hand, and rather less numerous in those of the foot.

26–28 *weeks*. The muscle fibres, although still slender, present a finished appearance; the nuclei are now peripheral and the whole fibre is filled with fibrils; the cross-striations in the fibrils look typical. The connective tissue between the fibres is not so marked. From this stage onwards the muscle fibres increase in length and in girth, without showing any real modification of structure. The nerves of the tongue muscles now show complex but unfinished motor plates, the terminal branchings and swellings being well differentiated and associated with numerous nuclei (see Plate II, fig. 1). The sensory endings of the limb muscles still have terminal knobs and are not yet completed. The motor endings in the forearm muscles are just beginning to differentiate.

Full term. The sarcolemma of the various muscle fibres does not seem to be complete until the motor end-plates are differentiated. It is very noticeable that all the muscles do not differentiate at the same rate, the tongue muscles being among the earliest and apparently completed in detail at birth: the extrinsic eye muscles are among the latest to differentiate, and at all stages the muscles of the foot and of the leg lag behind those of the hand and of the arm.

At birth the sensory nerve endings have a "finished" appearance, but the motor endings of the limb muscles, particularly of the foot and of the leg, are not yet nearly completed. The motor plates in the tongue, the intercostal muscles and the diaphragm are, on the other hand, highly differentiated at full term.

(2) *Differentiation of nerve endings*

(*a*) *In smooth muscle.*

10 *weeks.* The smooth muscle of the gut and of the bronchioles possesses numbers of isolated nerve fibres, but no endings were seen. The smaller blood vessels and capillaries already possess a rich plexus, which may be part of the connective tissue plexus (see below).

14 *weeks.* Auerbach's plexus is well developed although fine: Meissner's plexus is also present.

26–28 *weeks.* The media of larger blood vessels is innervated: probably these fibres have also been present in the younger specimens.

(*b*) *In cardiac muscle.*

10 *weeks.* The heart is richly innervated, particularly in the neighbourhood of the sino-auricular node: a plexus is present and fibres can be made out in the auricular walls, and in the interauricular septum; only very few fibres are present in the ventricles. At this stage the nerve endings appear varicose. It is not possible in these preparations to say whether the fibres are of sympathetic or of parasympathetic origin.

(*c*) *In epithelial tissue.*

7 *weeks.* There is a rich nerve supply just beneath the epithelium of the tongue.

8 *weeks.* The skin of the toes is supplied by a network immediately below the epithelial cells.

10 *weeks.* The lung alveoli and bronchiole epithelium is innervated by fine branching fibres just below the lining cells. The mucous membrane of the tongue has a rich network just below the surface epithelium. Free nerve endings are present between the specialised sensory epithelial cells of the cochlea and of the maculae of the inner ear.

12 *weeks.* The plexus beneath the skin epithelium is very complex, and is particularly well marked below the surface in the penis.

20 *weeks.* Free nerve fibres can be seen ending in the Malpighian layer of the skin. The tongue papillae are richly innervated, and although no definite taste buds were seen free nerve endings can be seen among the epithelial cells. The subepithelial plexus is very marked. The lingual glands possess nerve fibres, but apparently no nervous network nor terminations.

22 *weeks.* The developing sweat glands are innervated, but have no characteristic nervous plexus nor endings.

26–28 *weeks.* Free nerve endings are numerous among the epidermal cells, the dermis possessing a rich subepithelial plexus. The lingual glands now have a plexus innervation, while in the sweat glands this can hardly be made out.

Full time. The sweat glands are not yet fully developed, but a simple nervous plexus can sometimes be made out just outside the secretory cells.

(*d*) *Connective tissue.*

(i) *Free nerve endings.* Plexuses which are probably nervous (see below) are of very early appearance and widespread distribution: they are possibly autonomic in origin.

7 *weeks.* The corium of the tongue already possesses a plexus.

8 *weeks.* A primitive-looking plexus is present in the connective tissue between the developing muscle fibres of the limbs. Very few nerve fibres seem to be passing towards developing bone and cartilage.

10 *weeks.* The plexus between developing striped muscle fibres is very rich and fine (see Plate II, fig. 2): a similar plexus is present in the connective tissue in relation to the capillaries. Many of these plexus fibres appear to be related to cells with long processes that look like embryonic ganglion cells (see below).

12 *weeks.* The great capillary development of this stage is associated with an increasing complexity of the plexuses related to both the capillaries and the larger blood vessels: the fibres of the network possess varicosities, and some show terminal swellings. The plexus in the connective tissue of the muscles is interwoven with the muscle fibres, apparently lying entirely outside them.

At this stage also the sympathoblasts of the ganglia in the sympathetic chain show the rosette arrangement (12) so characteristic of their functional grouping and migration.

Full time. The plexuses of the connective tissue are well marked between the muscle fibres, and in fact in almost all places where connective tissue is found. The network is now finer, more spread out, and associated with fewer nuclei than before (see Plate III, fig. 1).

(ii) *Encapsulated nerve endings.* 20 *weeks.* Developing Pacinian corpuscles were first seen at this stage in the sole of the foot: they are of a relatively simple type.

26–28 *weeks.* Comparatively complex Pacinian corpuscles are present in large numbers among the muscle masses of the thumb.

Full time. Although still small, the Pacinian corpuscles in both hand and foot look very "finished" (see Plate III, fig. 2).

DISCUSSION

The histogenesis of striped muscle in the human takes place in the same way as that of the chick, which has been so fully described and illustrated by Tello (2). This worker has also followed out in great detail the differentiation of the main types of sensory and motor nerve endings in the chick, and the present investigation shows that in the human the order of their development and the different stages of differentiation are quite comparable. As, however, the whole period of development up to hatching in the chick is only 21 days, whereas in the human it is about 40 weeks, the variations in individual muscles and organs is much more readily seen in the latter. The accompanying table shows a comparison between the stages in the chick and in the human of histogenesis of striped muscle and of some sensory and motor nerve endings.

Striped muscle and tendon	Sensory nerve endings	Motor nerve endings	Human (approximate age) weeks	Chick (Tello) (approximate age) days
Beginning differentiation	—	—	7	5
Myofibrils appear	Exploratory fibres with bulbous endings penetrate into muscles		8	6
Great increase of connective tissue. Vast numbers of nuclei	—	—	—	6–7
Isolated "fat" fibres	—	—	10–12	7
	Varicose endings in contact with myotubes	—	10	—
Tendon fibres differentiate	—	—	12	10
	Beginning Pacinian bodies	—	20	11
Multiplication of myotubes	—	—	12–22	9–13
	Spindles differentiate	—	20	12
Dissociation of myotubes from one another. Nuclei become peripheral	Spindles become complex	—	22–26	13
	Pacinian bodies well developed	—	26–28	15
		Plates begin to differentiate	26–28 (very rudimentary except tongue)	18

Several points of interest emerge from a consideration of the relative times of development of the nerve endings. Perhaps the most striking fact, to which others have already drawn attention, is that the evolution of the sensory nerve endings is more or less completed before that of the motor endings has begun. In the human the motor plates begin to differentiate at about 26–28 weeks, but at first these are very rudimentary except in the tongue muscles: these muscles, which are among the first to differentiate, look more nearly completed than do those of the limbs at this age. Tello has suggested that there is a relationship between the formation of the motor endings and the functional activity of the muscle, and this is supported by the finding that at an age when the foetus is viable the tongue muscles possess complex motor endings while the limb muscles do not, and even at birth the motor endings in the diaphragm and intercostal muscles are considerably further developed than are those of the limbs.

The sensory endings in the tongue muscles cannot be very numerous: none were identified in this series, although they must be present.

The differentiation of the muscles and of the sensory innervation is further advanced in the hand and arm than it is in the foot and leg at all stages of development, the difference being noticeable even at 10 weeks and persisting until birth.

The innervation of the lingual glands and of the sweat glands is of interest. The nerve plexus arrangement that gives rise to fine, branching, varicose fibres that penetrate among the secreting cells, was not seen in the glands of the tongue until 26–28 weeks, that is the age at which the foetus can live and suckle, and the same age at which the real motor endings of the tongue muscle fibres are developing. Mucin-secreting cells are differentiated about this time also in the salivary glands (13). The sweat glands are not fully innervated even at birth, the external nerve plexus being still very rudimentary: this may be a contributory factor in the inability of the new-born child to regulate its own temperature, although sweat can be produced.

If the plexus of the connective tissue described here is the same as that described by Boeke (7, 8, 9) it is of very early appearance, being distinctly differentiated in the connective tissue between the developing muscle fibres by 8 weeks: the fibres are quite distinct from those supplying the future sensory and motor endings. He regards the plexus as "sympathetic" and describes it as "a syncytial arrangement of the protoplasm of the lemmoblasts with the neurofibrils imbedded in it—Lawrentjew's 'interstitial cells'": the plexiform anastomosing bands of neurofibrillae run along capillaries adhering to the endothelial wall, encircle and envelop striated muscle, make a network round clumps of gland cells and from this network spring the fine fibres which run between the gland cells: it is found in the connective tissue. It is not always easy to demonstrate this plexus, but the silver technique used in this investigation usually gives a good impregnation of an argentophil plexus. (The Fischer-Ranvier gold chloride method (14) will also frequently show up a similar plexus.) In the younger stages the impregnated cells of the plexus are relatively more numerous than in the older stages when the plexus fibres are presumably stretched out somewhat by growth. It is possible that this plexus is not nervous at all, but the impregnation of the fibres is of the same type as that of undoubted nerve fibres, and some of the cells connected with them have long processes and look as if they must be of nervous nature. If these elements are nervous, they probably represent the sympathetic elements (15). Further investigation of this point is in progress.

SUMMARY

1. The histogenesis of striped muscle takes place in the human in the same way as in the chick.
2. The differentiation of the various voluntary muscles occurs at different stages of foetal development.
3. There is a relationship between the stages of muscle differentiation and the development of the nerve endings in the muscle.
4. Sensory nerve endings are almost completely developed before the motor endings begin to differentiate.
5. Muscles and nerve endings are differentiated first in the tongue: at all stages the neuromuscular mechanism of the hand and arm is further advanced than that of the foot and leg.
6. An argentophil fibre plexus is already present at 8 weeks' development: this may perhaps be the fundamental sympathetic plexus.
7. By 20 weeks' development Pacinian bodies and spindles are well differentiated: by 28 weeks the motor plates are just beginning but are very rudimentary except in the tongue.
8. The functional significance of the ages at which certain nerve endings develop is discussed.

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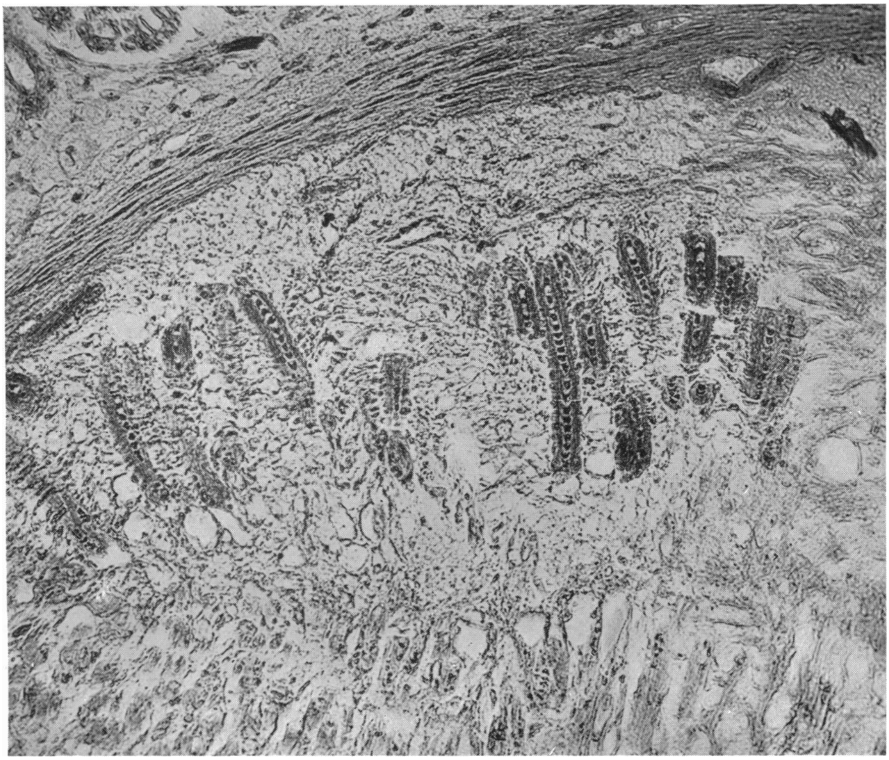


Fig. 1.

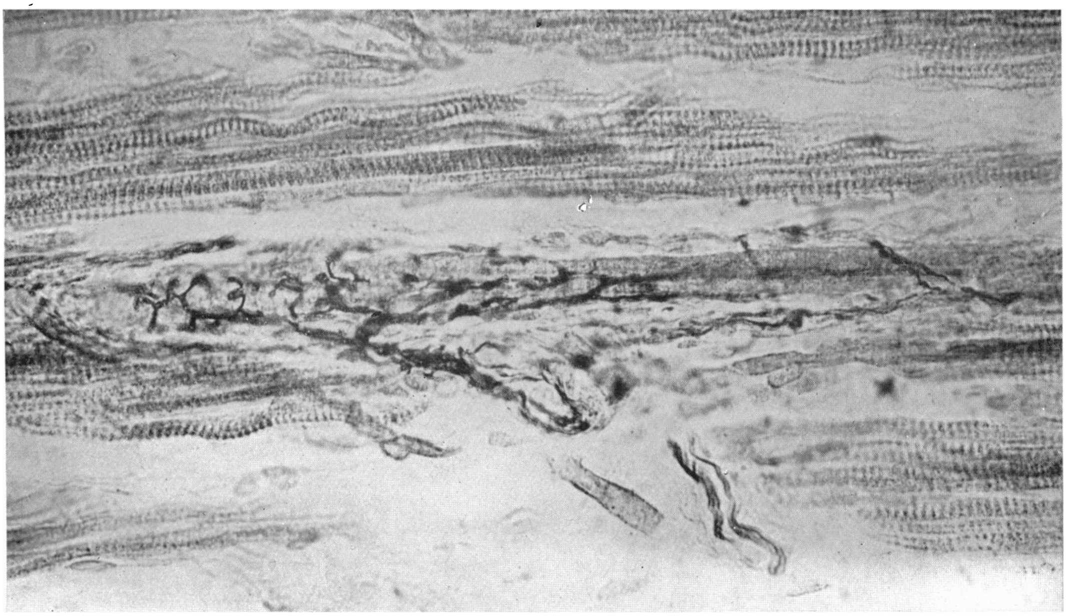
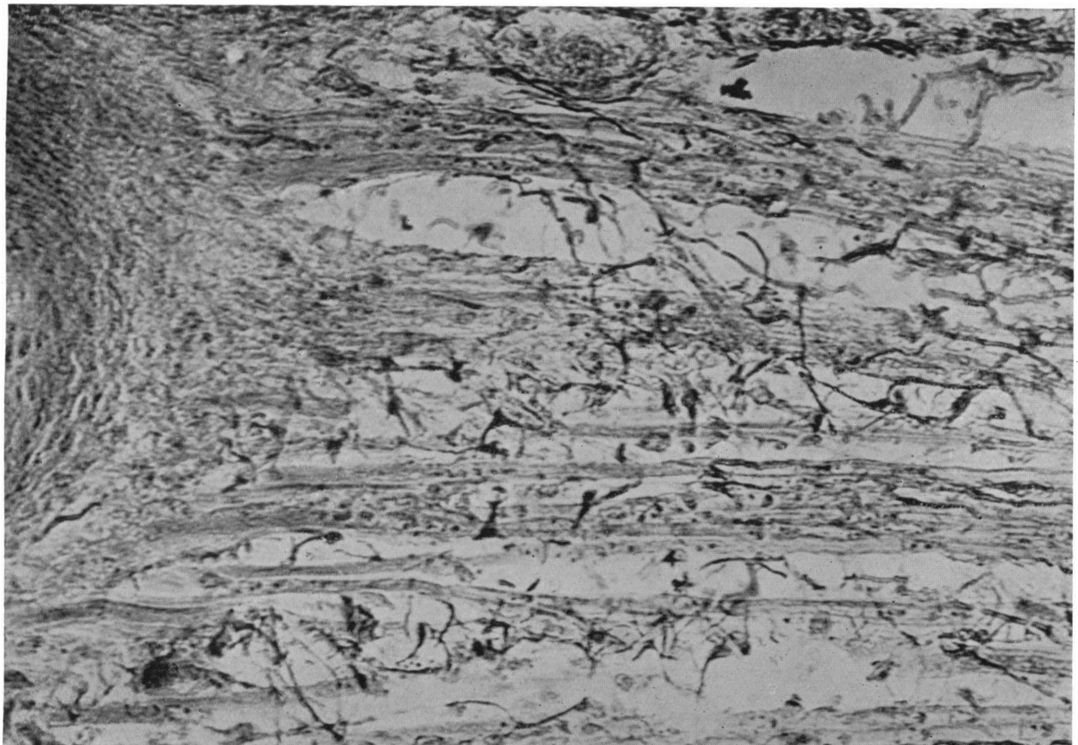


Fig. 2.

HEWER—Nerve Endings in the Human Foetus

Fig. 1.



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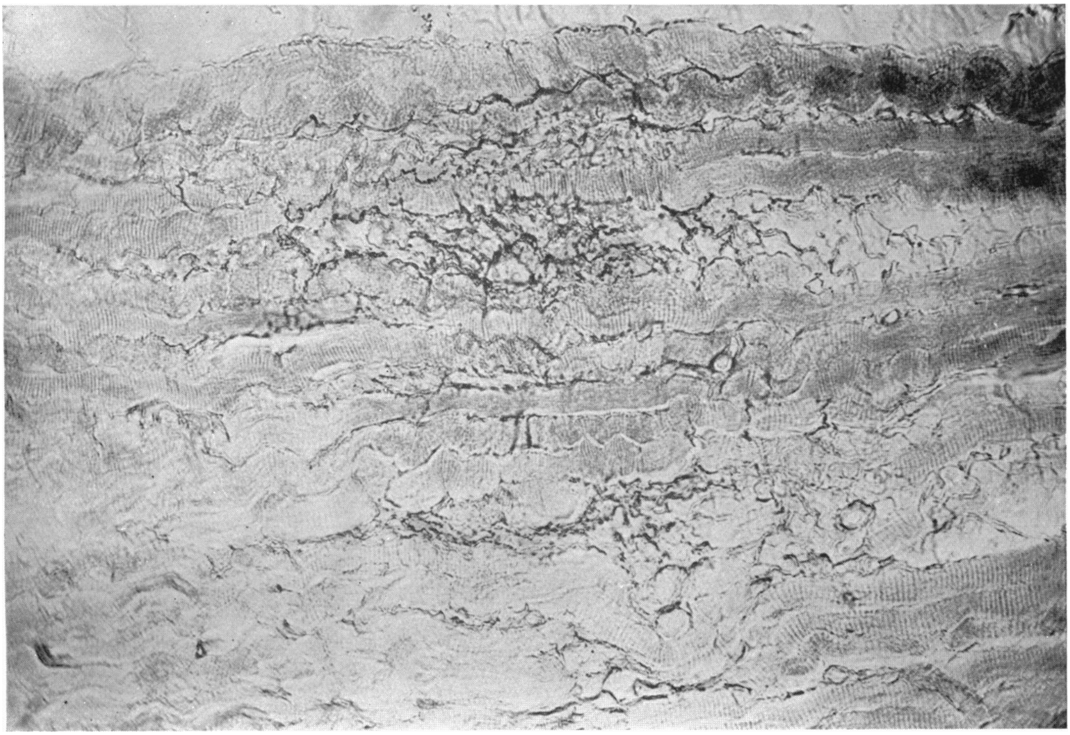

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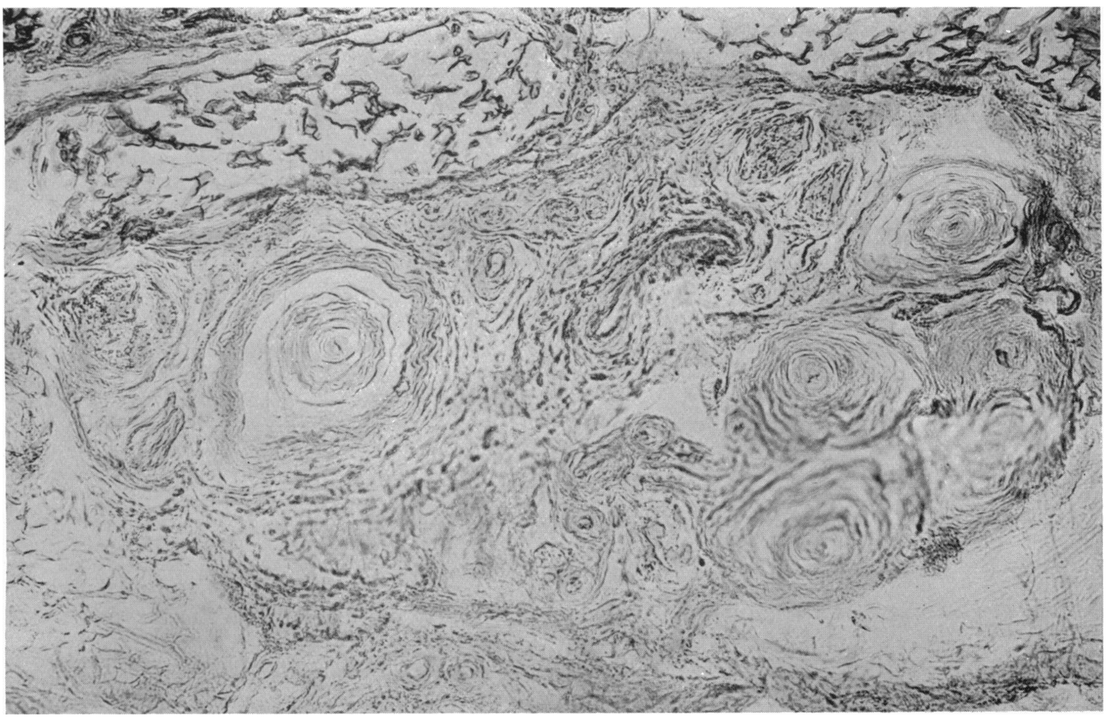

Fig. 2.

HEWER—NERVE ENDINGS IN THE HUMAN FOETUS

EXPLANATION OF PLATES I–III

(Untouched photomicrographs)

Plate I

Fig. 1. Developing striped muscle fibres. Foetus of 10 weeks. (No. 21.) × 250.
Fig. 2. Developing sensory nerve ending in the muscles of the forearm. Foetus of 20 weeks. (No. 60.) × 500.

Plate II

Fig. 1. Developing motor nerve endings in the muscle of the tongue. Foetus of 26–28 weeks. (No. 6.) × 500.
Fig. 2. Argentophil plexus among developing muscle fibres of the foot. Foetus of 10 weeks. (No. 67.) × 500.

Plate III

Fig. 1. Argentophil plexus among muscle fibres of the hand. Full-time foetus. (No. 80.) × 500.
Fig. 2. Pacinian corpuscles from the sole of the foot. Full-time foetus. (No. 80.) × 300.