THE GROWTH OF EMBRYONIC NEURITES

A STUDY ON CULTURES OF CHICK NEURAL TISSUES

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INTRODUCTION

That the tip of an embryonic growing nerve fibre terminates in a distinct expansion was first recognized in 1890 by Ramón y Cajal, who gave the name of 'cone d'accroisement' thereto. The amoeboid behaviour of this structure was first described by Harrison in 1910, in those papers which not only definitely established the neurone theory of Ramón y Cajal but also founded the technique of tissue culture, and so opened a wholly new approach to the experimental study of cells and tissues in general. Thus research on the growth of neural tissues in culture dates from the very beginning of the use of such methods in experimental biology (Harrison, 1907, 1910; Burrows, 1911; Lewis & Lewis, 1912). Since that time, tissue-culture methods have been used for all but one of the studies on the behaviour of the living amoeboid tip, the exception being the brilliant observations of Speidel (1932, 1933) on the growth of neurites in the tail of living amphibian larvae. Levi (1934) has summarized the studies on explants of nervous tissues in his general survey of the results obtained by tissue-culture methods. Many of the observations on cultures of the nervous system have been made by Levi himself; in 1917, for instance, he published a series of drawings of the changes undergone during a period of 5 min. by the tip of a neurite in an explant of chick mid-brain.

A recent account of the behaviour of living nerve fibres in culture is due to Lewis (1950), who describes some time-lapse films of nerve cultures presented at a Symposium on *Genetic Neurology* in March 1949, though no illustrations from these films are included in the published record to console the reader for absence on this occasion. The use of phase-contrast microscopy for the study of cultures of nervous tissues has been introduced by Prof. C. M. Pomerat (Costero & Pomerat, 1951), who has prepared by this means a number of time-lapse films on the behaviour of cells from the adult nervous system.

In this paper some observations are described which have been made of the tips of living neurites emerging from explants of neural tissues of the embryonic chick.

The behaviour of these structures has been recorded by phase-contrast ciné-microscopy, and the conclusions which have been reached are derived from the analysis of the resulting film records.

TECHNIQUE

Explants of spinal ganglia, segments of the spinal cord, and fragments of the cerebral hemispheres or optic lobes were made from 7–12-day chick embryos under the usual conditions of tissue culture. These explants were grown as hanging-drop preparations, usually in a clot consisting of approximately equal parts of diluted embryo extract and adult fowl plasma. Some were grown in a fluid medium; this was obtained by first preparing a clot of embryo extract and plasma, then chopping it into small pieces from which an exudate drained slowly away. This fluid was incubated for a period of an hour together with the clot fragments and was then collected for use.

The cover-slips bearing the cultures were mounted on culture chambers suitable for phase-contrast microscopy, consisting of a metal ring 1–2 mm. in thickness, on the underside of which was sealed a further cover-slip of the same size. The study and photographic recording of the behaviour of the neurites in such cultures was begun on the day following explantation. A fair proportion of cultures then usually showed the beginnings of the outgrowth of neurites, but sometimes they were as yet only to be found in the deeper layers of the plasma clot around the explant; further outgrowth during the course of the day would then bring them near to the undersurface of the cover-slip. Only those neurites at this level were suitable for photography.

The apparatus used for this purpose, and the technical details, both of the 16 mm. ciné-camera and the photographic methods employed, have already been described (Hughes, 1949), though recent improvements in the mechanism of the camera drive still remain to be published.

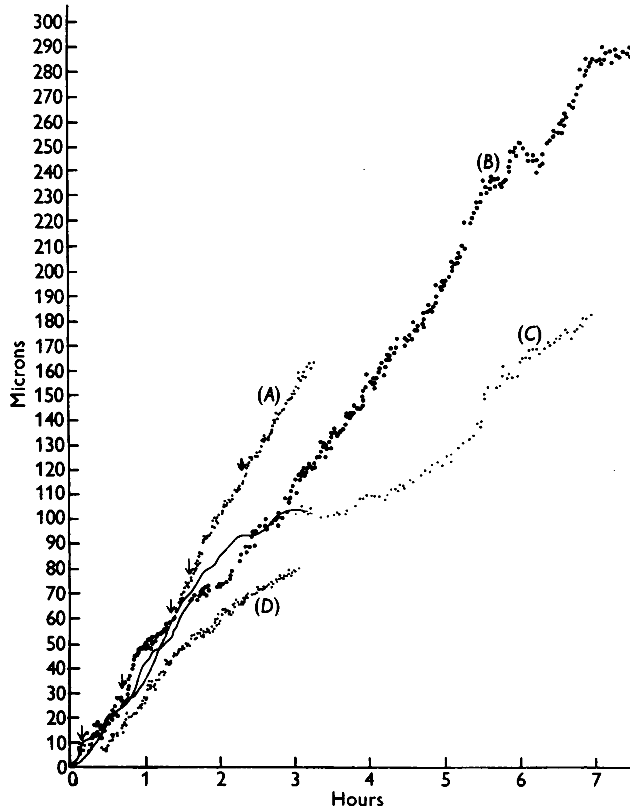
The rate of exposure of the film varied between eleven and one frames per minute; the actual speed chosen depended on the magnification used and the rate of movement with the field.

The films after development were analysed by means of a Yade animated viewer, to which was attached a device for counting the frames of the film which was being projected. The magnification on the screen of this instrument was measured and the growth of a neurite was plotted by measuring the distance moved against time, as recorded by the film counter. Whenever a neurite had grown across the field, it was moved back again by means of the mechanical stage of the microscope; these resettings were carefully noted and measured in the analysis of the film. Finally a continuous growth curve was prepared in which these movements were taken into account.

Special features of the technique connected with experiments on the micro-manipulation of cultures will be noticed in the appropriate section below (p. 156). For this work, cultures were mounted in liquid paraffin, in order to prevent evaporation of the culture from the open side of the culture chamber through which the micro-needle is inserted. It was thus necessary to check whether this treatment has any effect on the growth of the culture. No difference could be detected after growth for 48 hr. between normal hanging-drop cultures in contact with air and

others in which the air had been replaced by liquid paraffin. In Text-fig. 1 the growth of four neurites over periods of 3–7 hr. is shown graphically. Of these, two (*B* and *C*), were grown in contact with liquid paraffin.

One feature of the technique, however, does affect the growth of the neurite, namely, exposure to light. This sensitivity is generally a limiting factor in time-lapse ciné-micrography of living cells. Even a half-second exposure every minute



Text-fig. 1. The growth of four embryonic neurites in tissue culture, plotted from film records. *A*, the ganglionic neurite of Pl. 1, fig. 1. The arrows point to the occasions, plotted in Text-fig. 2, when vacuoles migrate backwards. *B*, *C* and *D* refer to neurites in cultures of mid-brain. The earlier parts of the curves for *B* and *C* are represented by continuous lines to avoid confusion. *B* and *C* were grown in contact with liquid paraffin.

is sufficient to prevent further growth of a neurite after several hours; the maximum time for which continuous growth was recorded was 7 hours. The rate of elongation is maintained nearly to the moment when growth ceases and the neurite breaks up. Fortunately, there is a clear difference between these periods for which normal growth can be recorded and the duration of survival of the experimentally severed neurites, the observations on which are described in a succeeding section of the paper (p. 156 below, Pl. 1, figs. 2, 3).

OBSERVATIONS

(*a*) *General*

The general features of the manner of growth of explants of chick neural tissues have often been described. The earlier literature on the subject is fully reviewed by Levi (1934). Since that time this author has published several further papers in this field, including a definitive study of spinal ganglia in culture (Levi, 1941). The growth of such explants differs from those of fragments of the brain and cord in several ways; the outgrowth consists not only of neurites, but also of fibroblastic cells of neural crest origin, with an extremely high mitotic rate; some of these are closely associated with the neurites in the manner of Schwann cells. Furthermore, after undisturbed growth for a week or so, the central part of the explant has become so thinned by outward migration that the cell bodies of the neurons in this region become accessible to observation (Weiss & Wang, 1936).

The association between neurites and fibroblasts is often very close indeed—much closer than that between a neurite and the surface of a cover-slip against which it is growing. In observing by phase microscopy a neurite growing over a fibroblast in the living state it is often impossible to discern their respective boundaries (Pl. 2, fig. 8). In time-lapse film records, however, one may readily distinguish between them by their distinct and separate types of movement.

Such films convey a striking impression of motion everywhere in the zone of growth. Fibroblasts migrate, and within them is a constant movement of the cytoplasmic inclusions; most neurites are elongating in growth, and exhibit at the tip the activities with which this paper is chiefly concerned.

The cell bodies at the centre of the culture are in complete contrast to this. Their cytoplasmic inclusions, which have a granular appearance under the phase microscope in the living state (Pl. 2, fig. 5), show no detectable movement in a time-lapse film. At the cell surface, however, there are occasionally signs of a rhythmic activity such as Pomerat (1951) has observed in films of explants of adult human brain, though of a much slower periodicity. In these embryonic cell bodies, the interval between successive pulsations is about 20–30 min.

(*b*) *The rate of growth of embryonic neurites*

From a comparison of the data on this subject in the literature certain conclusions may be drawn. The rate of growth is variable and does not remain constant for the individual neurite over periods of more than a few hours. Furthermore, the rate for neurites explanted in tissue culture is apparently higher than for those within the intact embryo.

In his original experiments on the growth of explants of the larval amphibian brain, Harrison (1907) measured rates of growth of 15·6 and 56 μ per hour. One neurite was followed for 53 hr. and grew at an average rate of 21·6 μ per hour. In the tail fin of the intact larvae of several Amphibian species Speidel (1933, p. 6) found that 'for 5 or 10 minutes a growth cone may extend at an average rate of 1 μ per minute, but this is never maintained. Over a period of 1 hr. an active cone might travel 30–40 μ, while over a 24 hr. period, 200 μ would represent active growth' (i.e. an average of 8·3 μ per hour).

For the growth of chick neurites in culture, Levi (1934, p. 604) quotes a mean figure of his own of 23·5 μ per hour at 38° C., and a further average of 33 μ per hour at 39° C., due to Mossa (1926), who found that this temperature was the optimal for neurite growth in the chick. The values over periods of 3–7 hr. obtained in this present work vary from 26 to 51 μ per hour (Table 1, Text-fig. 1).

An estimate of the rate of growth of nerve fibres in the intact chick embryo is given by Ramón y Cajal (1928, p. 365). He says that in the 3-day chick it 'approaches 10 μ per hour', though no further details are given.

Table 1. *Data on the growth of neurites in culture*

Source	Culture in contact with	Distance grown in time		Rate (μ/min.)
		μ	min.	
A. Unoperated neurites				
Lumbar ganglion	Air	162	192	0·85
Mid-brain	Paraffin	295	420	0·70
Mid-brain	Paraffin	148	240	0·62
Mid-brain	Paraffin	182	420	0·43
Mid-brain	Air	80	180	0·45
B. Cut neurites				
Mid-brain	Paraffin	80	110	0·73
Mid-brain	Paraffin	55	104	0·53
Mid-brain	Paraffin	54	160	0·34
Mid-brain	Paraffin	33	50	0·66
Mid-brain	Paraffin	33	100	0·33
Fore-brain	Paraffin	135	200	0·67
Fore-brain	Paraffin	30	66	0·34
Cervical cord	Paraffin	60	120	0·50

One factor which may be responsible for a slower average rate of growth within the embryo is the inhibitory influence of adjacent neurites upon each other. Neurites growing into the plasma clot of a culture are less crowded together than those within the neural tube of an intact embryo. An example from a culture of a fragment of the mid-brain of the growth of one neurite being hindered by another across its path is given in Pl. 2, fig. 9.

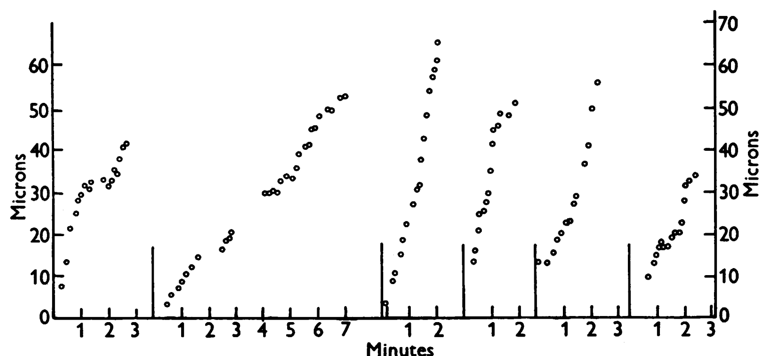
It is noteworthy that even the maximum rate of growth of embryonic neurites is well below the modern estimates of Gutmann, Gutmann, Medawar & Young (1942) for the 'rate of advance of the fastest axon tips' in the regeneration of adult nerves. For branches of the sciatic nerve of the rabbit once the latent period is passed, these authors state that this velocity is from 3·5 to 4·4 mm. per day (146–180 μ per hour).

The comparison of these rates in embryonic and adult tissues will be noticed further on a succeeding page.

(c) *Pinocytosis at the tip of the neurite*

In cultures of spinal ganglia in the first and second days after explantation, the early neurites which appear in advance of the fibroblastic outgrowth are easily distinguishable from those in cultures of the brain and cord by reason of their large size, and their complexity of structure at the tip. Fine, uniform regions of neurites from brain and cord explants in solid media are usually about half a micron in diameter; early ganglionic neurites are usually much more irregular in calibre and vary in width from one to several microns. At the tip in healthy examples of both,

fine pseudopodial processes in continual movement are extended from the terminal expansion. This structure is very much larger in the ganglionic neurite, and within it numerous vacuoles can often be seen, again in constant movement. The largest of these are about two-thirds of a micron in diameter, while the smallest which can be resolved in these ciné records are rather less than a half of this value. Such vacuoles have already been observed by Matsumoto (1920) in embryonic sympathetic fibres *in vitro*, and by Lewis (1950) in ganglionic neurites. A further feature of the tip of ganglionic neurites which Lewis also described, is an undulating membrane, comparable to that of a macrophage, which like that of other cells, can enfold vacuoles of external fluid by the process which was originally described by the same author as 'pinocytosis'. (Lewis, 1931).



Text-fig. 2. Migration of vacuoles towards cell-body in the ganglionic neurite of Text-fig. 1*A* and Pl. 1, fig. 1. Distances are measured from the neurite tip.

Figs. 6*a*, *b* and 8*a* of Pl. 2 of this paper illustrate the terminal undulatory membrane in a spinal neurite. It appears to form a web stretched between filamentous pseudopods, though these may possibly be folds in a continuous structure.

It is very probable that the entry of vacuoles into the tip of the neurite is thus a consequence of its protoplasmic activity. New vacuoles are formed almost continuously at the tip of an active neurite, though not always is a continuous undulatory membrane visible; filamentous pseudopods also seem to bring vacuoles into the neurite tip.

What, therefore, is the fate of these vacuoles which accumulate within the neuroplasm? Near the tip they seem mostly to move at random along the neurite but occasionally one is seen in the film records to move rapidly and continuously away from the tip, and towards the cell body. In one particular record of the behaviour of a ganglionic neurite over a period of 3 hr., this phenomenon was studied in some detail (Text-fig. 2). The limitation on the recording of the movement of one particular object of the size of such a vacuole is the extremely small depth of focus of the phase microscope at high powers. Even within a neurite closely applied to the underside of the cover-slip, a vacuole can readily move in and out of the plane of focus. However, six clear instances of the continuous movement of vacuoles in the direction of the cell body over distances of 12–28 μ were found in this record. Their velocities varied from 3·8 to 17 μ per minute.

Careful search has revealed examples of this centripetal movement in most of the records of the behaviour of neurites which have been obtained during the course of this work.

It cannot yet be decided whether periods of flow in each direction along the neurite alternate with each other, or whether streams of 'raw material' taken in at the tip, and of formed protoplasm proceeding from the cell body, can simultaneously flow in opposite ways along so small a structure as the embryonic nerve fibre.

(*d*) *Surface behaviour in other types of cell in neural explants*

Macrophages become increasingly evident as the age of all such cultures advances, and finally are still active when all other cells are moribund. Macrophages of a flattened epithelioid type are conspicuous in cultures of spinal cord grown in fluid medium in the first few days after explantation. In them, the process of pinocytosis is especially clear; a series of stages in the formation of a vacuole is shown in Pl. 2, fig. 4. Comparison of this figure with those of ganglionic neurites shows how much larger are the vacuoles in macrophages.

From the edge of an explant of spinal cord there often grows outward a coherent epithelium of supporting elements, the marginal cells of which have undulating membranes (Pl. 2, fig. 7), due to the activity of which small vacuoles are continuously entering this epithelium. It is not clear whether embryonic sustentacular cells normally behave in this way, or whether their pinocytic activity is here merely a response to the conditions of tissue culture.

(*e*) *The effect of severing a neurite from contact with its cell body*

The main paper in the literature on the micro-manipulation of cultures of nervous tissues is that of Levi & Meyer (1945). These authors summarize the results of previous studies of this kind. In 1926 Levi showed that if the nucleus of a neuroblast was pierced with a micro-needle, the cell body degenerated rapidly, but that there was an interval of time before the autolysis of its neurites. Levi & Meyer (1945) made cuts with a micro-needle through a plexus of neurites in the outgrowth of spinal ganglion cultures, and found that healing could take place within a few hours without degeneration of the region of the plexus distal to the cut.

The object of the present experiments was to sever a neurite near its emergence from the explant, and to follow the subsequent fate of the distal portion. A simple micro-manipulator which gave vertical and transverse motion in one other plane was constructed, and attempts were made to sever neurites in culture by means of a needle formed by honing a piece of 0·2 mm. stainless steel wire to a pointed tip a few microns across. However, it was found that the plasma coagulum could only be torn after a degree of disturbance which rendered the operated region of the culture unfit for further high-power observation. Fortunately, a way out of this difficulty was provided by the area of liquefaction of the clot which usually develops around explants of the brain and cord, which is due, as Weiss (1934) has shown, to the proteolytic activity of the ependymal layer. The neurites in this zone are slightly stretched across a fluid zone between explant and the unliquefied clot beyond, in which the neurites are still growing (Pl. 1, figs. 2*a*, 3*a*). It is comparatively easy to tear away neurites from this proximal zone, and to leave undisturbed their distal extremities.

When this operation is performed it is found that these neurites deprived of connexion with their cell bodies do not immediately die, but continue to grow for periods of one or more hours. Around cultures of spinal ganglia, the medium does not liquefy; both for this reason and because of the presence of Schwann fibroblasts in these cultures, it was decided to use only explants of the brain and cord for these particular experiments, although their neurites are less spectacular than those of spinal ganglia.

Explants of fragments of the brain and cord at 7–8 days were found most satisfactory for this type of experiment. From each batch of cultures it was necessary to choose examples in which the neurites were in an early stage of outgrowth, and where, next to the explant, there was a suitable zone of liquefaction of the medium from 100–200 μ in width. The $\frac{7}{8}$ in. square cover-slip bearing a suitable culture was mounted in ordinary medicinal liquid paraffin over a glass cell, which gave support on three sides, leaving the fourth open for the introduction of the micro-needle. In this way, drying-up of the culture during the experiment is avoided; this element of the technique is of course due to de Fonbrune who uses it for his exquisite micro-manipulator. No difference could be detected in the growth of cultures in contact with liquid paraffin and those sealed over air in the ordinary way.

The neurites were torn away from the zone of liquefaction under a $\times 10$ objective, the field of operation being photographed before and after the act. At higher magnification, the severed neurites in the plasma beyond were then inspected, and one was chosen in which there could be no doubt that contact with the cell body had been interrupted. Glial cells, together with a few neuroblasts, were usually to be found on the far side of the zone of operation, and so careful scrutiny was necessary to ensure that the neurite chosen for observation did not proceed from a neuroblast drawn away from the main body of cells in the explant. Sometimes it was necessary to operate on several cultures in succession before a suitable neurite was found.

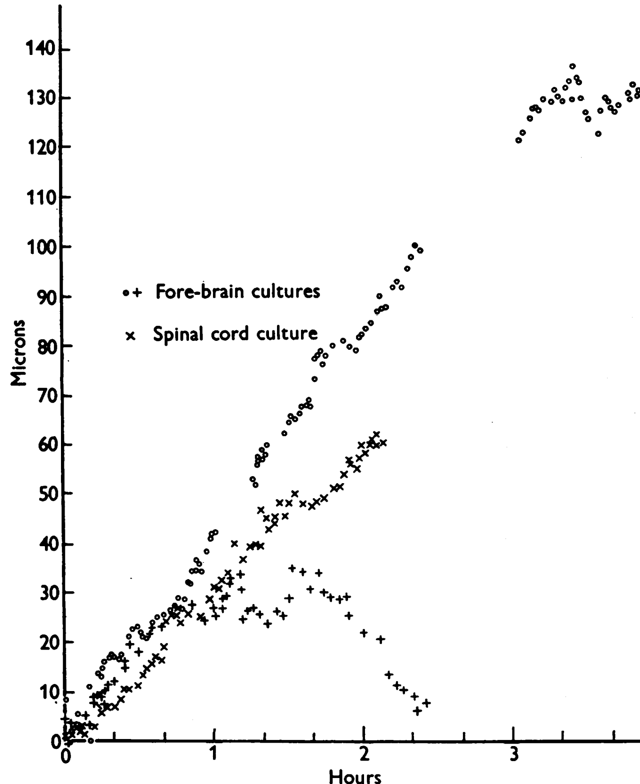
In different experiments, both ends of the isolated neurite were studied. The proximal end, where the break has been made, becomes club-shaped (Pl. 1, fig. 2*c*, *d*); vacuoles collect within it, and a film record of its behaviour shows that at the surface there is continual movement during its period of survival. During this time growth continues at the tip of the isolated neurite. Eight records were made of the growth of severed neurites; they advanced for distances which varied between 33 and 135 microns at rates of 0·33 to 0·73 μ per minute (Table 1, Text-fig. 3). This range of velocity suggests no difference from that observed in unoperated cultures.

Growth ceased in the operated neurites at periods of 50–200 min. after operation; fine pseudopodia were then no longer emitted from the tip, which usually began to retract. The outline of the whole became irregular, and sooner or later broke up into round protoplasmic drops (Pl. 1, fig. 3*c*).

DISCUSSION

The analogy of the tip of a living embryonic neurite with an amoeba has suggested a general picture of its movement and extension, although it may be doubted whether a complete description of the processes has yet been given. Usually (Weiss, 1941; Lewis, 1945, 1950) it is stated that fine pseudopodia are extended

from the nerve tip, most of which are later withdrawn; endoplasm, however, flows into one or perhaps two of these, and so the neurite advances by the enlargement of a pseudopodium into a new section of the neurite. Thus Weiss (1941, p. 175): 'once a pseudopodium has established itself and caused the mass of protoplasm to flow into it, the drain thereby exerted on the other pseudopodia produces their automatic withdrawal.' Lewis stresses the role played by the outer layer of contractile gel over the neurite, which forces endoplasm into the persisting process. He says: 'The weakest part—the weak gel layer of the terminal pseudopod—is expanded and advanced', though this inference, as he admits, is mainly based on the study of the locomotion of amoebae.



Text-fig. 3. Growth of three neurites after severing connexion with the cell body in the explant. Data on further experiments of this kind is given in Table 1.

Close observation of the film records of the growth of the neurites in spinal ganglion cultures in this present work has led me to question this view. In actual fact the rapidity with which the pattern of extrusion of the fine pseudopodia changes is far too great to allow for the persistence of any individual one of them. The main tip of the neurite advances discontinuously, in separate thrusts; during one of these forward movements several completely fresh sets of fine pseudopodia will be extruded and withdrawn. It is thus impossible for the terminal pseudopodia to serve as the means by which the neurite is anchored to its surroundings.

Again, the distal flow of endoplasm through the neurite is regarded as the immediate cause of the forward growth of the neurite (Weiss, 1944). In post-embryonic nerves, Weiss & Hiscoe (1948) have assembled an impressive body of experimental evidence on this question, mainly relating to the damming of axoplasm when a nerve is constricted along its course. They conclude that 'growth in the sense of production of new protoplasm occurs solely at the base of the fiber in the nucleated part of the cell body'.

For the embryonic neurite, however, the results of the isolation experiments described in this paper show that growth in the embryonic neurite does not immediately depend upon protoplasmic synthesis of this kind. The distal part of a neurite continues to grow at the normal rate for a period of one to several hours after its connexion with the cell body has been destroyed.

To what, then, is the growth of an embryonic neurite immediately due? The probable cause is the intake of fluid. In the large neurites of spinal ganglion cultures, and in those of the brain and cord grown in fluid media, the process is microscopically visible as terminal pinocytosis. In smaller neurites, where this process cannot be observed at the tip, it is probable that water is also being absorbed, though on a smaller scale, for visible vacuoles have been seen to collect at the proximal end of a neurite isolated from its cell body in a culture of mid-brain in a plasma clot (Pl. 1, fig. 2*c*, *d*).

After several hours' growth of a severed neurite it stains noticeably paler with haematoxylin than the unoperated neurites in the same culture. This observation suggests a comparison with the recent work of Brachet on halved amoebae (Brachet, 1952). This author divided *Amoeba proteus* into two portions, one of which contained the nucleus. Both halves were starved, and after 10 days the main difference between them was that the basophilia of the cytoplasm of that without a nucleus rapidly decreased. It would be of great interest to know whether Brachet's inference from this work, concerning the function of the nucleus in coupling oxidation and phosphorylation, is also true of the neuroblast.

One function, if not the main one, of the terminal pseudopodia of the neurite is to bring in water and dissolved substances by pinocytosis. The details of the process are not yet fully understood, but the analogy with the activity of the undulating membrane of macrophages is clear. In them, the membrane seems first to fold round and enclose a portion of the external fluid (Pl. 2, fig. 4). This passes in towards the main body of the cell, and it is not until then that the new vacuole becomes rounded and spherical by surface-tension forces; its area in the photomicrographs has then markedly decreased. It thus may well be that over the surface of the undulating membrance there is some surface-active substance such as has been proved to exist at the surface of marine eggs (Danielli, 1945).

At any rate, it is true to say that where no processes are being emitted at the tip of neurites, then there is no increase in length. There is, however, no uniform relationship between the rate at which a neurite advances, and the degree of pseudopodial activity at the tip. In fluid media, the latter is very marked, and yet the velocity of progression of the neurite may be low. This difference between growth in solid, and fluid media is particularly striking in the neurites from explants of the brain and cord.

The actual rate at which a neurite is growing must depend upon a number of factors: the intake of water at the tip and its passage in vacuoles towards the cell body; the contrary flow of synthetized protoplasm, the almost peristaltic movement of the neurite due to its contractile gel layer; and the tactile influences exerted on the neurite tip by its surroundings, must all play their several parts in the growth of the embryonic nerve fibre.

It may well be that there is a real difference in the growth of embryonic and adult nerve fibres in the degree to which elongation is immediately dependent upon the flow of axoplasm towards the advancing tip, and that this distinction is related to the difference in their respective rates of growth. It would be of great value if the regeneration of an individual nerve fibre could be observed in the adult higher animal.

SUMMARY

1. The growth of neurites in cultures of explants of chick neural tissues has been observed by means of phase-contrast ciné-microscopy. The rate of growth is of the same order which has been measured by previous authors.

2. The activity of the tip of the neurite has been studied. The intake of vacuoles of external fluid has been confirmed, and it is concluded that this is the main function of the movement of the terminal pseudopodia and undulating membranes.

3. Vacuoles at the tip have been seen to pass at intervals down the neurite, towards the cell body.

4. Cutting of the neurite close to the explant does not immediately lead to the death of the severed distal portion, which continues to grow for periods of 1–3 hr.

5. It is concluded that the immediate cause of the growth of embryonic neurites is the intake of water at the tip.

6. The suggestion is made that regenerating axons of the adult animal are more closely dependent on the flow of synthesized protoplasm from the cell body and that this postulated difference is correlated with their more rapid rate of growth.

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EXPLANATION OF PLATES

All figures are of phase-contrast photomicrographs of living cells, or parts of cells, from explants of the nervous system of chick embryos, grown in tissue culture. They are enlargements from single frames of 16 mm. time-lapse sequences. Figs. 2*a*, *b* and 3*a*, *b* are at low-power; for the remainder a 2 mm. immersion lens was used, and they are to a magnification of ×1400.

PLATE 1

Fig. 1*a*, *b*. Tip of neurite from a culture of a lumbar dorsal root ganglion of a 7-day chick, cultivated for 2 days. *a–d* and *e–h* are sequences separated by an interval of just over 1 hr. They each show the passage of a vacuole down the neurite in the direction of the cell body. (*b*), 1·1 min., (*c*), 3 min., (*d*), 5 min. after (*a*), (*f*) 0·8 min., (*g*) 1·0 min., (*h*) 1·2 min. after (*e*).

Fig. 2*a–d*. An experiment in which neurites were severed in a 24 hr. culture of 6–day optic lobe. (*a*) Part of the culture at low-power, showing the proximal zone of neurites in the zone of liquefied medium. (*b*) Same field 8 min. later after the neurites have been removed from this region. (*c*) Proximal end of one isolated neurite in this field under high-power, 13 min. after operation. It becomes club-shaped and vacuoles collected within. (*d*) Same field 204 min. after operation. The neurite still shows movement.

Fig. 3*a–e*. Observation on the tip of a neurite in a similar experiment. (*a*) Field under low-power before operation, showing neurites in zone of liquefaction adjacent to explant. (*b*) Same field 3 min. later, showing neurites removed from this zone. (*c*) Tip of one severed neurite in plasma clot beyond, under high-power; 9 min. after operation. (*d*) Same tip 98 min. after operation, after having grown outwards for a further 72μ. (*e*) Same tip 132 min. after operation, now degenerating.

PLATE 2

Fig. 4*a–d*. A macrophage from a culture of 6–day spinal cord in fluid medium, during the third day after explantation, to show the intake by pinocytosis of a vacuole indicated by an arrow. (*b*) 1·6 sec., (*c*) 7·5 sec., (*d*) 14·4 sec. after (*a*).

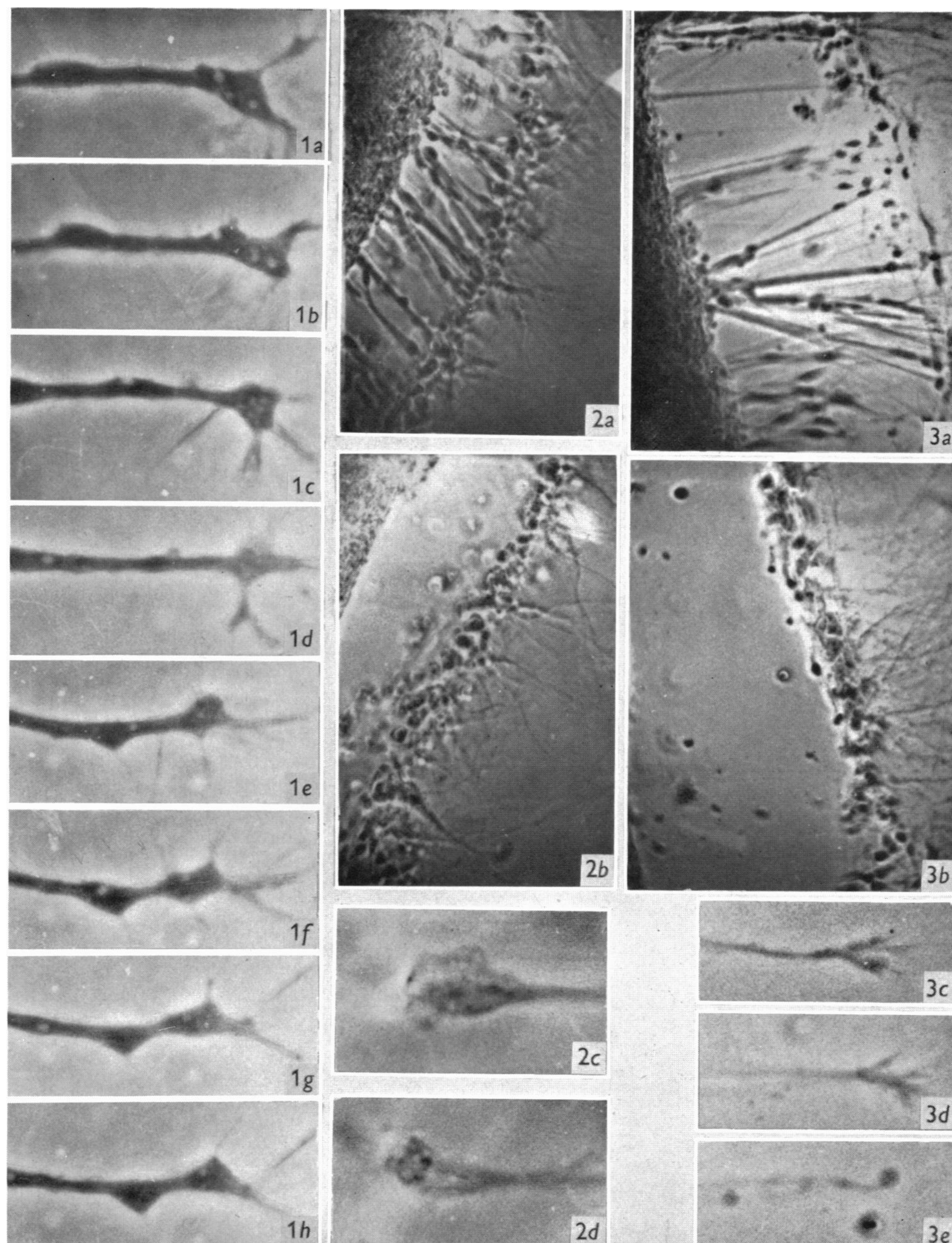
Fig. 5. Cell body in 3–day culture of 9–day lumbar spinal ganglion, to show neurofibrillae in perinuclear cytoplasm. They are granular in appearance by phase-contrast.

Fig. 6*a*, *b*. Ganglionic neurite in the same culture on the previous day, to show a terminal undulating membrane. (*a*) and (*b*) are 6 min. apart.

Fig. 7. The outgrowth from an explant of 9-day spinal cord grown in fluid medium for three days. Supporting elements have formed an epithelium, at the margin of which cells are actively pinocytic.

Fig. 8. The overgrowth of a Schwann fibroblast by a ganglionic neurite in the same culture as in figs. 5 and 6. (*a*) The neurite tip with undulating membrane is to the right of the cell nucleus. (*b*) 37 min. later. The neurite has covered the nucleus and much of the cytoplasm of the fibroblast.

Fig. 9. Part of the outgrowth of a 24 hr. culture of a fragment of 6-day optic lobe, to show the interaction of two neurites, which approached each other at right angles (*a*). (*b*) 9·4 min. later than (*a*); the two tips have met. (*c*) 50 min. later than (*a*); the first has retreated, while the second continues to grow. (*d*) 115 min. later than (*a*); the first has now grown over the second, the tip of which by now is approximately 50μ further than in (*a*).



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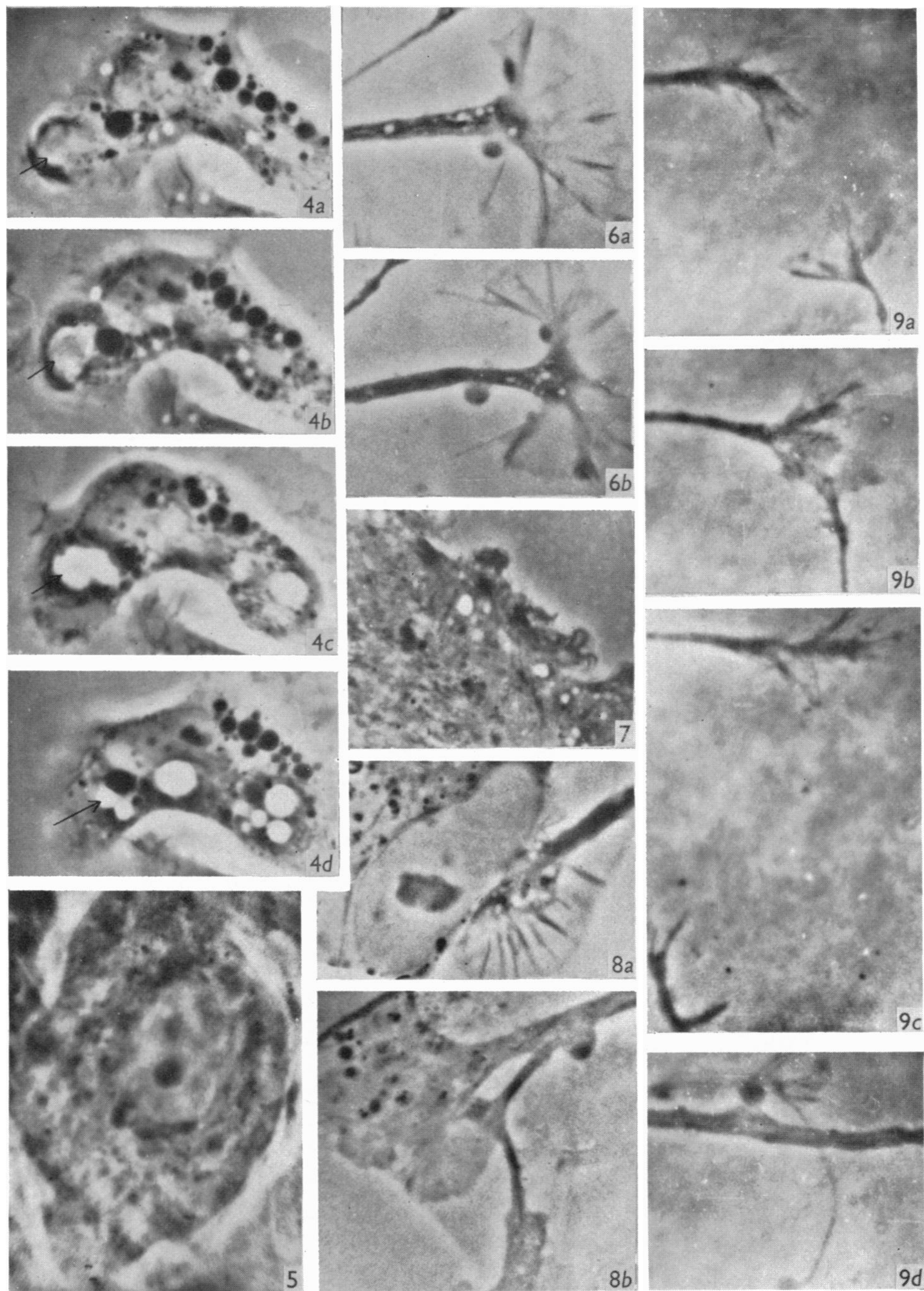

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