THE EFFECT OF FIXATION ON NEURONS OF THE CHICK

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INTRODUCTION

The literature relating to the intimate texture of the nervous system is certainly as large as in any other branch of histology or cytology. Some of its fundamental problems, however, such as the nature of neurofibrillae, and the action of fixatives on the neurocytoplasm, are still being debated without any immediate prospect of a final conclusion. The first of these topics is the older, and goes back as far as Remak (1844) and Parker (1929), before the use of fixatives in microscopy became general. From that time, there have been occasional cytologists such as Schultze (1870) and Held (1895) who studied the unfixed nerve cell which at the present time is again receiving attention. Furthermore, attempts at fixation by freezing, a method perfected only in recent years, were made as early as 1874 (Key & Retzius). Thus it may be said that comparison of the appearance of the living cell with that produced by various methods of preparation has been from time to time attempted, but that this approach has mostly been submerged by the development of particular techniques such as silver impregnation methods, and in recent years, electron microscopy, which are relevant only to fixed material. At the present time, however, advances in optics applicable to the living cell and to unstained preparations, such as phase-contrast and ultra-violet microscopy, have greatly increased the scope of this general approach and an increasing number of workers are at present taking advantage of these possibilities (Thomas, 1947; Koenig & Feldman, 1953; Adamstone & Taylor, 1953; and Taylor & Adamstone, 1953). My own interest in this field began with the observation of cell bodies of chick embryonic spinal ganglia in tissue culture. Under the phase-contrast microscope they have a granular texture

(Hughes, 1953), although a fibrillar appearance in them with ordinary microscopy has previously been described (Weiss & Wang, 1936; and Levi & Meyer, 1937). A comparable granular appearance was then observed in other immature unfixed neurons of the chick, although standard neuro-histological procedures on the same material produced the usual appearance of neurofibrillae. Experiments which have attempted to explain these discrepancies are here described.

TECHNIQUE

(1) *Observation of living neurons in dorsal root ganglia*

Spinal ganglia were dissected from 10- to 12-day chick embryos under sterile conditions and were explanted as hanging-drop cultures into a medium consisting of roughly equal parts of fowl plasma and chick embryo extract. In such cultures, Schwann cells migrate outwards in a fibroblastic form of outgrowth, accompanying the regenerating neurites. Many of the neurons within the explant degenerate, but in favourable cultures the remainder form a thin layer in the centre, which are accessible for microscopic observation after most of the Schwann cells have left. Usually, nearly a week of incubation is necessary before this stage is reached. Recently, however, at the suggestion of Dr H. B. Fell, it has been found that short treatment of the ganglia with 1% trypsin in Tyrode saline before explantation digests the intercellular matrix (Moscona, 1952) sufficiently to accelerate the separation of its constituent cells in culture, so that the cell bodies can often be observed much sooner after explantation than would otherwise be possible. Murnaghan (1941) found preliminary treatment with trypsin of use in making spreads of spinal ganglia.

(2) *The squash method for the cerebellum*

If fragments of the cerebellum of a chick are squashed between a slide and coverslip, and then examined under the phase microscope, Purkinje cells may be examined unfixed. They are distorted in form by the treatment and their processes are shorn off; nevertheless, the nucleus and cytoplasm of the cell body may be studied for a period of 15 min. or so without showing any degenerative changes. The method is applicable to chicks either before or after hatching. In the squashed preparation, it is necessary to search for the Purkinje cells amongst a jumble of fibres and smaller cells, and so the method is only suitable for nervous tissues in which one type of neuron is present in very large numbers. This technique can also be used for the detection of the first appearance within the central nervous system of myelinated fibres which are evident in the chick cerebellum after 18 days' incubation. At later stages, however, when myelination is far advanced, the presence of so much material of high refractive index completely obscures cellular detail elsewhere in the preparation.

The method can be used for the study of the effects of some fixatives on the texture of neurocytoplasm, by squashing a fragment of the cerebellum in a drop of a particular reagent. Protein precipitants, however, cause such marked increase in the refractive index of the cell constituents, that the use of this technique is mainly restricted to formalin and osmic acid which fix by other chemical processes. After fixation for a few minutes in either of these reagents, the preparation is washed in

distilled water, mounted in glycerol and then observed in the phase microscope. In order to retain the film of squashed material adherent to the cover-slip during these operations, it is advisable first to place on the microscope slide a small square of wet cellophane and to squash the fragment initially between this and the cover. This 'sandwich' of cover-slip, tissue and cellophane may then with care be slid off the slide and the cellophane peeled off in water. The film of squashed nervous tissue should then remain on the cover-glass during any subsequent operations. Staining of such films is thus possible.

(3) *Fixation by freeze-drying*

Dr Bell of the Zoology Department of King's College, London, was kind enough to undertake fixation by this method for me. Fragments no larger than 2 mm. across were dissected out of the cerebellum of chicks either before or within a few days after hatching. These were placed on aluminium foil and quenched in *iso*pentane at a temperature near 160° C. They were then dehydrated at −40° C. in the apparatus of Messrs Edwards designed by Prof. J. F. Danielli (Bell, 1952; Danielli, 1953). The specimens were then infiltrated with paraffin wax for a few minutes and were subsequently treated at Cambridge. Sections were cut at 8μ and mounted on clean slides thinly coated with celloidin. Frozen-dried material must be kept from contact with water, as the proteins therein have not yet suffered denaturation. For this reason the sections must be flattened in a non-aqueous medium in which paraffin wax does not dissolve. At the suggestion of Dr Bell, acetonitrile was used for this purpose.

Some slides of these sections were de-waxed in xylol and mounted in liquid paraffin for observation directly by the phase microscope. Others were first dipped in absolute alcohol to denature their proteins, then in 1% celloidin in absolute alcohol and ether to retain the sections on the slide and afterwards in 70% alcohol to harden the resulting celloidin film. They were then ready for staining by neurohistological methods.

The inevitable use of small fragments for freeze-drying meant that the sections were orientated at random on the slide. It was thus necessary to prepare a number of fragments and to select sections which happen to lie in the desired plane.

(4) *Chemical fixation*

Chick cerebella were fixed whole either in formol-saline or in Carnoy's mixture for 24 hr., or in absolute alcohol with 1% ammonia for 48 hr. when Ranson's method of silver impregnation was being used (Ranson, 1914). Before fixation in 2% osmic acid, the cerebellum was cut into fragments about 1 mm. square and left therein for 24 hr.

(5) *Staining methods*

The impregnation technique which was mainly used is that of Holmes (1947) in which mounted sections are silvered. With the chick cerebellum, it was effective either after fixation by freeze-drying, or in formol-saline or Carnoy's fluid. The method is also applicable to cultures of spinal ganglia. After fixation in osmic acid, although the cell bodies are coloured brown by silver, their fibres do not then take up the

metal. Ranson's method (Ranson, 1914) was also used on the cerebellum for purposes of comparison.

For staining Nissl substance, thionin was used in a buffer at approximately pH 3·7 as recommended by Windle, Rhines & Rankin (1943). A formate buffer was found satisfactory for the purpose when a solution of the dye (0·5 %) rather stronger than that recommended by these authors was found advisable. It was possible to combine in the same sections a light silver impregnation with subsequent staining of the Nissl substance.

(6) *Phase-contrast microscopy*

The living cultures of spinal ganglia or the unstained preparations of cerebellar squashes or of frozen-dried sections were studied with a Cooke, Troughton and Simms phase-contrast microscope, usually under the × 95 oil-immersion objective. To record these observations, photo-micrography is essential, and a rapid technique is desirable with evanescent preparations such as squashed unfixed films. A strong light source is required; the 250 W 'Mercra' high-pressure mercury arc of Messrs B.T.H. was used for the purpose with a Wratten mercury monochromatic filter no. 77. Exposures were made on Ilford 'Micro-Neg' Pan film by means of a 35 mm. reflex camera mounted over the microscope. In enlarging these negatives on to Bromide paper, it was often found necessary to use the most contrasty grades of paper.

RESULTS

(1) *Comparison of different methods of preparation and observation*

A generally granular texture is revealed by the phase microscope in the cytoplasm of neurons of the chick which have not been treated with chemical fixatives. This is true equally of living cells in cultures of spinal ganglia (Pl. 1, fig. 2) (Hughes, 1953, plate II, fig. 5); of unfixed Purkinje cells in squashes of the cerebellum (Pl. 2, figs. 9, 10); and in sections of the latter fixed by the freeze-drying technique. Probably more than one type of cell inclusion is represented by these granules, though some of this material is capable of impregnation with silver (Pl. 2, fig. 11).

Their form, size and arrangement differs in the different types of neuron. The finest granules of this type are seen in spinal neurons (Pl. 1, fig. 2), while the coarsest are those of the dentate nucleus of the cerebellum (Pl. 1, fig. 6). In squashed Purkinje cells, the granular inclusions of all types are dispersed to some extent (Pl. 2, fig. 9), while in sections of the frozen-dried cerebellum, they are tightly packed (Pl. 2, figs. 10, 11). They are arranged at random in the main body of the cell but tend to be orientated within the dendrite. Here the individual elements may take the form of short rod-like bodies (Pl. 2, fig. 10). After silver impregnation the appearance of the frozen-dried Purkinje cell is similar to that which it previously exhibited under the phase microscope, though some granules take up silver more heavily than do others. There is a slight tendency for their coalescence into short rows which in the dendrite stem are linearly arranged (Pl. 2, fig. 11). Here, this orientation is shared by particles of the Nissl substance, as seen in preparations stained with thionin (Pl. 2, fig. 13).

After fixation of the cerebellum in formol-saline or Carnoy's fluid, followed by silver impregnation by Holmes's method, occasional granules are still separate, but

the majority have formed continuous chains in some of which the constituent granules can be recognized (Pl. 2, fig. 12). The distortion which is caused by chemical fixation is even more strikingly seen within the nucleus, for the nuclear contents often become aggregated around the nucleolus, with an empty space between this whole mass and the nuclear membrane. This condition is in marked contrast to that in the frozen-dried neuron, where the nucleolus is discrete, with the rest of the nucleus filled with nucleoplasm, within which a few small chromocentres can be seen. Cell shrinkage is still greater after fixation in the absolute alcohol-ammonia mixture which is used in the Ranson method of silver impregnation. In such preparations, none of the impregnated substance within the cytoplasm is present as separate granules, and there are clear spaces between the chains of silvered material. In frozen-dried preparations, the neurons of the dentate nucleus contain argyrophil granules larger and fewer in number than those of the Purkinje cells (Pl. 1, fig. 6). In chemically fixed material, these coalesce completely into fibrils arranged in the reticular pattern characteristic of conventional illustrations of the neuron.

In the cell bodies of living ganglionic neurons in culture, more than one type of granule can be seen. Around the nucleus is a zone containing short rod-like bodies, which have been identified by Murnaghan (1941) as mitochondria. Elsewhere, the phase microscope reveals much smaller rounded granules, best seen at focal levels above and below that of the nucleus. Although continuous lines of neurofibrillar material are not to be seen within the cell body, yet in the neurite stem there can occasionally be observed a faint parallel striation (Pl. 1, fig. 2) distinct from the elongated mitochondria which Murnaghan found therein.

In the neurons of spinal ganglion cultures fixed in formalin and impregnated by Holmes's method, a bundle of neurofibrillae can be traced in the neurite stem (Pl. 1, fig. 3). They fan out as they enter the cell body, in the rest of which they are much less definite. There they form a rather vague reticulum on which little silver is deposited. After fixation in Carnoy's fluid, impregnation reveals a different picture, for the neurofibrillae then form an extremely coarse network, heavily loaded with silver. Other neurons in such preparations show a different and still more severe form of shrinkage; in them the cytoplasm is contracted to a small totally blackened knot, from which the nucleus bulges on one side.

The appearance of the neurofibrillar material in impregnated preparations, then, depends entirely on the method of fixation employed. The distribution and arrangement of the other main cytoplasmic constituent of the neuron, the Nissl substance, is known to be similarly affected by fixation. In sections of the frozen-dried cerebellum, the great bulk of this material is found as large amorphous and flocculent masses (Pl. 2, fig. 13). After fixation in Carnoy's fluid the Nissl substance has a generally reticular arrangement (Pl. 2, fig. 14). Its appearance in osmic-fixed squashes and spinal ganglion cultures is intermediate between these two conditions. The total amount of Nissl substance is much greater after freeze-drying; Dr Bell informs me that it is generally true that this method of treatment preserves a much greater degree of basophilia in other cells than is ordinarily found in chemically fixed material.

(2) *The effect of chemical fixatives*

Since the cytoplasmic texture of these neurons is so much affected by standard neuro-histological procedures, it is necessary to ask at what stage these changes originate. Observation of the action of chemical fixatives suggests that this first step is the one mainly responsible.

When a fragment of the fresh chick cerebellum is squashed in 5% neutral formalin and the squash mounted in glycerol after washing in water, the cytoplasm of the Purkinje cells under the phase microscope is then seen to be uniformly filled with vacuoles up to 2μ in diameter (Pl. 2, fig. 16). When 1 or 2% osmic acid is used, and the procedure repeated, the vacuoles are smaller, but are still uniformly distributed throughout the cytoplasm (Pl. 2, fig. 15).

If cultures of spinal ganglia are fixed in formalin and mounted in glycerol, vacuoles are also seen within the cytoplasm of the neurons, but are now as small as those produced in the Purkinje cell by osmic acid (Pl. 1, fig. 1). In silvered preparations of the same material fixed with the same agents, the meshes of the network of the neurofibrillae are roughly proportional in size and distinctness to the diameter of the vacuoles formed by the fixatives alone. Neutral formalin produced a loose network in the Purkinje cells, but a fine and rather vague one in spinal ganglion cultures; while after fixation in osmic acid, Purkinje cells do not exhibit a network, though here, the fixative considerably modifies the impregnation process.

This correlation suggests that the network of the fixed neuron is formed between vacuoles. In other cells, as Hardy (1899) showed, a fine network may be formed at fixation, though in such meshworks the interstices are too small to be distinguishable as vacuoles. When, however, these are formed within a cell at fixation, much of its water must be withdrawn from the cytoplasm; as the volume of the cytoplasmic phase decreases, particles suspended therein must be concentrated together. In the neuron, these changes confer a spurious definiteness on both the Nissl substance and the neurofibrillar material.

If this explanation is correct, then the interstices between the network of silvered preparations should be empty spaces in the sections, not occupied by cytoplasmic ground substance, or by any other cell constituent. This point has been checked under the phase microscope in mounted sections of the cerebellum fixed in formol saline, de-waxed but unsilvered. In such preparations, within the cytoplasm of Purkinje cells and of the neurons of the dentate nucleus, there are to be seen empty spaces corresponding to the interstices of the silvered network, though these spaces lack the sharp boundaries of the vacuoles in squashed preparations, for which dehydration, the extraction of lipoids and shrinkage in the course of preparation for sectioning may be responsible.

The formation of vacuoles within cells after fixation in formalin has already been described by Crawford & Barer (1951). In the neurons of the chick, however, they are produced in much greater numbers throughout the whole cell body. To establish any general validity for this hypothesis of network formation, it would be necessary to show that in general, neurons are specially susceptible to the formation of vacuoles on chemical fixation. Similar vacuoles were evident in the Purkinje

cells of the adult human cerebellum fixed in formalin, sectioned by freezing, and examined in the phase microscope.

DISCUSSION

The effect of chemical fixatives on the texture of the neurocytoplasm has been studied repeatedly, though in nearly all such studies the form of the Nissl substance has alone been considered. In the year following Nissl's description of the chromophilic granules of the nerve cell (Nissl, 1894), Held (1895) published a classical paper in which the effect of vacuolation on the form of the Nissl substance was clearly demonstrated. Held isolated fresh anterior horn cells from the spinal cord of the guinea-pig and studied them first unfixed but mounted in saline. This treatment by itself produced cytoplasmic vacuolation which was much exaggerated by treatment with water alone. After staining, it was seen that the Nissl material had been distorted into a reticulum whose meshes surrounded these vacuoles.

In 1911 Møllgaard attempted to avoid fixation artefacts by freezing, though at temperatures near enough to 0° C. for the formation of ice crystals of appreciable size between which a fine network of neurocytoplasm was produced. This artefact of the freeze-drying method which even with modern technique can appear in fragments of tissue too large for instantaneous quenching, had already been observed by Key & Retzius (1874). The effect of different chemical fixatives in producing various degrees of aggregation of the Nissl substance has since been studied by Hopkins (1924) and by Sheinen (1932). The form of the Nissl material in neurons fixed by modern freeze-drying technique has been demonstrated by Bensley & Gersh (1933) and by Hyden (1943). Living neurons of chick embryos in culture have been photographed in the ultra-violet by Koenig and Feldman (1953) who find 'that in the cytoplasm, most of the absorbing material is homogenous and unresolved'.

Although the effect of fixatives on the form of the Nissl material has been well investigated, there have hitherto been no comparable studies on the fixation artefacts of the neurofibrillae, even though these structures have been repeatedly studied in the perikaryon of the living neuron (Remak, 1844; Schultze, 1870; Weiss & Wang, 1936; Levi & Meyer, 1937; Murnaghan, 1941; and Thomas, 1947). Several other authors have described neurofibrillae of living nerve fibres, but here it will be as well to discuss separately the fibrillae in the cell body and in the nerve process.

Remak's paper in 1844 was the first to describe neurofibrillae; within a neuron from the ventral ganglionic chain of the crayfish he illustrates an arrangement of striae round the nucleus which follows the curve of the cell wall. Schultze (1870) gives a remarkable drawing of a ganglion cell isolated from the electric lobe of the brain of *Torpedo*. As each nerve process joins the perikaryon, parallel neurofibrillae fan out towards the nucleus, round which a number of concentric lines are also shown. The photomicrograph of living neurons from a culture of a spinal ganglion of the chick given by Weiss & Wang (1936) again shows both fibrillae round the nucleus and also parallel bundles within the neurites, all of which are considered by Murnaghan (1941) to be mitochondria. In none of these examples, moreover, is neurofibrillae shown in the reticular pattern characteristic of fixed and impregnated preparations. The first observer to use the phase-contrast microscope on a living

neuron was Thomas (1947), who examined cell bodies of the snail in which, by this method, he could see a very long chain of minute 'cocci'. This chain was looped round the nucleus with the two ends continued into the axon. In his preparations, Thomas examined the same field with and without phase contrast and stated that 'with ordinary transmitted illumination they [the "cocci"] cannot with certainty be recognized; but if a quick transition from transmitted to phase-contrast illumination be made on the same field, the impression of a very faint striation at once gives place to the well-defined picture'.

During the course of the present work, a similar comparison with ganglionic neurons of the chick in culture led to much the same conclusion. Sufficient contrast to examine or photograph a living cell body by the ordinary microscope is only obtained when the substage aperture is so far reduced that diffraction effects become prominent; when alternate light and dark bands parallel to surfaces of discontinuity such as the cell and nuclear membranes are produced. Hence by phase contrast the cytoplasm of the perikaryon of these neurons has a granular texture, but otherwise a more fibrillar effect is seen. It is thus advisable to regard with caution any appearance of parallel striations in living neurons unless and until the phase microscope has also been used to examine the same material. That there is ample scope for further research of this kind is at once obvious from a glance at the original figures of living neurons given by Remak and Schultze.

In living nerve fibres, however, it is very unlikely that the apparent neurofibrillae are always merely optical artefacts, for some of them at least do not run parallel with the surface membrane. Those of the nerves of the crayfish, for instance, which Remak (1844) originally described, have a wavy course within a straight fibre. Again, in the present work, one instance was seen of a chick ganglion cell in culture where, by phase contrast, a continuous neurofibril was seen lying obliquely across the course of a neurite entering the cell body (Pl. 1, fig. 2). In sections of the cerebellum fixed by freeze-drying, much the same distinction was clearly seen within Purkinje cells between the arrangement of the neurofibrillar material in the dendrite and in the perikaryon. Cowdry (1913), in his remarkable study of the effect of various fixatives and stains on the spinal ganglia of the adult pigeon, shows figures in which parallel neurofibrillae, stained by various methods but without silver impregnation, are restricted to the axon alone.

This difference in form of the same cytoplasmic material in cell body and nerve fibre is incompletely understood. It is shared also by other cell constituents, such as the mitochondria (Nicholson, 1916; and Murnaghan, 1941) and the Nissl material (Pl. 2, fig. 13). Smith (1952) finds that in sympathetic ganglionic neurons of the toad, homogeneous protein-containing droplets are spherical within the perikaryon, but are elongated in the axon hillock. Chambers & Kao (1951) find that oil drops and air-bubbles micro-injected into the gelated cortex of giant nerve fibres of the squid assume ovoid shapes, which these authors ascribe to the influence of a linearly arranged protoplasmic ultra-structure. This, however, has been shown to be qualitatively similar in some neurons in both cell body and nerve fibre by Chinn (1938), who studied the birefringence of living neurons from the leech and the frog.

Within recent years, the nerve fibre alone has been considered in nearly all those

papers which have dealt with neurofibrillae. Hoerr (1936), in the only previous work in which freeze-drying fixation has been applied in this field, found evident neurofibrillae in rabbit axons fixed in this way, either with subsequent silver impregnation or treated with osmic vapour. In most of the recent studies by means of the electron microscope on the nerve fibre, filamentous bodies have been found therein, usually beaded in form (Schmitt, 1950; Fernandez-Moran, 1950; and Duncan, 1951) though opinion is not unanimous on this question. Pease & Baker (1951) concluded that 'neurofibrils in the conventional sense are artefacts of fixation. When fixation is optional, coarse fibrillar structures are to be seen only in the immediate vicinity of the nodes of Ranvier in peripheral axones'. It is evident that not only do different authors have varying concepts of what constitutes a neurofibril but also that the state of aggregation of neurocytoplasmic material into fibrillae is not the same in all nerves nor, moreover, as the present work suggests, is it necessarily identical in cell body and nerve fibre in the same neuron. Questions of definitions and nomenclature must therefore be decided as further work in this field proceeds. Argyrophil elements within neurocytoplasm may be granular or fibrillar in form; they are thus not satisfactorily described by the term 'neurofibrillae'. This word would better be kept strictly for the impregnated filaments of the silvered preparation, with the implication that their form, to a varying extent, is of the nature of an artefact.

SUMMARY

1. The appearance has been compared of neurons from the chick prepared for microscopical observation in various ways.

2. In the cerebellum, Purkinje cells and the neurons of the dentate nucleus, have been studied at or near the hatching stage. Neurons in spinal ganglia of 12-day embryos have been observed in tissue culture.

3. Ganglionic neurons have been studied alive, and unfixed Purkinje cells observed in squashes. Fixation both by freeze-drying and by common chemical fixatives has been employed. The appearance of unstained preparations by phase microscopy has been compared with the effect of methods of silver impregnation.

4. The cytoplasm of the perikaryon either unfixed or frozen-dried has a granular texture. The granules are either rounded or rod-like, and vary in their affinity for silver. In nerve processes, they tend to be longitudinally arranged.

5. Chemical fixatives cause vacuoles to form within the cytoplasm of the neuron. They vary in size with different fixatives, and with different neurons. When large enough, the vacuoles distort the cytoplasm into a network. It is suggested that the typical reticular arrangement of neurofibrillae within the perikaryon in neurohistological preparations is produced by this means.

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EXPLANATION OF PLATES

All figures are photomicrographs of the cell bodies of neurons from chicks either embryonic or within 2 days after hatching. Unstained material photographed by 2 mm. phase-contrast objective; stained preparations by 2 mm. apochromatic objective N.A. 1.30.

PLATE 1

Figs. 1–4 are of unipolar neurons from cultures of spinal ganglia of 12-day chick embryos, grown for 3–6 days *in vitro*; × 1500.

Fig. 1. Fixed in 5% neutral formalin and mounted in glycerol. Very fine vacuoles are present throughout the cytoplasm.

Fig. 2. Phase contrast. Living culture mounted in liquid paraffin. In the neurite, fine neurofibrillae run somewhat obliquely.

Fig. 3. Fixed in 5% neutral formalin and silvered by Holmes's method. Neurofibrillae most distinct in neurite; in cell body, light deposit of silver on fine reticulum.

Fig. 4. Fixed in Carnoy's fluid and silvered by Holmes's method. Heavy silvering of coarse reticulum.

Figs. 5–6 are of neurons from the dentate nucleus in sections of the cerebellum of chicks near or soon after hatching; × 2200.

Fig. 5. Phase contrast. Fixed in 2% osmic acid. Sections mounted in D.P.X. without further staining. Dense granular texture of cytoplasm.

Fig. 6. Fixed by freeze-drying, and silvered by Holmes's method. Argyrophil material consists of granules and short filaments forming an incomplete reticulum.

Fig. 7. Fixed in Carnoy's fluid and silvered by Holmes's method. Reticulum of impregnated fibrils more definite than in fig. 6. Note shrinkage of nuclear contents.

Fig. 8. Fixed in alcohol-ammonia and silvered by Ranson's method. Coarse network of impregnated fibrils.

PLATE 2

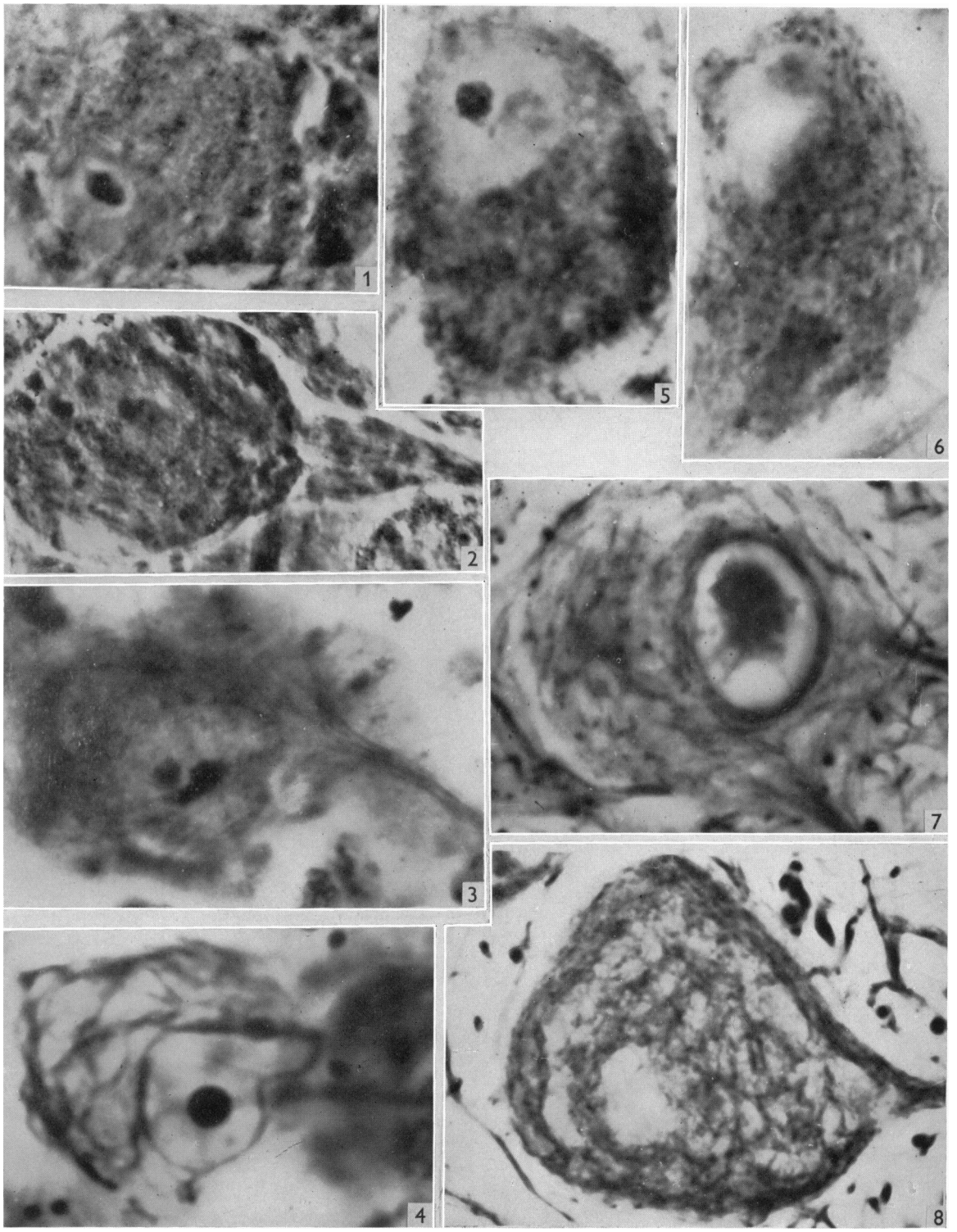
Figs. 9–16 are of Purkinje cells from the cerebellum of chicks near or soon after hatching; × 2000. Reduced 5 = 4.

Fig. 9. Phase contrast. Unfixed squashed preparation. Within the cytoplasm are seen granules or short filaments, which tend to an orientated arrangement towards the dendrite.

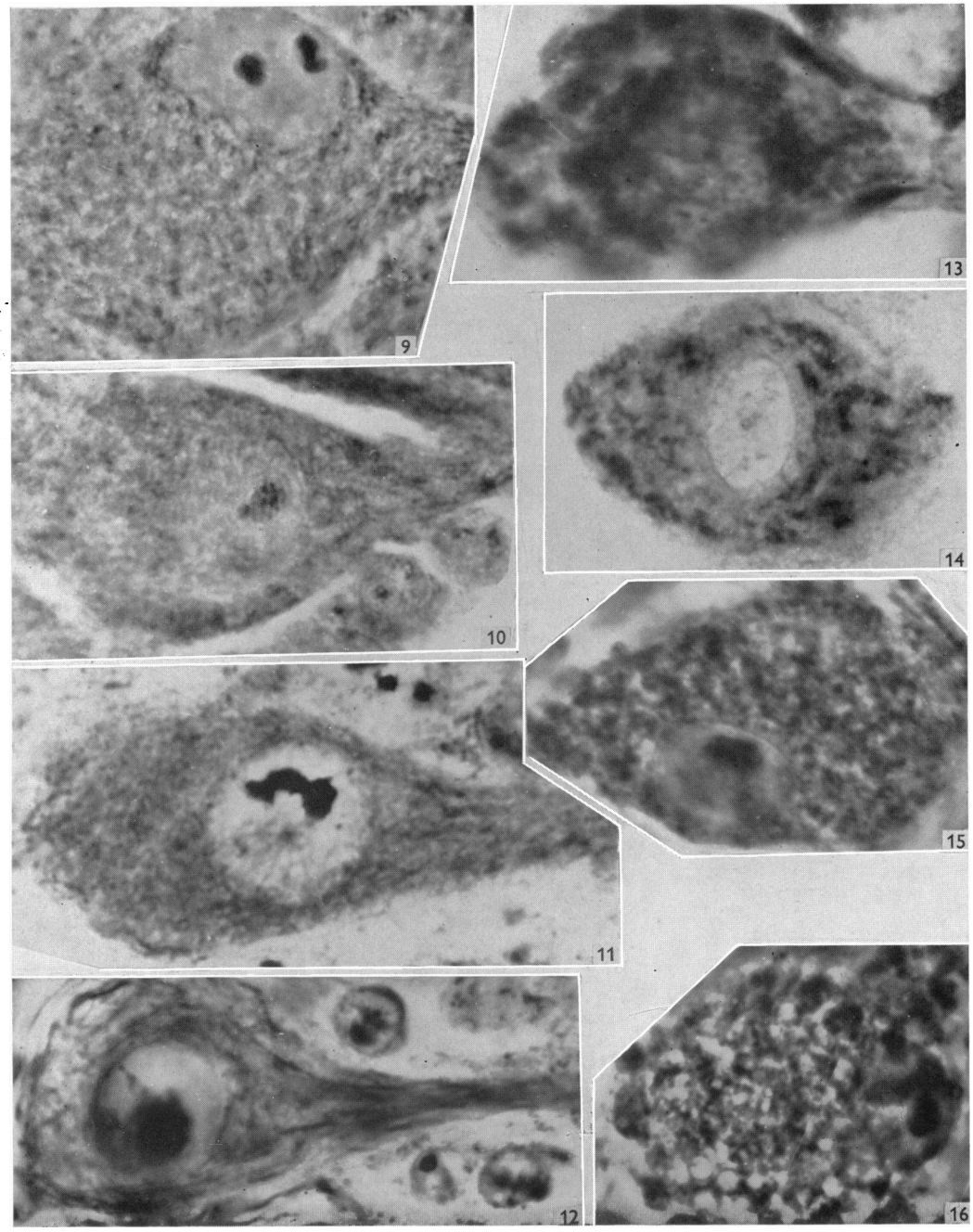
Fig. 10. Phase contrast. Fixed by freeze-drying, sectioned and mounted in liquid paraffin. Granular texture in cytoplasm, with linear arrangement towards dendrite.

Fig. 11. Fixed by freeze-drying, sectioned and silvered by Holmes's method. Similar texture to fig. 10; some granules, however, are more argyrophilic than others.

Fig. 12. Fixed in Carnoy's fluid and silvered by Holmes's method. Network of argyrophil material in cell body; straight filaments in dendrite, which has shrunk in diameter. Also notice shrinkage of nuclear contents.



HUGHES—The effect of fixation on neurons of the chick



HUGHES—THE EFFECT OF FIXATION ON NEURONS OF THE CHICK

Fig. 13. Fixed by freeze-drying and stained in buffered thionin. Nissl substance mainly floccular in texture with tendency towards linear arrangement in dendrite.

Fig. 14. Fixed in Carnoy and stained in buffered thionin. Nissl substance precipitated into definite filament, partly reticular in arrangements.

Fig. 15. Phase contrast. Squashed in 1 % osmic acid. Cytoplasm uniformly filled with vacuoles 1 μ or less in diameter. Note thickening of nuclear membrane.

Fig. 16. Phase contrast. Squashed in 5 % neutral formalin. Cytoplasm filled with larger vacuoles, some of which are more than 2 μ in diameter. Nucleus pushed to extreme right of cell.