AN EXPERIMENTAL STUDY OF THE AVIAN VISUAL SYSTEM

By W. M. COWAN, L. ADAMSON and T. P. S. POWELL

Department of Human Anatomy, University of Oxford

INTRODUCTION

In a recent experimental study of the thalamic projection upon the telencephalon in the pigeon (Powell & Cowan, 1961), an unexpected finding after telencephalic lesions was the absence of any retrograde cell degeneration in the diencephalic nuclei said to receive afferent fibres from the retina. Taken by itself this finding need not be viewed as evidence against a telencephalic projection in the avian visual system because the pathway from these relay nuclei to the telencephalon may be multisynaptic or their cells may be resistant to axonal section. In accord with the first of these alternative explanations are the electrophysiological observations of Bremer, Dow & Morruzzi (1939) who recorded a marked 'on-response' from the cerebral cortex in the pigeon on illumination of the contralateral eye.

Previous studies of the avian visual system have been made either on normal material or on material stained to show degenerating myelinated fibres (Kappers, Huber & Crosby, 1936). To exclude the possibility of a visual projection to other diencephalic areas, and in particular to those nuclei which have a telencephalic projection, it is desirable that the visual pathway be re-examined with the recently developed axonal degeneration techniques which have been used so successfully in the mammalian and reptilian brains. Until this has been done it must remain uncertain whether the avian visual system more closely resembles that of the mammal or that of the lizard in which there does not appear to be a direct diencephalic relay to the telencephalon (Armstrong, 1950; Powell & Kruger, 1960). That the silver degeneration techniques can be applied to the avian brain has been shown by the comparative study of Evans & Hamlyn (1956) on the relative advantages of the Glees and Nauta techniques. Their finding that axonal and terminal degeneration was as marked in the avian brain as in the mammal also suggested that the avian visual system might provide valuable material for examining the usefulness of other degeneration techniques, in particular of paraffin 'on-the-slide' methods.

In the present study the course and termination of optic nerve fibres has been investigated in the pigeon after unilateral enucleation of the eye using a number of silver methods. In addition we have paid particular attention to the question of centrifugal fibres to the retina and to the possibility of a direct projection to the hypothalamus from the visual system which might be concerned with the regulation of endocrine activity.

MATERIAL AND METHODS

The brains of 14 pigeons were used in this study. In 11 of these unilateral eye enucleation was performed under ether anaesthesia, and, with one exception, the birds were allowed to survive for periods ranging from 5 to 26 days; these brains were fixed by immersion in 10% formal saline. The remaining pigeon survived for 60 days and its brain was fixed in 70% alcohol and 2% acetic acid. Two normal brains fixed in 10% formal saline were used as controls. In addition a number of other brains with no involvement of the visual pathway which had been stained with thionine and Bodian's protargol method were available for comparison.

Table 1

Experiment	Survival period (days)	Plane of section	Preparation
		A. Frozen section methods	
AP 5	5	Coronal	Glees, Bodian, Nauta & Gygax (1954), cresyl violet
AP 6	8		
AP 3	12		
AP 4	15		
AP 1	18		
AP 7	22		
AP 2	26		
AP N	Normal control		
		B. Paraffin methods	
AP 3A	12	Horizontal	Marsland, Glees & Erikson, Bodian, Guillery *et al.*, Nauta & Gygax (1951), thionine
AP 1A	18	Coronal	
AP 2A	26	Horizontal	
AP N1	Normal control	Coronal	
EP 1	60	Coronal	Bodian, thionine

Most of the brains were sectioned on a freezing microtome at 12μ or 25μ. The sections were collected in groups of 4 and from each brain a 1 in 16 series, stained according to the Nauta & Gygax (1954) technique, and a second series with the Glees (1946) technique. Two additional series were mounted on gelatinized slides and stained with cresyl violet or, after a coating with thin celloidin, according to the Bodian method. Concurrent with each experimental series a corresponding 1 in 32 series of normal sections were mounted and stained. Three brains were embedded in paraffin wax and cut at 15μ; in each case a 1 in 10 series was stained according to the original Nauta & Gygax (1951) technique, the recent modification of this technique by Guillery, Shirra & Webster (1960), the method described by Marsland, Glees & Erikson (1954), Bodian's (1936) protargol method, and thionine. The alcohol fixed brain was embedded in paraffin wax, sectioned at 25μ and stained with thionine and protargol. The details of the preparation of the 11 experimental and 2 control brains are set out in Table 1.

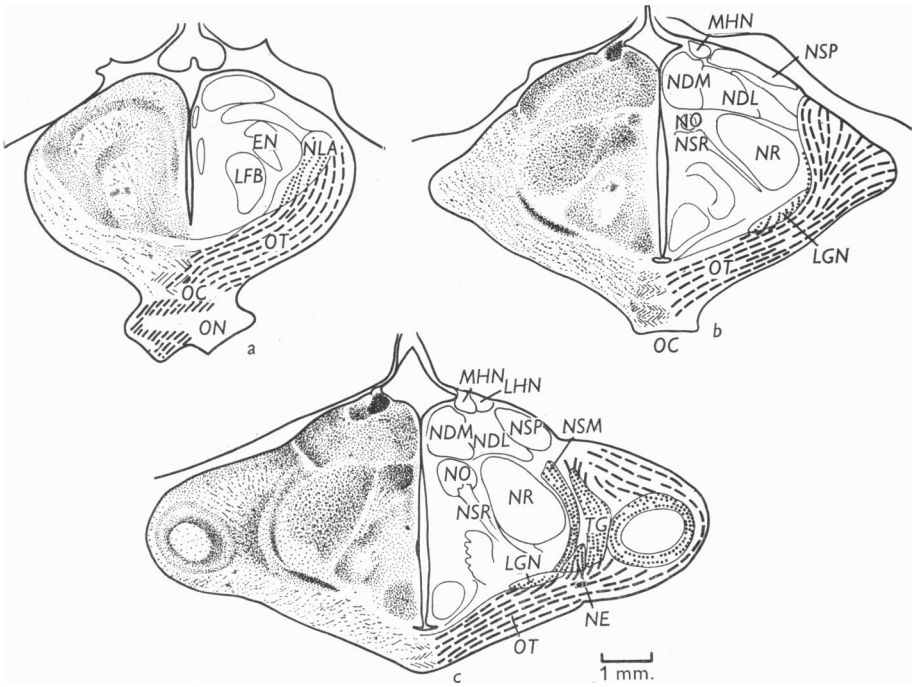
RESULTS

Apart from differences in the degree of axonal and terminal degeneration depending upon the survival period, the experiments are remarkably uniform in their results. For the description of the visual pathway, therefore, only one experiment of

moderate survival period need be described in detail. The differences found after differing survival periods and with the various techniques will be described in a following section.

The visual pathway in the pigeon brain

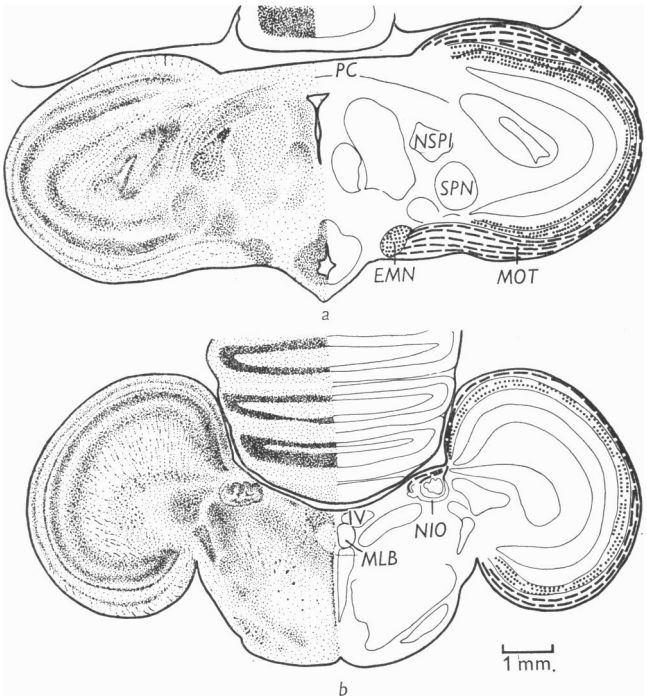
The course and termination of the fibre degeneration will be described as seen in successive antero-posterior sections cut in a coronal plane. The description of the course of the degenerating fibres is based on Nauta-stained sections, and the



Text-fig. 1. The disposition of the principal nuclei of the diencephalon and the related visual pathways at three antero-posterior levels (*a*, *b*, *c*). The cell masses are shown semi-diagrammatically on the left and in outline on the right; the sites of fibre degeneration (broken lines) and terminal degeneration (dots) are also shown on the right. The outlines in this and the subsequent figures were drawn with the aid of a low-power projection apparatus at an original magnification of 20 ×.

terminal degeneration upon Glees and Bodian preparations. Following enucleation of the right eye the optic nerve is severely degenerated throughout its cross-sectional area, and this degeneration can be traced through the optic chiasma where the fibres undergo complete decussation. While it is not feasible to exclude the possibility that a few fibres continue into the optic tract of the ipsilateral side careful examination of all our experimental material, and of the control sections from the normals brains, has failed to show any appreciable number of degenerating fibres on the ipsilateral side, and certainly there is no fasciculus as Armstrong (1950) has described in the lizard brain. The characteristic pattern of normal and degenerating fibres in the optic chiasma is shown in Pl. 1, fig. 2.

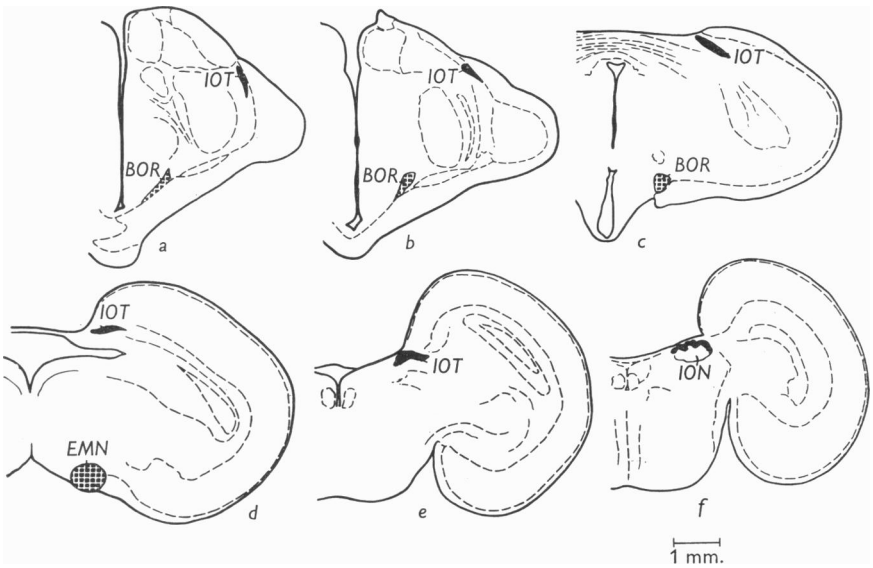
The degenerating fibres pass backwards and laterally into the optic tract, the ventral two-thirds of which is completely degenerated; in the dorsal third, adjacent to the ventral surface of the brain, the amount of degeneration is much less. In under-suppressed material a considerable number of normal fibres are seen in this dorsal part. More posteriorly, in what is known as the marginal optic tract these normal fibres form a distinct bundle along the ventral aspect of the brain and although they can be traced anteriorly into the region of the optic chiasma it has not been possible to determine their further course. The corresponding area in the ipsilateral marginal optic tract contains no degenerating fibres.



Text-fig. 2. Drawings of the nuclei associated with the visual projection at two representative levels (*a*) through the ectomammillary nucleus and (*b*) through the isthmo-optic nucleus at the level of the IVth nerve nucleus.

At the level of the anterior end of the thalamus a diffuse bundle of fibres sweeps dorsally and laterally above and in front of the lateral geniculate nucleus and below the lateral forebrain bundle towards the nucleus lateralis anterior. As it approaches this nucleus the fibres fan out and penetrate it from all aspects except posteriorly and laterally. Within the nucleus preterminal fibre degeneration is seen throughout its extent, and in the Glees and Bodian preparations numerous ring-like boutons are found around many of the cells; in the lateral geniculate nucleus of the opposite side only an occasional bouton can be seen. No degeneration is seen dorsal or posterior to the nucleus indicating that this bundle is terminating only in this nucleus (Text-fig. 1*a*).

More posteriorly, numerous fibres are seen turning dorsally at right angles from the tract to enter the overlying lateral geniculate nucleus (Text-fig. 1*b*, *c*). Within this nucleus most of the fibre degeneration is confined to the ventral part of the nucleus which contains only a few scattered small cells. However, most of the degenerating boutons, shown by the Glees and Bodian techniques, are found amongst the cells of the dorsal part of the nucleus. The degeneration in the lateral geniculate nucleus remains the same throughout its antero-posterior extent. In addition to the fibres which are terminating in the nucleus many degenerating coarse fibres pass through and along the dorsal aspect of the nucleus. Laterally these fibres unite with other large fibres from the optic tract to form the axillary or isthmo-optic bundle.



Text-fig. 3. Tracings at successive antero-posterior levels to show the course and relations of the basal optic root (stippled) and the isthmo-optic tract (solid black).

At the level of the anterior end of the optic tectum a considerable number of fibres are given off at right angles to the tract in the region of the nucleus externus and the so-called tectal grey. In addition to dense preterminal degeneration within these cell masses (see Pl. 1, fig. 4) distinct bundles of fibres can be seen passing dorsally along their medial and lateral aspects to reach the nucleus superficialis synencephali and the deeper parts of the tectal grey (Text-fig. 1*c*). With the appropriate stains numerous degenerating boutons are found in all these nuclei, and they are exceptionally dense in the nucleus externus. For the sake of convenience, it may be pointed out here that the dorsal part of the tectal grey also receives numerous degenerating fibres descending from the dorsal part of the marginal optic tract.

Just behind the level of the optic chiasma distinctly coarse fibres collect on the dorso-medial aspect of the optic chiasma to form the basal optic root (stippled in

Text-fig. 3). More posteriorly this forms a wedge-shaped bundle which passes backwards close to the medial edge of the optic tract to reach the ectomammillary nucleus which is filled with fragmented fibres. Although few boutons in the form of rings are seen, many large, solid end-bulbs are found in close proximity to the cells (Pl. 2, figs. 8, 9).

The isthmo-optic tract first appears as a distinct bundle in the region between the tectal grey and the nucleus rotundus. It arches dorsally and backwards beneath the dorsal margin of the optic tectum and then turns ventro-medially to reach the nucleus of the same name. Approaching the nucleus from its dorso-lateral aspect the fibres spread out to surround all but its ventral surface (Text-fig. 3). Although the fibres of the tract are fragmented right up to, and clearly outline, the nucleus, no fibre degeneration is seen within the limits of the nucleus or among the cells. Similarly, in neither the Glees nor the Bodian preparations is there any evidence of terminal degeneration within the nucleus, although again the fibres of the tract are clearly degenerated. The interpretation of these findings will be discussed later, but it may be noted that in no experiment, nor with any technique, is there any sign of terminal degeneration within the nucleus, and only after a survival period of 26 days is any change found in the neuropil of the nucleus.

Although a careful search was made throughout the diencephalon no evidence of terminal degeneration was found in any nuclei apart from those already mentioned. In particular, no projection of optic nerve fibres could be traced to the hypothalamus or to the nucleus rotundus and pretectal nuclei of the thalamus.

The principal part of the visual projection is undoubtedly to the optic tectum. The marginal optic tract, on reaching the antero-ventral aspect of the tectum, spreads out over its entire surface so that the fibres of the tract come to form the outer layer or stratum opticum of the tectum. Before describing the distribution of degeneration it is necessary to comment upon the terminology used in describing the layers of the optic tectum. The basis of most recent accounts of this region is the comprehensive study of Huber & Crosby (1933) on the reptilian tectum. Their subdivision into six primary layers has been followed by Jungherr (1945) whose description of the chicken tectum we have found to be sufficiently similar to that of the pigeon as not to warrant a separate description of our material (see Pl. 1, fig. 1). These layers are, from without inwards:

(1) stratum opticum;
(2) stratum griseum et fibrosum superficiale;
(3) stratum griseum centrale;
(4) stratum album centrale;
(5) stratum griseum periventriculare;
(6) stratum fibrosum periventriculare.

As no terminal degeneration is found deeper than the second of these layers no further reference will be made to the deeper layers. In the interpretation of the degeneration it is necessary, however, to further subdivide the stratum griseum et fibrosum superficiale. In Nissl-stained perparations (Pl. 1, fig. 1) the following layers can be recognized.

(*a*) a narrow layer of small cells;
(*b*) a slightly thicker, relatively cell-free layer;

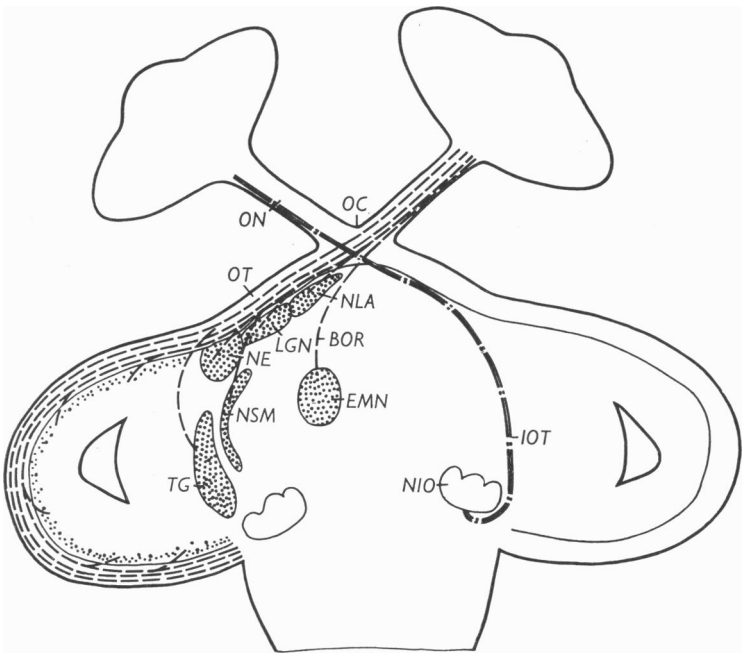
(*c*) a layer of medium and small-sized cells;
(*d*) a second almost cell-free layer;
(*e*) a single layer of medium-sized, pale-staining cells;
(*f*) a narrow cell poor layer;
(*g*) a very narrow layer of deeply staining and compactly arranged cells;
(*h*) a broad layer containing cells of variable size and occasional pyramidal neurons;
(*i*) a thicker layer of more compactly grouped cells of uniform size;
(*j*) a cell poor layer.

The only difference between this description and that of Jungherr (1945) is that the latter makes no mention of the outermost layer of small cells—layer (*a*).

In the experimental material the superficial part of the stratum opticum shows marked fragmentation of all the optic nerve fibres, but in the deepest part, adjacent to the stratum griseum et fibrosum superficiale, there is a considerable number of normal fibres remaining. The number of these normal fibres appears to increase further rostrally in the marginal optic tract. From the stratum opticum fibres enter the stratum griseum et fibrosum superficiale at an acute angle and ramify in its outer part, the appearance of the degeneration varying in the different laminae of this zone. In layers (*a*) and (*b*) the degenerating fibres form an irregular plexus, which appears to be most dense in the more superficial layer in which many of the fibres can be seen to run horizontally for some distance and to break up into fragments. There are comparatively few degenerating fibres in layer (*c*), but in laminae (*d*) and (*e*) distinct degenerative changes have occurred in the radially orientated fibres which form such a prominent feature of these layers in normal material. These fibres are nearly all broken up into isolated fragments but retain their radial arrangement. In the Glees preparations there is definite fragmentation of the fibres in the optic nerve layer, and in the immediately subjacent layer there are a number of degenerating fibres scattered irregularly amongst the normal fibres together with a moderate number of degenerating boutons. In layer (*b*) of the stratum griseum et fibrosum superficiale there are slightly more boutons than in (*a*), but apart from this the fibre plexus shows no change. The fragmented fibres in layer (*c*) are appreciably darker staining but only an occasional bouton is seen. The radial fibres of layer (*d*) are very irregular in both their course and their depth of staining, and many are coiled. The fibres are also distinctly shorter than on the normal side, and, especially in the deeper part of the lamina, appear to be beaded. Throughout the depth of this layer there is an exceptionally large number of boutons, and these are found mainly in the form of rings of variable staining intensity. Amongst the cells of layer (*e*) solid, deeply staining end-bulbs are present; the majority of these are seen in continuity with a short, deeply staining terminal segment of an axon. In the adjacent molecular layer (*f*) occasional boutons and degenerating terminals are seen. It is interesting that in both these layers the boutons are in continuity with fibres which are orientated either obliquely or parallel to those in the stratum opticum or in many cases curl back on themselves; no fibres have been seen to terminate without turning in one or other of these three ways. No boutons or degenerating fibres are seen deeper than this level.

The appearance of the outer layers of the tectum is essentially the same in the Bodian preparations, with the exception that there is no evidence of fibre fragmentation in the stratum opticum or in layer (*a*). In the other affected layers the only appreciable difference is the absence of beaded fibres in layer (*d*); the other degenerative changes in the layer, however, closely resemble those found in the Glees preparations (Pl. 1, fig. 3).

A common finding in all the material was the greater density of boutons in the lateral part of the tectum as compared with more medial regions. This is of interest in the light of the electrophysiological evidence of Hamdi & Whitteridge (1954) that the central portion of the retina projects to the lateral part of the tectum. To



Text-fig. 4. A schematic diagram to show the main features of the visual projection as determined in this study. The broken lines indicate pathways in which fibre degeneration has been traced and the dotted areas in which terminal degeneration is found. The isthmo-optic nucleus and tract are shown separately on the right for reasons given in the text.

confirm this qualitive impression of bouton density a series of counts of the boutons was done in one experiment. Using a graticule inserted into the eyepiece, all the boutons in an area 0·1 mm.^{2} were counted in the dorso-medial, ventro-medial and lateral sectors of the stratum griseum et fibrosum superficiale respectively. At the medial margins of the tectum the mean counts were 194 and 187, while in the lateral area there were 258 boutons. These counts indicate that in the central projection field of the retina there is an increase of approximately one-third in the number of boutons.

The description of the visual pathway which has been given above is typical of the findings in all the coronally sectioned brains, and is essentially similar to our

findings in horizontal preparations although these show some additional features of interest. First, the course of the fibres to the nucleus lateralis anterior is seen particularly clearly in horizontal sections, and in addition they show that the afferents to the tectal grey enter it from all aspects. Secondly, the course and relations of the basal optic root are seen distinctly because in several sections the tract can be traced throughout its entire antero-posterior extent. Another feature is the greater ease with which the nuclei externus, superficialis synencephali and tectal grey can be differentiated where they lie in the angle between the nucleus rotundus, the lateral geniculate and the tectum. One of the most striking features of the horizontal material is the clarity with which the bundle of normal fibres in the deeper part of the stratum opticum can be traced medially into the region of the optic chiasma.

Having given an account of the course of the visual pathways in the pigeon we shall now describe the differences in appearances of the degenerating nerve fibres and terminals after varying survival periods and as seen in material prepared with several silver impregnation techniques. Subsequently we shall present certain unexpected findings in this material bearing on the question of a possible centrifugal projection to the retina and on the interpretation of axonal degeneration in the nervous system.

Observations on the nature and time course of fibre degeneration

From the point of view of tracing the central connexions of the retina all the techniques used in this study have given essentially similar and equally consistent results. It would have been possible to determine the principal sites of termination of optic afferents with any single technique, but by using most or all of them on the same material (i.e. on successive sections) we have obtained both a clearer picture of the course and mode of fibre termination and also of the nature of the degenerative process.

The relative advantages of the Nauta and Glees methods as commonly used on frozen sections are well known (Glees & Nauta, 1955; Evans & Hamlyn, 1956; Bowsher, Brodal & Walberg, 1960). Thus, while the Nauta technique selectively stains degenerating fibres through the suppression of normal fibres it does not impregnate the finer axonal terminals and boutons; the Glees method, on the other hand, will demonstrate degenerating terminals and boutons in addition to the degenerating fibres, but this degeneration is considerably more difficult to distinguish as the normal fibre plexus is also impregnated. In our hands the technique for paraffin sections described by Marsland *et al.* (1954) gives essentially the same picture as the original Glees method, but the appearance of degeneration and of the normal fibre plexus in sections stained with either the Nauta & Gygax (1951) or the recent modification of the method (Guillery *et al.* 1960) differs in a number of important respects from the frozen section method as now commonly used (Nauta, 1957). Apart from the obvious advantage that serial sections are more easily obtained with paraffin embedded material, the most significant advantage of the latter technique is that it stains fine terminals and boutons as well as giving a considerable suppression of normal fibres. The principal fibre tracts are usually impregnated so that the appearance is more or less intermediate between the

conventional Glees and Nauta techniques. The staining of these fibre bundles is not necessarily disadvantageous; for example, in the thalamus this impregnation of normal fibres serves to delimit clearly many of the nuclei. The only thing that need be said about the use of the Bodian technique is that, while it has not generally been used for experimental purposes to show degenerating terminals, we have found that it stains degenerating boutons with even greater clarity than the Glees method, but is less useful for determining degeneration of fibre tracts.

The differences in appearance at varying survival periods with the Glees and Nauta techniques on frozen sections have already been described for the avian optic tectum by Evans & Hamlyn (1956). Our findings with these methods are in essential agreement with theirs, but it is necessary to describe the appearance of the other optic centres and to give the findings in the material prepared with the paraffin methods we have used. Because the findings in the lateral geniculate nucleus, the nuclei lateralis anterior, externus and synencephali and the tectal grey are basically the same, these nuclei may be grouped together in the following account.

It should be emphasized that very few boutons are seen in normal sections through these areas or on the ipsilateral side in the experimental material with any of the techniques used. As Armstrong (1950) found in the visual system of the lizard, and as is well known in mammalian experimental material, one of the earliest signs of degeneration in these nuclei is the appearance of numerous clear, ring-like boutons. Were it not for their absence in control material these early degenerating boutons would be indistinguishable from 'normal boutons' as commonly described. Subsequently the degenerating rings become thickened, enlarged and finally converted into solid end-bulbs.

The earliest degenerative changes are found in these areas 5 days after eye-enucleation. In the Glees and Bodian preparations a moderate number of boutons are found scattered throughout the nuclei together with increased argyrophilia of the terminal fibres. At this stage the Nauta sections show only an irregular impregnation and spindling of the optic nerve fibres, the control side showing a complete suppression of staining. The changes at 8 days are so little advanced as to require no further comment, but in the two animals which survived 12 days there is an appreciable change in the degree of degeneration. The brain of one of these animals had been embedded in paraffin and sectioned horizontally; in the other, frozen sections were cut in the coronal plane. In the frozen sections stained with the Nauta technique numerous degenerated fibres are seen either in a beaded or fragmented condition. In the lateral geniculate nucleus the degeneration is more conspicious in the outer plexiform layer and in the tectal grey degeneration is arranged in a definite peri-cellular pattern. In the Glees and Bodian preparations of these sections more degenerating boutons and terminals are seen than at any other survival period. The boutons are in all stages of degeneration from simple rings to solid end-bulbs, many of which are attached to a short segment of a terminal fibre. In addition, in the Glees sections many degenerating fibres are seen with a characteristic beaded and fragmented appearance. The most striking feature of the paraffin Nauta sections through these areas is the presence of a considerable number of degenerating boutons having essentially the same appearance as in the Glees

sections. This terminal degeneration is, of course, in addition to the fragmented fibres which can be traced into and through these areas.

At longer survival periods the number of degenerating boutons progressively decreases until at 22 days only an occasional ring is seen. The fibre degeneration, on the other hand, becomes progressively more severe up to 22 days after which there is an appreciable loss of neuropil in these nuclei.

The much larger myelinated fibres which pass through the basal optic root to reach the ectomammillary nucleus appear to degenerate more rapidly than the optic tract fibres. As early as 5 days after enucleation the Nauta technique on frozen sections shows some fragmentation of the fibres in the basal optic root and scattered argyrophilic droplets in the ectomammillary nucleus. In addition, there is, in and around the nucleus, a considerable number of coarse rings comparable to those described by Evans & Hamlyn (1956) in preparations subjected to 'lipid fixation' prior to treatment with fat solvents. These are quite distinct from the boutons seen in the paraffin Nauta sections after slightly longer survival periods. In the Glees preparations at 5 days there is some increased argyrophilia of the fibres of the basal optic root and in the ectomammillary nucleus there are many ring-like boutons. The Bodian preparations of the tract give no evidence of degeneration but there is an appreciable number of boutons in the nucleus. At 12 days the degeneration in this system has progressed to an advanced degree: all the preparations show a virtually complete break-up of the fibres of the tract into large deeply staining droplets, and in the Bodian material there is an appreciable fibre-loss (see Pl. 2, figs. 6, 7). Longer survival periods show a continuing fibre loss although there is still a good deal of degeneration in both the tract and nucleus even at 26 days.

Our findings on the time course of the degeneration in the optic tectum parallel those of Evans & Hamlyn (1956) and only a few significant differences need be described. The first of these is that the degeneration appears to occur more rapidly in the pigeon than in the chicken. At 5 days the Glees and Bodian preparations show not only an increased argyrophilia in the fibres of the outer layers of the tectum but also numerous boutons, especially in the deeper parts of the affected region. The Nauta degeneration is also fully developed by 12–15 days and the paraffin Nauta sections show a considerable number of rings and drop-like disintegration of the fibres at this stage. With longer survival periods the degeneration becomes more limited to the superficial layers of the tectum, and by 26 days there is a considerable loss of the radially orientated fibres in the stratum griseum et fibrosum superficiale. At this stage the Bodian preparations show a very severe fibre loss in the outer two-thirds of the stratum opticum, but in the deeper part of this layer there are numerous, apparently normal, fibres persisting. The number of these fibres increases in the ventro-medial part of the tectum where they can be traced quite clearly into the marginal optic tract and hence to the region of the optic chiasma. There is no loss of fibres in the comparable region of the ipsilateral tectum.

As mentioned above, no evidence of degenerating boutons or pericellular fibre fragmentation has been seen in the nucleus isthmo-opticus after any survival period or with any of the techniques used but there is some loss of neuropil and break-up of the coarse fibres in the nucleus after 26 days. Furthermore, although the diameter of the fibres in the isthmo-optic tract is slightly greater than those in the optic tract,

the time course of the degeneration is appreciably slower. No degeneration is seen until 12 days and at this stage it is present only in the part of the tract immediately adjacent to the nucleus, and can only be seen in the frozen Nauta sections. By 18 days the whole length of the tract is affected, but apart from the sections close to the nucleus the fibres show only increased argyrophilia and irregularity of outline. Beading of fibres along their whole length is not seen until 22 days and the degeneration is not fully developed until 26 days. These findings present a paradox: after short survival periods there is distinct degeneration in the tract close to the nucleus, and although the degenerating fibres surround the nucleus on all but its ventromedial aspect, no degenerating fibres or boutons can be seen within the nucleus. On the other hand, after long survival periods when the tract is degenerate throughout its extent slight fragmentation of fibres is seen within the nucleus but with no evidence of boutons (Pl. 3, figs. 12, 13). A second distinctive feature of degeneration in this nucleus is the fact that only here do cellular changes occur after eye enucleation. In thionin-stained preparations shrinkage and pallor of the cells has been found after 18 days. At 26 days this neuronal atrophy is more severe: the nucleus as a whole is smaller and there is appreciable gliosis throughout the nucleus. After 2 months severe cell-loss has occurred and the surviving cells are shrunken and poorly stained (Pl. 4, figs. 14–16). The possible interpretation of these findings will be discussed later.

One interesting finding in this material will be described although it has no direct bearing on the central projection of the retina but is relevant to the interpretation of the isthmo-optic tract. In all experiments with survival periods of 12 days or longer unequivocal fibre degeneration has been found in the IVth cranial nerve on the affected side. At 12 days the proximal part of the nerve from the dorso-lateral aspect of the nucleus to the decussation on the dorsum of the midbrain shows distinct axonal break-up. In the trochlear decussation and in the more distant parts of the nerve the axons appear completely normal. By 18 days the degeneration has extended into the decussation and here the intermingling of the degenerating axons with the normal fibres from the opposite side can be clearly seen. By 26 days the degeneration has extended into the nerve where it lies on the lateral aspect of the midbrain (Pl. 3, figs. 10, 11). This description of degeneration in the IVth nerve applies only to material prepared with the Nauta technique (frozen and paraffin). In adjacent sections stained with the Glees and Bodian methods no changes were found until 26 days when the Glees sections show increased argyrophilia and irregularity of the degenerating axons. Even at this stage the Bodian technique shows only slight narrowing and varicosity of the fibres. Although no fibre degeneration was seen within the IVth nerve nucleus at any stage between 15 and 26 days, the cells of this nucleus showed characteristic chromatolytic changes; by 2 months these cells have fully recovered their normal appearance. Similar cellular and fibre changes have been seen in the IIIrd cranial nerve but because of the bilateral distribution of the fibres in this nerve these changes have been more difficult to assess; no statement will be made about the VIth nerve as the plane of section of most of the material did not permit of a systematic study of the lower brain stem.

DISCUSSION

The account given of the central projection of the optic nerve fibres in this article is in close agreement with descriptions in the older literature based upon the study of normal and Marchi-stained material (Huber & Crosby, 1929). Although a variety of silver techniques has been used in this study almost any single technique would have proved adequate for tracing the central connexions of the retina, and either paraffin or frozen sections could have been prepared. Each technique has, of course, certain advantages and disadvantages for a study of this type. For example, in the Nauta & Gygax method (1951) and in the modification of this technique by Guillery *et al.* (1960), not only are the degenerating fibres clearly shown but the terminal boutons are also impregnated. On the other hand, while degenerating boutons are shown as clearly with the Bodian technique as with any of the others, it is much less useful than the latter for tracing degenerating fibres.

Taken together with the previous findings of a study of the thalamic projection upon the telencephalon (Powell & Cowan, 1961), it appears that there is no direct relay of visual impulses from the thalamus to the telencephalon. In addition, a re-examination of the same material has failed to give any evidence of a projection to the telencephalon from the mesencephalic nuclei which receive retinal afferents. In view of these findings the identification of the pathways mediating the visual responses in the cerebral hemisphere found by Bremer *et al.* (1939) must await further investigation. Two possibilities may be mentioned. In the first place, as we have discussed previously, absence of retrogade cell degeneration following removal of the cerebral hemisphere does not necessarily exclude a direct projection from one of these nuclei. Secondly, the projection from one of these visual relay nuclei to the telencephalon could be through an indirect, multi-synaptic pathway; for example, fibres may pass from the tectum or relay nuclei to either the dorsal or central nuclear groups of the thalamus. From these nuclear groups impulses originating in the retina could then be relayed to the cortex or striatum. It may be noted here that Bremer *et al.* (1939) were unable to determine whether the responses which they obtained were cortical or subcortical in nature.

Although the visual centres of the avian brain, and particularly the tectum, are more highly developed than in the reptile there appears to be a basic similarity in the organization of the retinal projection in these two classes of vertebrates. After enucleation of the eye in *Lacerta*, Armstrong (1950) found terminal degeneration in the lateral geniculate nucleus, certain pretectal nuclei and the tectum. A further similarity is that none of these nuclei were found to undergo retrograde cell degeneration after removal of the telencephalon (Powell & Kruger, 1960), suggesting again that there is no direct relay to the telencephalon. The organization of the visual system in both birds and reptiles is therefore completely different from that found in the higher mammals where the neocortical projection from the thalamus has assumed a more prominent role than that to the midbrain.

Apart from the probable absence of a direct telencephalic relay system, the avian visual pathway is at least as complex as that found in the mammal. In all we have found eight distinct sites of termination of the optic nerve fibres. Following the classification of Huber & Crosby (1929) five of these nuclei are thalamic: the

nucleus lateralis anterior, the lateral geniculate nucleus, the nucleus synencephali, the ectomammillary nucleus and the nucleus externus. Of these nuclei, the lateral geniculate and ectomammillary nuclei have many features in common with the lateral geniculate nucleus and nucleus opticus tegmenti respectively of the reptilian brain. Thus, in the case of the lateral geniculate nucleus it is not only apparent that it has a similar topographical position, being adjacent to the optic tract in the ventrolateral part of the thalamus, but that in these two species these nuclei resemble one another in their morphology, both being readily divisible into an inner cellular and an outer plexiform layer. The ectomammillary nucleus of the avian thalamus and the nucleus opticus tegmenti of the reptile also closely resemble each other in their topographical position, and in receiving optic nerve fibres through the basal optic root: moreover, in both cases these fibres are larger and degenerate more rapidly than those of the main optic tract. In the absence of experimental evidence on the efferent connexions of these nuclei the validity of these homologies remains uncertain. The suggestion of Armstrong (1950) that the basal optic root and the nucleus opticus tegmenti of the reptile subserve visual reflex functions may apply equally well in the pigeon, especially in view of the observation of Huber & Crosby (1929) that the ectomammillary nucleus is connected with the oculomotor nuclei. It is difficult to suggest homologies for the nucleus lateralis anterior or the nucleus synencephali. It is possible, however, that the nucleus lateralis anterior is the avian homologue of the nucleus ovalis of the reptile to which earlier workers traced optic nerve fibres, although Armstrong (1950) found no terminal degeneration in this region. Similarly, it may be suggested, on topographical grounds, that the nucleus synencephali is homologous with the dorsal part of the lateral geniculate of reptiles. Although the nucleus externus is the smallest and least well defined of these thalamic nuclei in Nissl-stained material, the severity of degeneration in this nucleus after eye enucleation is particularly striking. Indeed, the density of boutons in the nucleus externus is as great as we have seen in any of the visual centres.

As is well known, the optic tectum is the principal site of termination of retinal fibres. Our degeneration experiments have confirmed the older observations based upon Golgi-stained material that the optic nerve fibres, after ramifying on the surface of the tectum, penetrate quite deeply into the stratum griseum et fibrosum superficiale (Cajal, 1911). Unequivocal terminal degeneration has been found throughout the outer half of this area reaching inwards as far as layer (*f*) of the classification given above which corresponds with the seventh layer of Cajal's description (cf. his figure 132). The fibres which penetrate most deeply have been found to undergo the earliest degenerative changes, while the maximum degeneration in the outer layers is not found until much later. This difference in the time course of degeneration in the superficial and deeper layers could be due either to different rates of degeneration in fibres ending at the various levels or to the fact that the collaterals given off to the more superficial layers by an incoming fibre fragment more slowly than the terminal portion of the axon. These experimental findings, although in agreement with the description of earlier authors using Golgi-stained material are at variance with the more recent work of Cragg, Evans & Hamlyn (1954) who, in their Golgi–Cox material, could not find terminal ramifications at levels deeper than the superficial plexiform layer (equivalent to our layers

(*a*) and (*b*)). In a later experimental study, however, Evans & Hamlyn (1956) illustrate terminal degeneration in what is clearly the 'radial fibre layer' of their classification, i.e. the deeper parts of the stratum griseum et fibrosum superficiale.

One feature of our findings on the tectum which was made most clearly in the Bodian preparations is the persistence of a substantial number of fibres in the deeper part of the stratum opticum even 26 days after removal of the contralateral eye. These fibres are more numerous in the ventro-medial part of the tectum where they can be traced forwards and medially into the optic chiasma. Their further course and direction of conduction is unknown, but there are three possible sites to which they may be directed or from which they may arise: the thalamus and hypothalamus of the same or opposite side, or the contralateral tectum. Cajal (1911) has described fibres entering the stratum opticum from deeper layers of the tectum but leaves their further course unspecified. In the tectum these fibres are only seen clearly in the Bodian preparations. The ease with which these fibres could be seen and traced in the Bodian preparations, especially after long periods, illustrates an additional advantage of this technique which might be used more widely in other studies.

Our findings on the nucleus isthmo-opticus present a number of problems for discussion. First, while there is unequivocal evidence of fibre degeneration in the isthmo-optic tract no terminal degeneration has been found in the nucleus after any survival period. The normal fibre plexus in the nucleus has always been clearly impregnated, but careful examination of material stained with all techniques has failed to show boutons or preterminal degeneration; it was not until 28 days after eye enucleation that some fibre break-up was seen, and by this time the cells of the nucleus showed fairly advanced chromatolysis. Secondly, this nucleus is the only site in the visual projection pathway in which definite cellular changes have been observed. These changes are first seen 18 days after enucleation and proceed over the following 6 weeks to severe cell-loss. The time course of the early cellular changes in this nucleus parallel those in the cranial nerve nuclei, but the later changes differ in that the isthmo-optic nucleus shows cell-loss whereas the cells of the cranial nerve nuclei progressively recover. A third unexpected feature of degeneration in this system is the finding that the initial signs of axonal degeneration in the isthmo-optic tract occur in that portion of the tract nearest to the nucleus and after longer survival periods appear to proceed centrifugally towards the optic chiasma. Finally, mention should also be made of the difference in time of appearance of degeneration in this tract according to the staining technique used; for example, although degeneration is clearly seen in the tract as early as 12 days in Nauta preparations it is not until 26 days after enucleation that the Bodian technique gives evidence of fibre degeneration. These findings provide further evidence that the silver techniques used in the present study stain different components of the fibre (cf. Evans & Hamlyn, 1956). Before dealing with the interpretation of these findings it is necessary to discuss the incidental findings on the IVth nerve which have a bearing on these problems. With the exception of the degree of the late cellular degeneration, the changes in the IVth nerve and its nucleus parallel, in every respect, the changes in the isthmo-optic pathway (including the different appearances with the various silver techniques). As there can be no doubt that the changes in this cranial nerve are retrograde in character there is a strong *a priori* case for the hypothesis that the

isthmo-optic system forms a centrifugal pathway. This hypothesis provides the simplest explanation for the absence of terminal degeneration in the isthmo-optic nucleus and of the severe cellular degeneration in the nucleus. It was first put forward by Perlia as early as 1889 to explain the complete atrophy of the isthmo-optic nucleus after experimental eye enucleation in the chicken. His finding has been confirmed by Jelgersma (1896) and by Huber & Crosby (1929). Taken by itself the finding of cellular degeneration in the nucleus is, of course, inconclusive and might be regarded as being transneuronal in character. As little is known about transneuronal degeneration in the avian brain this possibility cannot definitely be excluded, but from what is known of this process in the mammalian visual system it seems unlikely that it would have proceeded to such severe cell-loss as early as 2 months after enucleation in an adult animal (see Matthews, Cowan & Powell, 1960). The most conclusive evidence in favour of the hypothesis that the isthmo-optic pathway is a centrifugal one is provided by the work of Wallenberg (1898). Following lesions in the region of the isthmo-optic nucleus this author traced Marchi degeneration in the isthmo-optic tract, through the optic chiasma and nerve into the ganglion cell layer of the retina. In view of the considerable interest in centrifugal pathways at this time (see Granit, 1955; Livingston, 1959; Brindley, 1960) it is of some importance that Wallenberg's experiments be repeated using the more refined silver degeneration techniques.

The interpretation of the isthmo-optic system as a centrifugal pathway taken together with our observations on the IVth nerve suggests that retrograde fibre degeneration does not necessarily spread sequentially from the site of section of a nerve centripetally towards the cell body. Although there is a considerable amount of evidence on the sequence of changes close to the site of section of a nerve (see Cajal, 1928; Young, 1942; Guth, 1956), and while it is known that in some cases this degeneration may spread as far back as the cell body, it does not appear to have been recognized that degeneration may also commence close to the cell body and then spread centrifugally towards the site of section. It is difficult to assess the extent to which the slight traction exerted upon the optic nerve and extra-ocular muscles during enucleation may have contributed to this finding. It is well known that avulsion of a nerve may result in more severe degeneration than simple section, but it may be pointed out that in our experiments the nerves in the orbit were cleanly sectioned and nothing comparable to avulsion occurred.

Our material provides no evidence for an ipsilateral projection of the retina to either the diencephalon or the tectum. This is in agreement with the findings of nearly all previous workers (cf. Huber & Crosby, 1929; Evans & Hamlyn, 1956), but Polyak (1957) has described two small bundles which leave the contralateral optic tract in the pigeon, recross the midline in the suprachiasmal region and enter the ipsilateral 'rotund core' and the lateral geniculate nucleus. Despite repeated examination we have never observed degeneration in the nucleus rotundus (? 'rotund core'), and as it is difficult to assess the significance of the few boutons seen in the ipsilateral lateral geniculate nucleus (comparable numbers being seen in normal material) we are unable to confirm Polyak's findings. Nor have we found any distinct bundle recrossing the midline in the manner he describes or passing into the ipsilateral optic tract as Armstrong (1950) has found in the lizard.

SUMMARY

1. The central projection of the retina has been studied in the pigeon using a variety of silver degeneration techniques.

2. Within 5 days of eye-enucleation the optic nerve is severely degenerated and the degenerating fibres appear to decussate completely in the optic chiasma to enter the ventral part of the contralateral optic tract. From the optic tract this degeneration can be traced into the following thalamic nuclei: nucleus lateralis anterior, the lateral geniculate nucleus and the nuclei externus and superficialis synencephali. More posteriorly fibres pass from the marginal optic tract to the outer layers of the optic tectum and to the tectal grey. Coarse degenerating fibres leave the dorsum of the optic chiasma to form the basal optic root; this can be followed along the ventral aspect of the diencephalon to its termination in the ectomammillary nucleus.

3. Severe cellular degeneration in the isthmo-optic nucleus and the unusual nature and time course of the fibre degeneration in the axillary or isthmo-optic tract are suggestive of retrograde degeneration in a centrifugal system, which is in agreement with the findings of earlier authors.

4. An appreciable number of normal fibres persist in the stratum opticum of the tectum and can be followed through the marginal optic tract to the region of the optic chiasma; their precise origin and termination are, however, unknown.

5. No degeneration has been found in the hypothalamus or in those thalamic nuclei which are known to project upon the telencephalon.

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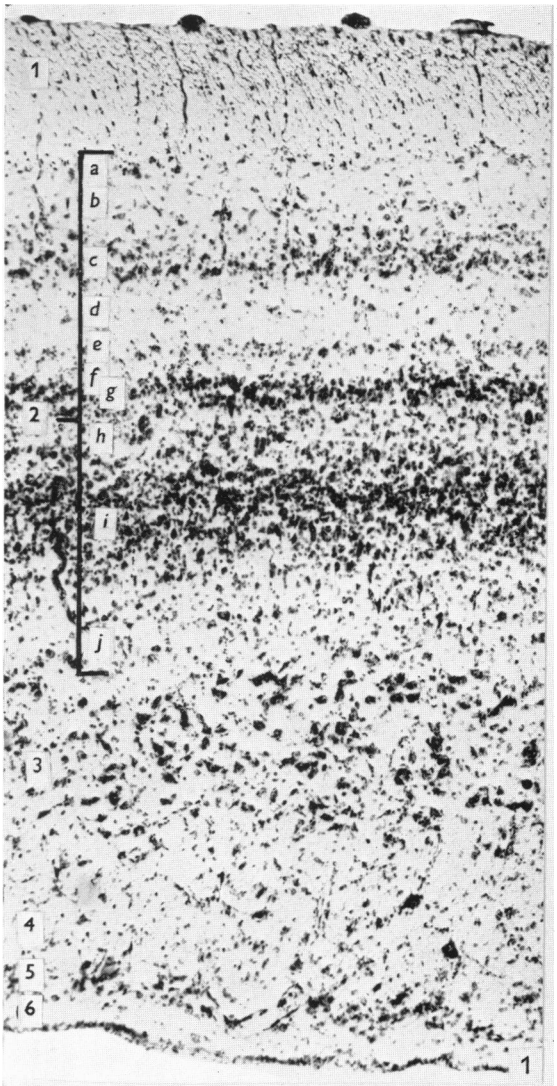
ABBREVIATIONS

BOR	Basal optic root
EMN	Ectomammillary nucleus
EN	Entopeduncular nucleus
IOT	Isthmo-optic tract
LFB	Lateral forebrain bundle
LGN	Lateral geniculate nucleus
LHN	Lateral habenular nucleus
MLB	Medial longitudinal bundle
MHN	Medial habenular nucleus
MOT	Marginal optic tract
NDL	Nucleus dorsolateralis
NDM	Nucleus dorsomedialis
NE	Nucleus externus
NIO	Nucleus isthmo-opticus
NLA	Nucleus lateralis anterior
NO	Nucleus ovoidalis
NR	Nucleus rotundus
NSM	Nucleus superficialis synencephali
NSR	Nucleus subrotundus
NSP	Nucleus superficialis parvocellularis
NSPI	Nucleus spiriformis
OC	Optic chiasma
ON	Optic nerve
OT	Optic tract
PC	Posterior commissure
SPN	Subpretectal nucleus
TG	Tectal grey
IV	IVth nerve nucleus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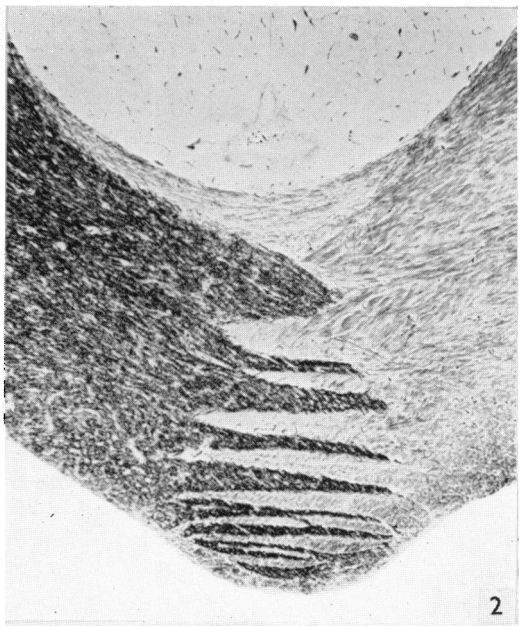

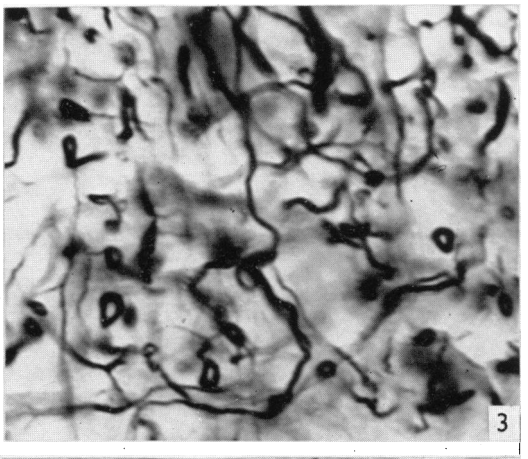

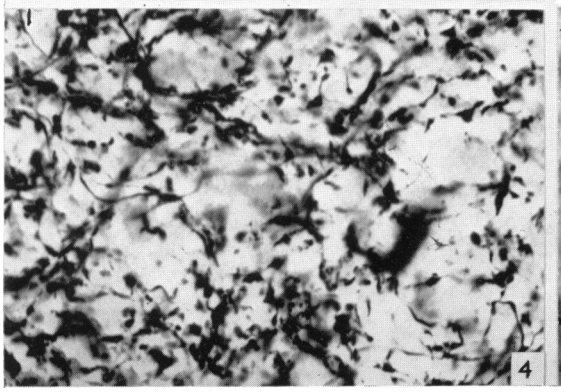

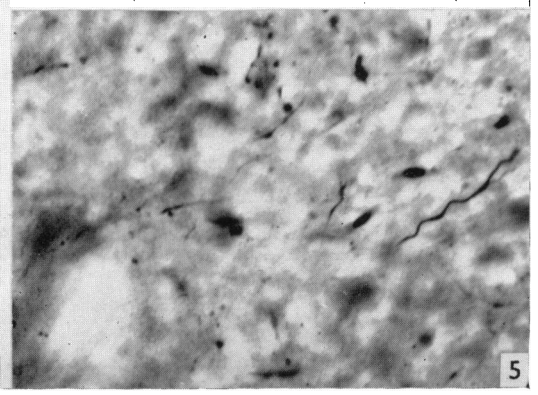

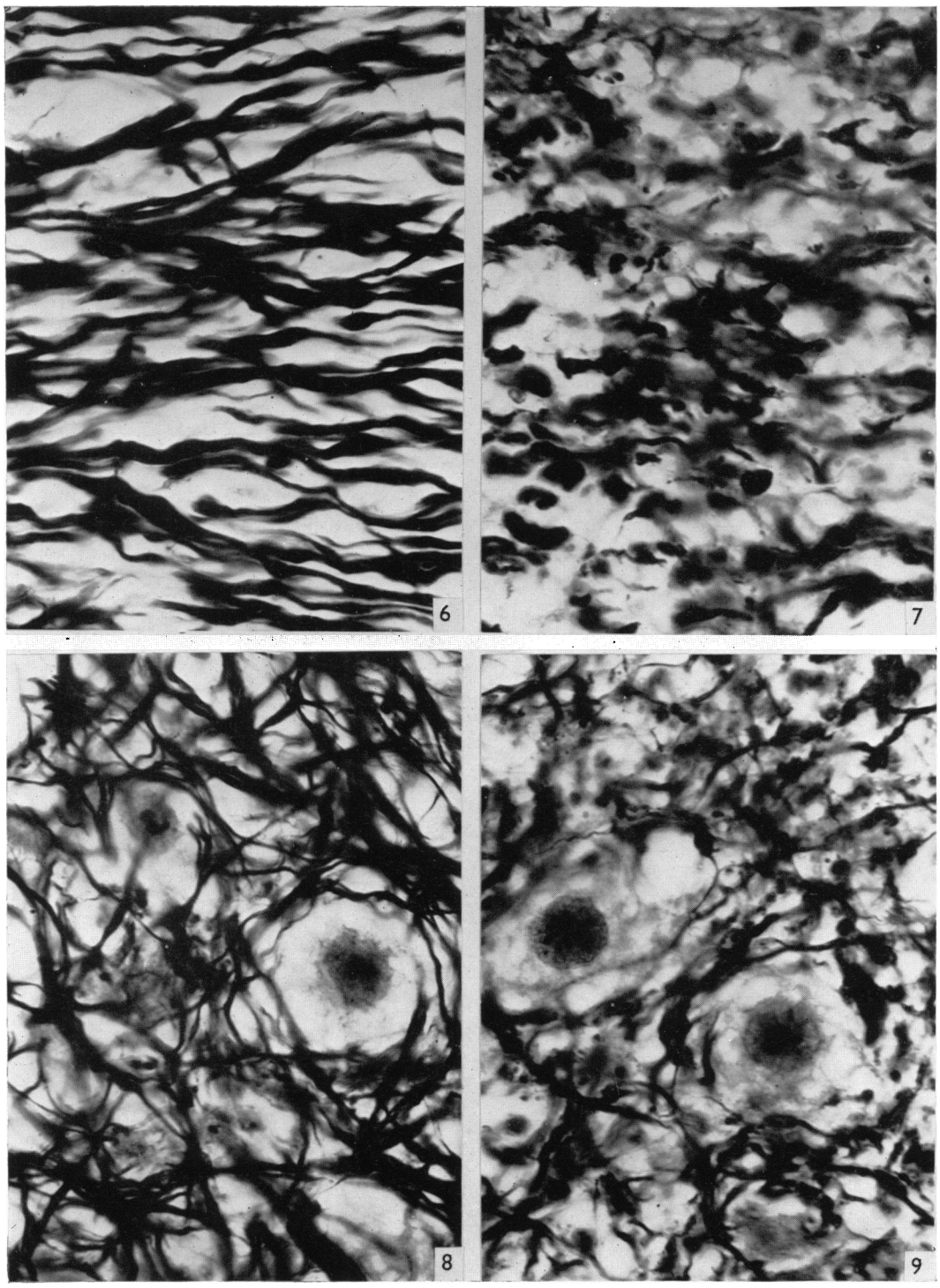

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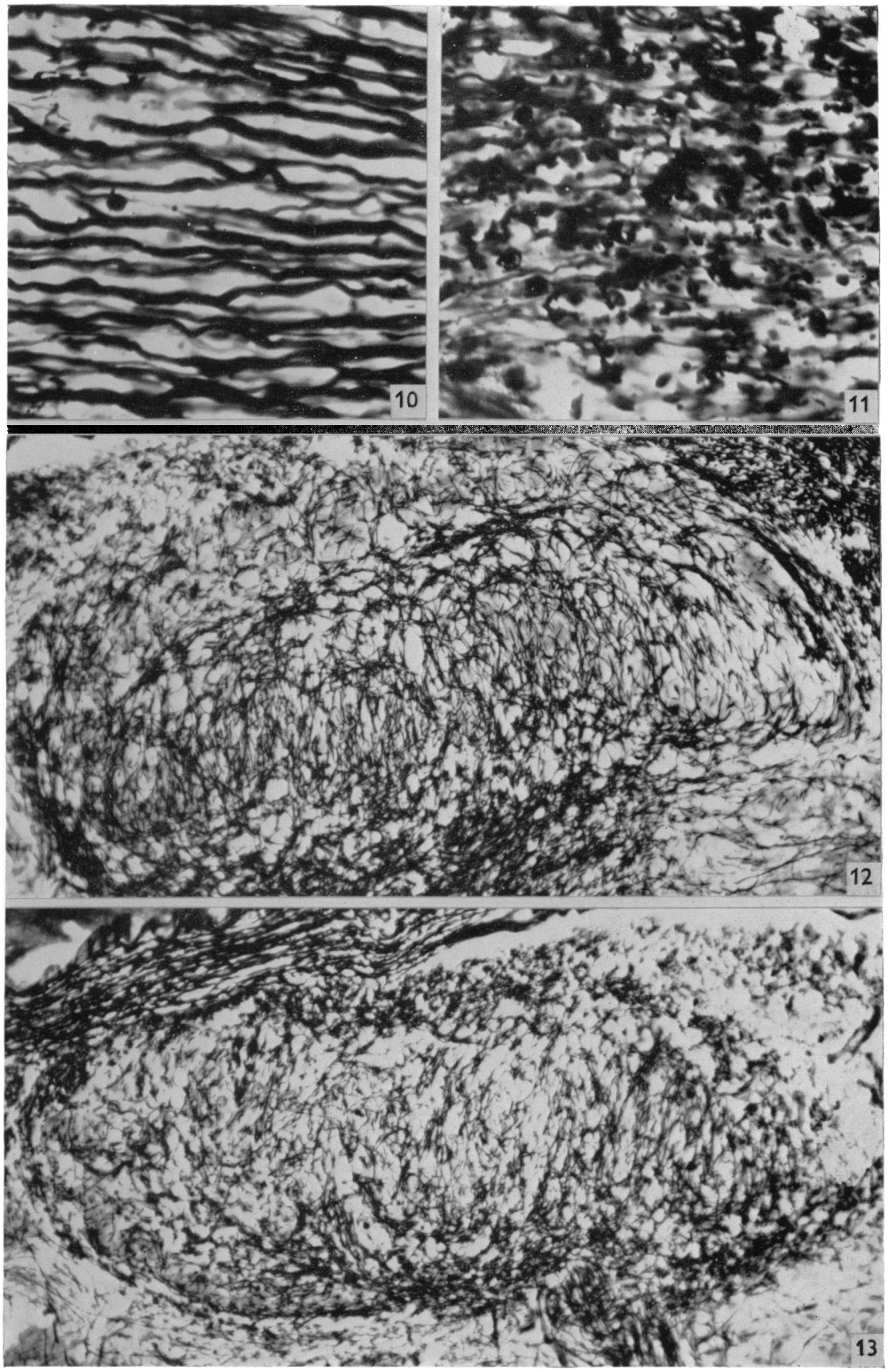
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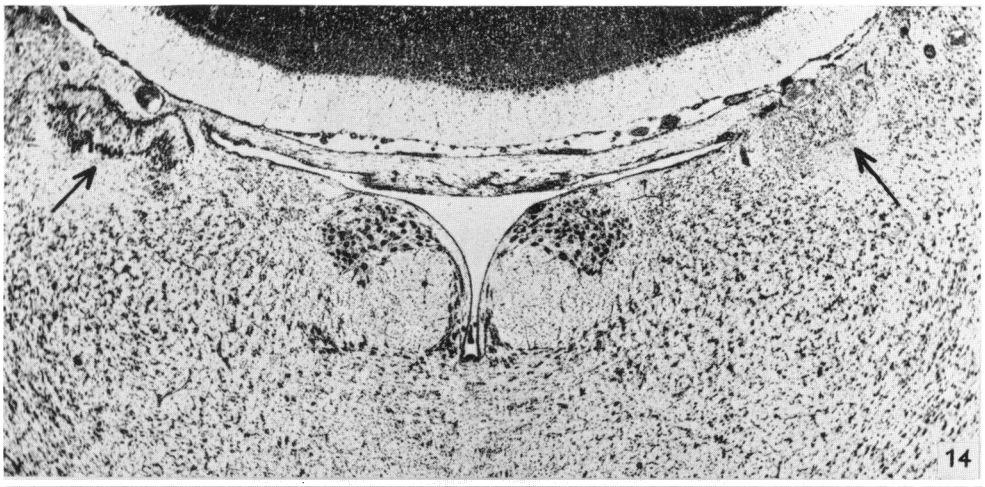
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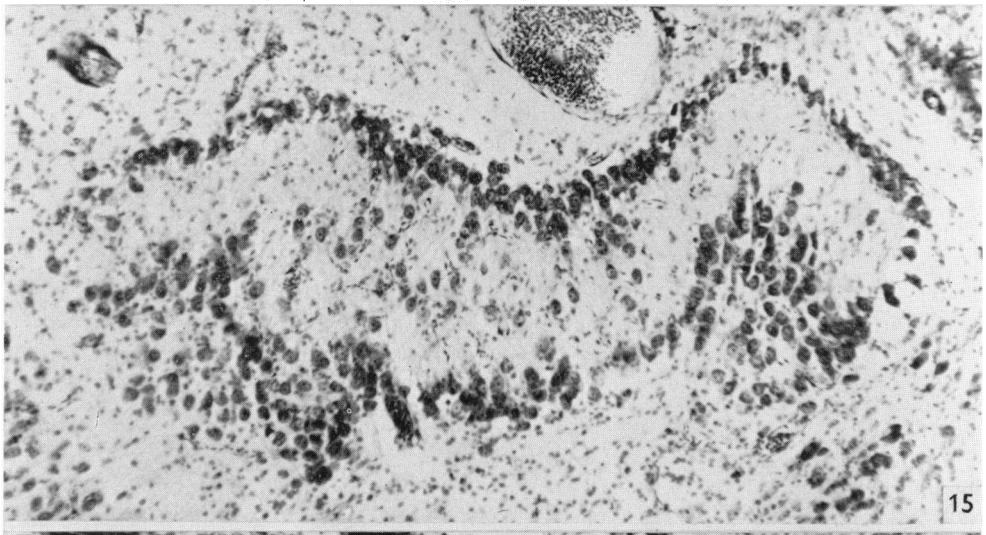
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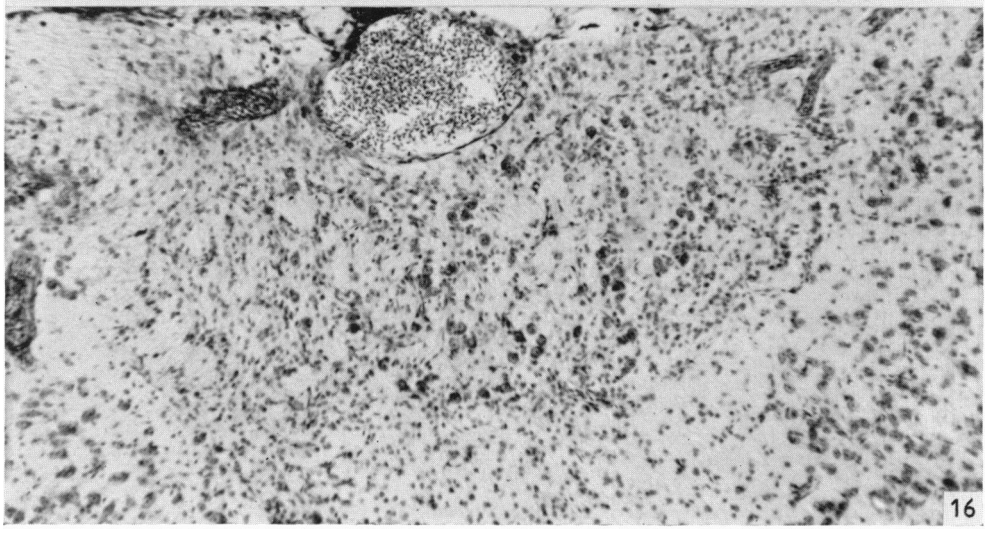
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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Section through the optic tectum of a normal pigeon to show the lamination described in the text. Thionine preparation. × 96.

Fig. 2. The optic chiasma 26 days after eye-enucleation as seen in coronal section in a paraffin Nauta preparation. × 32.

Fig. 3. Boutons in layers (*d*) of the stratum griseum et fibrosum superficiale of the tectum 12 days after enucleation of the contralateral eye. Bodian method. × 1720.

Figs. 4, 5. Nauta degeneration in the nucleus externus (fig. 4) with control from the opposite side (fig. 5). Expt. AP1 (18-day survival). × 630.

PLATE 2

Figs. 6, 7. Fibres from the normal basal optic root (fig. 6) and from the side contralateral to the eye-enucleation (fig. 7). AP3A (12-day survival). Glees and Marsland technique. × 1150.

Figs. 8, 9. The normal fibre plexus in the ectomammillary nucleus (as seen in the Glees sections) (fig. 8), and 12 days after eye-enucleation (fig. 9). × 1150.

PLATE 3

Figs. 10, 11. Fibres in the normal trochlear nerve (fig. 10) and on the affected side 26 days after section (fig. 11). Paraffin Nauta preparation. × 1150.

Figs. 12, 13. The normal fibre plexus of the isthmo-optic nucleus (fig. 12), and 26 days after eye-enucleation (fig. 13) to show loss of fibres in the central part of the nucleus. × 154.

PLATE 4

Fig. 14. Low-power photomicrograph through the midbrain at the level of the IVth nerve nucleus to show the position of the normal isthmo-optic nucleus (indicated by arrow on left) and of the degenerated nucleus on the right. Expt. EP2 (thionine preparation). × 22.

Figs. 15, 16. Photomicrograph of the normal (fig. 15) and degenerated isthmo-optic nucleus (fig. 16) from experiment EP2 at a higher magnification to show the severity of the cellular degeneration 2 months after eye-enucleation. × 140.