NERVE ENDINGS IN THE CONJUNCTIVA

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INTRODUCTION

Sensory nerve fibres in the integument of the body terminate peripherally in one of two ways: either in a system of diffuse, overlapping, arborizations (commonly called free nerve endings), or in various types of compact endings (commonly called encapsulated nerve endings). As far as we know, there has never been an attempt to subdivide the diffuse type of ending into morphological subspecies; on the other hand, a morphological classification of compact endings has been undertaken by numerous workers—without, it seems, achieving any measure of general agreement on the subject.

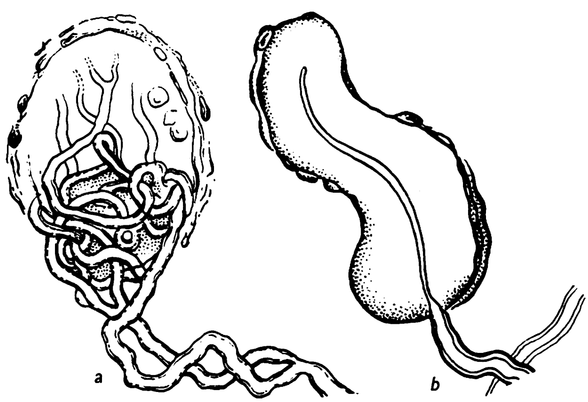
There are, indeed, three types of compact ending which are generally recognized as distinct morphological entities. These are: (1) the basket-like formations surrounding the shafts of hairs and vibrissae; (2) the 'tactile corpuscles' of Meissner, occupying the dermal papillae underlying the papillary ridges in the glabrous areas of the hand and the foot; and (3) the deeper-lying corpuscles of Pacini, with their characteristic onion-like lamellation. Apart from these there exists an ill-defined assortment of types of compact ending, many of which have been dignified with the names of eminent anatomists. Ruffini (1906) was one of the first to point out that the taxonomic classification of these 'types' was an arbitrary and even an unprofitable exercise, since each 'type' is found to merge insensibly, through a continuous series of forms, into other 'types'. This view has received support from modern workers (see Weddell, Palmer & Pallie, 1955). On the other hand, some investigators in the field of cutaneous sensibility, seeking to discover correlations between sensory 'modalities' and morphologically recognizable nerve structures, have focused attention on certain, as it were, bands of the spectrum, and claimed to show that certain 'types' are in fact specific receptors for particular forms of cutaneous stimuli. Curiously enough it does not seem to have been appreciated that in hairy skin, which covers the greater part of the body, the only compact nerve endings are those in specific relation to hairs (see Hagen, Knoche, Sinclair & Weddell, 1953).

The most assiduous workers in this field were von Frey and his school, who claimed to have demonstrated that a certain type of compact nerve ending, the 'Krause end-bulb', was the specific receptor for stimulation by cold. The crucial evidence for this view was a series of experiments carried out on the conjunctiva of the living human eye, where they believed, from the reports of various nineteenth-century histologists, that 'Krause end-bulbs' were abundant. The experimental part of this work will be discussed elsewhere; at this point, it is sufficient to say that von Frey's reading of the earlier literature was, to say the least, selective. As will be shown in the ensuing section, although many workers have in the past studied the innervation of the conjunctiva, general agreement has not in fact been reached either as to the precise morphological characteristics of a 'Krause end-bulb', or as to the regular occurrence, density and distribution of 'Krause end-bulbs' in the mammalian conjunctiva.

The present investigation was undertaken with the object: first, of discovering what lay behind the confused and contradictory state of the literature on this subject; and secondly, of finding an anatomical basis for the evaluation of experimental work on the sensibility of the eyeball. We concerned ourselves primarily with two questions: whether there is a specific morphological entity which can fairly be described as a 'Krause end-bulb', and whether 'Krause end-bulbs' or compact nerve endings of any kind are a constant feature of the conjunctiva in man and other mammals. As it happened, our findings led us to some unexpected conclusions, which will be discussed at the end of this paper.

LITERATURE

Krause (1859, 1860, 1861) was the first to describe compact nerve endings in the conjunctiva. He examined small pieces of tissue, either fresh or after maceration in dilute acetic acid, without fixation or staining. By this means he claimed to see, at the terminations of all medullated nerve fibres in the conjunctiva, characteristic 'organs' which he termed *Endkolben*. These were of two kinds. The first (Text-fig. 1*a*) which he found only in material from primates, was a spherical or ovoid



Text-fig. 1. Redrawn from Krause (1859) (*a*) end-bulb from human conjunctiva (cf. Pl. 3, figs. 31, 32); (*b*) end-bulb from bovine conjunctiva (cf. Pl. 1, figs. 6, 7; Pl. 2, fig. 16). Fresh, macerated specimens.

structure, 40–100 μ in diameter, with a capsule containing several layers of flattened nuclei, and served by one or more myelinated nerve fibres. Before losing their myelin sheaths and penetrating into the interior of the bulb, these fibres entered into a dense entanglement on the outside of the bulb. The second type (Text-fig. 1*b*) was characteristic of lower mammals, in particular the calf. Here the 'end-bulb' was several times longer than it was broad, and the nerve fibre supplying it, instead of forming an entanglement, ran directly in at one pole, losing its myelin sheath at the point of entry, and terminated in a more or less straight unmyelinated axonal segment in the centre of the bulb. The structure as a whole was reminiscent of a Pacinian corpuscle, but without its characteristic lamellation. This simple, elongated type was also found once or twice in conjunctivae from young children.

The distribution of *Endkolben* was somewhat irregular: in what he regarded as a typical human case, however, Krause counted 76 and 87 end-bulbs respectively in strips of conjunctiva 4 mm. wide, surrounding the margins of the right and left corneae. Nine per sq.mm. was taken as a rough average for the bulbar part of the conjunctiva, rather less for other parts.

Krause observed similar structures in various other exposed mucous surfaces, including the tongue and the external genitalia. In general, these were of the rounded, complex type in man and other primates, and elongated and simple in other mammals. In addition, he described, as a rare finding in the human conjunctiva, myelinated nerve fibres which, after forming a close *Knäuel* or tangle—not, apparently, enclosed in a capsule—continued in a further uncomplicated course without losing their myelin sheaths.

Krause's main findings were quickly confirmed by H. Frey (the histologist) (1859). But in 1862 Arnold published a rebuttal of their views. Using methods similar to those of Krause on human and bovine material, he saw myelinated nerve fibres ramifying in the conjunctiva, and was even able to trace their non-myelinated offsets to the epithelium, where they seemed to end blindly. Of the structures described by Krause he saw no trace. On the other hand, after much searching he did observe a few rounded, apparently structureless excrescences on the course of myelinated nerve fibres in the vicinity of a tear in the tissue, and found subsequently that by suitably maltreating his specimens, these appearances could be reproduced. He interpreted them as being effects of damage to myelin sheaths and of the flowing-out of the substance of a torn nerve fibre into an artificial tissue space. The fact that nerves, after entering such a 'bulb', commonly re-emerged from it and ran a further course, forced him to conclude that the 'bulbs' were not true nerve endings, but technical artefacts.

In the following year, Arnold was the victim of a polemical outburst by Lüdden (1862–3), a medical student in Kölliker's laboratory. Lüdden claimed to have seen end-bulbs, such as Krause had described, in man and calf, which could not have been produced in the manner suggested by Arnold and to which Arnold's artefacts bore no resemblance. His conclusions and drawings were accepted by Kölliker and incorporated in several editions (e.g. 1889) of his text-book of histology. Arnold made a dignified reply to the attack, but thereafter seems to have lost interest in the controversy.

In 1867 Mauchle reopened the subject. Using the newly introduced gold chloride and osmium tetroxide techniques, he was unable to obtain satisfactory preparations; but by treating tissues with acetic (or still better, sulphuric) acid he obtained results which confirmed Krause's and Lüdden's observations in man and calf. He noted that the end-bulbs were not confined to the vicinity of tears or cuts, and that divided nerve fibres showed no tendency to form bulbs or blobs. Mauchle extended his observations to rabbits, cats, pigs, dogs, rats and mice; but in none of these could he find end-bulbs. He confirmed Arnold's finding of free nerve endings, and differed from Krause's view that all myelinated fibres terminated in end-bulbs.

In the same year, Lightbody (1867), using caustic potash, acetic acid and carmine, saw in the subconjunctival areolar tissue peculiar rounded or oval bodies hanging on to the nerves "like a cherry on its stalk". He was apparently unaware of Krause's work: but Krause subsequently (1867) identified Lightbody's 'cherries' with his own *Endkolben*.

In the following year (1868) Rouget, using tissues macerated in dilute acid, found 'small, rounded bodies' situated at the tips of myelinated fibres in the human conjunctiva. He noted, as others had done, that these bodies were encircled by the myelinated pre-terminal portion of the nerve fibre, sometimes with a single turn, sometimes with more. Rouget disagreed with his predecessors on the subject of the connective tissue capsule, of which he saw no trace. The nuclei surrounding the central mass he confidently described as belonging to Schwann cells, and the 'capsule' itself as an expansion of the Schwann sheath; while the central mass appeared to be nothing other than a swollen blob of axonal substance. He noted, as did most subsequent authors, that the distribution of these bodies was very irregular; in one sector of an eye they were abundant, in another completely absent.

In 1870 Helfreich published a book on the nerves of the conjunctiva and sclera, in which he confessed that he had been unable to find end-bulbs in any of the animals he had investigated, with the exception of one unconvincing appearance in a frog. The only nerve endings he could see were fine unmyelinated fibres terminating close beneath the epithelium. Morano (1871) similarly described free nerve endings but was silent on the subject of end-bulbs. He was accused of piracy by Helfreich, and an undignified quarrel ensued.

In 1873 Ciaccio published a long and detailed article on the histology of the human conjunctiva. His account of the conjunctival nerves is based on pieces of tissue stretched out and gently scraped in order to remove the epithelium, and thereafter treated with either gold chloride or osmium tetroxide. He described a deep conjunctival plexus, which sends branches either to the cornea or to the conjunctival epithelium, where the fibres 'become pale', or end in 'Krause end-bulbs'. The latter are of various sizes, round or oval in shape, and of irregular distribution; in some areas there are none, in others many; in general, they are most abundant in the upper and outer quadrant of the bulbar conjunctiva. They consist of an outer investment of connective tissue, continuous with the sheaths of the nerve fibres supplying them, and an inner granular mass in which the 'pale' (i.e. non-myelinated) segments of the fibres terminate. These are served by one or more nerve fibres; most commonly there are two, deriving from a single fasciculus or as branches from a single stem fibre. In the calf, the fibre merely loses its myelin sheath and runs a straight course as a pale fibre in the centre of the bulb; in man, the myelinated segment forms a more or less intricate tangle outside the bulb (thereby obscuring its contents) before becoming pale and piercing it. The author was in no doubt as to the existence and the reality of these organs, and referred to Arnold's opinions with contempt. He also described a peculiar unencapsulated bush-like terminal expansion, which he encountered in a single preparation and to which he applied the term *fiocchetto*.

In the following year Waldeyer (1874) again cast doubt on the existence of end-bulbs in the eye. After a painstaking search he had been unable to find any, in either man or calf. He drew attention to the striking difference between the two morphological types of end-bulb which previous workers had claimed to find in these two species, and to the fact that Krause and others had failed to find them in many other mammals. In Waldeyer's view, the descriptions and illustrations given of the end-bulbs in man imposed a severe strain on histological credulity.

In 1875, however, Longworth, working in Waldeyer's laboratory, succeeded not only in displaying end-bulbs, conforming to previous descriptions, in the human and bovine conjunctiva, but also in convincing Waldeyer, who thereupon published a recantation of his previous view as a tailpiece to Longworth's paper. Longworth noted, once again, that not every eye examined contained end-bulbs, and that when they were present their distribution was very irregular. He considered that the 'human' type of end-bulb was related to the Meissner corpuscle and the 'bovine' type to the Pacinian corpuscle.

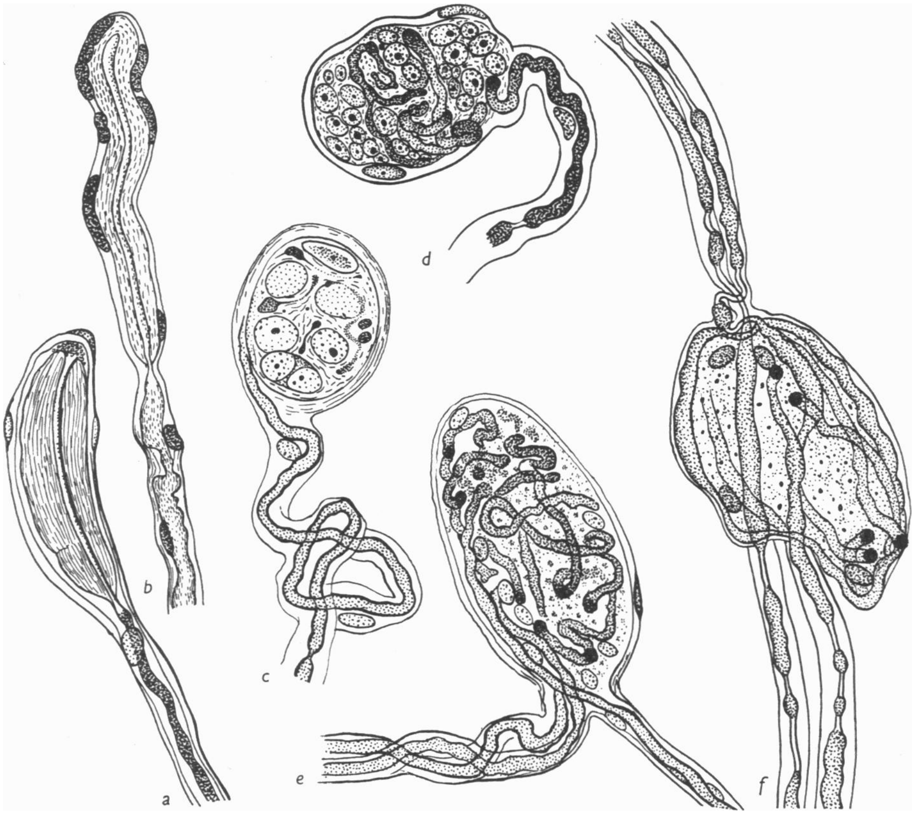
In the same year Poncet (1875) published a review of the literature and the results of his own findings, which closely supported those of Ciaccio. His drawings, from whole-thickness preparations of conjunctiva stained with osmium tetroxide, tally in detail with Ciaccio's from similarly mounted gold-impregnated specimens.

In 1876 Key & Retzius also reviewed the literature and gave a detailed, comprehensive and beautifully illustrated account of their own observations relating both to human and bovine material. Using tissues macerated in dilute acid as well as gold chloride and osmium tetroxide preparations, they described their findings in relation to what others had previously seen and described, with especial emphasis on the fact that they encountered great variations in form, amounting to a continuous series from the simplest club-like endings to large and complex end-formations, some of which appeared to consist of two or more end-bulbs stuck together (Text-fig. 2*a–f*). Like Poncet, they mention but do not explain the failure of some other workers to display end-bulbs.

In 1881 Krause himself published a general account of compact endings, with fresh illustrations which bear surprisingly little resemblance to what had now become the accepted picture of *Endkolben*. One sees what is presumably a myelinated fibre stopping abruptly at the edge of a solid ball of connective tissue cells; there is no suggestion of intricate tangles outside the 'capsule' or of fine ramifications inside. The text does not help to explain this discrepancy.

In 1884 Suchard gave a description, with illustrations, of 'human' and 'bovine' end-bulbs, confirming the observations of Ciaccio, Poncet and Longworth. His main concern was to show 'affinities' between the 'human' type and the Meissner corpuscle on the one hand, and between the 'bovine' type and the Pacinian corpuscle on the other.

A new era came with the introduction of methylene-blue staining. Dogiel published his findings in the eyeball as a whole in 1891. His general pictures of the conjunctival nerve plexus correspond well with those obtained by other methods; at various points, however, nerve fibres, after leaving the bundles, become varicose and enter into bizarre figures and convolutions (described as *Endknäuel* by the author) from which they emerge and run an uncomplicated course for a short way, only to enter into still other contorted figures (Text-fig. 3*a*). One fibre is depicted as passing through several such tangles, and eventually returning to and fusing with the parent stem, close to the point where it originated. In his description, he points out that this serial arrangement is the normal pattern; if a fibre seems to terminate in a tangle, this is due to incomplete staining. In some of his preparations, Dogiel used a counterstain to show up the connective tissue elements, and in many cases was able to see a lightly staining 'capsule' surrounding an *Endknäuel.* This led him to identify these structures with Krause's *Endkolben,* though a comparison of his pictures with those of previous authors makes it difficult to see how such dissimilar objects could be identified. Incidentally, Dogiel claimed to have seen similar structures in the outer 2 mm. of the corneal margin, and to be the first to describe them.



Text-fig. 2. Redrawn from Key & Retzius (1876). End-bulbs from conjunctiva of ox (*a*) and (*b*) (cf. Pl. 1, figs. 6, 7; Pl. 2, fig. 16), and from man (*c* to *f*) (cf. Pl. 3, figs. 31, 32). Osmium tetroxide.

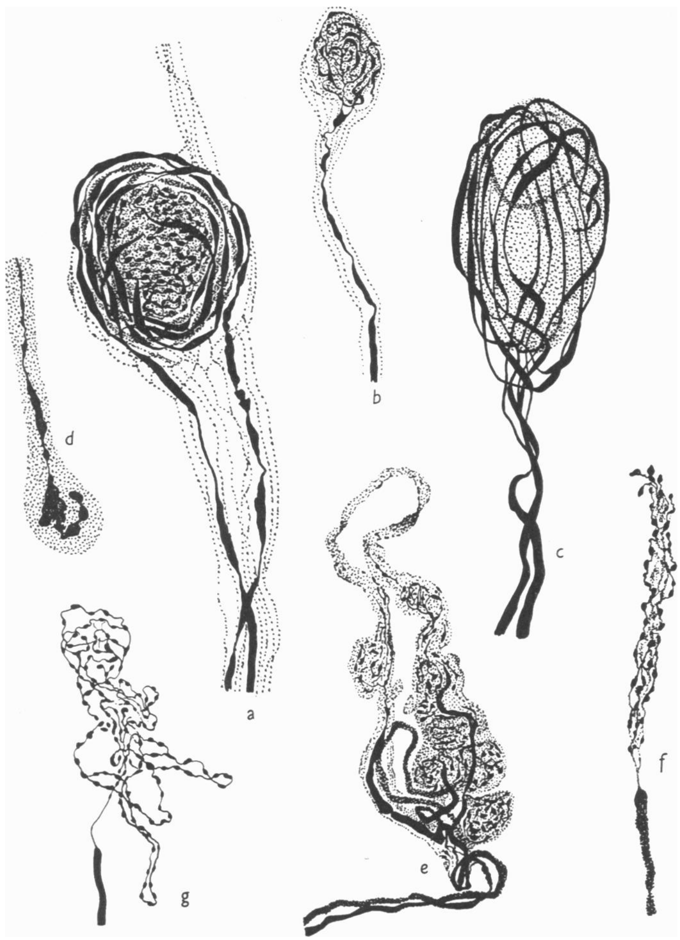
In addition to the pictures of encapsulated and unencapsulated tangles, Dogiel's paper gives a picture of an encapsulated structure which is strikingly different both from the traditional conception of an end-bulb and from the rest of his figures (Text-fig. 3*b*). Here there is no emergent fibre; a single fibre loses its myelin sheath and enters a connective tissue capsule, within which an axon of smooth contour but with fusiform swellings on its course branches and weaves in a complex but orderly manner. This single drawing has since become a standard for depicting the morphology of 'Krause end-bulbs', and reproductions of it appear in many text-books.

In 1903 there appeared a detailed and comprehensive paper on the innervation of the human conjunctiva by Crevatin. He used both gold chloride and methylene-blue techniques on human material, and his illustrations seem to be from preparations of both kinds. His description of the 'typical' end-bulb tallies with those of Ciaccio and others: in addition, he was able to observe, in the interior of the bulb, a closely woven entanglement of fine, branching, naked nerve filaments, which was only partially obscured by the outer, myelinated entanglement (Text-fig. 4*a–c*). His illustrations show a wide variety of forms, including a number of compound

Text-fig. 3. Redrawn from Dogiel (1891). (*a*) Serially innervated *Endknäue* and (*b*) encapsulated ending from human conjunctiva. (*b*) is commonly reproduced as type specimen for Krause end-bulbs. Methylene blue.

structures, consisting of 'nests' of two or three, more or less 'typical' end-bulbs (Text-fig. 4*e*). Crevatin also described various other forms of nerve ending, distinguished from end-bulbs principally in the fact of possessing no capsule. They included *striscette*, knobbed, club-like endings of coarse nerve fibres; *fiochetti*, elongated, tangled skeins of varicose nerve fibres, occurring either in isolation or in association with a group of end-bulbs (Text-fig. 4*f*); and *intrecci*, *plessicini* and *reticelle*, loose, irregularly-shaped types of nerve entanglement, bearing a general resemblance to the *Endknäuel* depicted by Dogiel (Text-fig. 4*g*).

After Crevatin, there seems to have been little original work in this field until 1922, when Knüsel & Vonwiller described the microscopic and slit-lamp appearances of the living human eye, subjected to vital staining. For demonstrating nerve elements, they used instillations of 1% aqueous methylene blue (without local anaesthetic) into the conjunctival sac. In this procedure, the first elements to appear were the most superficial nerve fibres, and end-bulbs, which could be seen hanging, like fruit from the twigs of a tree, at the ends of many of these fibres. The



Text-fig. 4. Redrawn from Crevatin (1903). (*a*) to (*c*) End-bulbs from human conjunctivae, showing outer, myelinated and inner, non-myelinated entanglements (cf. Pl. 2, figs. 18–23). (*d*) Simplest form of end-bulb (cf. Pl. 3, fig. 29). (*e*) Complex neural formation including several end-bulbs (cf. Pl. 3, figs. 25, 26). (*f*) *Fiocchetto* (cf. Pl. 1, fig. 4). (*g*) *Reticella* (cf. Pl. 1, fig. 3). Gold chloride and methylene blue.

size and distribution of the end-bulbs were variable. In general, they measured 40–50 μ in diameter, but in one subject they attained sizes of 80–200 μ. They were most frequent near the limbus, and more so in the upper sectors than in the lower ones. The largest number seen in a single eye was about 50. The appearance of the end-bulbs changed progressively as the dye took effect. At the stage of optimal staining, a fine nervous entanglement could be made out in the interior of the end-bulb. Occasionally, nerve fibres could be seen connecting one end-bulb with another, in the manner described by Dogiel.

These authors also made the experiment of stimulating end-bulbs with *von Frey* hairs under vision through a corneal microscope, both during and after the process of staining. No definite sensations of touch or pain were evoked; nor, it is presumed, of cold, although the point is not specifically mentioned.

A similar technique of vital staining was used by Strughold & Karbe (1925) who sought to demonstrate a correlation between 'cold spots' and end-bulbs. They confined their investigation to Dr Strughold's right eye, in which, during the course of three experiments, they found only five compact endings, three of which formed a serially innervated group.

In 1934 Egorow, using methylene blue in the manner of Dogiel, published an account of nerve endings in the guinea-pig eye. Her illustrations show a variety of more or less complex nerve terminals, including a large encapsulated tangle of fine, branching filaments, which was seen near the corneo-scleral margin.

It seems that little use has been made of silver impregnation for the demonstration of conjunctival end-bulbs. The only histological account based on silver preparations that we have come across is that of Boeke (1932). This author is not concerned with the differentiation of 'species' of compact endings—indeed, he shows the modern tendency to put Krause end-bulbs, Ruffini endings, corpuscles of Golgi–Mazzoni and the rest into a single group, and displays some impatience with the long differential descriptions of earlier writers. He is interested rather in finding a common structural principle in these endings. From his description and illustrations of end-bulbs from the human conjunctiva, it would seem that the tangle of terminal branches of the entering fibre lies free within a protoplasmic syncytium, containing a variable number of nuclei, which constitutes the 'core' of the structure: outside this core is a nucleated capsule, which is continuous with the endoneurium of the entering axon. No mention is made of an outer myelinated entanglement. The more complex of the two end-bulbs which he depicts bears a close resemblance to Dogiel's much-reproduced figure.

In summary, during the fifty years following Krause's original demonstration of compact nerve endings in the conjunctiva, eighteen workers published their findings on this subject. Among these there was general agreement on the existence of diffusely arranged free nerve-endings but some sharp disagreements on the existence of compact endings in the conjunctivae of men and oxen. Seven of those who reported their presence remarked that their distribution was always highly irregular, and that in some cases they could not be found at all. Six authors extended their observations to other mammals, but for the most part, their findings were negative. Only Krause claimed to have found compact endings in the conjunctivae of a wide range of animals.

Regarding the morphology of the compact endings, different authors give strikingly different accounts. Two authors, however, describe a wide variety of observed forms, from which it may be conjectured either that they had examined a larger number of specimens, or that they had examined their specimens more thoroughly than the others. It is unfortunate that not a single author actually states the number of specimens examined.

During the last fifty years, little more has been added to our knowledge of the innervation of the conjunctiva, but the subject has acquired theoretical importance from the work of von Frey and his collaborators, who have claimed to show that the compact endings of the conjunctiva (and similar endings elsewhere in the body) are specific receptors for the sensation of cold.

MATERIAL AND METHODS

(1) *Material for histological examination*

This was taken from 74 mammalian eyes. Table 1 indicates the distribution of the specimens among the animals examined as well as the manner in which the specimens were prepared for microscopical examination. The figures refer to the number of eyes in which the nerve elements were successfully stained or impregnated throughout large areas of bulbar conjunctiva. The eyes from laboratory animals were obtained before or shortly after death from an overdose of Nembutal. The eyes of sheep and oxen were taken within an hour of slaughtering. Human material was taken from cadavers, 6–48 hr. after death.

Table 1. *Material examined*

	(1) Whole mounts of bulbar conjunctiva dissected from eyeball				(2) Sections cut from eyeballs with bulbar conjunctiva *in situ*			
Animal	Methylene Blue	Osmium	Silver	Gold	Methylene blue	Osmium	Silver	Total
Albino mouse	2	.	.	.	.	.	.	2
Albino rat	6	.	3	.	.	.	1	10
Rabbit	6	.	1	.	.	1	2	10
Cat	1	.	1	.	1	1	2	6
Sheep	.	.	1	.	.	.	1	2
Ox	4	2	4	.	.	1	4	15
Monkey	1	1	2	1	2	1	2	10
Man	1	.	8	.	.	1	9	19
Total	21	3	20	1	3	5	21	74

The specimens for microscopical examination took the form either of sections cut from the fixed, undissected eyeball on the freezing microtome, or of whole mounts of pieces of dissected conjunctiva. Sections were cut in a variety of planes, including the vertical and tangential. Whole mounts were obtained as follows; after fixation, the bulbar conjunctiva was gently stripped from the sclera in five or six sectors, each attached to a sector of cornea. The pieces were pinned out with the epithelium downwards, and the loose areolar tissue, which contains the main nerve bundles but not the conjunctival nerve plexus, was carefully snipped away with fine scissors. Much depends on this procedure, for the areolar connective tissue fibres attract an irregular deposition of metallic silver, which may completely obscure the nerves running between them and the epithelium.

In heavily pigmented specimens, as for instance those from monkeys and oxen, the epithelium was gently scraped away from the conjunctival connective tissue. Artefacts, due to the recoil of nerve fibres stretched to breaking point, are sometimes observed as the result of these procedures. Such artefacts are usually easy to recognize.

In the case of albino rats and mice, it was found possible to prepare the anterior halves of the eviscerated eyes as whole mounts, for the tissues are thin enough to allow translucent specimens to be obtained even following silver impregnation. A number of radial incisions enabled flattening to be achieved without notable distortion of nerve fibres.

(2) *Histological methods*

(*a*) *Methylene blue*

For methylene blue staining, a spreading factor, 0·025 % hyaluronidase (Hyalase, Benger) was first injected beneath the conjunctiva. This was followed by a sub-conjunctival injection of 0·02–0·05 % methylene blue (Merck) in physiological saline. Whenever possible these were done in the living, anaesthetized animal; good results were obtained, however, in animals which had been recently killed, and in one case in a man who had died 6 hr. previously. After 15–30 min. staining, the anterior half of the eyeball was immersed in ice-cold 7 % ammonium molybdate for 12–16 hr. To obtain whole mounts, the conjunctiva was removed from the sclera, rapidly dehydrated in several changes of redistilled absolute alcohol, and cleared. To obtain sections, the specimens were removed from the ammonium molybdate, washed and placed in 10 % formalin neutralized with calcium carbonate, where they remained for 1 or 2 days before sections were cut on the freezing microtome. The sections were then washed, and rapidly dehydrated.

The advantage of methylene blue staining is that in zones where the concentration of dye is optimal it gives a very clear and undistorted picture of nerve fibres of all calibres. However, it is very difficult to obtain, consistently, specimens which are evenly stained over a wide area, for conditions leading to optimal staining are very critical. Overstaining, whether from excessive concentration of dye or from too prolonged application, leads to gross distortion and even disruption of nerve fibres as well as to non-specific staining, such elements as blood vessels, lymphatics and reticular and other connective tissue fibres readily taking up the dye. Local patches of understaining, some of which are probably due either to uneven spreading of the dye or to decolorization during processing, are unfortunately common.

(*b*) *Osmium tetroxide*

Myelinated sheaths were stained by first injecting a 1/3 % solution of osmium tetroxide subconjunctivally into an enucleated eye and then immersing the whole eyeball in a solution of the same concentration for 12 hr.

(*c*) *Gold chloride*

Gold chloride impregnation after the manner of Ranvier proved to be an unsatisfactory method in our hands. Usually, the capillary network showed up with extreme clarity, but the nerve fibres never had the sharpness of outline seen in good methylene blue or silver preparations.

(*d*) *Silver*

In the case of human eyes, we had to rely chiefly on silver impregnation. For this, a method was needed which gave consistent results and was specific enough to enable us to examine thick preparations satisfactorily. In the first place, various modifications of the Bielschowsky technique were tried, but in the end we adopted a technique developed in our laboratory by Schofield (1957). This gave the most consistent and rewarding results and enabled us to examine whole mounts satisfactorily. Axis cylinders of all calibres are specifically impregnated, and appear black against a light brown background, which yields sufficient details of the tissue elements present to render counter-staining unnecessary. There is little, if any, distortion of the axons and little haphazard precipitation of metallic silver on other than neural elements. This point is of considerable importance in view of the possible existence of pathological changes in the nerve fibres of the specimens at our disposal.

In only a few cases can we claim to have made a complete examination of the whole of the bulbar conjunctiva in any given eye. A certain amount of material is invariably wasted in specimens obtained by cutting frozen sections, and complete and uniform staining throughout the tissue by methylene blue is rare. In whole mounts impregnated with silver, there are nearly always a few patches where the tissue is not translucent, owing to incomplete removal of areolar tissue or slight folding. We can claim, however, that in all of the 74 eyes listed above, we were able to examine a reasonably large area of the bulbar conjunctiva, at least in its circumcorneal region.

(3) *Vital staining with methylene blue*

(*a*) *The material* consisted of volunteers, five male and three female, between the ages of 9 and 49 years. On these, a total of fourteen experiments was carried out.

(*b*) *Staining technique.* The eye was first bathed for about 30 sec. in an eyebath with a 2 % solution of cocaine hydrochloride containing 1 part in 2000 of adrenaline hydrochloride. Bathing was repeated at about 5 min. intervals, until the conjunctiva was anaesthetic to light touch and completely blanched. This took about 10 min. The eye was next bathed with a solution of 1 % methylene blue (Hoechst, Medicinal) in distilled water diluted with 4 % cocaine hydrochloride to give a final concentration of dye of between 0·3 and 0·5 %. This was also repeated two or three times during the next half an hour.

Thereafter, the progress of the staining could be followed by means of a hand-lens. For closer inspection, the adjustable brow-pad and chin rest of a slit-lamp microscope were used to support the head. The stained conjunctiva was examined through a microscope fitted with lenses having focal lengths of 3·0 and 4·5 cm. respectively, and an eye-piece magnifying ten times. Photographs were taken by means of a 35 mm. photomicrographic camera attachment in the eye-piece position and a ring-shaped flash tube fitted around and a little behind the microscope lens.

This method proved to be an invaluable complement to the histological techniques described above. Although the magnification which could be usefully employed was too small for the finest structural details to be observed, it gave an over-all view of the pattern of innervation of an intact conjunctiva, of a kind well-nigh impossible with histological methods. It also provided a check on our histological material, in that the eyes examined were those of healthy subjects, not of hospital patients in the course of a disease. Most important, it enabled us to observe changes in the innervation of an individual eye at varying intervals of time.

Regarding the completeness of staining of nervous elements by this method, we regard it as comparable with the best silver or methylene blue preparations of whole mounts. Thus, in every case (except one, in which the inner half of the conjunctiva remained unstained) we were able to see, in all parts of the bulbar conjunctiva, the details of the conjunctival nerve plexus precisely as we had seen it in histological specimens. In some respects, in particular in evenness of staining and in the absence of non-specific staining of connective tissue fibres, the vital method showed considerable advantages over the conventional histological methods. In many cases, it was noticed that the staining of the conjunctiva did not progress simultaneously in all areas; thus, different areas might reach the stage of optimal staining at different times. This effect is presumably due, among other things, to local differences in the permeability of the tissue, and one may infer that the same factor may be responsible for the uneven staining so often observed in methylene blue preparations of detached pieces of tissue. Here, again, the vital method shows up to advantage, in that all areas pass at some period through a stage of optimal staining.

The one case in which staining was clearly incomplete was that of a child of twelve. A second attempt was made some months later on the same eye. On this occasion, no staining whatever occurred—not even of epithelial cells, which are normally the first to take up the stain. We were unable to account for this phenomenon.

In view of some earlier accounts of the intense discomfort caused by methylene blue staining, it may seem strange that we used children as subjects. In fact, it was soon found that the procedures described caused no severe pain. The treated eye usually begins to ache within 2 hr. of the application of the dye; but this aching is completely stopped in a matter of seconds by bathing with cocaine. Repeated applications of cocaine are required during the next 4 hr. or so, after which the aching disappears. On the following day, the conjunctiva is a little hyperaemic but not painful. It is usually anaesthetic to light touch, and remains so for 2 or 3 days, after which sensibility returns to 'normal' (i.e. light touch feels the same as it does in the control eye). No untoward after-effects have been observed.

The prolonged period of anaesthesia is probably to be ascribed to the action of the dye on the nerves, since it is well known that the effect of cocaine wears off in a few hours.

OBSERVATIONS

A. *Histological*

(1) *Laboratory animals*

The first specimens which we examined were of fresh material from young healthy animals kept under artificial conditions in a laboratory animal house. They came from mice, rats, rabbits, cats, macaques and baboons. These showed nerve bundles emerging from the sclera at various points, and running, in company with major vessels, in the subconjunctival connective tissue, where they formed a characteristic plexus, with many radially-directed small bundles of fibres coursing towards the limbus. The more anterior bundles crossed the limbus to form a plexiform network in the more superficial layers of the cornea. Offsets from the conjunctival plexus passed into smaller nerve bundles which they eventually left as individual axons of various calibres. These, after branching extensively, became thinner, lost first their medullary sheaths, then their (visible) Schwann sheaths and finally tapered to invisibility in relation either to the walls of blood vessels or to the conjunctival epithelium (Pl. 1, fig. 2). As our primary concern was with the presence or absence of end-bulbs, we have not attempted a detailed or quantitative analysis of the pattern and distribution of the nerve fibres ending freely in the conjunctiva. From what we have seen, however, we have found no occasion to disagree with the qualitative descriptions given by Ciaccio and others on this subject.

Exceptions to this mode of termination by branching and tapering to invisibility were found in only five instances in thirty-eight specimens examined. In one specimen, a methylene-blue preparation from an apparently healthy rat, a terminal axon formation was seen (Pl. 1, fig. 3), which closely resembled what Crevatin described as a *reticella* (cf. Text-fig. 4*g*). This, as can be seen, consisted of an irregular entanglement of branched, varicose fibres, with no sign of encapsulation. In the second specimen, a gold chloride preparation of a macaque conjunctiva, there were two comparable, though somewhat elongated, formations of branched, varicose fibres, again without capsules (Pl. 1, fig. 4). These appeared to us to correspond to Crevatin's *fiochetti* (cf. Text-fig. 4*f*). The third specimen, a silver preparation from a cat, showed two small 'end-bulbs' similar to, but smaller than, those said to be classical for the ox (Pl. 1, fig. 5, and compare Text-figs. 1*b* and 2*a*). As the innervation of the remainder of these eyes conformed to the diffuse pattern described above, we felt compelled to regard these five instances of compact endings as exceptions to the normal rule. We were encouraged in this view by the fact that several previous workers had been unable to find end-bulbs in the conjunctiva of animals other than ox and man. The possibility that they were technical artefacts will be discussed later.

(2) *Slaughterhouse material*

Two eyes from sheep, and fifteen from young oxen, were examined. All the animals were slaughtered less than an hour before the specimens were collected. The eyes were either placed in fixative or treated with methylene blue on the spot. In these, a precisely similar pattern of innervation was seen to that found in laboratory animals, namely a plexiform network of fibres giving rise to individual axons (Pl. 1, fig. 1), terminating diffusely in relation either to blood vessels or to cells of the epithelium.

No compact or encapsulated endings were found in either of the sheep's eyes, or in those of the first eight bovine eyes examined, all of which were from young calves. However, on examining material from a batch of eyes from 2-to 3-year-old bullocks after silver impregnation, we at last had some confirmation of what had been described in the older literature. In randomly scattered regions, unusually thick myelinated nerve fibres of irregular contour running just beneath the epithelium ended in characteristic sausage-shaped 'organs' (Pl. 1, figs. 6, 7; cf. Text-figs. 1*b* and 2*a*, *b*). On the outside of each there was a 'capsule', which was seen to be a continuation of the endoneurial sheath of the fibre supplying it. Underlying this were elongated and somewhat flattened nuclei, indistinguishable, apart from the flattening, from those of Schwann cells in the neighbourhood. Within this outer layer was a structureless granular substance; and in the centre, the densely staining club-like termination of the axon. There was generally a marked constriction of the axon at the point of entry, where the myelin sheath ended abruptly. Outside the 'organ', connective-tissue cells sometimes showed a slight degree of concentric orientation, giving the impression of a passive thrusting aside of connective-tissue elements by an expanding body.

This 'classical' form of bovine 'end-bulb' was not the only type observed. In some cases, a number of short thick branches took the place of the single central axonic club (Pl. 1, figs. 8, 9); in others, one or two fine fibres, arising from the club-like axonic swellings, could be seen running in the interior of the 'bulb' (Pl. 1, fig. 10); and in one specimen two 'bulbs' were seen in which fine fibres sprouted from the sides or tip of a terminal club and formed a more or less spirally-wound skein inside the 'bulb' (Pl. 1, fig. 11). There were also 'bulbs' in which the terminal club was clearly defined, but the 'bulb' as a whole was smaller and the surrounding sheath and its nuclei were only faintly discernible.

In some instances, large irregular myelinated nerve fibres which terminated in typical bovine 'end-bulbs' gave off collaterals which behaved quite differently. In one case the collateral arose from a nodal point, but rapidly changed direction and pursued an irregular course towards the epithelium. Shortly before reaching it, it lost its myelin sheath, arborized into numerous branches which, in turn, lost their Schwann sheaths and finally terminated diffusely (apparently by attenuation) in relation to the cells in the epithelium. In other words, one and the same axon gave rise to both a compact and a diffuse terminal. The axis cylinders forming such collaterals usually contained fusiform swellings (of the kind frequently seen in regenerating nerves) which were largest at points where the axon changed direction abruptly. An example of an arrangement of this kind is given in Pl. 2, fig. 16. In this instance, the axis cylinder, derived from the collateral having the largest number of swellings, ended in a small conical mass reminiscent of a growth cone (Pl. 2, fig. 16, arrow on montage).

The dimensions of the bulbs were in agreement with previous descriptions—namely, 65–140 μ in length by 25–35 μ in breath. In the matter of distribution, our findings also agreed with those of most previous authors. In a single eye, there were areas where 'bulbs' occurred with a density of 4 per sq.mm. and other areas, of a square centimetre or so, where there were none. In some eyes, as already mentioned, we found none at all; in others, only one or two.

Two additional features were observed which we believe to be significant. In the first place, the nerve-fibres supplying the 'bulbs' were without exception 'abnormal' in appearance. In some cases, the visible abnormality consisted merely in varicose irregularities in the axon. These were either smooth in outline and fusiform in shape or irregular in both shape and outline (Pl. 2, figs. 12, 13; compare Text-fig. 4*a*, *b* and *d*). In others, the impregnation of the axon was discontinuous, and the myelin sheath, which is not usually impregnated at all, was diffusely impregnated and very irregular in calibre (Pl. 2, fig. 14). This feature was very conspicuous in the specimen which provided the biggest yield of 'end-bulbs'. Here, of course, the suspicion of technical artefacts arises; we concluded, however, that there was more to it than this, since not only were there normal-looking fibres of even calibre in the same preparation, but in individual nerve bundles there were both smooth and irregularly varicose fibres running side by side with normal-looking fibres of coarse and fine calibres; and wherever an abnormal fibre could be traced to its termination, it was found to end in a 'bulb' (Pl. 1, figs. 6, 7; Pl. 2, figs. 12–14).

The second observation was that, in at least three cases, the 'bulb' was clearly intra-neural, that is, included along with a number of passing nerve fibres within the perineurium of a nerve bundle, which was locally distended to accommodate the 'bulb' (Pl. 2, fig. 15).

In short, we have found 'end-bulbs' of the bovine type in the bulbar conjunctiva of five young bullocks' eyes, locally abundant in some cases, sparse in others, We failed to find them in two young bullocks' eyes and eight young calves' eyes. In general, they conform to previous descriptions; but to these we would add our observations of more complex forms, the occurrence of intra-neural 'bulbs' and the abnormal appearance of the nerve fibres serving them. These observations are summarized in Table 2.

(3) *Human material*

We were unable to obtain perfectly fresh material. Six eyes were obtained from cadavers, between 6 and 48 hr. after death. We were also supplied with a number of formalin-fixed eyes, nine from diabetics, and four from non-diabetic patients. In most cases fixation had begun 36–48 hr. after death. The subjects were mostly elderly or middle-aged.

It was found that late fixation did not prevent the successful silver impregnation of nerve axons. Even when the epithelium itself showed post-mortem changes such as exaggerated intercellular spaces, the axons were generally almost normal in appearance, being of relatively even calibre over long distances. They were arranged in a precisely similar pattern to that found in the other eyes which we had examined, namely a plexiform network ending diffusely in relation either to blood vessels or to epithelial cells. Thick fibres of markedly uneven calibre, when they could be followed to their termination, were nearly always found to enter into some peculiar type of end-formation. In our single osmium tetroxide preparation (from a 12 hr. cadaver), the myelin sheaths showed irregularities of outline, which from their appearance may well have been due to post-mortem changes.

No characteristic differences were observed between the eyes from diabetics and the others, except that in four of the 'diabetic' eyes there were places where the nerves emerging from the sclera, instead of running in simply-branched bundles, formed loose and much less formal entanglements (Pl. 2, fig. 17). This feature was not observed in any 'non-diabetic' eyes; but the material was too limited to enable one to judge whether these entanglements were in any way attributable to the particular disease process from which the patients were suffering.

In two of the eyes examined, no compact nerve-endings were seen. In two, single solitary compact endings were found: in four, a solitary group of three or four such endings: in others, there were several such groups, while in four specimens compact endings were locally abundant—up to three or four per low-power field —though even in these, large areas were apparently devoid of them. On account of the technical difficulties mentioned earlier, precise figures of the distribution of these endings in different eyes cannot be given; it became obvious, however, that great variations occur as between one eye and another, and between different areas of the same conjunctiva. There was no correlation between numbers of complex endings and the ages of the subjects, all of whom had died from disease after the age of 30. Lacking material from young and healthy persons, we did not consider it profitable to seek for correlations with the patients' medical histories. It may be mentioned here that compact endings were never seen in any part of the cornea. These observations are also summarized in Table 2.

The variety of the forms assumed by these endings was very striking. It has been mentioned that Crevatin described, illustrated and classified a wide variety of forms. We have seen endings which fall neatly into each of his pigeon-holes; but we have also seen enough intermediate forms to convince us that Crevatin's classification is an artificial one. In general, we can say that the specimens in which compact endings are most abundant show the widest scatter of forms, while in those which show only a few, these tend to be similar in form, and of a comparatively simple type.

Thick, irregular fibres ending in knobbed or thorny 'clubs', corresponding to Crevatin's *striscette*, have been seen, though it is often difficult to be sure that these constitute the true terminations of fibres. These 'clubs' may possess a few short twigs sprouting near their tips (Pl. 3, fig. 29; compare Text-fig. 4*d*). Such forms merge into what Crevatin would have described as a simple 'end-bulb'—a cluster of short twigs forming a knot less than 20 μ in diameter. Pl. 2, fig. 18, is an example of a simple 'end-bulb' which has an additional point of interest; the axon from which it springs has fusiform swellings along its course, and gives rise to a collateral which pursues a complicated course towards the surface, where it ends in a diffuse arborization (cf. Pl. 2, fig. 16, and Text-fig. 4*b*, *d*). From such an 'end-bulb' there is a continuous series of endings in which fibres are more branched, and the branches perform more complicated contortions (Pl. 2, figs. 19, 20). In 'end-bulbs', which may be 80 μ or more in diameter, the thick parent fibre or fibres (there may be two or more) are often seen to wrap themselves round the 'bulb'; their main branches, still thick and presumably myelinated, form an investing entanglement either in the periphery of the 'bulb', or at or near its hilum; and the fine terminal branches are seen weaving intricate patterns in the interior (Pl. 2, figs. 21–23). This last type clearly corresponds with the forms described by the earlier workers (compare Text-figs. 2*c*, *d*; 3*b*; 4*a–c*). In a few cases we have seen compound 'end-bulbs' in which up to four 'bulbs' of the more elaborate type lie closely packed together (Pl. 3, figs. 24–26). These too were described by Crevatin (Text-fig. 4*e*).

An equally natural series appears to link the simple 'end-bulb' with the elongated forms described by Crevatin (which, incidentally, would probably be classified by some workers as 'Ruffini endings'). These are sometimes isolated, sometimes closely associated, at their hila, with 'end-bulbs' from which they seem to differ in shape and size alone. Similarly, there does not appear to be a sharp distinction between the 'end-bulb' with its densely woven fibres and globular shape, and the looser, irregularly shaped entanglements called *reticelle*, *intrecci*, or *plessicini* by Crevatin (Pl. 3, figs. 27, 28; cf. Text-fig. 4*b*, *g*).

On one point we are unable to follow Crevatin, and that is the question of encapsulation. According to him, there is an investment of one or two layers of connective tissue surrounding each 'end-bulb', but no such investment around any of the other types of ending. In our material we have seen no clear evidence of non-nervous tissues entering intimately into the structure of 'end-bulbs'. As with the bovine material, Schwann nuclei are generally numerous in the periphery of the 'bulb', and sometimes occur inside it; in the larger specimens, there may be some roughly concentric arrangement of connective-tissue cells which give the appearance of having been thrust aside by an expanding body; but this apparent 'capsule' is more often than not deficient on one side. The point is of some theoretical importance, and will be discussed later,

Another point of difference from Crevatin is that, whereas he was of the opinion that fine terminal nerve twigs (even those served by axons of even calibre) are almost always coarsely beaded, they do not appear so in our silver preparations. In discussing this point Crevatin concluded that the beads were 'natural' since they were seen equally in gold chloride and methylene-blue preparations. We are inclined to regard beading of this kind as a technical artefact, for both the nerves and other tissue elements are less distorted (i.e. correspond more closely to those in the fresh cornea under phase-contrast conditions) in specimens impregnated by the silver technique we are using than is customary in specimens prepared by other silver methods which, in turn, are far less distorted than those impregnated with gold or stained with methylene blue. We ourselves have regularly encountered beading of the finest terminal nerve twigs but only in gold chloride and methylene-blue stained material (see Pl. 1, figs. 3, 4; and compare Pl. 1, fig. 2. and Pl. 3, fig. 28).

It was observed that every compact ending was served by at least one thick, myelinated fibre, usually of uneven calibre; often, however, there were fine, even-calibred fibres running in association with these and apparently joining in the terminal entanglement. The degree of varicosity of the thick fibres seemed to be generally related to the complexity of the ending; thus, the fibres serving the simpler endings were often even in calibre over a long stretch, more or less normal in appearance but of unusually large diameter. The varicosities referred to are either fusiform swellings, reminiscent of those seen along the course of regenerated axons where they mark the sites of temporary obstruction (Weddell, 1942), or irregularly shaped swellings resembling those seen either in the early stages of nerve degeneration or following prolonged compression of nerve bundles (Denny-Brown & Brenner, 1944*a*, *b*). It is worth mentioning here that varicose fibres of both kinds, though not mentioned in the literature, are clearly delineated in many of the illustrations, including those of Dogiel and Crevatin (see Text-figs. 3*b* and 4*a*).

We were unable to answer the question, first raised by Dogiel, whether some or all 'end-bulbs' possess both entrant and emergent nerve fibres. Most commonly, one or more fibres enter (or leave) the 'bulb' at one place, which one may term the 'hilum'; sometimes fibres are seen leaving (or entering) in another part of the 'bulb'; but whether these are independent fibres, or the continuation of one of the others, cannot be determined owing to the complexity of the entanglement within the bulb. In one or two specimens, however, we have seen a fibre forming either a simple unbranched entanglement or a more complex branched entanglement at some point in its course, thereafter continuing as a single axon for a short distance before terminating in an 'end-bulb'. These entanglements would correspond to what Krause called *Knäuel*. Pl. 3, fig. 30, is an example of such a formation. The axon '*a*' enters the entanglement '*b*', where it gives off a number of abbreviated offshoots which end in small club-like processes. The stem axon itself proceeds to the end of the entanglement where it divides into two. Both daughter axons then pursue a recurrent course and emerge from the entanglement at '*c*'. Here they fuse into a single axon which after a short straight course terminates by branching in a complex manner and forming a large 'end-bulb' (cf. Text-figs. 2*e, f*, and 4*a*).

In addition to silver impregnation, we made spreads of a human conjunctiva stained with methylene blue, and sections of an eyeball fixed and stained with osmium tetroxide. The former showed no compact endings. In the latter, we found a group of three in a single section, which closely resembled forms described by the earlier workers (Pl. 3, figs. 31, 32; cf. Text-fig. 2*d, e*). In these, the myelin of the axons and of their branches ramifying on the outside of the 'bulb' could be clearly seen. A feature of this, which can be seen in several of the illustrations of Key and Retzius and others, is that the tangled nerve fibres are only intermittently myelinated—that is, a myelinated segment often comes between two non-myelinated segments (Text-fig. 2*f*). The arrow in Pl. 3, fig. 31, points to a non-myelinated segment of an axon encircling one of the 'bulbs' in our preparation. The irregular contours of the myelin sheaths may well be a post-mortem change.

In summary, we have seen, in material from cadavers, compact nerve endings of a wide variety of types, most of which have been described at one time or another in the past. We were able to confirm previous observations on the extremely irregular distribution of these endings. We have the impression that the fibres ending in this manner are in some way 'abnormal'; that the terminations themselves consist of nervous and Schwann elements only; and that their 'capsules', when they are present, are merely prolongations of the normal coverings of the nerve fibres entering into their formation. Finally, in Table 2, we have summarized all our histological observations.

Table 2. *Summary of histological observations*

	Eyes		
Source	No. examined	No. with compact endings	No. of compact endings seen
Laboratory animals	38	3	5
Slaughterhouse material	17	5	1 eye: more than 20 2 eyes: 5–20 per eye 2 eyes: 1–2 per eye
Human autopsies	19	17	4 eyes: more than 20 per eye 7 eyes: 5–20 per eye 4 eyes: 3–4 per eye 2 eyes: 1 per eye

B. *Observations* in vivo

The first elements to take up the blue stain are randomly scattered superficial cells of the epithelium. Fortunately, the colour fades during the course of the experiment, so that when nerve staining is most complete the stained epithelial cells have largely become invisible again. Next, after an interval varying from

20 min. in some subjects to over an hour in others, isolated segments of the conjunctival nerves are seen as blue threads. At about the same time, compact endings, if they are present, begin to show themselves; however, they cannot be identified with certainty until their parent axons take up the dye selectively. More and more 'normal' axons are seen, including the deeper-lying bundles and the finer superficial branches, which appear to end by attenuation, until after a period which varies between $\frac{3}{4}$ and $2\frac{1}{2}$ hr., the whole conjunctival nerve plexus is stained. As has been mentioned before, it often happens that staining in one sector lags behind that in another. The nerves of the cornea are also stained, but the nerve bundles entering it appear to be cut short at the limbus unless slit lamp conditions are substituted for direct illumination. In one case, a few capillaries containing blue-stained erythrocytes were seen in the conjunctiva; apart from these, and the epithelial cells mentioned above, there was no specific staining of non-nervous structures. Pl. 4, figs. 33 and 34, give a general impression of the pattern of the conjunctival vessels and nerves in two young subjects in zones free from large axons and 'end-bulbs' after staining *in vivo*.

As regards compact endings, Table 3 gives a summary of our findings. It will be seen that they reinforce the histological observations in several respects, particularly in showing the gross disparity in numbers as between different subjects (over 50 in one subject, none at all in another) and between different sectors of the same eye (for example, 27 in one quadrant, 6 in another, and none in the remaining half). As a general rule, we were able to confirm the observation of Ciaccio and others, that compact endings tend to occur most frequently in the upper and outer quadrant of the conjunctiva. In the matter of shape and size, too, gross differences were observed. In the case of very small (under 20 μ) endings, it was sometimes difficult to decide whether they were in fact nerve endings or epithelial cells which had not become completely decolorized; and it is possible that some of our counts were too low, owing to our having ignored some of the smallest and simplest end-formations. Admitting this, we were left in no doubt whatever as to the extreme variability of compact endings in the matter of size, form and distribution. The fact that the compact endings regularly showed a strong affinity for the dye, by staining earlier and more densely than the neighbouring nerve fibres, renders it very improbable that the observed irregularity in their numbers was due to either uneven or incomplete staining.

Another point in confirmation of the histological findings was the almost invariable association of compact endings with axons of large diameter and irregular contour. Not every ending was served by an obviously abnormal fibre, although minor degrees of abnormality would not be visible under the magnifications available; and, in one case, in an eye which was being stained for the second time, some fibres having an exceptionally large diameter were seen which, towards their terminations, became suddenly very fine and apparently ended by attenuation. Apart from this, the association of fibres of exceptionally large diameter and compact endings was too close to be fortuitous.

In every case of repeated staining, there was a striking diminution in the number both of compact endings and of exceptionally large nerve fibres. The larger endings had disappeared completely, and only a sprinkling of smaller compact terminals was

Table 3

Subject	Sex	Age	Eye	Date	Findings
AGMW	M.	49	L	20. iii. 57	40 compact endings counted, of very varied shapes and sizes, irregularly grouped in all quadrants, but most plentiful in the upper outer quadrant. Many abnormally thick axons served the 'end-bulbs' but many normal axons seen, apparently ending by attenuation.
DRO	M.	42	L, 1st expt.	11. vi. 56	50 compact endings counted, of very varied shapes and sizes, irregularly grouped in all quadrants but most plentiful in the upper outer quadrant. Many abnormally thick axons served the 'end-bulbs' but many normal axons were seen apparently ending by attenuation.
DRO	M.	42	L, 2nd expt.	3. vii. 56	10 compact endings counted, irregularly scattered in the outer quadrants. All the 'end-bulbs' were small and rounded in shape. No abnormally large axons served them nor were any seen elsewhere in the bulbar conjunctiva. A full complement of normal axons was seen apparently ending by attenuation throughout the conjunctiva.
DRO	M.	42	L, 3rd expt.	21. ix. 56	5 compact endings counted, randomly scattered in the upper quadrants. 4 of the 'end-bulbs' were small and 1 of them was large. 3 abnormally large axons served the 'end-bulbs'; there was a full complement of normal axons apparently ending by attenuation throughout the bulbar conjunctiva.
DRO	M.	42	R, 1st expt.	24. x. 57	40 compact endings counted, of very varied shapes and sizes (although 16 of them were exceptionally large and arose from abnormally thick axons.) The 'end-bulbs' were irregularly grouped in all quadrants but most plentiful in the upper outer quadrant. There was a full complement of normal axons throughout the bulbar conjunctiva.
DRO	M.	42	R, 2nd expt.	30. i. 57	4 small rounded compact endings counted in the upper outer quadrant. 5 abnormally thick axons were seen, 3 of which appeared to end by attenuation. The other 2 served 'end-bulbs'. Some capillaries filled with immobile blue-stained red cells were seen in the early stages of this experiment.
PPL	M.	28	L	25. x. 56	33 compact endings counted, of varied shapes and sizes. 27 were seen in the upper outer quadrant and 6 in the lower outer quadrant; none were seen in the inner quadrants. Some abnormally large axons served them. There was a full complement of normal axons apparently ending by attenuation.

Table 3 (*continued*)

Subject	Sex	Age	Eye	Date	Findings
BP	F.	23	L	5. vii. 56	7 compact endings of varied shapes and sizes arose from a single axon of abnormally large diameter in the upper outer quadrant. No 'end-bulbs' or abnormally large axons were seen elsewhere in the conjunctiva. There was a full complement of normal axons which apparently ended by attenuation.
BP	F.	23	R	28. viii. 56	3 compact endings counted in a single group in the upper outer quadrant. No 'end-bulbs' were seen elsewhere; nor were any abnormally large axons encountered. There was a full complement of normal axons which apparently ended by attenuation.
JD	F.	18	L	13. iii. 57	11 small rounded compact endings counted. There were 10 in the upper outer quadrant and 1 in the upper inner quadrant. No 'end-bulbs' were seen elsewhere; nor were any abnormally large axons encountered. There was a full complement of normal axons which apparently ended by attenuation.
CNO	F.	14	L	29. viii. 56	25 compact endings of varied shapes and sizes counted, arranged in 6 groups sited irregularly around the limbus. A single abnormally thick axon was seen; it appeared to terminate by fraying out into a tassel-like formation. There was a full complement of normal axons which appeared to end by attenuation.
RNO	M.	12	R	19. ix. 56	14 compact endings of varied shapes and sizes counted in the upper outer quadrant; none were seen elsewhere. No abnormally thick axons seen. Full complement of normal axons was seen in the outer quadrants. Staining of the inner quadrants was incomplete.
SJO	M.	9	R	20. ix. 56	0 compact endings counted in the bulbar conjunctiva. No abnormally thick axons were seen. There was a full complement of normal axons throughout the bulbar conjunctiva.

seen. There appeared to be no decrease in the density of the innervation of the conjunctiva in general. Thus, one must suppose either that the endings had become refractory to the stain, or that they had been destroyed by the dye. The latter alternative appears more plausible, for methylene blue is known to interfere with tissue respiration, and in general those structures which are most vulnerable from this point of view become blue first.

In summary, the following points emerge from our observations on vital staining with methylene blue:

(1) There are gross disparities in numbers of compact endings as between individuals and as between different sectors of a given eye. When present, they tend to be commonest in the outer and upper quadrants. Too few subjects have been examined to make a clear correlation between age and numbers of compact endings; however, it is worth remarking that the largest number was seen in the oldest subjects, and the smallest number in the youngest.

(2) Compact endings are usually, though not invariably, associated with thick nerve fibres having an uneven calibre.

(3) When the same eye is stained for a second time, there is a striking diminution in the number of compact endings although no obvious decrease in the local density of innervation.

Pl. 4, figs. 35–41, illustrate some of the compact endings which we saw in the stained human eye.

Pl. 4, fig. 39, shows a single compact ending seen in the upper outer quadrant of the bulbar conjunctiva of the right eye in a male aged 42 years. The axon serving it is large, irregular in outline and pursues a tortuous course; the 'normal' axons in the nerve bundles on the left of the picture are in sharp contrast. More than forty compact endings, of which sixteen were 100 μ or more in diameter, were seen in this eye. Under low magnification they looked very like those in Pl. 4, fig. 40, which is of a small area of bulbar conjunctiva in the upper outer quadrant of the left eye in a male aged 49 years. More than forty compact endings were counted in this eye also. The photograph (Pl. 4, fig. 40) was taken soon after staining; thus some of the epithelial cells were still dark blue and many 'normal' nerves had not yet taken up the dye.

Pl. 4, fig. 35, shows one of four small compact endings seen in the upper outer quadrant of the same eye as that from which Pl. 4, fig. 39, was taken, but after an interval of 14 weeks had elapsed from the time the eye was first stained. In addition to the four compact endings a full complement of 'normal' nerves was seen which apparently terminated by attenuation in the usual way. In other words, the 'abnormal' axons, together with all but four of the 'end-bulbs' they served, had disappeared in the interval between the experiments. Another point to be noted is that the terminal shown is small and served by a fine axon, which is presumably non-myelinated, for all that can be seen is a pathway of Schwann cells whose nuclei can be seen at regular intervals along its course. This is what was once known as a Schwann 'band'. In methylene-blue preparations of skin examined with the light microscope, Schwann 'bands' are *only* seen when fine regenerating axons have either reached them or are within a few microns of them (Weddell, 1942).

DISCUSSION

The two most striking features of the earlier work on the innervation of the conjunctiva are: first, the contradictory findings of different authors; and secondly, the extreme variability of form and distribution of compact nerve endings reported by many of them. It seems highly probable that the first was the direct result of the second—in fact, that the contradictions arose from limited observations on material which was liable to great variations. Even so, this very variability, which is far in excess of what is found in the innervation of other parts of the integument, and which is amply borne out by our own observations, is an interesting fact, which seems to call for some attempt at explanation. Hitherto, so far as we know, the attempt has not been made.

Regarding the biological significance of compact nerve-endings, it seems to have been assumed on all sides that they are of the nature of sensory end-organs, playing a specialized rôle in the sensibility of the integument. The assumption is a natural one, and in the case of hair follicles and the corpuscles of Meissner and Pacini, we have no desire to quarrel with it. The exact sensory function of these structures is still not altogether clear, but there is no question of their regular occurrence in characteristic zones, or of the relative stability of their numbers and their morphology.

The case is different with the compact nerve endings of the conjunctiva, and we now believe there is evidence to justify us in casting doubt on the view that they are 'sense organs' in the usual meaning of the term, and in proposing an alternative view.

It is well known from the work of Perroncito (1905), Cajal (1928) and others, that following experimental section of a peripheral nerve, a certain proportion of the fibres in the proximal stump are obstructed in their efforts to grow peripherally, as a result of the closure of the distal end of the so-called digestive chamber. The axonal stump may thicken and assume bizarre forms, and the digestive chamber may become filled with sprouting collaterals, with growth cones at their tips. These 'sterile' endings may assume a large size—up to 40 μ in diameter, according to Cajal—but sooner or later they degenerate and are absorbed into the surrounding tissue. There are, in addition, endings of similar appearance, which Cajal describes as 'semi-fecund'. In these, one or more collaterals are seen escaping from the digestive chamber into the surrounding tissue, and terminating some distance away in typical growth-cones.

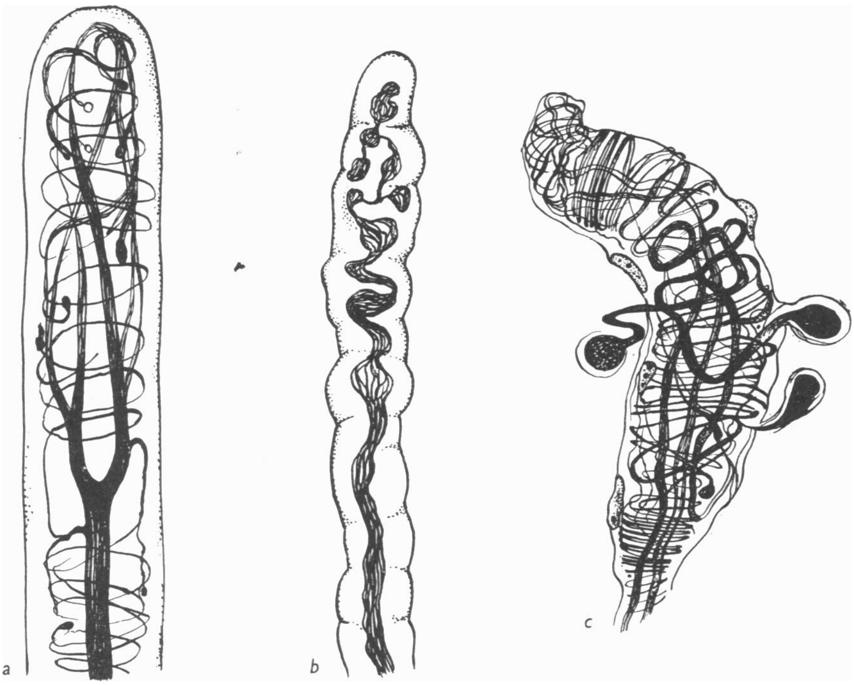
A very characteristic form of 'sterile' ending is the apparatus of Perroncito. Here the digestive chamber forms a closed, elongated bag containing the thick axonal stump, and a number of fine, collateral fibres which pursue a spiral retrograde course, seeking, as it were, to escape by coursing round and round their prison wall (Text-fig. 5*a*). In a second type (Text-fig. 5*b*) no fine collaterals are seen, but the axonal stump is irregularly thickened and shows numerous short club-like excrescences.

Of a third type, the 'nervous spool', Cajal writes: "[It] is in reality nothing more than a persistent apparatus of Perroncito, whose central and spiral fibres have grown very much in thickness and in length, have acquired partially or completely a sheath of Schwann and a medullary sheath, and terminate either inside or outside the sheath of the old tube, in medium-sized or large capsulated balls" (Text-fig. 5*c*).

We have selected these three types of 'sterile' ending from the wide variety described by Cajal, because we have seen structures closely resembling each of them in our present material. Thus, the bovine 'end-bulb' shown in Pl. 1, fig. 11, shows a structure strongly reminiscent of the Perroncito spiral (Text-fig. 5*a*); those of Pl. 1, figs. 8 and 9, are very similar to the clubbed endings of Text-fig. 5*b*; while the 'nervous spool' (Text-fig. 5*c*) has obvious features in common with the typical human 'end-bulb' (Pl. 2, fig. 20; Pl. 3, fig. 27).

It was by structural similarities of this nature that we were led, in the first place, to consider the possibility of an alternative view of conjunctival 'end-bulbs'—namely, that they might represent stages in a continuous or intermittent process of growth, decay and regrowth in the distal ends of peripheral nerve-fibres.

Taken alone, of course, such structural resemblances prove nothing (we would point out, incidentally, that the assumption which we are calling in question—namely, that 'end-bulbs' are sense organs—was itself based on no more than a real or fancied structural resemblance between 'Krause end-bulbs' on the one hand and the corpuscles of Meissner and Pacini on the other). There are, however, certain additional points of evidence which strongly suggest that the similarity between 'end-bulbs' and the 'sterile' or 'semi-fecund' spools described by Cajal is more than superficial.



Text-fig. 5. Redrawn from Cajal (1928). (*a*) Apparatus of Perroncito (cf. Pl. 1, fig. 11). (*b*) Tuberose end-formation (cf. Pl. 1, figs. 8–10). (*c*) Nervous spool (cf. Pl. 3, fig. 27). Silver impregnations.

In the first place, there is the appearance of the axons supplying a large proportion of compact endings. The thickening and irregularity, which is seen both in silver preparations and in vitally stained human material, must be taken as *prima facie* evidence of a disturbed physiological state. In addition, the 'human' type of compact ending shows a remarkable feature in that the myelin sheath of the terminal portion of axon, and of the outer entanglement of thick fibres, is frequently discontinuous, so that the fibres exhibit alternate myelinated and unmyelinated segments. This feature is clearly brought out in Key and Retzius's illustrations (Text-fig. 2*f*), and we have observed it in our material (Pl. 3, fig. 31, at arrow head). It is probably of some significance, in view of the fact that intermittent myelination is a well-recognized feature of growing nerve fibres (see Speidel, 1932, 1933, 1935).

The second observation is the finding of 'bovine' endings embedded in the substance of a nerve bundle (see Pl. 2, fig. 15). Their presence here is rather difficult to explain on the old assumption that they are sense organs. This difficulty does not arise if they are regarded as mere by-products of some process of neural growth.

It will be appreciated that the theory we are putting forward would remove the 'Krause end-bulb' and other compact endings in the conjunctiva from the category of sensory end-organs which includes the corpuscles of Meissner and Pacini. We believe, in any case, that there are good morphological grounds for doing so. In the case of Meissner's and Pacini's corpuscles there is a definite framework, composed of non-nervous elements, among which the sensory nerve terminals insinuate themselves (Cauna, 1956, 1958). Thus, it is legitimate to speak of a Pacinian corpuscle as an organ, which is innervated. In the 'end-bulb', on the other hand, we have so far failed to detect any elements other than axis cylinders, myelin and Schwann sheaths and the terminal expansion of the endoneurium which constitutes the capsule of the 'end-bulb'. In other words, the innervation constitutes the whole 'organ'. A reservation must be made in the case of the bovine 'end-bulb', which contains a granular, apparently acellular core surrounding the central axonal stump. The nature of this material is unknown. If our theory is correct, it is probably the cytoplasm of enlarged Schwann cells containing the 'digested' débris of an earlier Perroncito formation.

Two problems arising out of the work of Dogiel have received little attention from subsequent histologists. These consist in his finding, as a regular occurrence in the conjunctiva and elsewhere, of serially innervated *Knäuel* (see pp. 325 and 326) and of branched fibres, one branch of which terminates in a *Knäuel* and the other as a diffuse arborization. If the *Knäuel* in question are to be regarded as specialized nerve endings, Dogiel's findings can hardly fail to be puzzling, as they would involve a radical revision of our conception of the anatomy of peripheral nerve endings. We ourselves reject the easy explanation which regards these particular formations described by Dogiel as artefacts resulting from mechanical distortion, partly because it is difficult to envisage how myelinated nerve fibres of the size depicted could be locally twisted into such bizarre shapes in the course of preparation for microscopical examination, and partly because we ourselves have seen neural formations of a similar kind which we have good reason to believe are not artefacts. For one thing, the entanglements have certain morphological characteristics which would not be seen in artificially coiled and twisted nerves (see pp. 338 and 339 and Pl. 3, fig. 30). These appearances, we repeat, are hard to explain if the 'end-bulbs' in question are sense-organs. If they are the result of 'obstruction' in the course of regeneration, the problem disappears. It will be remembered that Cajal described, in the proximal stump of a divided nerve, a type of 'semi-fecund' ending in which a collateral (rarely two) succeeded in penetrating the capsule of the nervous spool, and ran a further straight and direct course, to end as a typical growth cone in the scar. There is no difficulty, then, in supposing that in the conjunctiva such an emergent collateral might either form a normal diffuse arborization and terminate in the epithelium, or meet a further obstruction and produce another 'sterile' or 'semi-fecund' ending (see pp. 335 and 337, and Pl. 2, figs. 16, 18).

A question, which has so far not been satisfactorily answered, arises in relation to the marked structural difference between the typical 'bovine' and the typical 'human' type of ending. It has been suggested by several investigators that the former is a modified Pacinian, and the latter a modified Meissner corpuscle. There are obvious difficulties in this view. On the other hand, if one is prepared to abandon the assumption that they are specific 'end-organs' and consider their possible analogy with 'nervous spools' and other formations described by Cajal, one can see how both types might develop from an initial state corresponding to the spiral of Perroncito. In the case of the 'bovine' type, the spiral fibres would wither away, leaving either a simple or a branched axonal stump in the centre; while in the 'human' type, the spiral fibres would persist, or even multiply, while the preterminal portion of axon, restricted by the endoneurium in its efforts to increase in length, would lay down successive coils in the outer layer of the 'spool'. As to why the one process should be more favoured in the ox and the other in man, we can offer no suggestion; but we find it easier to believe that certain tissue differences between man and ox result in somewhat different expressions of a similar growth process, than that the conjunctivae in the two species are equipped with different types of sense-organ.

One would also like to know why men and oxen, as compared with common laboratory animals, should be especially favoured with 'end-bulbs'. The only observation we can make on this point is that, of the species we have examined, only man, and to a lesser extent the ox, expose a large area of bulbar conjunctiva to the outside world. In all the rest, the limbus is almost completely concealed by the eyelids. Other mammals will have to be examined and experimental investigations carried out before this question can be satisfactorily answered.

There remains the question why there should be such wide variations in the number of compact endings in the conjunctivae of different members of one species, and in different regions of one conjunctiva. We do not know the answer; on the other hand, we regard this variability as one of the most significant pieces of evidence that the endings in question are to be regarded as stages in a process, rather than as specialized sense-organs. One does not find a similarly haphazard distribution in the taste-buds of the tongue, the hairs of the skin, or the tactile corpuscles of the finger tips. The most natural supposition is that the structures in question are labile (our experiments with repeated methylene-blue staining have shown this to be so) and may come and go in response to changes in either the physical environment or in the *milieu interne*.

The conception of lability in the peripheral nervous system is not new. Speidel (1940–1) has made direct observations of alternate progressive and regressive changes occurring in the nerves of the tail of the tadpole. Zander & Weddell (1951) showed that after a partial keratotomy nerve fibres spread into the denervated area from the neighbouring segments of cornea, only to regress as the normal innervation became re-established. Weiss (1941) has generalized these and similar observations into a concept of a dynamic process by means of which the density of innervation of any given area remains approximately constant. This concept is probably applicable particularly to the system of diffuse (free) endings which is found over the whole integument; and it involves the notion of a continuously alternating process of advance and withdrawal of the tips of the terminal arborizations of this system. In the present context, we are making the additional suggestion that in certain mucosal surfaces, including the conjunctiva, it may happen from time to time that a fibre is obstructed in its distal growth, and responds by the formation of 'sterile' or 'semi-fecund' terminals.

Mention has already been made of the experiments of Strughold & Karbe. In these, Dr Strughold's right eye was first explored for 'cold spots' and subsequently subjected to vital staining on three separate occasions, In the result, fifty-two 'cold spots' were identified, evenly distributed over the bulbar conjunctiva, and recorded on a chart. Vital staining revealed a total of five compact endings, three of which formed a compact group. This group, and the other two endings, were each found to lie within a 'cold spot'. These findings were held to confirm von Frey's view that the 'Krause end-bulb' was the specific receptor for sensations of cold. It is difficult to see how these experiments can be regarded as evidence for such a theory. It would seem, on the contrary, that only one of two conclusions could be drawn from them—either that the staining technique was inadequate to dispay the fifty-odd 'end-bulbs' postulated by the theory, or that sensations of cold can be evoked without the mediation of 'end-bulbs'. The second alternative has in fact been shown (Lele & Weddell, 1956; Sinclair, Weddell & Zander, 1952) to hold good in the case of the human cornea and hairy skin, and in experiments (at present incomplete) in the case of the conjunctiva.

Thus, although many questions remain to be answered, we feel that we are in a position to give a general answer to the questions which we originally proposed.

First, as to the occurrence of compact nerve endings in the mammalian conjunctiva, it seems that such endings are absent or rare in some animals, and relatively common in others. Among the latter, there are gross variations between one individual and another, and between different areas of a single conjunctiva.

Secondly, these endings do not conform to any constant morphological type. The 'end-bulbs' described by Krause are merely two forms selected from a wide variety of compact endings.

Thirdly, the assumption which has prevailed hitherto, that these endings possess some specific sensory function, must be regarded as unproven. It follows that experimental work based on this assumption must be regarded with suspicion.

SUMMARY

1. The literature on compact sensory nerve endings in the conjunctiva is reviewed.

2. Histological examination of the conjunctivae of various mammals, and vital staining of the human conjunctiva with methylene blue, lead to the following conclusions:

(*a*) The great majority of the nerve fibres entering the conjunctiva end as diffuse arborizations (free nerve endings) in relation to blood-vessels or to the epithelium of the conjunctiva and cornea.

(*b*) In the conjunctivae of common laboratory animals compact nerve endings are rare.

(*c*) Compact endings of many types, which include the 'end-bulbs' described by Krause, occur in very variable numbers in the conjunctivae of men and to a lesser extent oxen.

(*d*) When present, they are very irregularly distributed.

(*e*) Various lines of evidence suggest that these endings represent stages in cycle of growth and decay in certain peripheral nerve fibres rather than specialized 'sense-organs'.

(*f*) Various problems arising out of the older literature are considered in the light of this theory.

We are very grateful to Dr Norman Ashton and Dr Philip Bedford for supplying us with human eyes: to Mr Frank Blackwell, for his help on the photographic side: to Miss Mary Hickman for technical assistance: and to Mrs Mary Dagley for help in preparing the manuscript. This work was supported by a grant to one of us (Dr G. Weddell) by the Rockefeller Foundation, which is gratefully acknowledged.

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EXPLANATION OF PLATES

PLATE 1. Figs. 1 and 2 from frozen sections, remainder from whole mounts. Fig. 3 is from methylene blue, fig. 4 from gold chloride preparation. Remaining figures from silver preparations.

Fig. 1. Offsets from conjunctival plexus passing to epithelium. Ox.

Fig. 2. Fine nerve filaments ending by attenuation in conjunctival epithelium. Macaque.

Fig. 3. Loose neural tangle. Note coarse beading of fibres in this and following figure. Rat. (Cf. Text-fig. 4*g*.)

Fig. 4. Elongated formation of varicose nerve fibres. Macaque. Whole mount. (Cf. Text-fig. 4*f*.)

Fig. 5. Two sausage-shaped 'end-bulbs'. Cat.

Figs. 6, 7. Large sausage-shaped 'end-bulbs', with simple club-like central axons. Ox.

Figs. 8, 9. 'End-bulbs' with branched central axons. Ox. (Cf. Text-fig. 5*b*.)

Fig. 10. Club-like axonal swellings giving rise to fine fibres inside 'end-bulb'. Ox. (Cf. Text-fig. 5*c*.)

Fig. 11. Fine collateral fibres running spiral course inside 'end-bulb'. Ox. (Cf. Text-fig. 5*a*.)

PLATE 2. All figures from silver preparations. Figs. 20 and 23 from frozen sections, rest from whole mounts.

Figs. 12, 13. Normal and thick varicose nerve fibres lying side by side in small bundles. The varicose fibres terminated in 'end-bulbs'. Ox.

Fig. 14. Impregnation of myelin sheath with defective impregnation of axis cylinder. Arrows point to nodes of Ranvier. Vertical fibre terminated in 'end-bulb'. Ox.

Fig. 15. Intra-neural 'end-bulb'. Ox.

Fig. 16. Enlargement of 'end-bulb' in Pl. 1, fig. 7. Montage shows collateral emerging proximal to 'end-bulb' and terminating diffusely. Arrow indicates 'growth-cone'. Ox.

Fig. 17. Loose entanglement of axons in conjunctival nerve plexus. Diabetic patient.

Fig. 18. Simple 'end-bulb'. Note collateral fibre. This seen to end diffusely in epithelium. Human.

Figs. 19–23. 'End-bulbs' of increasing complexity. Human.

PLATE 3. Figs. 31 and 32 from whole mounts stained with osmium tetroxide, rest from silver impregnated whole mounts.

Figs. 24–26. Compound 'end-bulbs'. Human. (Cf. Text-fig. 4*e*.)

Figs. 27–29. Various 'atypical' end formations. Human.

Fig. 30. Complex entanglement (*Knäuel*) arising on course of nerve fibre which terminated in 'end-bulb'. *a* axon proceeding to entanglement, *b* entanglement, axon gives rise to abbreviated offshoots which end in club-like processes then divides into two; *c* two emergent axons fusing, single axon proceeds on straight course for short distance before terminating in 'end-bulb'. Human.

Figs. 31, 32. 'End-bulbs' with myelinated fibres ramifying around central core. Arrow on Fig. 31 is pointing at non-myelinated segment. Human. (Cf. Text-figs. 1*a* and 2*c*–*f*.)

PLATE 4. All figures flash photographs of conjunctivae of living persons, stained with methylene blue.

Fig. 33. Lower inner quadrant bulbar conjunctiva; corneal margin on right. Nerve bundles radially arranged; axons have a regular contour and pursue a direct course. Neither large axons of irregular contour nor 'end-bulbs' are seen. Female, aged 18 years.

Fig. 34. Upper outer quadrant bulbar conjunctiva: axons arising from conjunctival plexus and serving diffuse terminals. Neither coarse axons of irregular contour nor 'end-bulbs' seen. Male, aged 9 years.

Fig. 35. Upper outer quadrant bulbar conjunctiva: one of only four 'end-bulbs' seen. Axon is certainly non-myelinated, the pathway in the picture is a series of Schwann cells and their nuclei (Schwann 'band'). 14 weeks previously this same eye was full of 'end-bulbs' (740). Male, aged 42 years. (Cf. fig. 39.)

Fig. 36. Inner quadrant bulbar conjunctiva at limbus: 'end-bulb' served by large coarse fibre of regular contour pursuing a straight course. Male, aged 14 years.

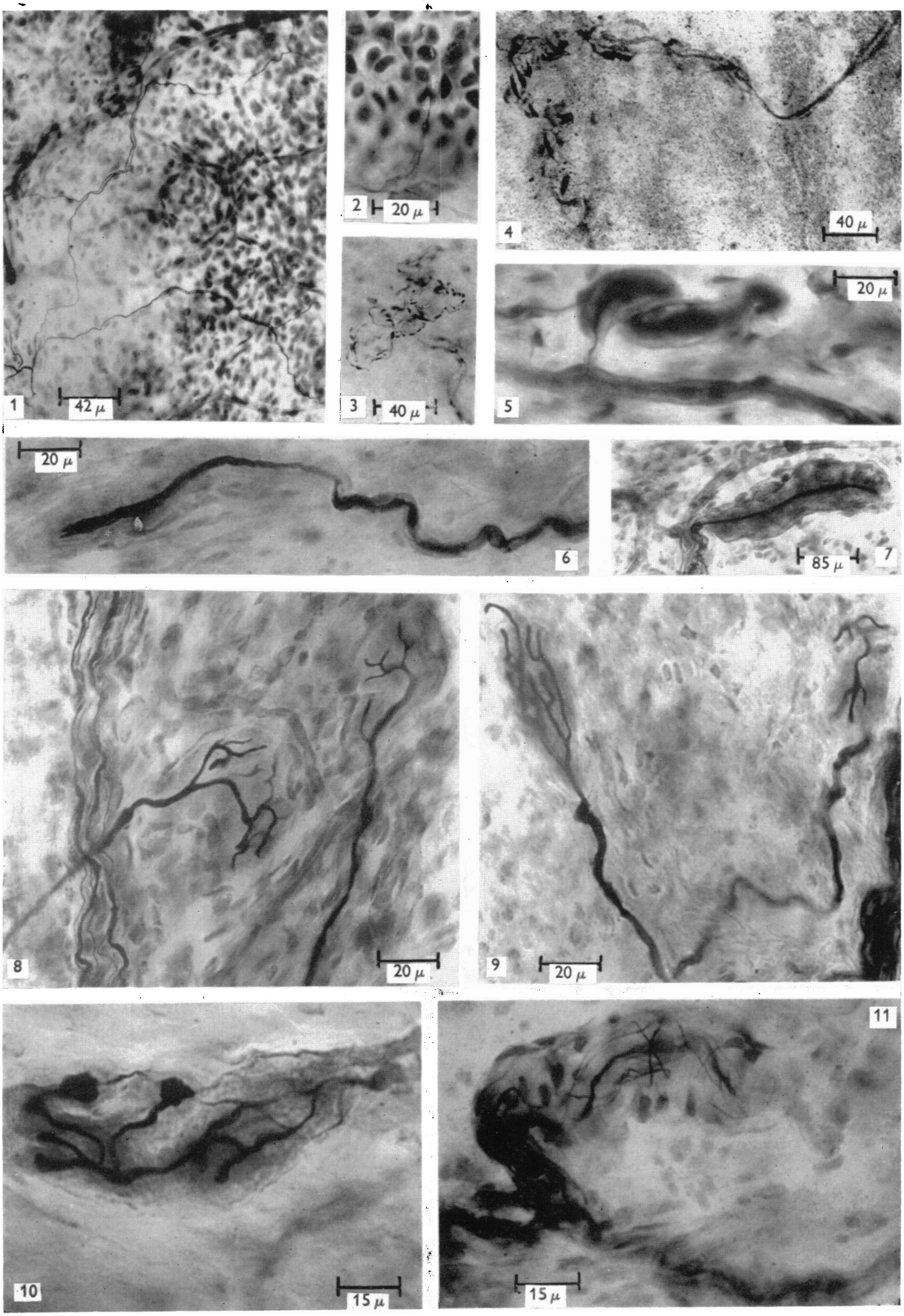
Fig. 37. Upper outer quadrant bulbar conjunctiva: one of 14 'end-bulbs'; it is small and deeply stained. The fibre serving it pursues an irregular course. Compare fig. 4 from same eye. Male, aged 12 years.

Fig. 38. Upper outer quadrant bulbar conjunctiva: clusters of 'end-bulbs', of various sizes and complexity, served by fibres of irregular outline pursuing a relatively straight course. Male, aged 12 years.

Fig. 39. Upper outer quadrant bulbar conjunctiva: single large 'end-bulb' served by a coarse fibre of irregular contour pursuing a very tortuous course. On the left there are bundles of normal nerve fibres; they are much finer, regular in contour and pursuing a straight course. More than 40 'end-bulbs' were counted in this eye: 14 weeks later, only 4 'end bulbs' were seen, but a full complement of 'normal' nerves. Male, aged 42 years. (Cf. fig. 35.)

Fig. 40. Upper outer quadrant bulbar conjunctiva: many 'end-bulbs' of different sizes served by large axons of irregular contour, pursuing tortuous courses. Picture taken shortly after staining, some epithelial cells are still dark blue. Male, aged 49 years.

Fig. 41. Upper outer quadrant bulbar conjunctiva: one of 14 'end-bulbs'. It is very large, and served by an axon of irregular contour pursuing a somewhat irregular course. Compare fig. 37, which shows another 'end-bulb' less than 2 mm. distant. Male, aged 12 years.



OPPENHEIMER, PALMER & WEDDELL—NERVE ENDINGS IN THE CONJUNCTIVA

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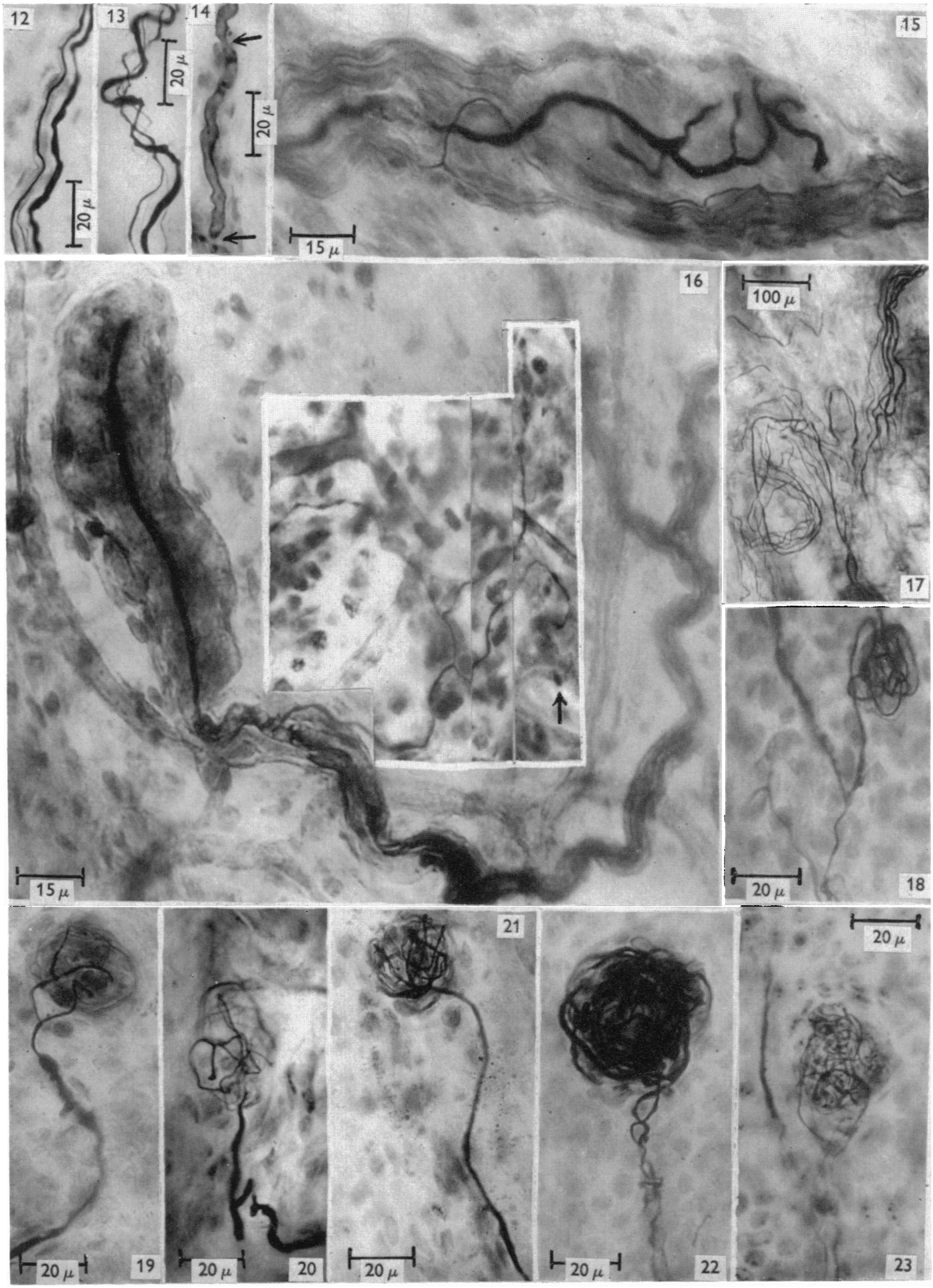
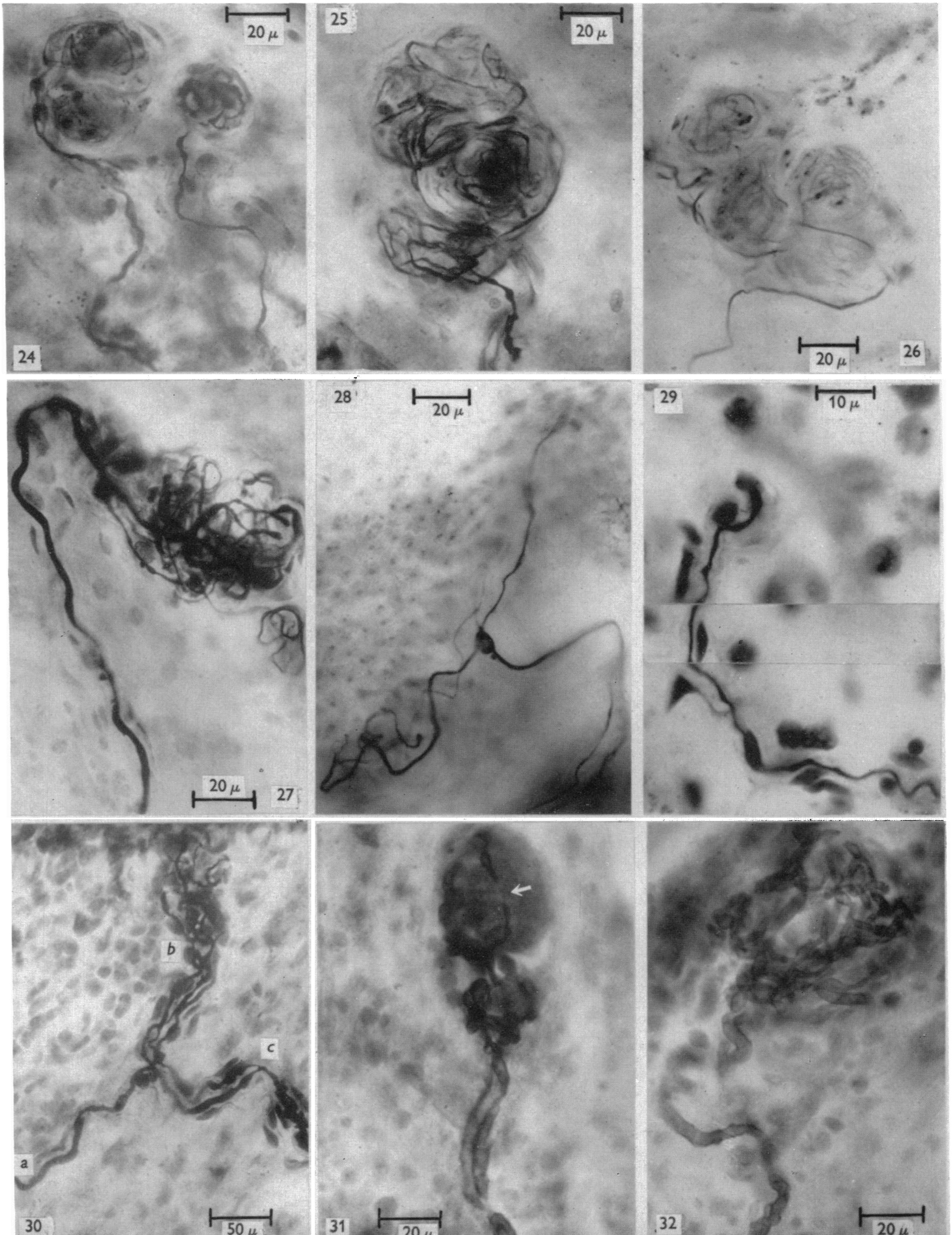
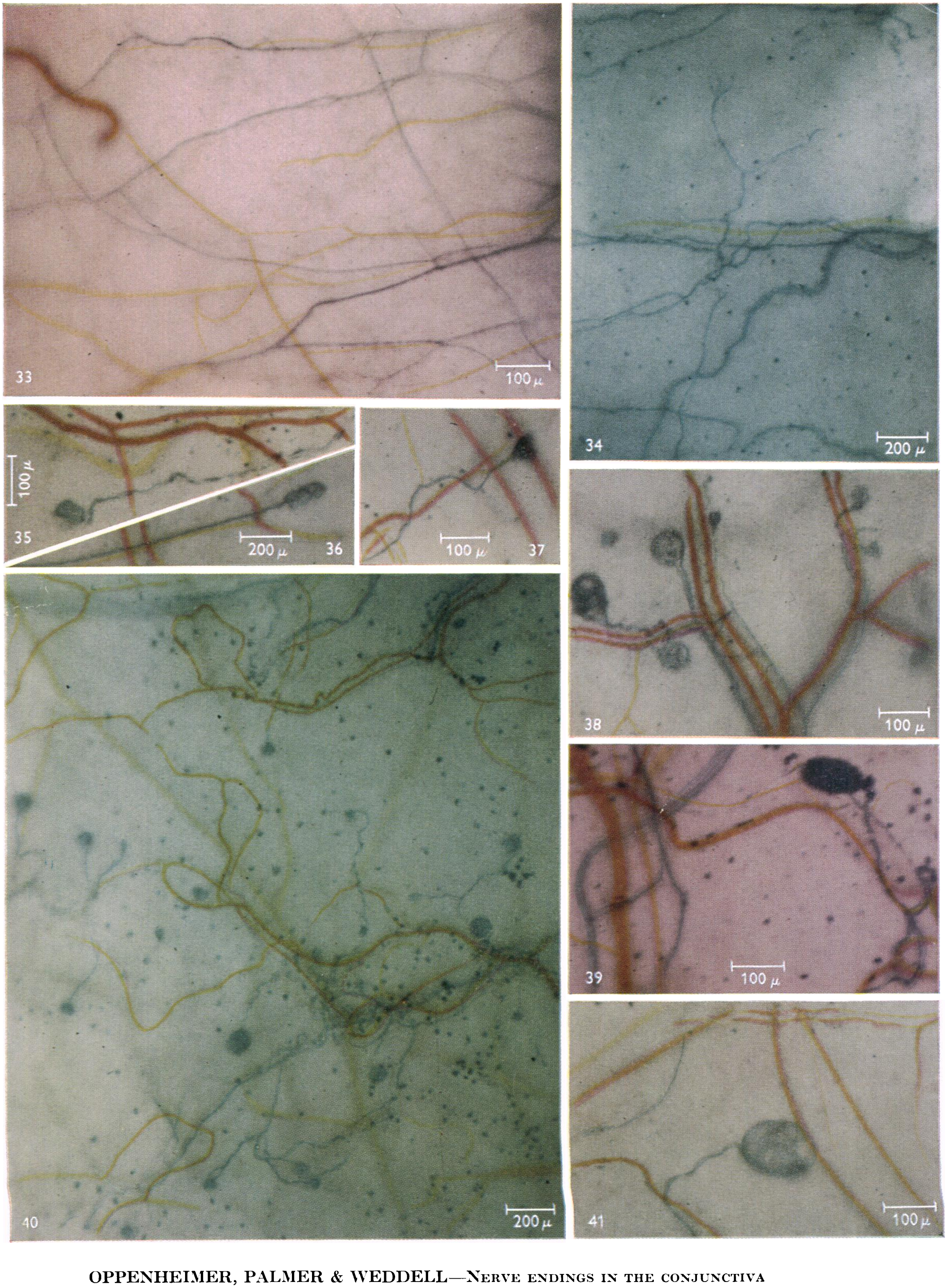


OPPENHEIMER, PALMER & WEDDELL—NERVE ENDINGS IN THE CONJUNCTIVA



OPPENHEIMER, PALMER & WEDDELL—NERVE ENDINGS IN THE CONJUNCTIVA



OPPENHEIMER, PALMER & WEDDELL—NERVE ENDINGS IN THE CONJUNCTIVA