For substances for which the size of the molecules in the vapour and the liquid phase is equal or about equal f is about 3, so that the value 4,221 indicates that for the violet phosphorus the average size of the molecules in the coexisting vapour and liquid phases is different.

A discussion of the vapour tension line of the white phosphorus will be reserved for another paper.

Anorg. Chem. Laboratory of the University.
Amsterdam, Dec. 24, 1914.

Anatomy. — *"On the termination of the efferent nerves in plain muscle-cells, and its bearing on the sympathetic (accessory) innervation of the striated muscle-fibre.* By Prof. J. BOEKE.

(Communicated in the meeting of December 30, 1914).

In recent years it has been demonstrated for a large number of different types of striated muscle-fibres of vertebrates, that the motor nerve-endings, which carry the nervous impulses towards the muscular fibres, are hypolemmal in position, i.e. are not lying outside the sarcolemma of the muscle-fibres as maintained by most of the earlier observers, but pass through the sarcolemma, which becomes continuous with the neurilemma, penetrate into the sarcoplasm of the muscle-fibre, which is considerably thickened as the site of formation of the end-organs, and it was further assumed, that here the neurofibrillar expansion of the motor nerve-ending is directly continuous with a reticulum in the sarcoplasm, the so-called "periterminal network" [1]).

At the same time it was shown, that beside the common motor nerve-ending there must be distinguished another afferent nerve-ending on the striated muscle-fibres of vertebrates, which may either be found imbedded in the same granular bed of sarcoplasm or "sole" of the motor endorgan of KÜHNE, or reaching the muscle-fibre independent of this, but always having a hypolemmal position in the muscle-fibre [2]). This "accessory" nerve-ending is always found at the end of a non-medullated nerve-fibre. As it could be shown, that these accessory nerve-endings did not degenerate after the cutting of the efferent muscle-nerve near the place of exit from the central nervous system, before the entering of the sympathetic nerve-fibres

[1]) J. BOEKE. Beiträge zur Kenntniss der motorischen Nervenendigungen. I. II. Internat. Monatschr. f. Anatomie und Physiologie Bd. 28. 1911.

[2]) J. BOEKE. Die doppelte (motorische und sympathetisch) efferente Innervation der quergestreiften Muskelfasern. Anat. Anzeiger. 44. Band. 1913.

(for example the accessory nerve-endings of the eye-muscle after the cutting of the nervus trochlearis near the mid-brain) which causes the motor nerve-endings of KÜHNE to degenerate, there was room for the conclusion, that the accessory nerve-fibres and their endorgans were of sympathetic origin, and perhaps had something to do with the tonic innervation of the striated muscle [1]). In my first paper, which dealt with these nerve-fibres (Congress Utrecht, April 1909), this conclusion was already drawn by me [2]). In the years 1913 and 1914 strong supporting evidence has been given to it by the admirable physiological experiments of DE BOER [3]).

In Fig. 1 is shown a degenerated motor nerve-ending (*m*) in a

Fig. 1.

Nerve-endings in muscle-fibres of the musc. obl. sup. oculi of the cat, $3^1/_2$ days after section of the nervus trochlearis.
af = accessory (sympathetic) fibre with end-organ, not degenerated.
m = degenerated motor nerve-fibre with degenerated end organ.

muscle-fibre of the superior oblique muscle of the eye-ball of a cat some days after section of the nervus trochlearis near the mid-brain. The accessory non-medullated nerve-fibre (*af*) is not degenerated,

[1]) See note 2, p. 982.

[2]) Proceedings of the 9th phys. and medical Congress. Utrecht, April 1909. See also the more detailed description in the Anat. Anzeiger 44. Band, 1913.

[3]) S. DE BOER. Folia Neuro-biologica 1913 and 1914.

and shows a normal hypolemmal nerve-ending in a muscle-fibre at the end of one of the non-medullated nerve-fibres.

If thus we may assume this so called accessory innervating apparatus of the striated muscle-fibres to be of a sympathetic (parasympathetic) nature, then the following question arises immediately: both the motor nerve-ending and the accessory nerve-endings are hypolemmal in position, i.e. the nerve-fibre passes through the sarcolemma and enters the muscle-fibre, being imbedded in the granular substance of the sarcoplasma, aud directly continuous with the intraprotoplasmatic reticulum of the periterminal network. Now it is generally assumed, that the efferent sympathetic nerve-endings do *not enter* the plain muscle-cells, but terminate by tapered or bulbous extremities which are applied to the outer surface of the cells. Why should there exist such a curious contradiction between equivalent elements? Why should the accessory nerve-ending in the voluntary muscle-fibres (when of sympathetic origin) be hypolemmal, and the sympathetic nerve-ending in plain muscle-cells remain on the outside of the innervated elements? We will try to show, that there is no such contradiction, and that the efferent nerve-endings in plain muscle-cells have exactly the same position as the accessory nerve-endings, which give the striated muscle-fibres their tonic impulses.

In general the modes of termination of the efferent nerves in involuntary muscles are rather difficult to study. The Golgi-method and staining with methylene blue often procure splendid results, but these methods do not allow to give a definite answer as to the exact relations between the nerve-endings and the plain muscle-cells. Apart from earlier aberrant accounts, that localised the terminations of the nerve-fibres inside the cell, even inside the nucleus and the nucleolus (THANHOFER, lastly OBREGIA in 1890), all the observers agree (I need only mention the names of KÖLLIKER, LÖWIT, ERIC MÜLLER, HUBER and DE WITT, S. RETZIUS), that the efferent nerves of the plain muscle-cells form complicated plexuses between the elements of the involuntary muscular tissue, in which the nerve-fibres bifurcate and give off branches at frequent intervals, and then, either united with those from adjoining nerve-fibres or not, come into close relation with the plain muscle-cells themselves, either terminating by tapered or bulbous extremities which are applied to the surfaces of the cells, or ending in networks and loops, without having free extremities.

Thus even the last observer in this field, AGABABOW [1]), who studied

[1]) A. AGABABOW. Ueber die Nerven der Augenhäuten, v. Graefe's Archiv für Ophthalmologie, 83. Bnd, 2. Heft. 1912,

the distribution and mode of termination of the nerve-fibres in the corpus ciliare and the adjacent membranes of the human eyeball, states, that "in keinem einzigen seiner Praeparate je etwas zu sehen war, was als ein unmittelbarer Zusammenhang des Nervenfädchens mit dem Protoplasma, dem Kern oder gar dem Nucleolus der Muskelzellen zu deuten wäre" [1]).

AGABABOW, as I mentioned before, studied the innervation of the corpus ciliare. This led him chiefly to a confirmation of his earlier observations on the same subject in the year 1897. In the corpus ciliare of human eyes and of the eyeballs of albinotic cats AGABABOW demonstrated the presence of the following nerve-terminations: 1. endorgans of the motor nerves in the plain muscle-cells of the musculus ciliaris, 2. vasomotor nerves for the ciliary bloodvessels, 3. terminations of afferent sensory nerves in the corpus ciliare, 4. terminations of afferent nerves in the lamina suprachorioidea, which covers the corpus ciliare as a loose soft membrane of areolar connective tissue at the outside. As for the motor nerves of the musculus ciliaris, they appeared in his preparations of cats' and mens' eyes (Golgi- and methylene blue-preparations) as numerous fine varicose threads running between the muscle-cells; at different points, always outside the cell-boundary, the nerve-fibres terminate and the ends are applied to the cell-surface. This however is after the observations of AGABABOW not yet the real ending of the nerve-fibres, for sometimes he could see one of these delicate fibres bifurcate again and the two exceedingly delicate terminal branches could be followed around the muscle-cell, encircling it on both sides. From these observations AGABABOW draws the conclusion [2]): "dass eine jede Muskelzelle von einem Netze sehr feiner Nervenfäden umflochten wird; hierbei stehen die Nervennetze der Nachbarzellen noch durch 2—3 Fädchen unter einander in Verbinding Diese Endigungsart der motorischen Nerven in Gestalt eines perizellulären Netzes, welches eine jede Muskelzelle umspinnt (o h n e terminale Anschwellungen) ist mit grosser Wahrscheinlichkeit eine fur die motorischen Nerven der glatten Muskulatur (Sphincter iridis, Gefässe, Darm) allgemeine Erscheinung".

AGABABOW studied the innervation of the corpus ciliare by means of the Golgi-method and intra-vitam staining with methylene blue. Now this account of AGABABOW coincides entirely with what I have seen in a number of Golgi- and methylene blue preparations of the

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[1]) l c. p. 355.

[2]) l.c. p. 358. cf. TIMOFEJEW 1896.

muscular coat of the intestine and of bloodvessels. The pictures only vary on account of the presence or of the distribution and number of the so-called terminal buds. Preparations after these methods are not sufficient however to study the intrinsic relations between nerve-endings and muscle-cells. For this we need thin (5—10 μ) serial sections of material sharply stained after the neurofibrillar staining-methods of CAJAL or BIELSCHOWSKY and counterstained by Haematoxylin and Eosin or Orange G. Unfortunately these staining-methods generally give only mediocre results, when applied to involuntary muscle-cells, and even in the best preparations the nervous plexuses between the muscular elements may be stained very sharply, but generally the final terminations are either not stained at all or take such a light stain, that it is impossible to draw any conclusions about the real relations between the nervous and muscular elements from them.

Some time ago however I got at my disposal, thanks to the kindness of Prof. P. TH. KAN, the director of the oto-laryngological section of the academic Hospital of our University, a freshly-enucleated normal human eye, which immediately after having been enucleated, had been put into a large quantity of neutral formaline-solution of 12 %.

Parts of the corpus ciliare and iris of this object were treated by the method of BIELSCHOWSKY, and these preparations turned out to have taken such a splendid stain as I had never yet met with in any of my neurofibrillar preparations of plain muscle-cells. Beside a very good preservation of the different histological elements the sections showed a perfect and strong colouring of the nervous elements, of which even the finest terminal fibrils and endings were visible as extremely delicate black lines, the thinnest of which were scarcely visible except under the highest power, but still stained a dark brown.

In this object I studied the relations between the nervous elements and the muscle-cells of the corpus ciliare. Serial sections (4—20 μ) were made through the corpus ciliare and iris in a transverse or a tangential direction. As a counterstain for the nuclei and the protoplasm were used haematoxylin and eosin or orange G. Especially the tangential sections through the musculus ciliaris were very instructive.

Letting alone for the moment the sensory innervation of the surrounding tissues, which need not be described here, we find, on turning our attention to the musculus ciliaris, two different systems of nervous terminations between the muscle cells. In the first place a

loose plexus (at places perhaps a network) with wide meshes of nervous non-medullated or partly medullated fibres, that end in distinct sheathed bulbous or coiled-up terminations; some of these are shown in fig. 2 (*a* and *b*). Since these nerve-fibres and their terminations lie in the connective-tissue around bundles of the plain muscle-cells and remain entirely independent of the muscle-cells, they must be regarded as the free nerve-endings of sensory nerves, which are already described by AGABABOW as being distributed in large numbers throughout the whole of the corpus ciliare.

In the second place we see in the musculus ciliaris a very fine plexus and network of very fine non-medullated nerve-fibres, with small meshes, lying between the muscle-cells, which at first sight seems to be of a bewildering complexity. Only gradually one learns to find one's way in the mass of extremely delicate black-stained threads running to and fro between the muscle-cells, and then it becomes clear, that this plexus contains in the first place the network described by AGABABOW, consisting of fine varicose nerve-threads, running between the muscle-cells, surrounding these cells, encircling them with smaller and longer meshes of extremely delicate fibrils and more or less thickened points of junction. At these points, visible in methylene blue-preparations as knots of a homogeneous blue colour, the neurofibrillar apparatus appears, when studied under the highest power, to be broken up into an extremely fine network of fibrillae. In fig. 2 at *c* a mesh of this network, magnified 2100 diameters is drawn. This network is the terminal network of AGABABOW. But now a close study of the sections soon reveals the fact, that this network, which encircles the muscle-cells, is *not* the terminal nervous apparatus. From the nerve-threads composing the meshes of this network, lying between the muscular elements and encircling them, are branched off at all points extremely delicate neurofibrillae, fine filaments having only a diameter of several millimicra, but appearing, thanks to the splendid impregnation of the sections, as distinctly visible black-stained threads of the greatest tenuity. Only these threads, that form a second network or plexus, exhibit the ring-shaped varicosities, the end-rings and small terminal nets, which must be regarded as the real terminations of the nervous apparatus. Some of these end-rings are drawn from the sections in fig. 2, *d*—*f*. But the fact, which interests us chiefly here, is that these end-rings (the termination of these final nervous branches is chiefly in the form of small rings or loops) are found lying *intraprotoplasmatically* inside the muscle-cells, and the fine fibrillae, composing this second network, form a reticulum in the protoplasm of the muscle-cells, encircling

the nucleus, running between the myofibrillae of the cell, and thus showing their intracellular position, finally giving off branches, that are so exceedingly fine and form such small meshes, that they cannot be distinguished from the protoplasmatic reticulum of the cytoplasm of the muscle-cells itself. Indeed, one gets the impression, that these finest terminal branches, given off by the delicate threads of the intraprotoplasmatic neurofibrillar reticulum, described above, are in the end nothing else but the protoplasmatic reticulum.

That this intraprotoplasmatic neurofibrillar reticulum with its ring-shaped varicosities and end-rings or terminal nets at the end of short twigs in reality lies inside the cell in the cytoplasm and not applied to the surface at the outside of the cell, may be demonstrated in the first place by the following fact. The muscle-cells of the musculus ciliaris are not always compact, but especially in the inner part more loosely arranged, so that in tangential sections through the inner parts of the corpus ciliare one often sees muscle-cells lying entirely isolated in the connective tissue. In these cases it is easy to determine whether the second neurofibrillar reticulum, mentioned above, lies inside the cytoplasm of the muscle-cell or simply surrounds the cell at the outside, and the intraprotoplasmatic position of this second reticulum with its ringshaped varicosities and end-rings, together with the very delicate threads passing from this netwerk into the protoplasmatic reticulum could be established with accuracy. Still, there is room for doubt, for a bundle of two muscle-cells might be cut lengthwise and it might be possible, that what was thought to lie in the cytoplasm, in reality was lying just between the two muscle-cells.

But the conclusion that the terminal branches of the neurofibrillar apparatus together with the terminal rings are intraprotoplasmatic in position is placed on a perfectly sure basis, when cases are found as are figured in fig. 2 *g* and *h*. Here we see the small terminal rings and nets of the second neurofibrillar network lying so close to the nucleus of the muscle-cell, that they even make an indentation into the nucleus, and thus are found lying in a shallow hole in the side or on the top of the elongated nucleus (fig. 2 *g* and *h*). Such cases are not rare in my preparations, indeed in nearly every section through the musculus ciliaris were found one or two of them; they can only be explained by adopting an intraprotoplasmatic position for the neurofibrillar rings. In many of these cases the connection of the rings, lying in the indentation of the nucleus, with the neurofibrillar network could be observed with perfect accuracy, and in several cases the direct connection of this intraprotoplasmatic neuro-

J. BOEKE: "On the termination of the afferent nerves in plain muscle-cells, and its bearing on the sympathetic (accessory) innervation of the striated muscle-fibre."

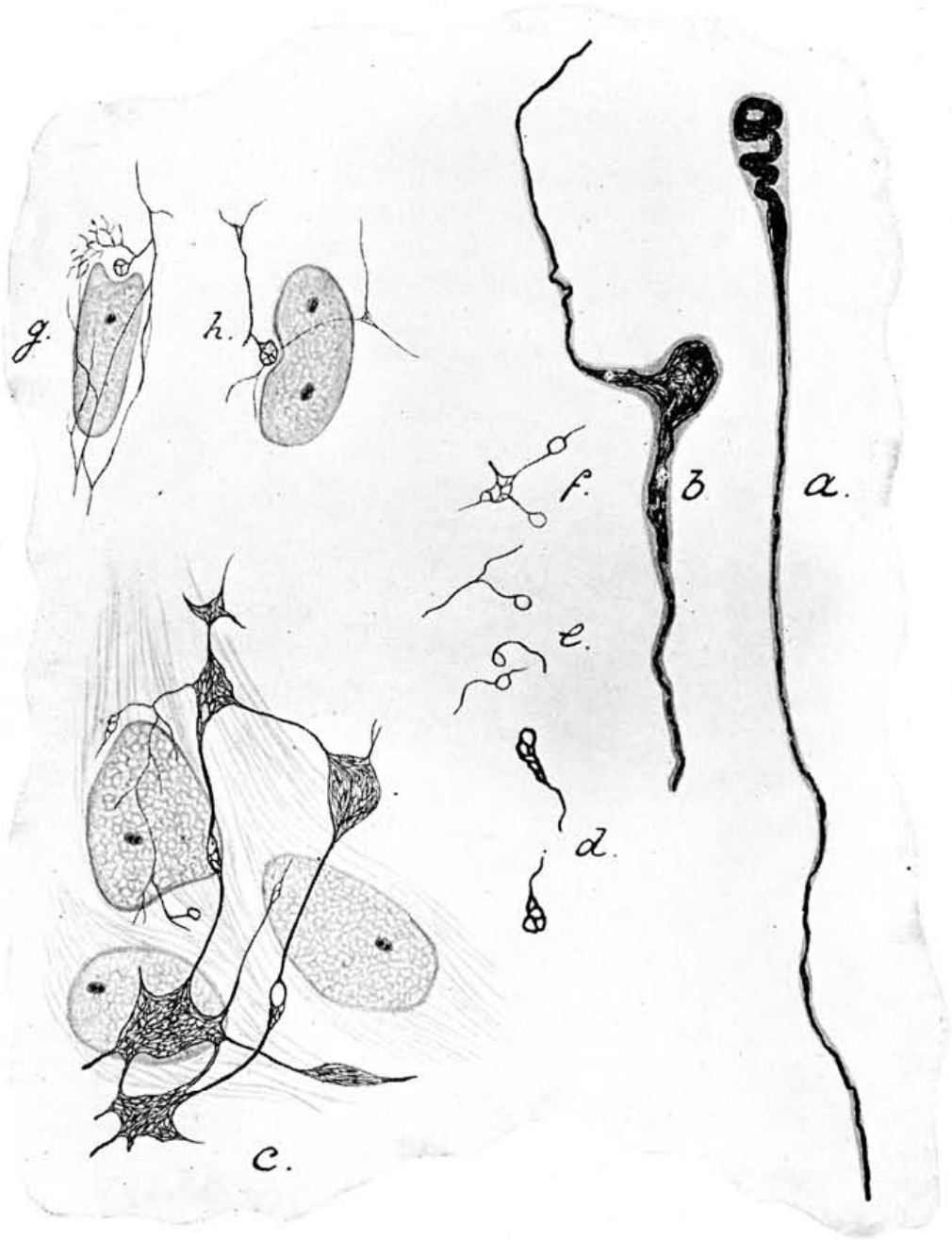

Fig. 2.
Terminations of the different nerves of the Musculus Ciliaris of the human eye.
ab = sensory terminal buds.
c = neurofibrillar network between the musclecells.
d—*h* = efferent motor intracellular endings.

fibrillar network with the network lying between the muscle-cells on one side, with an exceedingly fine protoplasmatic reticulum on the other side, losing itself in the cytoplasm, could be seen with great clearness.

Thus the apparent controversy between the relations of the accessory nerve-terminations and the striated muscle-fibres on the one hand, of the sympathetic nerve-terminations and the plain muscle-cells on the other hand, is seen to disappear. In the plain muscle-cells we find the same identical relations of nerve-endings and sarcoplasm as in the striated muscle-fibres. In both elements the neurofibrillar apparatus penetrates into the protoplasmatic (sarcoplasmatic) cell-body, forms the terminal nerve-endings inside the cell-body as small end-rings and loose netlike extremities or varicosities, and is in continuous connection with a very delicate protoplasmatic (or intraprotoplasmatic) reticulum, the periterminal network.

Leiden, December 1914.

Anatomy. — "*On the mode of attachment of the muscular fibre to its tendonfibres in the striated muscles of the vertebrates.*" By Prof. J. BOEKE.

(Communicated in the meeting of December 30, 1914).

Where the cross-striated musclefibres end in a tendon, the tendon becomes subdivided into as many small bundles as there are fibres in the end of the muscle, and each separate musclefibre has its separate small bundle of tendonfibrillae, to which it is attached. It often seems at first sight as if the tendon-fibres are directly continued into the muscular substance, but until recently it was generally admitted, that the fibres of each tendon-bundle ended abruptly on reaching the rounded or obliquely truncated often somewhat swollen extremity of a muscular fibre, and are only so intimately united to the prolongation of sarcolemma which covers the rounded extremity of the muscular fibre entirely, as to render the separation of the two difficult if not impossible, while the muscular substance, on the other hand, may readily be caused to retract from the sarcolemma at this point as at other points of its course.

While thus it was until recently generally admitted, that the extremity of a muscle fibre was covered entirely by the uninterrupted sarcolemmal membrane, in the year 1912 O. SCHULTZE [1]) and after him several of his pupils published the results of observations of a

[1]) O. SCHULTZE. Ueber den direkten Zusammenhang von Muskelfibrillen und Sehnenfibrillen Arch. f. Mikrosk. Anatomie. Bd. 79. 1912 pag. 307–331.