NOTES ON NEMAS*

(1) Segmentation of Their Organs, Illustrated by three new free-living marine genera. (2) Intravitam Color Reactions, (3) the Nema population of Beach Sand, (4) Locational Terms for the Cytology of Descent, and (5) Functions of the Amphids.

By N. A. Cobb

CONTRIBUTIONS TO A SCIENCE OF NEMATOLOGY V

1

SEGMENTATION IN NEMATODES

OBSERVATIONS BEARING ON THE UNSETTLED QUESTION OF THE RELATIONSHIP OF NEMATODES TO OTHER BRANCHES OF THE ANIMAL KINGDOM

I have long been impressed by certain evidences of segmentation in nematodes. My first impressions arose from a study of the distribution of the setae on aquatic forms. This distribution was in those days, and is even yet, described as irregular; the setae are said to be "scattered" on the body. Charting all the setae on a given specimen led to the conclusion that they were not scattered ("zerstreut"); that, rather on the contrary, they constituted a series of more or less harmonious groups. The cephalic setae, it is well known, have an orderly arrangement. The study of a large number of cases leads me to the conclusion that those setae, some distance behind the cephalic setae, denominated subcephalic setae, are also orderly in arrangement, and might, in some instances at least, be regarded as repetitive of the cephalic setae.

Later I was able to show that the transverse striae of the cuticle are retrorse on the posterior half of the body, and the reverse on the front half. (See Fig. 1.) This reversal in the cuticle at the middle of the body, or thereabouts, occurs in a very wide range of genera, is independent of age and of sex, and seems a character of fundamental significance.

Fig 1

Fig. 1. Diagram of the reversal of the striae of the cuticle of a nematode. The reversal is shown just above the letter F.

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Recently I have discovered that the principal cephalic organs are made up of segments which, while simple in character, bear no small resemblance to corresponding features in segmented organisms. The nature of these segmented appendages will be more easily understood by consulting the illustrations in Fig. 2.

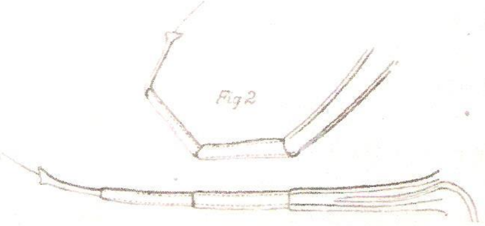
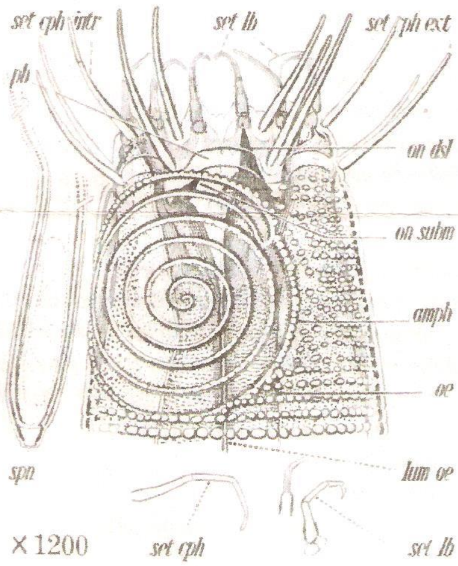


Fig. 2. Cephalic seta of a nematode, showing segmentation. Two different attitudes of the same seta.

The articulations in the cephalic organs of nemas are not easy to discover, owing to the small size of the organs and the transparency of the tissues. Some of these segmented organs are under muscular control, and can be extended and inflexed. This is true of some of the labial organs, which unfortunately are usually so small as to be difficult to observe. The cephalic setae, however, are larger, being particularly well developed on some marine forms, and in this case observation on living specimens affords evidence of the articulations when they might be overlooked if they were sought by other methods; for if a seta is obstructed it takes on the attitude natural to an organ composed of flexible joints and more or less inflexible segments, as shown in the upper illustration, Fig. 2. Here again, once having established the fact and learned how to make the observations, it proves that the setae of a wide range of genera are jointed, though the number of segments is often reduced to only one or two.



$$\frac{1.5}{1.6} - \frac{6.2}{1.9} \equiv \frac{21.}{1.9} - \frac{M}{1.7} - \frac{92.7}{1.5} \longrightarrow 1.8 \text{ mm.}$$

Fig. 3. *Pomponema mirabile* n.g., n.sp., a nema with jointed cephalic organs, and resembling *Cyatholaimus*, but with 3 onchi, more complicated amphids, long, jointed, labial palps, wings as in *Spilophora*, spicula as in *Cyatholaimus*, with 20 chromadoroid, male supplementary organs. Characters of *P. mirabile*, type species, given in the illustrations and formula.

One recalls that a number of observers have noted the presence of longitudinal series of repetitive organs in the lateral fields of nem-

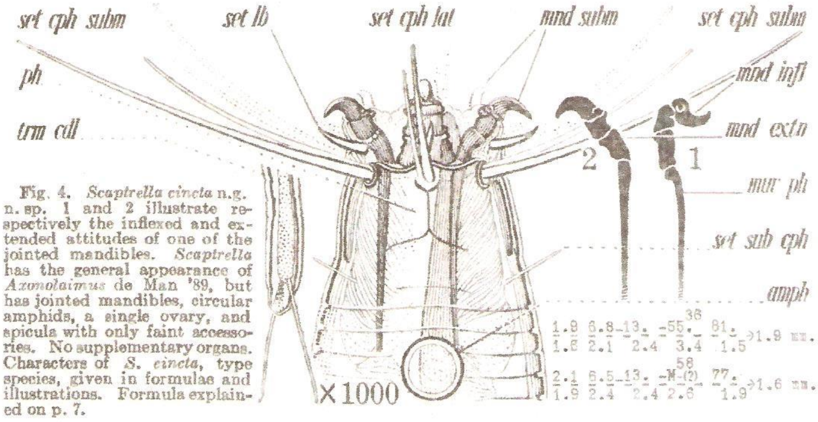


Fig. 4. *Scaptrella cincta* n.g. n. sp. 1 and 2 illustrate respectively the inflexed and extended attitudes of one of the jointed mandibles. *Scaptrella* has the general appearance of *Axonolaimus* de Man '89, but has jointed mandibles, circular amphids, a single ovary, and spicula with only faint accessories. No supplementary organs. Characters of *S. cincta*, type species, given in formulae and illustrations. Formula explained on p. 7.

atodes, though attention has never been called to the fact that these organs on opposite sides of the body may be symmetrical to each other. Sometimes they are exactly so.

How is the prominent trilateral symmetry of the nematode head to be expressed in terms of bilateral symmetry? *Selachinema* and *Cheironchus* assist in answering this question. A second species of *Selachinema*, not yet described, has little or no trace of the vestigial dorsal jaw present in *S. vorax* (p. 113), so that the two projecting mandibles become practically lateral, and are bilaterally symmetrical. An even more complete transformation occurs in *Cheironchus* (Fig. 5), where by a complete disappearance of the dorsal sector of the pharynx the submedian ones have become truly lateral. In both these genera the resulting transformation to a two-jawed animal gives rise to symmetrical mandibles, acting from side to side.

It was, of course, conceivable that any such transformation might take the form of a union of the submedian

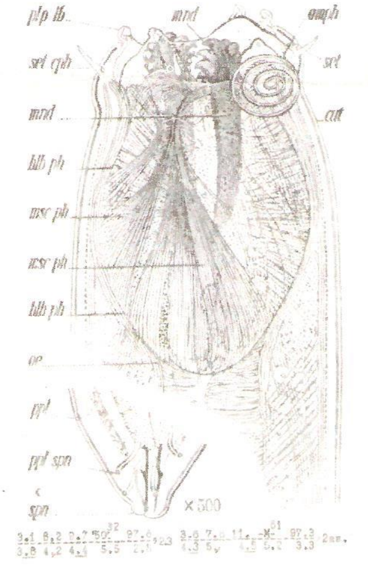


Fig. 5. *Cheironchus vorax* n.g., n.sp., a nema whose bilaterally symmetrical mandibles have arisen from the ventrally submedian sectors of the head at the same time that the dorsal elements of the pharynx have disappeared. *Cheironchus* has the general appearance of *Selachinema* but has no vestigial dorsal jaw, and presents both pharyngeal and cardiac bulbs. Characters of *C. vorax*, type species, given in the illustrations and formulae.

sectors, these united elements then acting in opposition to the dorsal sector, the two jaws thus developed becoming one dorsal and one ventral. There is no evidence of such a transformation.

2

INTRA VITAM COLOR REACTIONS IN NEMAS

We have slowly come to have great confidence in the specificity of certain physiological actions. The known cases of specificity are relatively few, and have been discovered largely by accident. We do not know the exact nature or cause of this specificity. We introduce into an organism certain substances, and definite results follow; about the only thing we know in the matter is that these definite results follow with certainty. What the reactions are that bring about the result we do not know. Our ignorance is so great that even our theories are very vague. In such cases, if only we could see what it is that happens while it is happening, it seems certain that important advances would be made in our knowledge of nutrition, growth, and decay,—of physiology, pathology and medicine.

If substances giving color reactions in living tissues could be applied to small, transparent, varied and highly complex living organisms, under circumstances that would permit microscopic examination while the reactions are in progress, we might hope for more light on this exceedingly important subject. Experiments I have made lead to the belief that many of the conditions requisite for success in this line of investigation can be much better realized than hitherto by feeding colored substances, notably coal-tar dyes, to free-living nematodes.

These minute, transparent animals are comparatively highly organized; not only this, but also extremely varied in their habitats and mode of life. Some are exclusively vegetarian, others exclusively carnivorous, and others omnivorous. They constitute a group composed probably of hundreds of thousands of species, embodying an almost inconceivable number of kinds of physiological action. Their organs are enclosed in a thin transparent cuticle, and are strung out so as to make them unusually suitable for intra vitam examination. Under slight pressure the nema flattens out more or less without losing its vitality sufficiently to preclude satisfactory intra vitam examination under the highest powers of the microscope.

Observing certain precautions, I find that a great variety of coal-tar compounds and other colored compounds can be fed to nemas, apparently without interfering materially with their normal metabolism. Some nemas are resistant to chemicals that to most organisms are poisonous. Often I have had the best results by cumulative action, using small quantities of color dissolved in the medium in which the nema lived, and allowing the dye to act for days or weeks.

Not infrequently the dyes prove to be highly specific in their action. Only certain cells, or only definite parts of certain cells, exhibit visible reactions in the form of colorations. The results obtained by the use of any given dye may be quite varied. It is evident in many cases that the dye is digested and assimilated, thereby undergoing molecular changes by which it is converted into new compounds in a manner analogous to the processes exemplified in chemical laboratories devoted to the production of aniline dyes. Thus, a dye may give rise to several different colors, none of them like that of the dye itself, and all of them very likely due to new compounds. I have seen considerable evidence pointing to the conclusion that in some cases the dye fed is converted into colorless compounds during the process of digestion (a reduction phenomenon?), and these colorless compounds re-converted into colored substances after they arrive at certain destinations or conditions. The number of changes these "living laboratories" can ring on the molecular structure of a given dye must in some cases be very considerable. Two or more dyes fed simultaneously sometimes produce results more or less independent of each other. The spectacles are very brilliant.

Using these methods I have been able to demonstrate within the confines of a single cell the existence of an unsuspected number of kinds of "granules," manifestly playing different rôles. After the differences among these bodies have been shown in this way, it is sometimes possible to perceive corresponding morphological differences; but without the aid of the color reactions the differences would never have been suspected.

The main thing to bear in mind is that on the basis of our present more complete knowledge of the chemical and physical properties of coal-tar compounds these color reactions in living nemas may be made the index of physiological characters possessed by the cells and their components. In view of the great variety of the known coal-tar derivatives, and the wonderfully varied metabolism of the free-living nemas, it seems to me a very reasonable hope that researches directed along this line will lead to important results, and that the nemas may become classical objects in cell and general physiology, as they have already become in sex physiology.

A new and rather extensive nomenclature will become necessary. It will be needful to distinguish between the results of *intra vitam*, *intra mortem* and *post mortem* staining; for these three terms, the last two new in this connection, represent as many different phases in the reactions that take place during the course of the experiments. As the cells lose vitality, new color reactions occur, and the death of the cell is followed by further equally marked changes in the reactions.

The cell elements I have mentioned vary in size, but most of them are exceedingly small, many so small that they are on the limits of visibility using the very best instruments with the greatest skill and under the most favorable conditions. On the other hand, some of them are large enough so that they can be examined in considerable detail and their structures made out. Among them are the bodies currently referred to under the name mitochondria, and other more or less synonymous words.

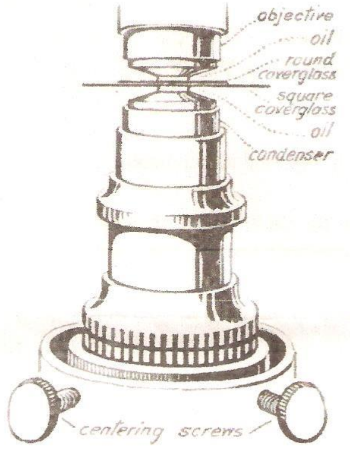


Fig. 6. Diagram of a pair of objectives, the lower used as a condenser for the upper.

As it will be some time before we can establish a rational nomenclature for these numerous intracellular structures it is desirable meanwhile to adopt terms that will permit intelligent discussion of our discoveries as they are made. While the principles underlying such a nomenclature are easily defined, it is by no means easy, in the present condition of things, to suggest suitable short and expressive roots to be used as a basis. In the long run there'll be less confusion if meanings of the terms first employed relate to form, size, and position rather than function.

Investigations of this character are not unlikely to stimulate further research in connection with aniline derivatives. Present efforts are directed toward the discovery of dyes of greater or less permanency. Permanency, however, is of little moment in these investigations; what is of moment is the chemical and physical nature of the dyes. No doubt dyes of a greater range of composition can be produced if permanency be disregarded. Furthermore, as already hinted, colorless compounds may be used if in the course of the metabolism they are converted into colored compounds. The results of recent studies of dyes as chemical indicators come into play, and give valuable evidence in determining acidity and alkalinity.

I am almost ready to express the opinion that a small army of investigators should be engaged on the problems opened up in this way. The equipment needed by the investigator is as follows: (1) He must be a good microscopist, versed in physiology, cytology, and histology. (2) He should be conversant with the chemistry of the coal-tar compounds, not so much from the viewpoint of the maker of dyes as from that of the broad-minded chemist, freed from the economic domination of the dye industry, for, as before remarked, fugitive dyes, and even colorless compounds, are possible factors in such investigations as are here under discussion. (3) He should have a working knowledge of nemas.

Illumination

In order to distinguish with accuracy among *intra vitam* color reactions it is necessary to be very particular about illumination. The most perfectly corrected lenses must be used, both as condenser and objective, and the light used must be as nearly white as possible. The best source of light known to me for these researches is bright sunlight reflected from a plane, matte, white reflector. The reflector should be several feet across, and placed at a distance from the microscope several times its own diameter. It should be universally adjustable, so that it can be set to reflect a maximum of light to the mirror of the microscope,—all the better if it is heliostatic. A good surface for the screen is made by whitewashing a rather finely woven cotton cloth.

The best optical arrangement I have tried is the use of one apochromat objective as a condenser for another apochromat. I have been using with success a 2 mm. apochromat as a condenser for a 2 mm. or 1.5 mm. apochromat objective. These precautions are necessary if fine color distinctions are to be made with the greatest possible accuracy. If these precautions

Fig. 7. Microscope slide used for *intra vitam* examinations as indicated in Fig. 6.

are taken, it will be found that fine distinctions can be made with such precision as to dispel all doubt as to the existence, side by side, in the same cell, of bodies of quite different character that it otherwise would be either impossible or exceedingly difficult to distinguish from each other.

The use of an ordinary apochromatic objective as a condenser necessitates the use of a special object slide, consisting essentially of a carrier, and two cover glasses. The object is mounted between the cover glasses. Such a slide is shown in the accompanying illustration. The substage of the microscope should have a centering arrangement and a rack and pinion, or screw focusing adjustment. A little experience with an apparatus of this sort, in which all known precautions are taken to remove color from the optical system leads one to distrust the ordinary Abbé substage condenser when fine distinctions are to be made between colors in the microscopic object, especially if the colors are of similar character.

3

NEMATODE POPULATION OF BEACH SAND

Through the courtesy of the U. S. Bureau of Fisheries I was able to make quantitative observations during the summer of 1916 on the nemas of ordinary beach sand, between tide-marks, at Woods Hole, Buzzard's Bay, U. S. A. Nemas were plentiful; it was calculated that on one beach in the top 3 inches of sand there were at least 527 millions per acre. On another beach there were at least 1040 millions in the topmost inch of sand. The nemas varied from a fraction of a millimeter to 10 millimeters in length, averaging 2 to 3 millimeters. On muddy shores where organic matter is more abundant, the nematode population is much more dense,—thousands of millions per acre.

Many of these nemas were strictly vegetarian, and fed on microscopic plants present in the beach sand,—plants both green and colorless. Experiment showed that a considerable amount of light penetrates average beach sand to a depth of $\frac{3}{4}$ inch, thus rendering possible the growth of green protophytes and various algae at slight depths in the sand. Some of the nemas were strictly carnivorous, feeding on protozoa and other small animals.

On trial it proved that the top layers of sand, between high and low tides, under ordinary circumstances, afford the nemas a habitat of considerable stability, since the shifting of these layers during the rise and fall of the tides is so little as to interfere in no way with the life activities of the nemas. On open ocean beaches, where the force of the breakers is greatest the nemas take on forms and acquire habits that protect them from destruction,—a tougher cuticle, and the habits of burrowing and of coiling themselves into a "ball."

Beach nemas in their turn are devoured by the larger animals dwelling in and on the sand, and thus form one of the links in a chain from the most minute forms of life to those of largest size.

Beach nemas lead a very active life, winding in and out among the grains of sand as do snakes in a pile of stones. The earth's hundreds of thousands of miles of beach sand, far from being barren, must be reckoned as a productive area of some little importance.

4

LOCATIONAL TERMS FOR THE CYTOLOGY OF DESCENT

There is no satisfactory locational terminology connected with parthenogenesis, hermaphroditism and bisexuality; in other words, with the space relationships of the reproductive cells and their essential elements,—a terminology enabling us to answer succinctly such questions as, "How are these cells and their elements located with reference to each other?" Such nomenclature as we have for this purpose has accumulated, bit by bit, through successive contributions of more or less independent investigators, and, naturally enough, has become a very heterogeneous mixture of terms and phrases.

Aside from standing open to the criticism of being inadequate and an incongruous mixture, such terms as are in current use, at least a considerable portion of them, date from a time when our knowledge of the chromosomes and their relationships to each other and to heredity was either non-existent or much less complete than at present. Most of these terms, therefore, are based on the assumption that the body or soma is the principal or predominant feature of the organism, and, philologically speaking, they take little or no account of the modern view of the importance of the gametes and of the rôle they play.

As we seem in need of a more adequate and homogeneous set of terms based on the relationship to each other in space of the gonads, the gametes, and the chromosomes and other intra-cellular elements, I call attention to the following series of terms, positional rather than physiological, I have been using to meet this need: Just as we have "cone" and "conic" evolved from the Greek *κονος*, I derive the words "gone" and gonic" from γονος. Primarily the word "gone" refers to the generative portion of a sexual organ. By metonomy "gone" designates an organism or species having gones; thus we have two kinds of organisms—"gones" and "agones."

From "gone" come the verb "to gone," and the substantives "syngone," "digone," "amphigone," "homogone," and "heterogone." From syngone come the words "syngonic," syngonically," and "syngony;" and corresponding words from digone, amphigone, homogone and heterogone.

Gone. To produce gones.
Gonic. Of or relating to a gone.
Syngonic. Having macro-("female") and micro-("male") gametes in the same gone; e.g. as in many nemas.
Digonic. Having macro-("female") and micro-("male") gametes in separate gones in the same individual; e.g. as in many hermaphrodites.
Amphigonic. Having macro-("female") and micro-("male") gametes in separate gones that are in separate individuals; e.g. as in all bisexual forms.
Homogonic. Having gones all of the same kind.
Heterogonic. Having gones of various kinds; e.g. as in a species presenting both syngony and amphigony.
Syngone. A gone bearing both macro-("female") and micro-("male") gametes. By synecdoche syngone also designates an organism or species containing, or characterized by, syngones; similarly with the following four terms.
Digone. A digonic individual or species.
Amphigone. An amphigonic species.
Heterogone. A species presenting both amphigony and syngony, or both digony and amphigony, etc. A heterogonic species.
Homogone. A species or individual presenting uniformity in the space relationships of its gonic cells. A homogonic species.
Kinetogone. A gone whose gametes are active, aggressive, or "male."
Statogone. A gone whose gametes are passive or "female."

Entering now a more or less theoretical domain whose permanency will depend on the results of future investigations, and carrying the analysis a step farther by taking into consideration the space relationships of the chromosomes and other intra-cellular elements that according to an increasingly prevalent modern belief themselves carry, or determine the factors of descent, and using the Greek word κυστος as a basis, I derive, as may be necessary, "syncyst," "amphicyst," 'heterocyst," etc., and their adjective, adverbial, and substantive derivatives (e.g., syncystic, syncystically, syncysty) to aid in expressing positional relationships. Thus the word "syncyst" refers to a gonic cell, such as a parthenogenetic ovum, containing all the elements, factors, or determinants necessary to the production of an individual (except, of course, the environmental ones), of which the chromosomes are the familiar example, expressed in some physical form, often, though not necessarily, of definite conformation. It is assumed that these factors belong to two classes, having opposite, vital, chemical, or physical properties. By synecdoche the word syncyst may also designate a gone, individual or species containing, or characterized by syncysts; similarly throughout the series of terms. In dicysty the inheritance mechanism is such that the full complement of factors necessary to the production of an individual is distributed to functional cells of two kinds, the macro-("female") and micro-("male") gametes, neither normally capable of producing an individual, but which, pairing, form zygotes capable of producing an individual.

Syncystic. Of, or relating to, a gonic cell containing all the factors necessary to the production of an individual,—as in the parthenogenetic ovum. The word syncystic may also be applied to a gone, individual, or species bearing syncysts. Syncysty,—state of being syncystic; and so with the following five words.

Dicystic. Of, or relating to, a gone bearing in separate cells the different factors necessary for the production of an individual; as in syngones. The word dicystic may also be applied to individuals and species.

Amphicystic. Of, or relating to, an individual in which the different groups of factors necessary for the production of a new individual occur in separate cells that are in separate gones; as in many hermaphrodites.

Telecystic. Of, or relating to, amphicystic species in which the different groups of factors necessary for the production of an individual are borne in separate cells in separate gones that are in separate somas or individuals; as in all bisexual forms.

Homocystic. Having or producing gonic cells all of the same kind.

Heterocystic. Having or producing gonic cells of more than one kind.

Syncyst. A gonic cell, or by synecdoche, a gone, individual, or species, presenting syncysty.

Dicyst. A gone, or by synecdoche, an individual or species, presenting dicysty.

Amphicyst. An individual or species presenting amphicysty.

Telecyst. A species presenting telecysty.

Heterocyst. An individual or species presenting both amphicysty and syncysty, or both dicysty and amphicysty, etc.

Homocyst. A species presenting uniformity in the space relationships of its different kinds of gonic intracellular reproductive elements.

Kinetocyst. A gonic cell whose elements are active, aggressive, or "male." A "male" gamete; a spermatozoon.

Statocyst. A gonic cell whose elements are passive, or "female." A "female" gamete; an ovum.

Digones, amphigones and heterogones, and many syngones, are dicystic. The word syncysty and its immediate relatives may be used to designate the conditions present in parthenogenetic organisms, in which single gonic cells (not zygotes, at least not in the ordinary sense of the word) contain all the factors necessary to the production of an individual. My (thus far theoretical) cryptogenetic organisms are syncysts.

5

FUNCTIONS OF THE AMPHIDS

My published observations emphasize the junction of each amphid, by means of a duct, with a chain of internal lateral organs. Bütschli and de Man each record an instance of definite outflow from the amphids. I now find this outflow in many different genera, when specimens are fixed with Flemming solution; from a definite part of each amphid there issues a coiled, or irregular "string" or "ribbon," the volume of which precludes attributing it to an evagination. Occasionally an "axis" is seen in the "string," but nothing warrants the belief that this "axis" is other than a coagulation phenomenon, just as it is in the similar coagulation "strings" occasionally seen at the spinneret. I mention evagination because some investigators declare the amphids to be supplied with special nerves, and because it is conceivable that death spasms might so act on a nerve organ as to cause an evagination. However, my numerous observations do not at all support the idea that the appearances I have studied are evaginations. In many cases I have traced inward and backward from the amphids structures whose histology in no way suggests nerve organs, but does correspond in every respect with the histology of ducts, especially those of nemas.

These new observations of mine afford, I think, a better basis for speculation as to the function of the amphids, and lead away from the idea that they are simply organs of sensation. I observe in *Mononchus,* and other genera, that invariably there is an innervated papilla very close to the amphid. May not confusion have arisen here through different observers having studied similar-looking, but unrelated structures?

I have instances of amphids so obscure that it would have been impossible to discover them had it not been for the issuance from them during fixation of the fluid matter described.