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OF THE  
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**WITH FIFTY PLATES.**

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over thirty-four years. The Society has one copy only of each of the first three numbers, and consequently only one complete set. The destruction of the Society's records up to September, 1882, has deprived us of any knowledge of the circumstances which led up to the adoption of the practice of publishing a Monthly Abstract; and the matter is not referred to in the address of the President, at the following Annual Meeting in January, 1883. It was doubtless intended to keep Members and Societies at a distance in touch with the Society's work; and this it certainly did, because the "Zoologischer Anzeiger," up to the time of the outbreak of war, very kindly was accustomed to republish the zoological portion of each monthly number. "Nature," also, for many years has helped us in a similar manner by publishing the abstracts of the papers read. The Society's indebtedness to these two journals, for their co-operation in diffusing a knowledge of the work done by the Society, is considerable, and may here be gratefully acknowledged. When conditions again become normal, the question of the resumption of the publication of the Monthly Abstract may be expected to receive the consideration of the Council.

As regards the Proceedings, the Society's printer has been able to obtain the necessary amount of paper so far required, but at a substantial increase in cost. Printing paper is not manufactured in Australia and we are dependent on British supplies. Now that the nation is mobilised for war, and the Government has placed restrictions on the production of various commodities for the sake of economy in man-power and tonnage, we are likely to feel the effect; and, just at present, the Council does not know what are the prospects of being able to carry on our publishing work on the usual scale in the immediate future. At any rate, I take the opportunity of reminding Members of the Council's appeal for all possible curtailment in the size of papers and in the notices of exhibits, otherwise it may necessitate their being declined, or being subdivided, and taken in instalments. The ability to deal with long papers is deteriorating until conditions again approach the normal; and, meantime, economy in space is

ON THE *CHÆTOSOMATIDÆ*, WITH DESCRIPTIONS  
OF NEW SPECIES, AND A NEW GENUS FROM  
THE COAST OF NEW SOUTH WALES.

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(Plates xlv.1., and 59 Text-figures.)

INTRODUCTION.

The *Chætosomatidæ* are a group of curious, little, free-living, marine worms, which appear to be related to the Nemathelminthes, more especially to the Nematoda, though they cannot at present be directly included in any recognised class. They have been found only along the shore line, and at shallow depths off the coast, where they live on the rocky or stony sea-floor, among clumps of seaweed and shell-fish; and creep along by curious, looping movements like those of a leech. In this movement, they are assisted by adhesive setæ situated in rows on the undersurface of the body towards the posterior end, and in a fringe round the anterior margin of the "head-swelling." These setæ, and the enlargement of the anterior end of the body to form a head-like swelling, constitute two of the most prominent characteristics of the animal, by which it is distinguished from any other known worm. All the *Chætosomatidæ* are very small, rarely exceeding 1 mm. in length, and cannot be detected by the unaided eye.

HISTORICAL.

They escaped observation until 1863, when Claparède(1) discovered a single female specimen on the coast of Normandy. To this, he gave the name *Chætosoma ophiocephalum*.

Three years later, Metschnikoff found them in considerable numbers in the Mediterranean Sea, near Salerno; and, in 1867, published a brief description(2) of a species differing slightly from that found by Claparède.

Between 1867 and 1881, four zoologists, Greef(3), Barrois(4), Panceri(5), and Levinsen(6) recorded the finding of *Chaetosoma* in such widely separated localities as the Canary Islands, Brittany, Ischia, and Greenland, but added little or nothing to the description given by Metschnikoff, though Panceri added another species, *C. tristicochaeta* Panceri. He himself considered this to be a new genus, to which he gave the name *Tristicochaeta inarimense*; but it is now thought that the difference on which he based his classification is not of generic importance. Nothing further is recorded of *Chaetosoma* for twenty-six years. Then, in 1907-1908, Schepotieff(7-8) published a brief, general account of the group, including a description of two new species, which he had found at Bergen and Naples. Seven years later, a short, systematic description of two additional species appeared in a paper by Southern in the Clare Island Survey Series(8).

I have not had an opportunity of seeing the original papers by some of the earlier workers(4-6), but Schepotieff states that they are all brief and superficial, and based almost exclusively on studies of whole preparations.

Levinsen's description is not made clear by illustrations, and is so imperfect otherwise, that identification of the new species he reported is uncertain. According to Schepotieff(8), it is probably identical with *Chaetosoma tristicochaeta*. Greef(3) confines himself to the statement that he frequently found *Chaetosoma* in different localities on the coast of the North Sea and the Canary Islands, but, beyond a few new species (which he does not describe or name), he could add nothing of importance to Metschnikoff's description. His paper, on some remarkable forms of Arthropod- and Worm-Types, deals mainly with the consideration of the systematic positions of this and other genera, which he designates as remarkable creatures, new or incompletely described, which bear in themselves the characteristics of different classes of animals, without inclining, with any decision, to one or other of them. He considers that the study of these yet living transition-forms between different classes of worms offers a great attraction to investigators, and demands as complete an investigation as possible, from every point of view,

of their organisation and life-history. Schepotieff's papers(7-8) deal with what he terms "The Nematoid representatives of the microscopic fauna of the rocky sea-bottom," including, in this term, the *Desmoscolecidae*, *Echinoderidae*, *Chaetosoma*, *Rhabdogaster*, and *Trichoderma*. His description is based on collections made on the coasts of the Adriatic, the Gulf of Naples, and the fjords of Norway. In the latter place, he had made extensive dredgings on a submarine reef running across the Byfjord near Bergen, and obtained *Chaetosoma* in large numbers in the coast-zone (10-15 mètres), in the years 1903 and 1905.

In his first paper(7), he gives a brief description of the external features only, explaining that the internal organisation is very difficult to investigate, owing to the strong development of the external cuticle, and its impenetrability to reagents. A year later, 1908, he published a fuller account(8), including in it observations on the internal anatomy made by means of transverse sections of a single species, *Chaetosoma longirostrum*. This, apparently, was the first time a successful attempt had been made to section the worm. But the drawings are lacking in detail, and a much fuller description is required to complete our knowledge of the anatomy of this group. As yet, too, no work at all has been done on the life-history.

Until 1914, the *Chaetosomatidae* were not known to occur outside the waters of the North Atlantic and Mediterranean Seas, though Schepotieff expresses the opinion that they are cosmopolitan, and belong to the most numerous and most typical representatives of the microscopic fauna of the rocky sea-bottom. In that year, Professor Haswell found a few individuals of two new species in Port Jackson, N.S.W.; and, some time later, he drew my attention to them.

During the past year, I have collected and examined specimens of these, and two additional species from the sea-shore in the vicinity of Sydney. The present paper is a record of that work, and an attempt to follow Greef's advice(3), and give as complete an account as possible of the anatomy of these curious, little creatures.

## MODE OF OCCURRENCE.

I have not found the *Chætosomatidæ* anywhere in the great numbers mentioned by Greef (3) and Schepotieff (7), but I have not been able to make dredgings on the sea-floor at depths of 10-15 mètres, where Schepotieff found them to be most numerous in the Byfjord. My search has been confined to depths accessible from the shore, from lowwater-mark to 5-6 feet below it, obtaining material from this depth by means of a long-handled net, and a bucket.

Long and careful searching has revealed a few scattered representatives in most of the material collected from clumps of molluscs and seaweed in different parts of Port Jackson and Broken Bay, and along the ocean-shore between these two bays. Round Circular Quay, they are to be found in fair numbers on the growth on the jetties; but, in the very dirty water of Darling Harbour, they appear to be almost entirely absent. I found only one in a very large quantity of material collected from an old pile, 40 feet long, which had just been pulled up, this specimen coming from a depth of 16-18 feet below lowwater-mark. Further up Port Jackson than this, (a distance of some  $4\frac{1}{2}$  miles from the ocean), I have not found any. In all, I have secured about 100 specimens as a result of collections extending over six to eight months in 1916 and 1917. The majority of these came from Circular Quay, Cremorne, and Vaucluse, in Port Jackson; and Lion Island and Pitt Water, in Broken Bay. Most of them, I have found at very shallow depths, a few actually above lowwater-mark, and all of them among the growth of Algæ, Sponges, Molluscs, etc., on rocks or piles. They do not appear to be present where the sea-floor is composed of pure sand or mud, nor in coralline zones.

## METHODS OF COLLECTING EMPLOYED

The methods of collecting adopted varied only slightly with the different species. When dealing with large quantities of material, the clumps of seaweed, sponges, etc., were transferred to a large bucket of sea-water, broken up, and thoroughly washed. The fine mud in suspension in the water was washed off by means of a siphon-funnel, the mouth of the funnel dipping into the

bucket being covered by miller's silk, to prevent the escape of anything but the finest sediment. Fresh water was continually added, till that in the bucket became fairly clear. It was then poured off into another vessel through a coarse wire-sieve to get rid of the more bulky material (some of which was kept for later examination), and concentrated by siphoning or filtering off most of the water through miller's silk.

The constant choking up of the silk by the fine sediment caused a good deal of difficulty in the use of the siphon. The same difficulty was experienced in filtering, when the finer quality of miller's silk was used, but I was uncertain whether the coarser quality would catch the *Chaetosoma*. It was necessary, therefore, to keep the filtrate from the coarser silk, filter it through the finer, and examine the material caught on this, and the water which passed through it. I was also uncertain whether the washings given to the bulky material collected were effectual in detaching the worms adhering to the seaweed, etc.

Until these points were settled, it was necessary to make four separate examinations under the dissecting microscope of each lot of material.

- (1). Seaweed, shales, sponges, etc.
- (2). Material caught on coarser silk.
- (3). Material caught on finer silk.
- (4). Filtrate from the finer silk.

I have never found anything but finely suspended mud in No.4, and, so far, no *Chaetosoma* in No.3; so that I think the coarser silk, which filters much more rapidly, can be used with safety. When dealing with small quantities of material gathered along the shore, I shake and wash it thoroughly in a jar, and filter off the water directly through the coarser silk, without making use of the siphon. The material caught on the cloth is either washed into a small dish of sea-water for immediate microscopical examination, or fixed for later examination.

Whether the washing is effective or not, seems to depend on the species of worm present. *Notochaetosoma tenax* clings so firmly to any support, that it is most difficult to detach it. I had found several worms of this species in material which had

been fixed before washing, but six hours' search through a second lot of material (fresh) from the same spot yielded only a single specimen. A careful examination of shells and seaweed revealed the living worms still clinging to them, after a very thorough washing. It is most difficult to detect the worm against the dark background of seaweed, so that, unless living specimens are required, the material collected is fixed before washing. With *C. haswelli*, there is not the same difficulty, as they wash off much more readily.

#### METHODS OF PREPARATION.

Various fixatives have been tried—formalin, sublimate-acetic, Carl's, glycerine-alcohol, and 70% alcohol, hot and cold. Formalin or sublimate-acetic, which for bulk-fixing are the most convenient, seem to give as good results as any.

Stains used in the preparation of whole mounts include Erlich-hæmatoxylin, picro-hæmatoxylin, picro-carmin, borax-carmin, and para-carmin. The picro-stains give very poor results. Erlich-hæmatoxylin and borax-carmin seem to be the most satisfactory; but the worm requires prolonged immersion in these, especially in the first, owing to the thickness of the cuticular covering. The length of time required varies with the species; three or four days for the species which have a very thick cuticle.

Clearing is a very difficult process, as most clearing agents, unless very carefully applied, shrivel and distort the animal. The more delicate *C. haswelli* mounts well in glycerine-jelly, when cleared for some time in glycerine-alcohol (glycerine, 10%; alcohol, 90%) under a bell-jar; but for the species with thicker cuticle, clove-oil or cedarwood-oil must be used, and the mounting done in Canada balsam. I have found clove oil the best, but it is necessary to add it very gradually, drop by drop, to the absolute alcohol, bringing up to pure clove-oil only after a day or two.

For section-cutting, the double embedding, paraffin-celloidin method was used. A few worms were embedded direct in paraffin, after clearing in cedar-oil, but the result was unsatisfactory; as was also the case when a celloidin-block was dispensed with, the worm being transferred direct from  $\frac{1}{2}\%$  celloidin-solution to

chloroform. In these cases, the handling of the minute specimens was a great difficulty. In all cases, I found it necessary to stain the worm slightly before embedding, in order to locate it with the dissecting microscope when cutting out the celloidin or paraffin block.

The mounted sections were afterwards double-stained with Erlich-hæmatoxylin and eosin. Satisfactory sections are difficult to obtain. *Chaetosoma haswelli* is so delicate, that the body-walls tend to fall together during the embedding process; and, when this occurs, the transverse sections take the form of a narrow thread, in which it is impossible to make out details of internal structure. *Chaetosoma falcatum* and *Notochaetosoma tenax*, which have a very thick, external cuticle, and, consequently, a firmer outline, sometimes give good transverse sections, but they tend to tear out of the block, especially in longitudinal section.

For many of the specimens examined, I am indebted to Professor Haswell, whom I have to thank for the direction of the work, and in whose laboratory it was carried out.

I have also to thank Acting-Professor S. J. Johnston for the valuable assistance he has given me in the preparation, both of the material and the paper; and Mr. R. J. Tillyard for his kindly and helpful criticism and interest during the progress of the work.

#### STRUCTURAL FEATURES OF THE CHÆTOSOMATIDÆ.

All the *Chaetosomatidæ* have an elongated, cylindrical, worm-like body, tapering posteriorly to a sharply pointed tail, and usually swollen anteriorly to form a more or less distinct head.

For purposes of classification into genera and species, the distinguishing features are the shape and size of the head-swelling, and the extent of its demarcation from the trunk; the character of the pharynx; the number of rows of ventral and head-setæ, and their position; the character of the setæ; the relative proportions of the trunk; the thickness of the external, cuticular covering; the variations in the character of the transverse striations, and in the size and arrangement of the body-hairs with

which it is beset; and the general shape which the worm tends to assume, when fixed.

In all species hitherto described, the head-area is clearly distinguishable into three regions.

(1). The frontal part, known as the rostrum, on which the cuticle is not striated. This, as a rule, bears the anterior "head-hairs" or setæ, and a pair of curious, laterally situated, spiral grooves.

(2). The middle part, always the narrowest of the three regions, which is ringed by cuticular striations, much coarser and more strongly marked than on any other part of the body.

(3). The hinder part, only distinguishable from the trunk by its greater thickness, and the "neck"-constriction which marks it off from the latter.

But in two of the New South Wales species now described, there is little or no demarcation of a head-region. The pharynx lacks the typical, swollen "bulbs," and, in consequence, the anterior end of the body is very little, if at all, enlarged. The worm can be distinguished from a Nematode only by the rows of ventral setæ, and its characteristic creeping mode of locomotion.

The absence of a head-swelling would seem to suggest that these worms are more nearly allied to *Rhabdogaster* (2, 10) than to *Chætosoma*. But, in the general structure of the body, they differ considerably from *Rhabdogaster*; and are very similar to other species of *Chætosoma*. There is no median constriction of the trunk; the ventral setæ are stout, hollow rods, with distal segments, not delicate and crook-like, as in *Rhabdogaster*; and the female genital pore and genital organs are situated in the anterior, not the posterior half of the body.

I have, therefore, placed these two worms as a new genus, *Notochætosoma*, in the family *Chætosomatidæ*. The typical rostrum is present in both species of *Notochætosoma*, but there is no special "banded" area of coarsely striated cuticle behind it. This banded area is also absent in one of the New South Wales species of *Chætosoma*, described as *C. falcatum*.

The number of rows of ventral, locomotor setæ is of considerable systematic importance. All four New South Wales species

have four rows. Of the *Chaetosomatidae* hitherto described, three species have two rows; three species, three rows; and two species, four rows. It has been suggested that these three types should be placed in three distinct genera.

The number of setæ in each row varies in all cases with the age of the animal, and is, therefore, not altogether a specific character; but the relative extent of the body covered by the rows varies considerably in the different species, as does, also, the character of the setæ.

Two species have been described in which the setæ are all simple; in the others, either the setæ are all compound, with a small distal segment, or both simple and compound setæ are present.

In all species, the part of the trunk on which the setæ are situated serves as a sole, the ventral setæ become firmly attached to some support; and the worm rears itself up and stretches the anterior part of its body forward, when about to move. Schepotieff states(7) that it then attaches itself, and draws itself forward by means of mouth-teeth. But in all cases in which I have been able to observe the *Chaetosomatidae* alive, it is the dorsal 'head-hairs,' or setæ arranged in a semicircle on the hinder part of the rostrum, which perform this function. In the case of *N. tenuis*, I have been able to make out small, distal segments on the dorsal setæ, very similar to those on the ventral setæ. The adhesive power of these setæ seems to be very great, and the animal can only detach itself by a sharp jerk after each forward-movement.

The character and arrangement of the trunk-hairs varies considerably in the four New South Wales species. In *Ch. falcatum*, they are so short and scattered as to be hardly noticeable; while, in *Ch. haswelli*, they are very long and prominent, and are markedly swollen at the base.

The internal organisation is simple. There is a body-cavity, and the alimentary canal is a simple, straight tube extending through the body from the mouth, at the anterior end, to the anus on the ventral surface close up to the posterior end of the body. A glandular mass, lying dorsal to the posterior end of

the alimentary canal, and opening by a pore at the posterior extremity of the tail, is evidently excretory in function. Glands of some kind are also present in the head-region of some, if not all, the species, lying above the pharynx. They appear to be connected with the hollow, dorsal, locomotor setæ.

Some species show traces of a nervous system, in the shape of an ill-defined ring round the pharynx. The peculiar, lateral grooves on the rostrum are probably sense-organs of some kind. In the possession of a 'tail-gland,' and these lateral grooves, the *Chætosomatidæ* resemble many free-living, marine Nematodes. The resemblance is discussed in the detailed description of *Ch. falcatum*.

The sexes are separate. In the male, a single, simple tube extends from the anterior region to open with the rectum at the anus, and two, equal, penial setæ are present. The female sexual organs consist of paired ovaries, anterior and posterior, each opening by an oviduct into a common uterus. The female genital pore is situated on the ventral surface, about or in front of, the middle of the body. The ova are relatively few, and fairly large; and the animal appears to be oviparous.

#### DESCRIPTIONS OF THE SPECIES.

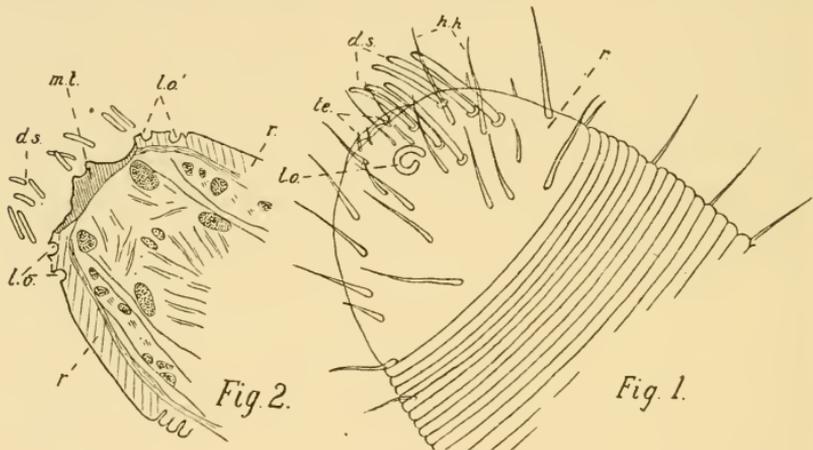
##### CHÆTOSOMA FALCATUM, n.sp. (Plates xlv.-xlvi.).

A few individuals of this species were found in 1914, by Professor Haswell, in material collected at Cremorne, Port Jackson; and the majority of the specimens, which I have obtained since, have come from the same locality. I have obtained only four specimens elsewhere; one in material from Vacluse, in which, also, I found *N. tenax*; and three from the rocks between tide-marks at Long Reef, a very exposed portion of the ocean-coast. At Cremorne, they were all taken from among the growth of shells and seaweed on a vertical rock-face, at a depth of from 4.5 feet below low water mark. They are scattered very sparsely through the material, and the search for them is a lengthy one, necessitating a preliminary fixation; and I have not yet been able to obtain any living specimens.

*Ch. falcatum* is an elongated, approximately cylindrical, worm-like animal, greyish-white in colour. In length, it does not

exceed 1.10 mm., and proportionally it is unusually broad, the trunk, in the largest specimens, attaining, at its widest part, a width of 0.092 mm. Dorsal and ventral surfaces are recognisable, and anterior and posterior ends. Anteriorly, the body is enlarged to form a head-like swelling (Pl. xlv., *hd.*) marked off from the trunk by a well-defined neck-constriction (Pl. xlv., *nk.*). Posteriorly, the trunk terminates in a short, sharply pointed, downwardly directed tail (Pl. xlv., *tl.*). The mouth-opening (*m.*) is anterior and terminal, and the anus (*a.*) is situated on the ventral surface, a short distance in front of the tail (0.06 mm. to 0.09 mm., according to the length of the animal). The sexes are separate, and, in the female, the external genital aperture is situated on the ventral surface, a little in front of the middle of the body (Pl. xlv., ♀, *g.p.*). When fixed, the animal usually assumes the shape of a query-mark, or a sickle, the anterior half of the body being strongly arched, with the concavity of the arch directed ventrally; while the posterior half is straight, or only very slightly bent in the opposite direction (Pl. xlv.-xlv.). The female genital aperture is situated close to the beginning of the anterior arch, in the region where the body attains its greatest width. Even in sexually immature worms, the anterior half of the trunk is always slightly wider than the posterior half, which is further distinguished by the presence, along the whole length of its ventral surface, of four longitudinal rows of stout, locomotor rods or setæ (Pl. xlv.-xlv., *i.r.*, *o.r.*). The whole body is enclosed in a thick, opaque cuticle, which, except at the extreme anterior and posterior ends, is divided up by close-set, transverse grooves, into numerous minute annuli or striæ (Pl. xlv.-xlv., *cut.*). The grooves are broad, and penetrate to nearly the full depth of the cuticle (Text-fig. 12, *cut.*). For the most part, they encircle the body in parallel lines, but, occasionally, adjoining grooves anastomose. On no part of the head or trunk is any variation in the size and thickness of the striæ to be recognised. The head-swelling is large in proportion to the size of the trunk, attaining a length of  $\frac{1}{4}$ th to  $\frac{1}{3}$ th that of the trunk; and a breadth usually slightly greater than the average breadth of the trunk. It is elongated-oval in shape, and is bluntly

rounded off anteriorly. Here, for a length of about one-fourth the total length of the head, the cuticle is devoid of striae, and is beset with numerous, very long hairs (Pl. xlv.-xlv.; Text-fig.1). Three transverse rows of setæ are arranged in a semicircle far forward on the dorsal side of the rostrum. The corresponding setæ of the three rows are in line, and form portions of longitudinal rows; the remaining portions of which are formed of unmodified hairs, extending from the junction of the striated cuticle with the rostrum, to its anterior margin (Text-fig.1, *d.s.*). A second row, consisting entirely of unmodified hairs, is inserted close beside each of these rows (Text-fig.1, *h.h.*). The hairs are



Figs.1-2.—*Chaetosoma fulcatum*, n.sp.

Fig.1.—Surface-view of rostrum, showing arrangement of hairs and setæ; ( $\times 630$ ).

Fig.2.—Longitudinal section through the rostrum in the plane of the lateral sense-organs. The cut ends of the dorsal setæ show above the section; *m.t.*, tissue surrounding the mouth; *te.*, mouth-setæ.

For other lettering in these and other legends, see *postea*, under Explanation of Plates.

long and slender, having an average length of 0.022 mm., and taper to a fine point. They project vertically up from their point of insertion in the rostrum, but the setæ, which are stout and blunt, and about 0.015 mm. long, are inserted by broad bases in depressions in the cuticle; and are strongly arched forward and downward, bending over the anterior margin of the

head, and forming a semicircular fringe above the dorsal side of the mouth-opening (Text-fig. 2, *d.s.*). These 'setæ' probably correspond to the hinder head-hairs described by Schepotieff (7-8) for other species of *Chatosoma*, but do not correspond in position with either the hinder circle of head-hairs or the frontal head-hairs mentioned by him. Unmodified hairs, similar to those on the rostrum, 0.022 to 0.026 mm. long, occur on the posterior part of the head, and appear to be arranged more or less regularly in longitudinal rows. The cuticle of the rostrum stops short of the extreme anterior end (Text-fig. 2, *r.*), leaving a circular aperture through which the body-tissue projects in a lip-like arrangement surrounding the mouth. On this, there is a circle of very minute hairs or setæ (Text-fig. 1, *te.*), apparently six to eight in number, but, on account of the extremely small size of all these parts, details are difficult to determine with accuracy.

On either side of the rostrum, very close to the anterior margin of the cuticle, there appears a curious cuticular marking in the form of an open, spiral groove, the open end being directed posteriorly (Text-figs. 1, 2, *l.o.*). Similar cuticular markings are mentioned by Metschnikoff (2) and Schepotieff (7-8) as occurring on all species of *Chatosoma* hitherto recorded. Schepotieff seems to think they are peculiar to the *Chatosomatidae*, and compares them (7) with the wing-like head-appendages of the *Desmoscolecidae*. But I have seen similar markings on some small marine Nematodes, and Bastian has figured them in some of the illustrations to his monograph on the *Anguillulidae* (11), notably on *Comesoma vulgaris*, though he does not mention them in his description. De Man, in his 'Nordsee Nematoden' (12), also records them as occurring in *Enoplus*, *Oncholaimus*, *Anticomus*, and *Tripolyoides*, in conjunction with the "paired lateral organs," which, he suggests, may be either excretory or sensory in function. So far as I have been able to determine, they are purely superficial cuticular structures. I cannot detect any sign of ducts leading away from them, nor any connection with the deeper layers of the body. But it is possible that such connection does occur, being difficult to detect on account of its extreme minuteness.

Immediately behind the 'head,' the trunk is sharply constricted to form a narrow 'neck' which is less than one-third the width of the head (Pl. xlv.-xlv., *nk.*). Behind the neck, the trunk rapidly broadens to its widest part in the anterior region of the body, becoming slightly narrower again in the posterior region, which bears the locomotor rods. The terminal portion of the trunk, distinguished as the 'tail,' is very short in this species,

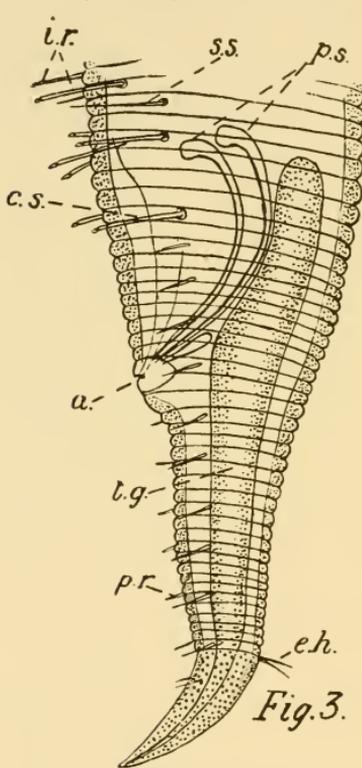


Fig. 3.—*Ch. falcatum*.

Posterior end of male; ( $\times 520$ ).

being not more than 0.051 mm. long in the largest individuals. While devoid of striæ, its cuticle is not quite smooth, being minutely pitted all over (Pl. xlv., fig. 2a; Text-fig. 3, *tl.*). A few, very fine hairs are scattered over the surface of the trunk (Pl. xlv., *th.*). These are never more than 0.007 mm. long, except towards the posterior end, where three or four pairs of much longer hairs occur on the dorsal surface. The last and longest of these, 0.026 mm. long, are inserted very close together just at the junction of the striated trunk-region with the tail (Text-fig. 3; Pl. xlv., xlv., *eh.*). The ventral rows of locomotor setæ extend from behind the anus nearly half-way up the trunk, to the beginning of the anterior curve. The number of setæ in each row varies with the size and

age of the animal; but the relative extent of ventral surface covered by them remains about the same in specimens varying in length from 0.528 mm. (the smallest) to 1.104 mm. (the largest obtained by me). The two inner of the four rows are situated close together along the middle of the ventral surface, each row consisting of from 12 setæ in the smaller to 23 in the larger

individuals (Pl. xlv., xlv., *i.r.*). The setæ (0.018 mm. to 0.022 mm. long) are all compound, each consisting of a stout, tubular rod inserted by a broad base in the cuticle, bearing, at its distal end, a very small, leaf-like, movably-jointed segment (Pl. xlv., fig. 2*b*). They are separated by fairly wide intervals posteriorly, but are more closely crowded anteriorly, especially in the older individuals. In front of each inner row, and directly continuous with it, there are, in individuals of all ages, three or four, small, simple setæ of the same length (0.007 mm.) as the trunk hairs lying in front of them (Pl. xlv., *s.*). The trunk-hairs continue the ventral rows to the anterior end of the trunk (Pl. xlv., *t.h.*). The two outer rows of locomotor setæ begin, as a rule, about three setæ further back than the inner rows (Pl. xlv., *o.r.*), and are situated ventro-laterally, separated by a fairly wide interval (0.018 mm.) from the inner rows. In the male (Pl. xlv.), they consist of simple and compound setæ, alternating fairly regularly, there being, in the adult worm, about twelve compound and seven simple setæ in each row. The compound setæ (*c.s.*) are quite similar in size and character to those of the inner rows; the simple setæ (*s.s.*) are the same length, but are very slender and taper to a fine point. In the female, the outer rows are composed entirely of compound setæ, about twenty-one in number in the adult (Pl. xlv.). The inner rows cease before the anus, but the outer rows are continued back on either side of it, almost to the junction of the trunk with the tail. In the female, the postanal portion of the row consists of three or four compound setæ exactly like those further forward (Pl. xlv., fig. 2*a*). But, in the male, the compound setæ stop some little distance in front of the anus, and the posterior portion of the row consists of eight or nine simple setæ, shorter than those between the compound setæ, but stronger, stouter, and very sharply pointed. The last pair are frequently inserted right on the tail-point (Pl. xlv., fig. 1*a*). They appear to correspond closely with the double row of setæ present in the region of the anus, in some of the free-living marine Nematodes, where they constitute one of the external characteristics of the male. They are described by Bastian (11) for the genera *Anticomæ*, *Enoplus*, and *Comesoma*; and in greater

detail by De Man(12), for several species of these genera, *e.g.*, *Enoplus communis*, in the male of which are found, "between the anus and the aperture of the accessory organ, eighteen setæ on each side of the ventral middle line."

In the adult female, the trunk is circular in cross-section in front (Text-fig.5), slightly flattened dorso-ventrally into a more oval form in the region of the genital organs (Text-fig 20), and posteriorly is broadly ovate (Text-fig.15), the narrowest part lying between the ventral locomotor setæ. The cuticle, which

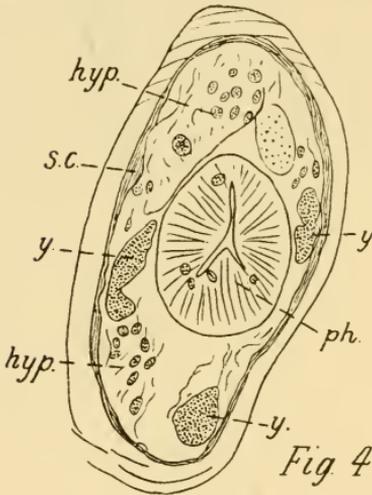


Fig 4

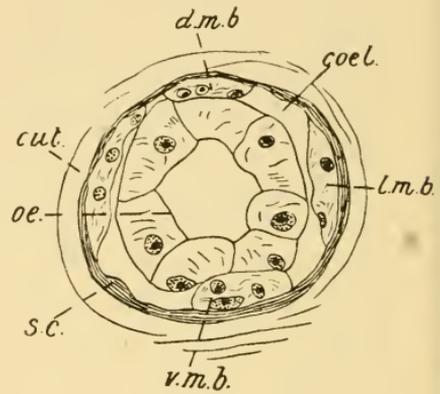


Fig 5

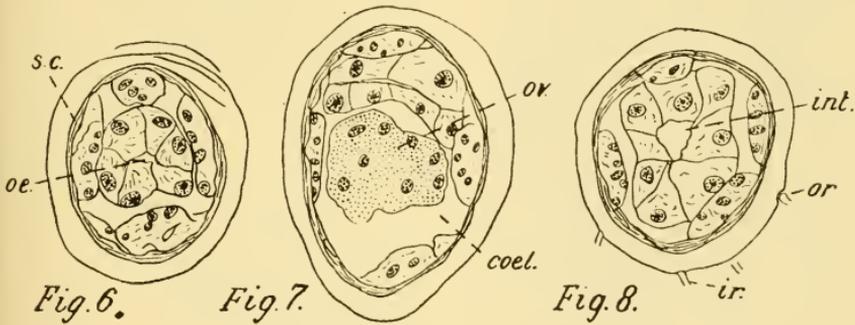
Figs.4, 5.—*Ch. falcatum*

Fig.4.—Transverse section through head in the region of the anterior pharyngeal bulb; ( $\times 610$ ).

Fig.5.—T.S. through anterior part of trunk of same specimen; ( $\times 610$ ); *hyp.*, hypodermis; *s.c.*, subcuticular layer of body-wall; *l.m.b.*, lateral mesodermal band; *y.*, glandular masses round pharynx.

forms the external covering of the body, is structureless, and quite homogeneous. It forms a relatively broad layer (0.004 mm., in a worm which has an average diameter of 0.048 mm.), and is of uniform thickness throughout (Text-figs.5-8, *cut.*). Beneath the cuticle is a much narrower layer (*s.c.*), in which it is impossible to make out any definite structure. It is strongly refractive, and appears bright yellow in sections stained with hæmatoxylin and eosin. Its internal outline is very irregular, both

in transverse and in longitudinal sections, due to variations in its thickness. In places, it projects into the body-cavity in the form of sharply angular thickenings (Text-fig.20, *s.c.*), but these thickenings are not constant in position, and there is no indication whatever of definite longitudinal lines in it. No cell-boundaries and no nuclei are visible within it, but scattered nuclei may occasionally be seen, lying along its inner surface (Text-fig. 12*a*). Four very definite thickenings of mesodermal tissue lie along the inner surface of this, and are respectively dorsal, ventral, and lateral in position (Text-fig.5, *d.m.b.*, *v.m.b.*, *l.m.b.*). They extend throughout the length of the trunk, from the neck-constriction to the beginning of the tail-region, forming four longitudinal ridges on the body-wall (Text-figs.12, 13). They appear to consist of a clear, net-like protoplasm, containing



Figs.6-8.—*Ch. fulcatum*.

Tr. secs. through trunk of a young specimen, showing the four, large mesodermal ridges on the body-wall, and the structure of the alimentary canal; ( $\times 830$ ).

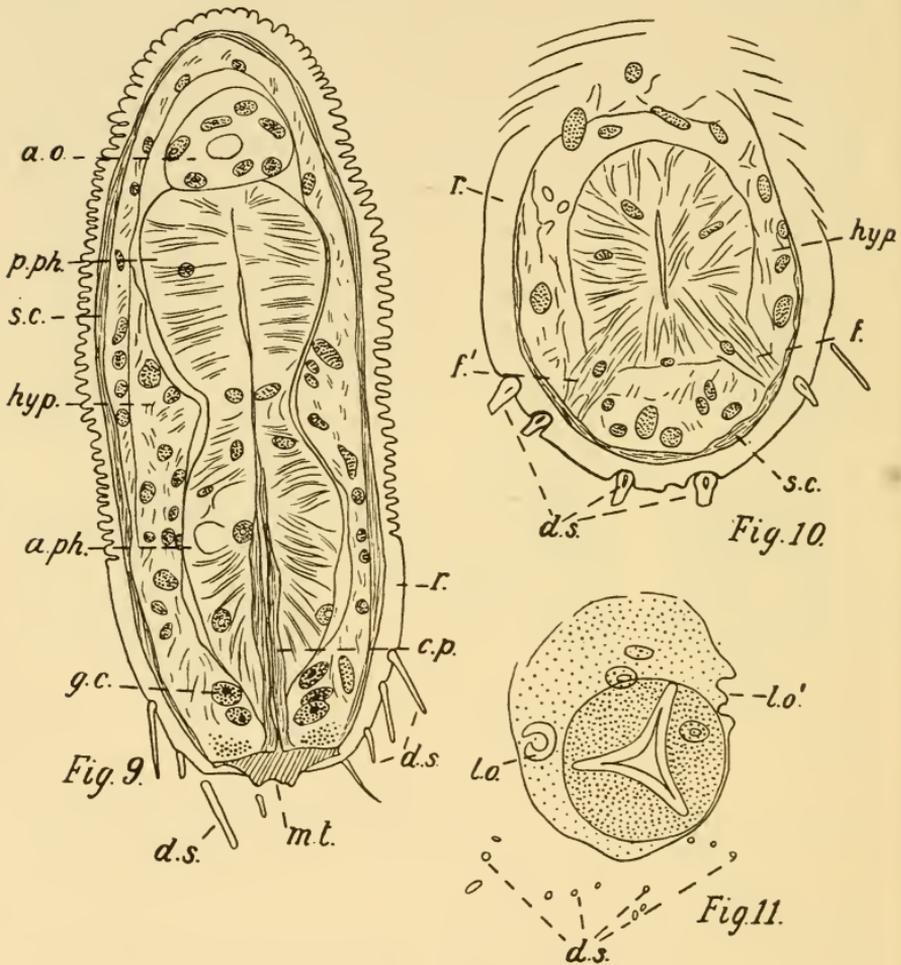
Fig.6.—Section through anterior region.

Fig.7.—Section through middle region, showing rudiment of genital system.

Fig.8.—Section through region of the ventral setae.

granules and nuclei, the latter being crowded along the inner surface of the ridge. These ridges are most clearly distinguishable in a young and immature worm, where they are relatively much larger than in the adult (Text-figs.6-8). In a worm, in which the alimentary canal and genital organs completely fill out the cœlomic cavity, and press closely against the body-wall

in the middle region of the body, the ridges, especially the lateral ridges, are only recognisable as thin strands containing a few



Figs. 9-11.—*Ch. falcatum*.

Fig. 9.—Horizontal longitudinal section through head; ( $\times 830$ ).

Fig. 10.—T.S. through rostrum, showing insertion of dorsal setæ.

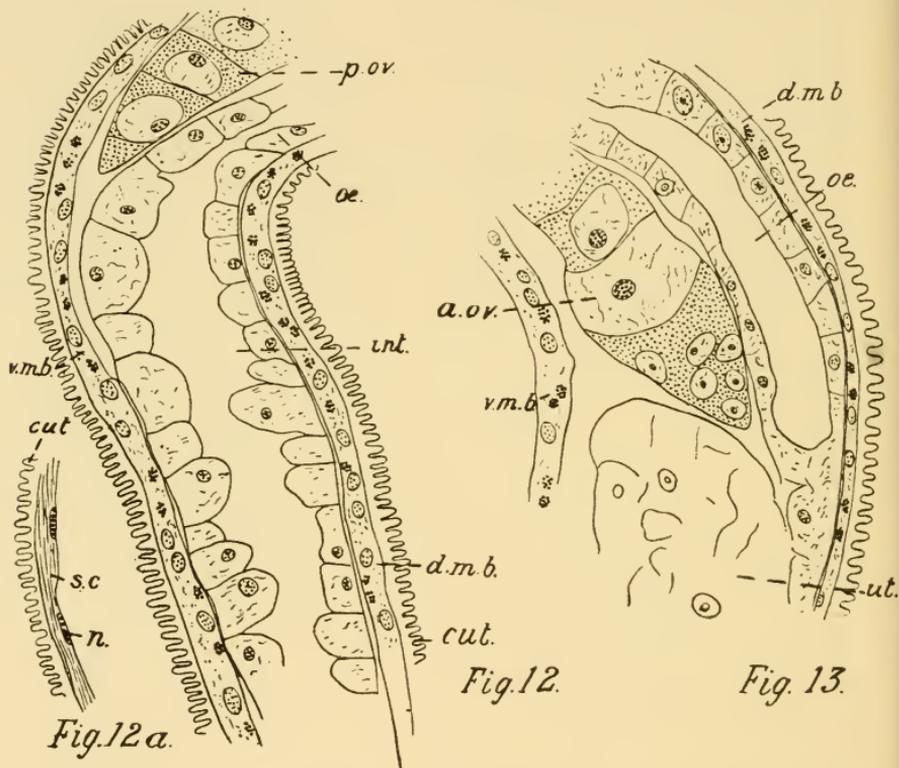
Fig. 11.—T.S. through anterior extremity of head, showing mouth-opening, and cut ends of dorsal setæ; *c.p.*, cuticle lining pharynx; *f, f'*, bands attaching pharynx to body-wall; *g.c.*, glandular cells surrounding anterior end of pharynx; *mt.*, tissue surrounding mouth-opening.

deeply stained nuclei and granules (Text-figs. 14, 15). The dorsal

ridge is more definite in outline and appears to be in contact with the alimentary canal throughout its length. In the head, the space between the pharynx and the body-wall is almost completely filled out with a loose, hypodermal tissue (Text-figs.9, 10, *hyp*), but, in the trunk, a definite cœlome is present, between the enteric canal and the body-wall. In the young worm, this space is relatively large, and extends uninterruptedly from the neck to the tail-region, where it is again filled out with protoplasmic tissue. But, in the adult, it is extensive only in the region of the œsophagus (Text-fig.5, *cœl.*). Further back, it becomes almost completely occluded by the growth of the genital organs and intestine. The enteric canal is a straight tube running through the length of the body, from the mouth, at the anterior end, to the anus near the posterior end. By variations in its width, and in the thickness of its walls, it is distinguishable into pharynx, œsophagus, intestine, and rectum.

The three-rayed mouth-opening (Text-fig.11) leads, through a very short and narrow passage, into a rounded bulb with thick, muscular walls, the anterior pharyngeal bulb (Text-fig.9, *a.ph.*). Its narrow cavity is rayed in cross-section, and is lined by cuticle (*c.p.*) which, everywhere thick, is thickest in the anterior portion, where the lumen is slightly larger than it is further back. Its walls are composed of a complicated system of circular, longitudinal, and radial muscle-fibres. Bands of muscle-fibres (Text-fig.10, *f, f'*.) attach its anterior end to the cuticle of the body-wall dorso- and ventro-laterally, and further back, towards its posterior end, there appear to be finer strands of fibrils running forward to attach it laterally. The anterior pharyngeal bulb extends through a little more than one-third of the length of the head, and is followed immediately by a second rounded swelling of equal length and width, the posterior pharyngeal bulb, the walls of which are composed of a less complicated system of radial muscle-fibres. Its lumen is still very narrow, and is lined with cuticle (Text-fig 9, *p.ph.*). It is divided by a well-marked constriction from the œsophagus, which is slightly swollen in the head-region to form a third, much smaller bulb (Pl. xlv., *a.o.*). This, however, differs from the pharyngeal bulbs in having non-

muscular walls, and a fairly wide cavity not lined by cuticle (Text-fig.9, *a.o.*). Behind the neck-constriction, the œsophagus extends back to the region of the genital organs as a cylindrical tube of uniform diameter, occupying a large part of the space enclosed by the body-walls (Text-fig.5, *œ.*). In an adult female



Figs. 12-13.—*Ch. falcatum*.

Fig. 12.—L.S. through female, in region of ventral setae; ( $\times 610$ ).

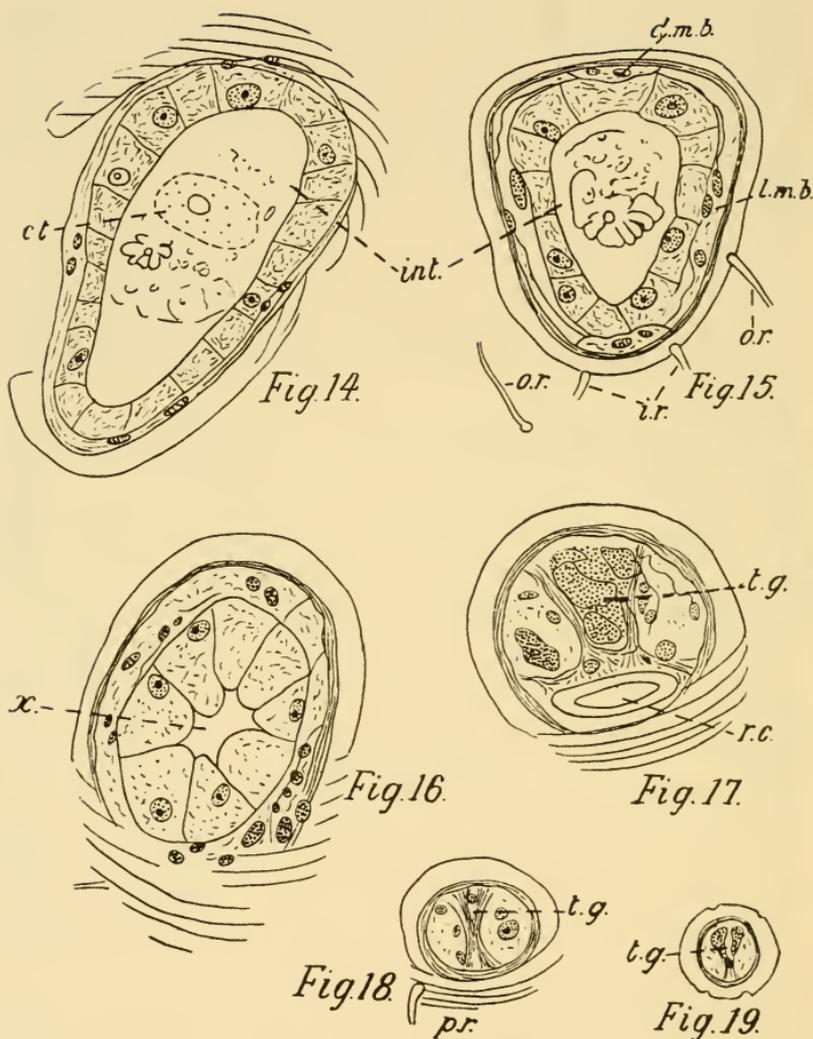
Fig. 12a.—L.S. through body-wall between the mesodermal bands, showing the subcuticular layer with nuclei (*n.*).

Fig. 13.—L.S. through body in region of anterior ovary and uterus. At the back of the uterus, the section passes through wall of intestine only.

worm, which is 0.055 mm. in cross-section, the diameter of the œsophagus is 0.033 mm. The walls are relatively thick, and composed of a single layer of large, rectangular cells, with thin

but easily recognisable walls and loose, granulated protoplasm. Each cell contains a large, oval nucleus, having a single, more darkly staining nucleus in it. There are from six to eight cells in cross-section. Further back, the entire canal becomes crushed up against the dorsal body-wall by the growth of the genital organs, and the walls become thinner (Text-figs.12, 13, 20-22, *æ.*). Behind the genital pore, where it passes backward to the left of the posterior ovary, its walls are very thin, and the cell-boundaries and nuclei are difficult to distinguish, while the protoplasmic contents become denser (Text-figs.23-24, *æ.*). Vacuoles (Fig.24, *v.*) and numerous rounded bodies, staining deeply pink with eosin, are present.

Immediately behind the posterior ovary, at the level where the first setæ of the ventral rows appear, it completely fills up the cœlomic cavity (Text-figs.12, 14, 15, *int.*). Here, the walls are still thin, so that the lumen is relatively very large, and this part of the canal may, perhaps, be regarded as a stomach. The walls increase in thickness as it passes back, though still formed of a single layer of from 15-20 cells, which vary considerably in size. A short distance in front of the anal aperture, it passes suddenly into the rectum through a narrow passage surrounded by eight or nine very large, wedge-shaped cells with clear, protoplasmic contents, and large, round nuclei (Text-fig.16, *x.*). Behind the constriction, the walls are extremely thin (Text-fig.17, *rc.*), the passage is dorso-ventrally compressed, and lined with cuticle, and the anus, by which it opens on the ventral surface, is a transverse slit situated on a slight projection. A good deal of food-matter is present in all parts of the enteric canal in the adult worm, but it consists of a kind of granular *débris*, in which it is difficult to recognise anything definite. I have seen, however, a Desmid, a small Foraminiferal shell, and several chains of minute Algal cells among the *débris*. In an immature female worm, the cells forming the wall of the enteric canal are relatively very large, and few in number; and the lumen is very small, in parts almost completely closed-up (Text-figs.6-8). Surrounding the anterior end of the pharynx, there is a group of large cells staining deeply blue with hæmatoxylin, which pro-

Figs. 14-19.—*Ch. fulcatum*.

Series of Tr. Secs. through trunk of female from middle region to tail; ( $\times 610$ ).

Fig. 14.—An oblique section through body, just behind posterior ovary.

Fig. 15.—Section in region of ventral setæ.

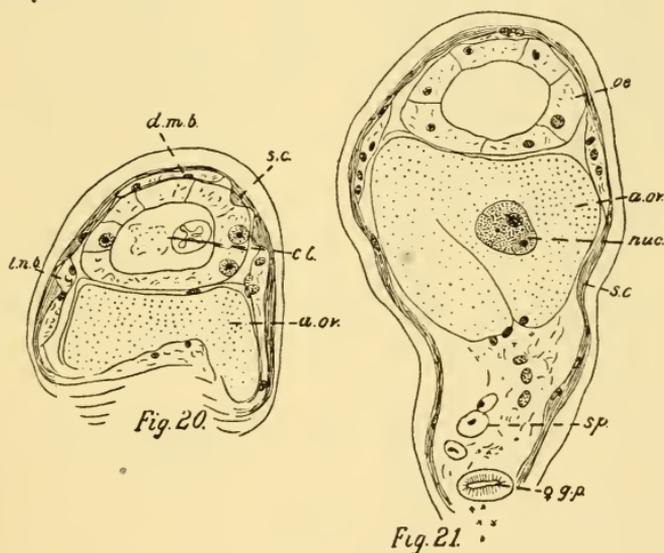
Fig. 16.—Section in region of constriction (*c.*) between intestine and rectum.

Fig. 17.—Section just in front of anus.

Fig. 18.—Section behind anus.

Fig. 19.—Section through tail; *c.t.*, contents of intestine.

bably function as digestive glands (Text-fig. 9, *g.c.*), and in addition, there are several granular masses of a yellow colour embedded in the hypodermal tissue immediately surrounding the mouth, which may be glandular. Possibly the pink-stained bodies and the vacuoles in the wall of the stomach have a glandular function. In addition, there is a large, granular mass of tissue, staining deeply blue with hæmatoxylin, situated immediately above the rectum (Text-figs. 3, 17-19, *t.g.*). It occupies most of the space between the dorsal wall and the rectum, and extends as a longitudinal strand into the tail-region, opening by a duct at the extremity of the tail. This may correspond to the tail-gland described in many marine Nematodes, and thought to be excretory in function.



Figs. 20-21.—*Ch. falcatum*.

Fig. 20.—T.S. through body of female at beginning of anterior ovary; ( $\times 450$ ).

Fig. 21.—Very oblique section through region of female genital aperture, showing portion of anterior ovary and uterus.

There is no trace of the longitudinal lateral vessels found in Nematodes, and the only indication of a nervous system is in the shape of an ill-defined granular mass of tissue round the constriction between the two pharyngeal bulbs.

In the female, there are two ovaries lying in the cœlome below the enteric canal, one behind, and one in front of the genital aperture (Pl. xlv., *a.ov.*, *p.ov.*). They are thick and short (about 0.066 mm. long), and the free end of each appears to be bent over on the remainder. The portion of each furthest removed from the genital pore, consists of finely granulated substance in which a few, very large nuclei are scattered (Text-figs. 20-21, *a.ov.*). The remaining part is divided by distinct walls into large cells, each

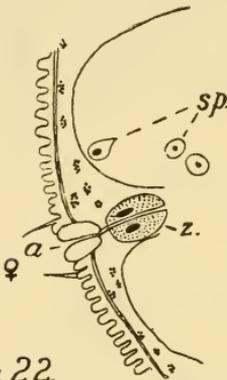


Fig. 22.

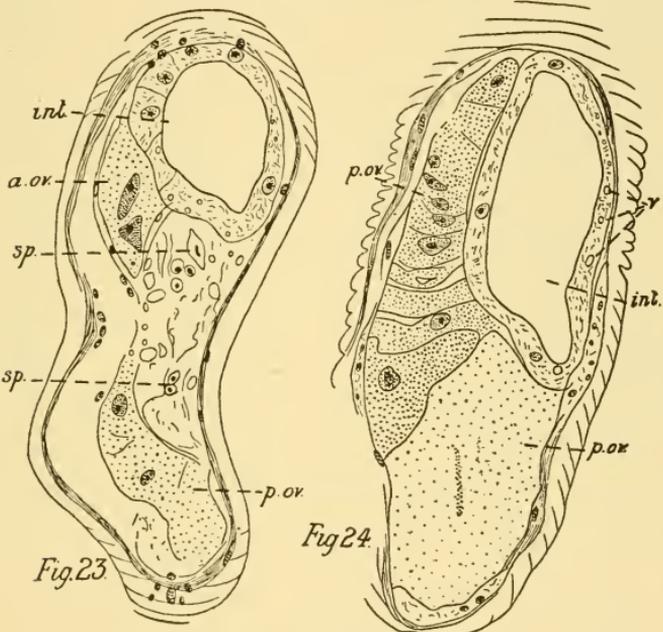
Fig. 22.\*

containing a large, round nucleus. These are probably ova in process of formation. The ovaries open into a single large uterus, which, in the adult, always seems to contain numerous sperms. The genital pore, by which it opens on the surface, is a transverse slit, 0.01 mm. wide, situated on a slightly raised papilla. It appears to be surrounded by very small setæ, and has thick, chitinous walls. Immediately below the aperture, lie two, thick, elongated cells, with very large nuclei, apparently enclosing the uterine passage (Text-fig. 22). Embedded in the wall of the uterus, and lying free in its cavity, are numerous rounded spots, stained bright pink with eosin.

In the male, the genital apparatus is in the form of a straight tube, lying ventral to the alimentary canal, throughout a greater part of the length of the trunk (Pl. xlv.). It is distinctly divided into three portions. At the anterior end, beginning 0.055 mm. from the neck, is the testis (*t.*), a narrow cord consisting of a solid mass of granular matter not divided into cells. Further back, cells become gradually differentiated, and pass into a wide, tubular cavity, the vas deferens, where they lie loose. At the level of the anterior end of the rows of ventral setæ, the vas deferens passes very abruptly into a narrow,

\* L.S. through female genital aperture (*a.*), showing the large cells (*z.*) surrounding the passage into the uterus; ( $\times 610$ ); *sp.*, sperms in uterus.

thick-walled duct, the vesicula seminalis (*v.s.*), which runs back to open with the rectum at the anus. At the junction of the vas deferens with the vesicula seminalis, the wall of the latter is very thick, opaque, and finely granular, and probably functions as a gland for secreting a seminal fluid. The lumen of the vesicula seminalis is difficult to make out. In all fixed specimens, it is filled with what is probably a coagulated fluid. The sperms are small and rounded, with a very distinct nucleus, but show no trace of the characteristic tail of the typical sperm.



Figs.23-24.—*Ch. fulcatum*.

Fig.23.—Oblique section through middle region of trunk of female, showing portion of uterus, and anterior and posterior ovaries; ( $\times 450$ ).

Fig.24.—Section through the same worm, a little further back, showing cellular portion of posterior ovary (*p.ov.*); *sp.*, sperms in uterus; *v.*, vacuoles in wall of intestine.

Two, equally long, curved, penial setæ, and a small, accessory piece lie dorsal to the rectum. The setæ are bow-shaped, and have enlarged, proximal ends (Text-fig.3, *p.s.*). Between the sperm-duct and the ventral body-wall, there is a row of block-

like, granular bodies, staining deeply blue with hæmatoxylin. Similar structures appear to be present in the female, connected with the ventral ridge of protoplasmic tissue. They probably have some relation to the locomotor setæ, though I cannot find any trace of communication between them.

Type-specimens, Nos. W, 452, 453; in the Australian Museum, Sydney.

*Chætosoma falcatum*, n.sp. Measurements in mms.

	♀	♂
Total length ... ..	0·888	0·840
Length of head ... ..	0·144	0·168
Length of trunk ... ..	0·744	0·672
Length from tip of tail to anterior end of rows of ventral setæ ... ..	0·408	0·360
Length from tip of tail to anus ... ..	0·092	0·077
Length of tail ... ..	0·051	0·033
Length from neck to ♀ pore ... ..	0·312	—
Greatest width of head ... ..	0·063	0·063
Greatest width of trunk ... ..	0·092	0·081
Width at level of ventral setæ ... ..	0·048	0·055
Width at level of neck-constriction ... ..	0·026	0·026
Length of rostrum ... ..	0·040	0·033
Length of setæ on rostrum ... ..	0·018	0·015
Length of hairs on head ... ..	0·022	0·022
Length of ventral setæ ... ..	0·018-0·016	0·018-0·016
Number of setæ in ventral rows—outer...	21 compound.	12 compound, 7 simple, 9 anal.
Number of setæ in ventral rows—inner...	23 compound.	17

CHÆTOSOMA HASWELLI, n.sp. (Plates xlvi.-xlvii.).

In 1914, Professor Haswell obtained a few individuals of this species from material collected along the shores of Port Jackson and Broken Bay, N.S.W. These were the first of the *Chætosomatidæ* found south of the Equator. In the following year, he drew my attention to them, and afforded me the opportunity of examining these and other specimens, which he subsequently collected. During the last twelve months, I have myself collected some fifty of the same species from various localities round the shores of these bays, and along the ocean-coast in the vicinity. *Ch. haswelli* appears to be the most generally dis-

tributed of the New South Wales species. I have found it in the same material with each of the other three species; and, in addition, at nearly every spot, where a search has been made, to a distance as far up the harbour as Circular Quay, and to a depth of 18 feet below lowwater-mark. No collections were made at a greater depth than this; and the majority of the specimens were obtained just below tide-marks.

I have been able to observe a fairly large number of living individuals. In one case, all the specimens were found alive in material which had been collected four days previously. Two were kept alive for fourteen days in a crystal-dish, by changing the water every day; and were then accidentally crushed during an examination under the microscope. A third worm, a young specimen, was kept alive for five weeks; and was still active, and apparently quite healthy, when it was lost during transference to fresh seawater. It had not then reached mature size. All the specimens found alive were very dirty, being covered with grit. They required frequent cleaning with a very fine camel's hair brush, as it was found that they continued to accumulate grit every day during the time they were alive, the dirt apparently adhering to a sticky secretion over the whole surface.

*Ch. haswelli* is the largest of the species found here. The length of the largest male obtained was 1.32 mm., and of the largest female, 1.44 mm. Sexual organs had not developed in the smallest specimen found, which was 0.60 mm. long. The worms are mostly S-shaped when fixed (Pl. xlvi.). They are long and slender, and narrower, in proportion to their length, than the other species. The females are, on an average, longer than the males; and, in both sexes, the head is broader than the average width of the trunk. In the male, there is very little variation in the width of the trunk, though there is a slight increase in size in the middle region. The female is markedly wider in the middle. Typical measurements are given in the accompanying Table p.798).

The head is oval in shape, and well marked off from the rest of the body. In length, it is only one-ninth to one-tenth of the

total body-length. The tail is fairly long and slender, tapering gradually to a fine point; and is longer in the male than in the female. The body is covered by a thin, delicate, semitransparent cuticle, which is marked by very delicate, transverse striations, except at the two extremities, where it is quite smooth; and in

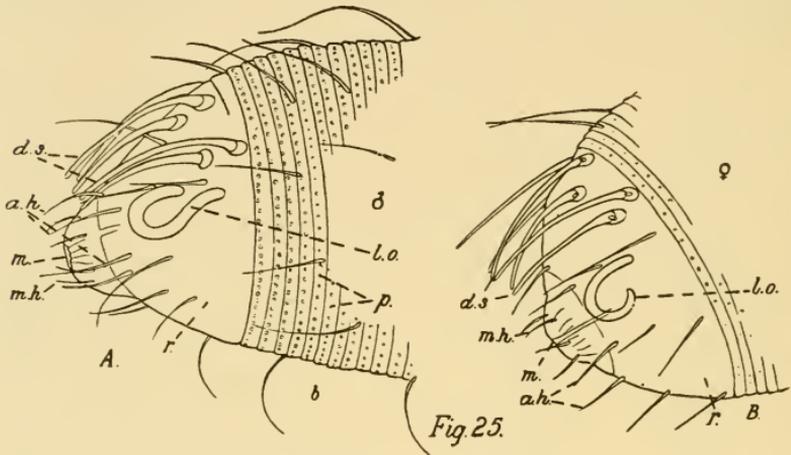


Fig. 25.—*Ch. haswelli*, n.sp.

A., Anterior extremity of head of male, showing details of structure of rostrum (*r.*), and banded area (*b.*), lateral view.—B., similar view of head of female, to show difference in form of lateral sense-organ (*l.o.*); ( $\times 630$ ); *m.h.*, hairs surrounding mouth; *a.h.*, terminal hairs of the rows extending down the trunk; *p.*, pores in the striae of banded area.

the middle region of the head, where there are from eight to fifteen much thicker ridges, forming a well marked band. The head, therefore, is distinguishable into three regions, a smooth, anterior end or 'rostrum' (Pl. xlvii, *r.*); a banded area (*b.*); and a broader region behind it, on which the transverse striations are inconspicuous. The rostrum is short, about one-fifth to one-sixth the total length of the head, and is blunted off in front. The banded area is of about equal length, and appears to constrict the head, which broadens out considerably behind it. The ridges forming this band slightly overlap one another from behind forward (Fig. 25, *b.*); and, in each ridge, continuing right round its circumference, there is a single row of fine pores set very

close together (Fig.25, *p.*). The pores are very deeply embedded (Fig.30, *p.*), but show, on the surface, as a row of fine, bright dots. They appear to be present only in this region, though the surface of the rostrum also is very finely, but irregularly, pitted all over.

The lateral, spiral grooves on the rostrum are large and conspicuous; the spiral is simple and very open, and is turned away from the dorsal hairs in the female, and towards them in the male (Fig.25, *l.o.*). Embedded in the hypodermis, below the groove on each side, is a peculiar cell, probably sensory in character (Fig.31, *x*), which may have some relation to these lateral organs, though I cannot trace any direct connection between them. The dorsal semicircle of stout 'head-hairs' or setæ, in the adult, consists of five or six pairs arranged in two rows close to the posterior margin of the rostrum (Fig.25, *d.s.*), the setæ of the posterior row being wedged against the most anterior of the ridges of the banded area. They have an average length of 0.025 mm. All the setæ are bent forward to such a degree, that they lie close to the surface of the rostrum, and follow its shape. They are enlarged at the base, and are long enough to project over the anterior end, where there is a lip-like arrangement surrounding the mouth (Fig.25, *m.*).

One immature female, without a genital pore, was found, in which there were only four setæ in a single row (Pl. xlvii., *b.*). In this case, the two inner setæ were inserted very close together in the dorsal mid-line; and the outer ones, separated from them by a fairly wide interval, are just dorsal to the spiral groove on each side. Close to the outer side of each seta is a long, fine hair.

The ventral setæ (Pl. xlvi., *i.r., o.r.*) are confined to a small portion of the trunk, usually about one-seventh the total length of the body, whether this is 0.60 or 1.44 mm. The setæ are arranged in four rows, beginning some little distance in front of the anus, about 0.051 mm., in mature worms of both sexes, and 0.034 mm., in young worms.

The two inner rows are situated very close together, and con-



had only a single row of head-setæ, and only three pairs of ventral setæ (Pl. xlvii., *b*). But it is possible that this was a damaged specimen, since the setæ in some of the others examined broke off with handling.

The setæ of the inner rows (Fig. 27*a*) are much shorter than those of the outer rows (Fig. 27*b*), which are often longer than the width of the body in that region; and longer in the male than in the female, averaging, in the former, 0.045 mm., and 0.040 mm. in the latter. The longest setæ are at the anterior end of the rows. Here, they are more closely crowded than they are further back. All the compound setæ are similar in character. Each consists of a proximal, elongated, hollow rod, and a small, expanded, movably-jointed end-segment, with a deep indentation in its distal margin. The simple setæ, which alternate with them in the male, are of equal length, but very slender and delicate, and tapering to a fine point. The body-hairs are a very conspicuous feature in this species. They are very long, relatively to the width of the trunk; and all have a bulb-like swelling at the base (Figs. 26, 27, 35, *t.h.*). They are arranged in eight longitudinal rows, two lateral rows on each side, two dorsal, and two ventral, which are all continued over the head to the anterior end of the rostrum (Pl. xlvi.). The dorsal and dorso-lateral rows extend down to the margin of the tail, but the ventral and ventro-lateral rows only to the beginning of the ventral setæ, with the inner and outer rows of which they are respectively continuous. The number of hairs in each row is limited, and appears to be fairly constant. On the trunk, there are, in the dorsal rows, 20-30 long hairs; dorso-lateral rows, 19-27; ventro-lateral, 15-17; and ventral, 15-23. On the head, there are, on the hinder region, 3-5 hairs; on the banded area, two; and, on the rostrum, 3-4, the terminal hair of each of the eight rows being situated right at the anterior margin of the cuticle, so that there are eight hairs forming a circle surrounding the lip-like prominence, in the centre of which, the mouth is situated (Fig. 25, *a.h.*). Immediately round the small mouth-opening, there is another ring of small hairs, about six apparently,

but the number is difficult to determine (Fig.25, *m.h.*). On the dorsal side of the rostrum, the rows of hairs are distinct from, and lie between, the stout 'dorsal hairs,' or setæ, already described. On the trunk, in each row, there are, alternating fairly regularly with the long hairs, very short, blunt hairs (Fig.26, *t.h'*), which have a similar bulb-like swelling at the base. This, in all the hairs, seems to be hollow. Particles of dirt are frequently found adhering to the hairs, and it appears probable that these are glandular, and secrete some sort of mucilage. The 'long trunk-hairs' of the dorsal rows are longer than those of the other rows, especially over the region of the genital organs in the female, but the variation in length is not marked. In different specimens, the length varies from 0.025 mm. to 0.05 mm., and seems to average about two-thirds the average width of the body.

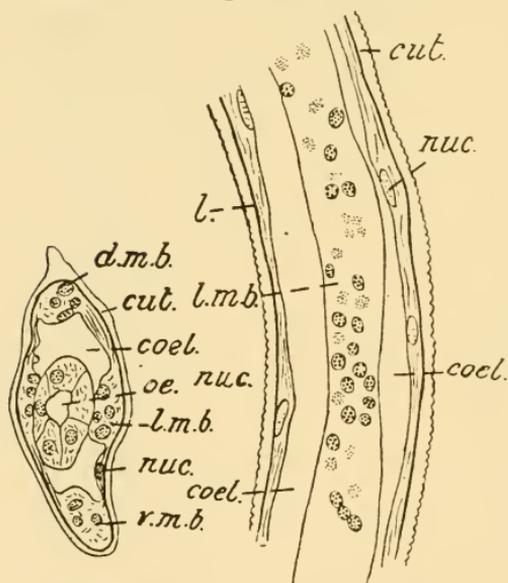


Fig.28a. Fig.28.

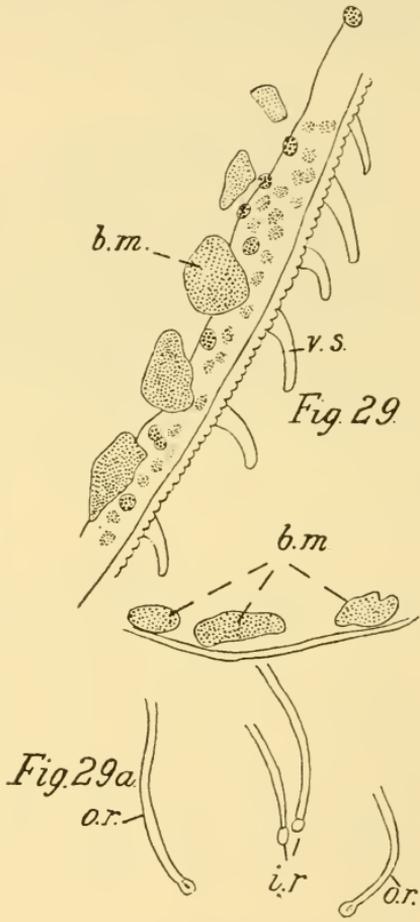
Figs.28, 28a.—*Ch. haswelli*.\*

level of the anus and the beginning of the tail. The cuticle of

In the male, there are, in the region of the anus, several hairs differing in character from the ordinary trunk-hairs, stronger and stouter, and without the basal swelling. These appear to be very constant in position, *i.e.*, two inserted very close together about half-way between the outer, ventral row of setæ and the anus (Fig.26, *pr.s.*), two on each side of the anus, one behind, and one in front of it, in the mid-ventral line (Fig.26, *a.s.*), and one or two others between the

\* Fig.28.—L.S. through body-wall, cutting through one of the mesodermal bands, and the subcuticular layer on either side of it; ( $\times 950$ ). Fig.28a.—T.S. through anterior region of trunk of a young *Ch. haswelli*; ( $\times 950$ ).

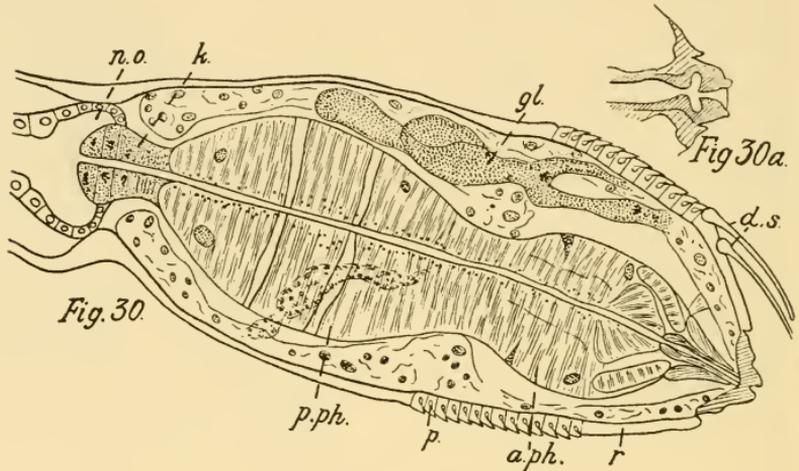
the body-wall is a thin, homogeneous layer, about 0.001 mm. thick (Fig. 28, *cut.*). An extremely thin, dark line (*l.*), which, however, becomes much thicker in the head, divides it from the next layer. This is of about the same thickness as the cuticle. It is difficult to make out any structure in it, but it appears to be of fibrous composition; and, lying along its inner border, there are, at wide intervals, elongated, oval nuclei (*nuc.*). Below it, at about equally spaced intervals, there run four longitudinal bands of mesoderm, containing numerous, crowded, round nuclei (*d.m.b.*, and *l.m.b.*). These bands are continuous from the neck to the tail, and form the innermost layer of the body-wall. Within it is a wide cœlomic cavity (*cœl.*), in which lie the enteric canal and the genital organs. In the head, a loose sort of protoplasmic tissue fills up a great part of the cavity, between pharynx and body-wall. Below the ventral body-wall, in the region of the ventral setæ, there are four rows of block-like, finely granular masses, staining deeply blue with hæmatoxylin (Pl. xlvii., *b.m.*). They are quite separate from one another, about ten to twelve in each



Figs. 29, 29a.—*Ch. haswelli*.\*

\* Fig. 29.—L.S. through body-wall in region of ventral setæ; (×950). Fig. 29a.—T.S. in same region; (×610). The section has been flattened out in cutting; *v.s.*, bases of ventral setæ.

row; and they lie directly under the rows of locomotor setæ throughout their length (Figs.27, 29, *b.m.*). It is probable that they have some relation to the setæ, since they are found only in this region; but I cannot trace any direct connection between them.



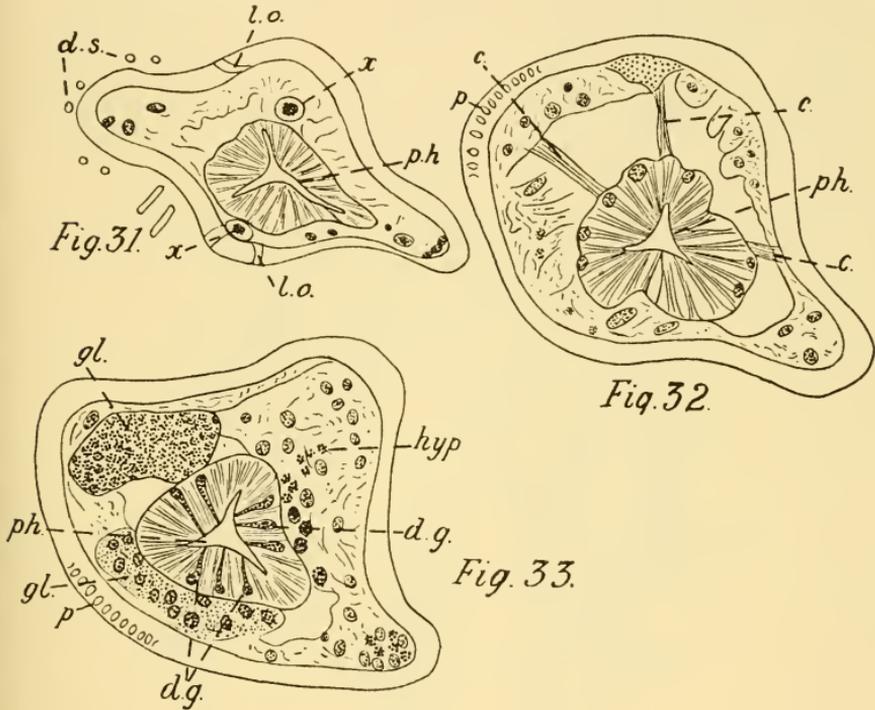
Figs.30, 30a.—*Ch. haswelli*.

Fig.30.—Sagittal, longitudinal section through head; ( $\times 660$ ).

Fig.30a.—Section through mouth-opening; ( $\times 760$ ).

The mouth-opening is surrounded by a circular projection, which seems to be protrusible, and bears a circlet of fine teeth (Fig.30a). The mouth leads directly into the pharynx, which has very thick, muscular walls, and extends through the full length of the head. It is constricted in the middle to form anterior and posterior bulbs, of which the posterior is the larger (Fig.30, *a.ph.*, *p.ph.*). Both bulbs are slightly larger in the female than in the male, corresponding to a difference in the size of the head. The walls of the anterior pharyngeal bulb consist of a complicated system of muscle-fibres, the arrangement of which is shown in Fig.30. The passage is narrow, and lined with cuticle, which is thickest towards the mouth-opening. Immediately surrounding the passage, there is a mass of finely pigmented granules; and deeply embedded in the muscle-fibres are numerous, small glands, probably digestive, which open into the

pharynx by fine ducts (Fig.33, *d.g.*). Strands of tissue connect the bulb with the inner wall of the rostrum, both at the anterior end and further back (Fig.32, *c.*). The muscle-fibres in the posterior bulb are all radial, and are divided into three sections by two radial gaps, which are very constant in position in different



Figs.31-33.—*Ch. haswelli*.

Series of tr. secs. through the head; ( $\times 950$ ). The head has been distorted in shape during the process of embedding.

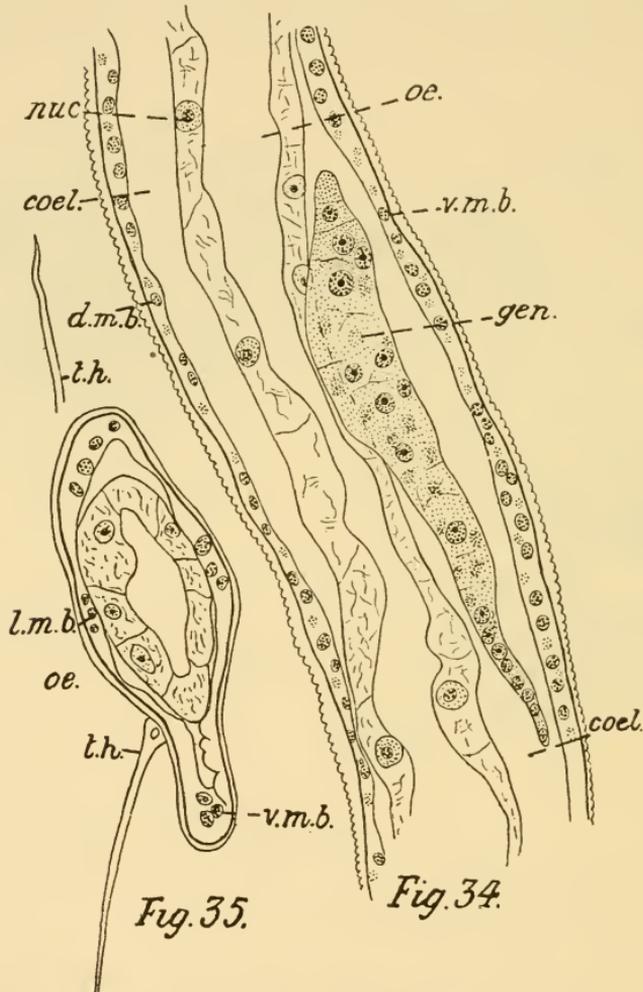
Fig.31.—Section through the rostrum.

Fig.32.—Section through the 'banded' area.

Fig.33.—Section through the hinder part of the anterior pharyngeal bulb; *x.*, sensory cells; *d.g.*, digestive glands; *p.*, pores in the striae of the banded area; *c.*, fibres from pharynx to body-wall.

specimens. This gives the appearance of a broad band surrounding the middle region of the bulb. The gaps contain a granular substance, which is probably glandular in character. The terminal portion of the pharynx consists of a knob (Fig.30, *k.*),

formed of a little group of dark granular cells, which projects into a wide, thin-walled chamber formed by a swelling of the œsophagus in the neck (Fig. 30, *n.o.*). This swelling is supported



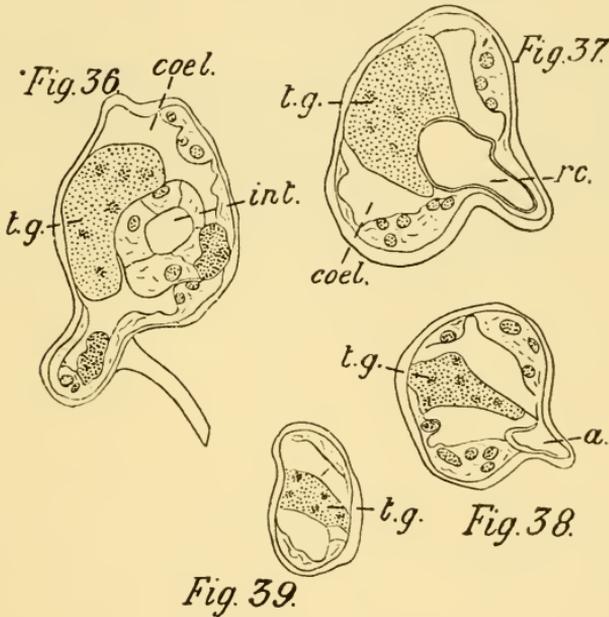
Figs.34-35.—*Ch. haswelli*.

Fig.34.—L.S. through trunk of a young specimen in middle region, showing rudiment of a genital system (*gen.*); ( $\times 950$ ).

Fig.35.—T.S. through trunk anterior to genital system; ( $\times 950$ ).

by inward projections of the body-wall. Behind it, the lumen of the œsophagus is narrow throughout its length, and the wall

is one cell thick (Figs.34, 35, *ae.*). The cells are thin-walled, and roughly rectangular in cross-section, measuring about  $0.014 \times 0.007$  mm. The protoplasmic contents are clear; and a large, round nucleus (*nuc.*) is situated about the middle of each cell. In the mid-region of the body, in the female, the intestine is constricted to a narrow tube pressed up against the dorsal wall by the genital organs. Below the posterior ovary, it widens so as nearly to fill the coelome. The cells of the wall are here very large, and the cell walls, forming the lining of the passage, are



Figs.36-39.—*Ch. haswelli*.

Series of tr. secs. through posterior region. Like most of the transverse sections, they have become distorted in shape during embedding; ( $\times 950$ ).

Fig.36.—Section through constriction between intestine and rectum. Fig. 37, through rectum. Fig.38, through anal aperture. Fig.39, through tail.

slightly thickened. The cells vary in shape, and project into the intestinal cavity, leaving only a narrow passage. The tube is constricted again in the region of the ventral setæ (Fig.27, *int.*).

About the level of the last of the ventral setæ, the intestine

passes, by a narrow constriction, into the rectum (Fig.37, *rc.*), a wide, thin-walled tube lined with cuticle. This opens by the anus (Fig.38, *a.*) on the mid-ventral surface, at an average distance of 0.080 mm., from the posterior end, in the female; and 0.099 mm., in the male.

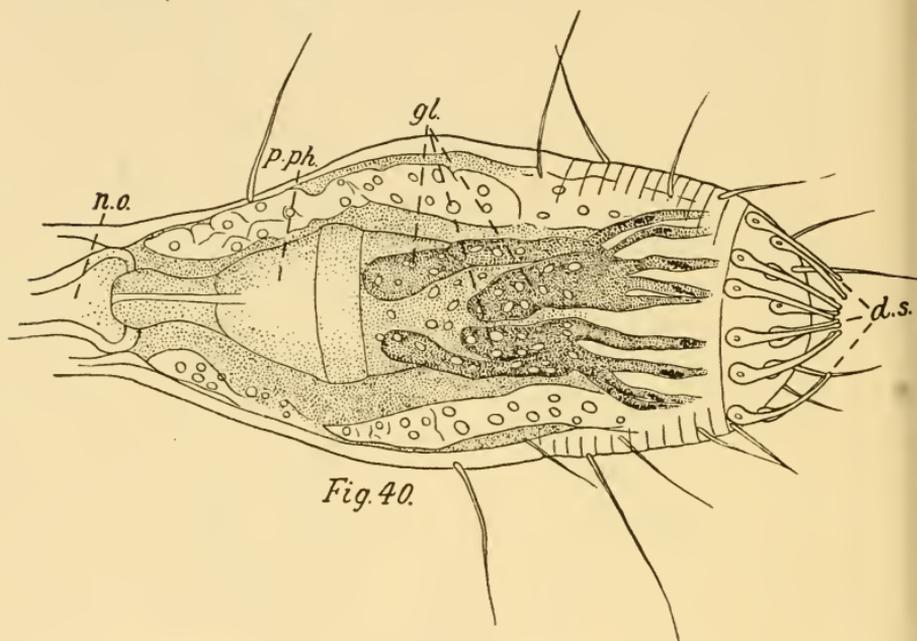


Fig.40.

Fig.40.—*Ch. haswelli*.

Dorsal view of head, showing arrangement of dorsal setæ, and the glandular bodies (*gl.*) lying above the pharynx; ( $\times 660$ ).

Nerve-cells and nerve-fibres are distinguishable in the protoplasmic tissue in the head. They seem to form an ill-defined ring surrounding the constriction between the two pharyngeal bulbs, and give off strands forward and backward.

Lying above the pharynx, and embedded in the hypodermis, there is a group of densely granular bodies (Figs.30, 40, 41, *gl.*), staining dark blue, from each of which, a strand runs forward, and apparently connects with the base of one of the dorsal setæ. One or several thickenings occur in each of these strands, towards the anterior end. I have been able to observe these bodies only in a few specimens, and have failed to determine their exact

nature. The thickenings on the strands seem to suggest nerve-matter, but I think it more probable that they are glandular in character, and secrete a fluid which assists the hollow, dorsal setae in their locomotory function.

Another body, of a glandular character, lies in the posterior end of the trunk, just dorsal to the rectum. It is club-shaped, composed of several, large, finely granular cells containing numerous vacuoles and large nuclei, and is bounded by a distinct wall (Figs.27, 36-39, *t.g.*). It is connected with

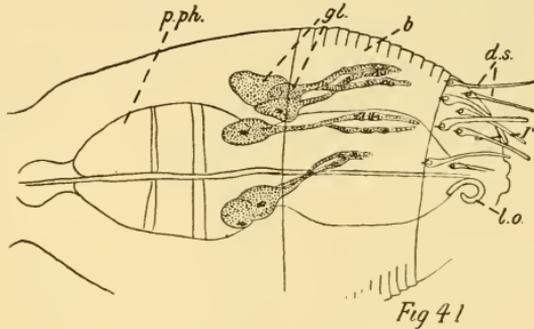


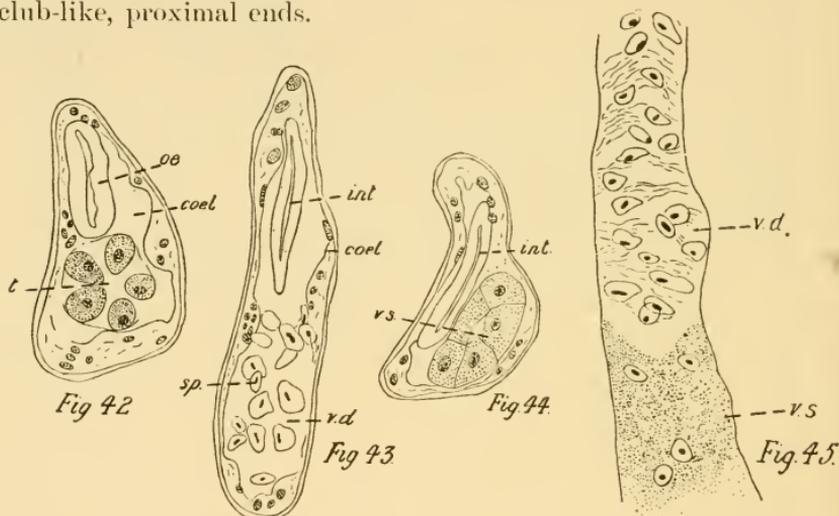
Fig.41.—*Ch. haswelli*.

Lateral view of dorsal setae and glands (*gl*) in the head; ( $\times 400$ ).

a fine duct, which runs back through the tail, and opens by a fine pore at the posterior extremity. Large, bright drops of fluid are distinguishable in the duct, in some specimens; it seems probable that this organ is an excretory 'tail-gland.'

In the male, the reproductive apparatus consists of a single cord, which begins at a distance of 0.074 to 0.140 mm., from the neck, and extends straight back below the alimentary canal, to open with it at the anus. The anterior portion is solid, and terminates in a fine point (Pl. xlvi., *t.*). It has an average width of 0.007 mm., and consists of a closely packed mass of small cells enclosed by a firm wall. Further back, the cells become larger and more loosely arranged, and take on the definite character of sperms, of rounded or somewhat irregular shape, with fragments of connective tissue between them (Figs.42-43, 45). This portion, which has a width of 0.014 mm., passes abruptly, towards the middle of the body, into a definite tube, with very wide, cellular walls (Fig.44, *r.s.*). The lumen is extremely small, and, in cross-section, is surrounded by four or five, large cells, with very large nuclei. In longitudinal section, it has the ap-

pearance of being a solid cord, but the dense, finely granular substance filling it, is probably a spermatic fluid, which has become coagulated in the fixing. It is impossible to examine it in the living state, owing to the difficulty of keeping the actively moving worm in the field, under a high power. Posteriorly, the sperm-duct diminishes in width. There are two, equal, penial setæ (Pl. xlvii.; Text-fig.26, *p.s.*), and an accessory organ. The setæ are long, and are curved into a bow-shape, with enlarged, club-like, proximal ends.



Figs 42-44.—*Ch. haswelli*, ♂.\*

Fig.45.—*Ch. haswelli*.†

The female genital pore (Figs.46, 47, ♀ *g.p.*) is a transverse slit, with prominent lips, situated on the ventral surface just about the middle of the trunk. There are two, large and well-developed ovaries (Fig.46, *a.ov.*, *p.ov.*) situated, one anterior and one posterior to the genital pore. The end of each, furthest from the pore, is roughly conical in shape, about 0.074 mm. long,

\* Series of sections through the trunk; ( $\times 630$ ). The sections have been laterally compressed, and the internal structure somewhat distorted. Fig.42—A section through the anterior part of testis. Fig.43, through the vas deferens. Fig.44, through the sperm-duct, in the region of the ventral setæ.

† L.S. through male genital tube, in region between the vas deferens and the vesicula seminalis, showing the finely granular matter in which the sperms are embedded at the junction; ( $\times 630$ ).

and 0.026 mm. broad at the widest part; and contains large nuclei embedded in finely granular substance. It is continuous

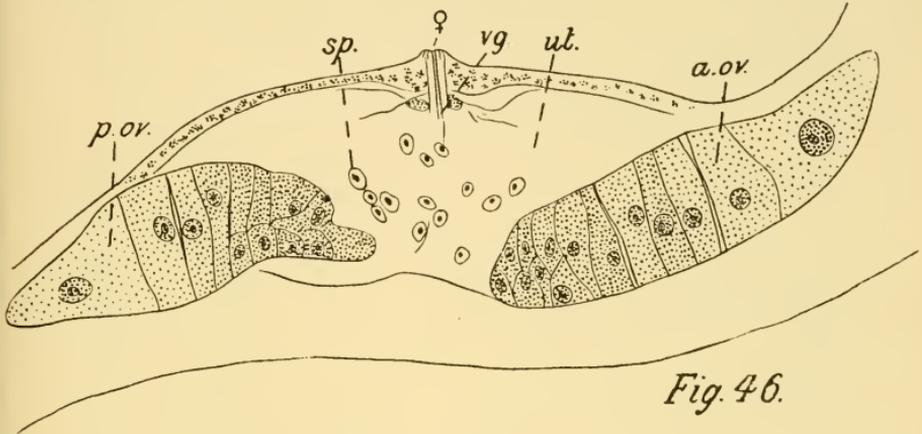


Fig. 46.

Fig. 46.—*Ch. haswelli*, ♀. Genital system; ( $\times 400$ ).

with a much broader portion, where there is a differentiation of the contained matter into long, thin cells, cut off in a transverse direction. There are distinct spaces between some of the cells, which appear to be ova in process of formation. This part may, perhaps, be looked upon as an oviduct. A definite, tubular duct, connecting it with the uterus, is not discernible in any of the specimens, which I have examined. Some individuals examined had a single, large, oval egg in the common uterus (Fig. 47, o.). The uterus measures 0.092  $\times$

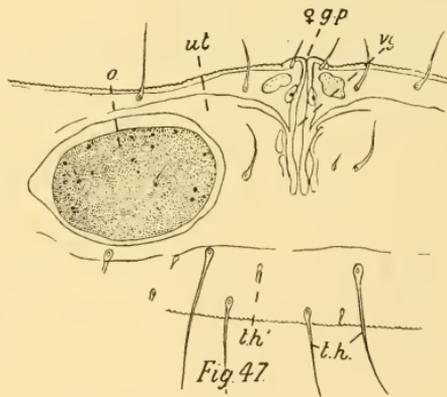


Fig. 47.—*Ch. haswelli*. Ovum in uterus; ( $\times 320$ ).

0.055 mm. A large receptaculum seminis seems to be a diverticulum from it. It lies to one side of the oviducts, and extends out towards the anterior and posterior ovaries. In all the specimens examined, it is full of sperms (Fig. 46, sp.). The narrow passage, leading from the uterus to the vulva, has thick walls,

and, at its junction with the uterus, is surrounded by a few large cells, which probably act as a sphincter (Fig. 47, *vy.*). The development of the fertilised ovum has not been followed.

Type-specimens, Nos. W, 454, 455; in the Australian Museum, Sydney.

*Chatosoma haswelli*, n.sp. Measurements in mms.

	♀	♂
Total length ... ..	1.39	1.128
Length of head ... ..	0.144	0.120
Length of trunk... ..	1.248	1.008
Length of trunk covered by rows of setæ ... ..	0.185	0.166
Length from tip of tail to ant. end of rows of setæ... ..	0.333	0.333
Length from tip of tail to anus ... ..	0.092	0.107
Length of tail ... ..	0.037	0.037
Length from neck to genital pore ... ..	0.600	—
Greatest width of head ... ..	0.070	0.066
Greatest width of trunk ... ..	0.085	0.059
Width at level of ventral setæ ... ..	0.047	0.030
Width at level of neck-constriction ... ..	0.029	0.033
Length of rostrum ... ..	0.026	0.022
Length of setæ on rostrum ... ..	0.022	0.022
Length of hairs on trunk ... ..	0.037	0.025
Length of ventral setæ, outer rows ... ..	0.044	0.055
Number of setæ in ventral rows—outer ... ..	13	7+4
inner ... ..	15	12

NOTOCHÆTOSOMA, gen.nov.

The two species, included in this genus, differ considerably from any of the Chætosomatidæ hitherto described, in that a well marked head-swelling is absent. There is a corresponding difference in the structure of the pharynx, which lacks the typical pharyngeal bulbs, and is only slightly swollen posteriorly. The structure otherwise is very similar to that of the Chætosomatidæ, but the differences indicated seem to be of sufficient importance to constitute a new genus of this family.

Genotype, *N. tenax*, n.sp.

NOTOCHÆTOSOMA TENAX, g. et sp.nov. (Plates xlvi. xlix.).

Only a few specimens have, so far, been obtained, all taken from a single locality in Port Jackson. I first found four in the

winter of 1916, in material collected at Vacluse, on the rocks between, and just below tide-marks. Since then, about thirty worms of the same species have been obtained from the same place; but extensive searches, in other localities, have proved fruitless.

The ventral, adhesive setæ are very powerful, and it is difficult to dislodge the animal from its support, so that I have been able to observe only five living individuals. These were taken from the concentrated washings of some thirty jars of fresh material. Two of the worms were kept alive in a crystal-dish, with two changes of sea-water, for a period of eight days, and a third for nine days. They have the characteristic, creeping mode of locomotion of the Chaetosomatidæ; and are more easily distinguishable from marine Nematodes when alive, than after death. When fixed, the general appearance of the body so closely resembles that of a small Nematode, that it is only possible to detect them by means of the minute setæ on the head and ventral surface.

The largest individuals attain a size of 1.0 mm., but the average size is somewhat less than this, from 0.8 to 0.9 mm. Male and female appear to be of the same length. The shape assumed, when fixed, is not constant; but the posterior third of the body is usually straight, while the region in front is more or less arched in a dorsal direction. The anterior end is only slightly enlarged, and is distinguishable as a head-region by its curvature, rather than by any definite neck-constriction. It is usually more or less bent in a ventral direction, but the curvature varies considerably in different specimens, as will be seen in the Plates. The cuticle covering the body is very thick, and, from the rostrum to the beginning of the tail, is transversely striated. There is no special banded area behind the rostrum, the striæ being all broad and deep, of the same thickness all over the body, though there is a difference in character between the striæ of the head-region and those behind, the former overlapping one another, from behind forward, to a much greater extent than the striæ on the trunk (Fig. 53, *cut.*). The rostrum (Figs. 48, 53, *r.*), is short, 0.014 to 0.018 mm. long; and the smooth cuticle

covering it is very thick, and has a serrated anterior margin. Beyond it, is a projection of tissue, 0·011 mm. long, with a rounded extremity, in the middle of which the very small, circular mouth-opening (Fig. 48, *m.*) is situated. The width of the body, just behind the rostrum, is 0·040 to 0·050 mm., which decreases, at the level of the neck-curve, to a width of 0·033 to 0·037 mm. The length of this anterior, ventrally curved portion varies, in different individuals, from 0·122 to 0·150 mm. Behind it, the body gradually increases to a width, in the male, of 0·050 to 0·060 mm., and, in the female, in the region of the genital organs, of 0·070 to 0·096 mm.

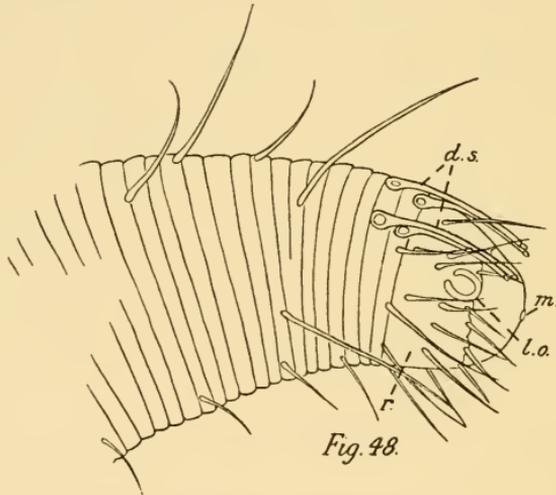
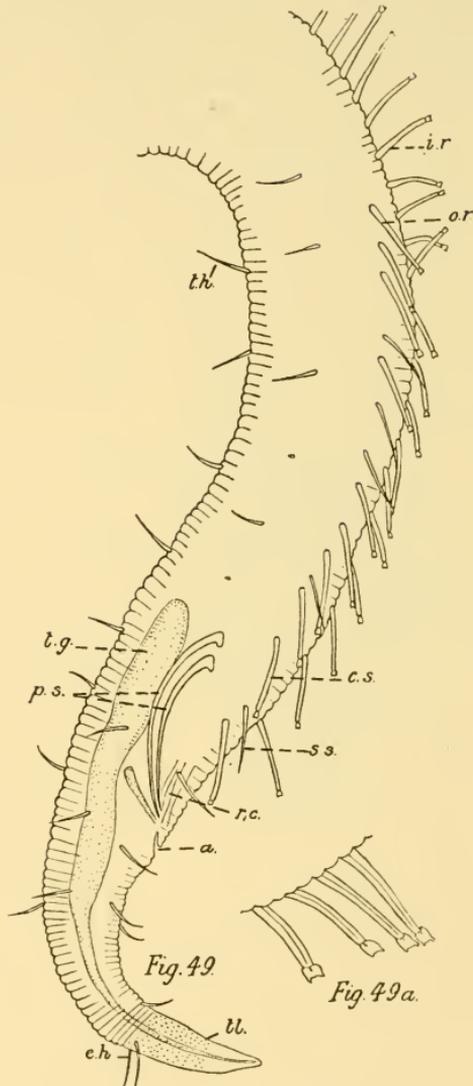


Fig. 48.—*Notochaetosoma tenax*, n.sp.  
Lateral view of anterior extremity; ( $\times 630$ ).

The tail (Fig. 49, *tl.*) is narrow, and sharply pointed. In the male, it averages 0·085 mm. from anus to tip; in the female, 0·077 mm. Cuticular striations are absent from the terminal portion (0·040 mm. long), and the anterior half of this part is closely pitted. A pair of long hairs is inserted on its dorsal surface, just at its junction with the striated area (Fig. 49, *e.h.*). As in the other species, the anus is situated on the mid-ventral surface, close to the posterior end (Fig. 49, *a*). The ventral setae are arranged in four rows, and cover a length of about one-sixth

to one-fifth the total length of the body. The rows begin about 0.045 mm. in front of the anus, and extend forward for a distance, which varies from 0.092 to 0.188 mm., according to the age of the animal, and the number of setæ. The outer rows (Figs. 49, 50, *o.r.*) are laterally situated, separated by a wide interval from the inner rows (*i.r.*), which are inserted very close together, and in pairs anteriorly. Further back, they alternate irregularly, and the two rows are not so clearly distinguishable. They usually begin further forward, and their setæ are much shorter than those of the outer rows.

In the male, the outer rows are composed of compound and simple setæ alternating irregularly, the simple setæ (Fig. 49, *s.s.*), being fewer in number; and shorter than the compound (*c.s.*), and very slender. Four or five, short, simple setæ continue the outer rows back on either



Figs. 49, 49a.—*N. tenuis*, ♂.\*

\* Posterior region of trunk. The tail-gland and penial setæ are seen in outline through the body-wall; ( $\times 386$ ).

Fig. 49a.—Compound setæ of ventral row; ( $\times 630$ ).

side of the anus. The number varies, in the specimens examined, from seven compound and five simple, to fifteen compound and seven simple setæ. In the inner rows, the setæ are all compound, and vary in number from 10-17.

In the female (Pl. xlix.), all the setæ, of both outer and inner rows, are compound, and vary in number, from nine in the outer and ten in the inner, to twenty in the outer and twenty-two in the inner rows. All the compound setæ in this species are comparatively short and stout. The proximal segment is hollow;

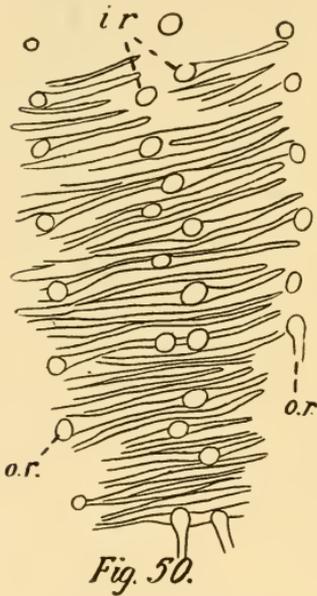


Fig. 50.—*N. tenax*.

L.S. through cuticle, and bases of ventral setæ; ( $\times 830$ ).

while the distal segment is very broad, with two, curious, lateral processes, which are very characteristic (Fig. 49a). In some of the younger specimens, two, distinct, inner rows of setæ are not fully developed. The head-setæ are not inserted on the rostrum, but immediately behind it, on the margin of the striated area (Fig. 48, *d.s.*). They are arranged in a semicircle on the dorsal side of the head; and, in most adult worms examined, are eight in number, forming two rows of four each. But, in one female specimen, there is a third row of two behind the other rows, the two being inserted in a line with the middle two of the rows in front. Several young specimens have only one row of four setæ, with a fine hair

inserted close to the outer margin of each. In one case, a second row appears to be just in process of formation, in front of that which is fully developed. In specimens deeply stained with hæmatoxylin, a small, jointed, distal segment is clearly discernible on each seta, the only case in which I have been able to distinguish this; though, in the other species examined, I have thought it probable that the head-setæ, as well as the ventral

setæ, were compound. The distal segment is much smaller than in the case of the ventral setæ, and can be seen only with the aid of an oil immersion lens. The setæ are hollow, and slightly swollen at the base. They are bent forward, arching over the rostrum, but do not reach quite to the anterior margin.

The trunk-hairs (Fig.49, *t.h.*) are short and slender, not more than 0.012 mm. long, and are not swollen at the base. They are arranged in eight longitudinal rows, the two rows on each surface, dorsal, ventral, and lateral, being situated close together. Each hair is inserted in a pit-like depression in the cuticle, from the base of which, a fine canal runs inward to the inner body-wall (Fig.51, *t.h.*). The rows extend over the rostrum to the serrated margin of the cuticle, where the terminal hairs form a circle surrounding the prominence on which the mouth is situated (Fig.48). The hairs become longer towards the anterior end (0.022 mm.), and are especially long on the rostrum, where there are three strong hairs in each row. Those on the ventral side of the rostrum are nearly as stout as the dorsal setæ, so that, under low magnification, they give the appearance of a circular fringe of stout hairs surrounding the anterior end. The ventral rows of trunk hairs are continuous with the rows of ventral setæ. The lateral grooves (Fig.48, *l.o.*) lie between the two lateral rows of hairs on each side of the rostrum. They are large, and horseshoe-shaped or hook-shaped, with the open end directed posteriorly.

On account of the toughness of the cuticle, satisfactory longitudinal sections are difficult to obtain, but some transverse sections show the internal structure fairly well. The thick cuticle (Fig.51,

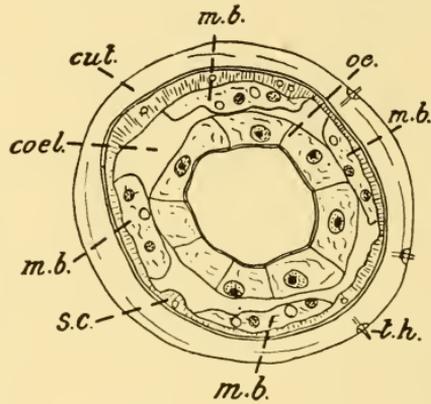


Fig. 51.

Fig.51.—*N. tenax*.

Transverse section through anterior region of trunk; ( $\times 950$ ).

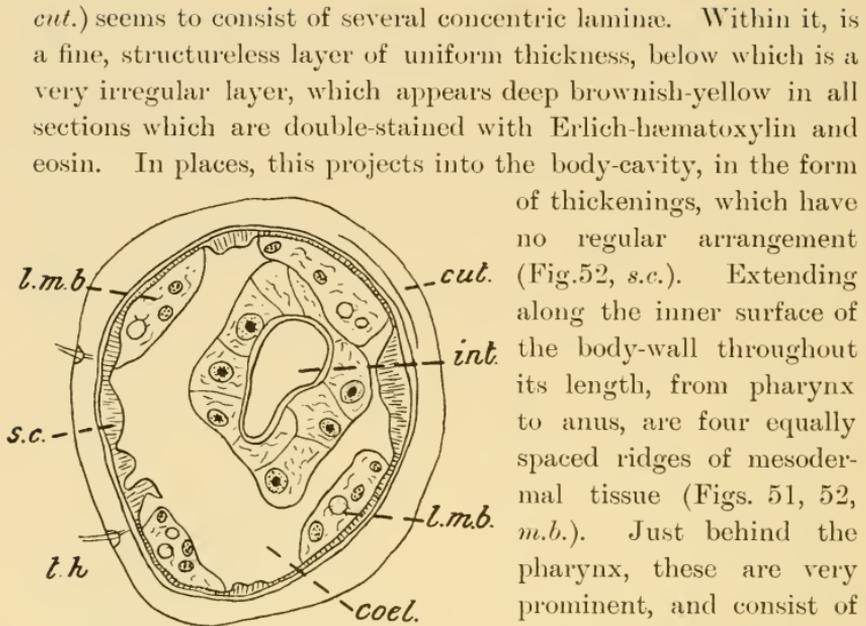


Fig. 52.

Fig. 52.—*N. tenax*.

T.S. through posterior region of trunk;  
( $\times 950$ ).

but, towards the posterior end, they again become very prominent, especially the two lateral ridges, which are here very large, and enclose large spaces which, in places, are almost filled-out with fine granules, staining a deep pink with eosin.

The body-wall encloses a coelomic cavity (Figs. 51, 52, *coel.*), which is extensive in the anterior region of the trunk, but, further back, becomes nearly filled up by the alimentary canal and genital organs.

The alimentary canal extends straight through the body, from mouth to anus. The mouth leads into a muscular pharynx, which is elongated and slender (Fig. 53, *ph.*). The posterior end is slightly enlarged, but there is no definite bulb. Its walls are one cell thick, the cells being few in number, and very large, with a large nucleus in each. Round its anterior end, is a group of elongated, slender, yellowish bodies, which seem to open into the

mouth-cavity. They are probably digestive glands (Fig. 53, *d.gl.*). The pharynx is separated from the remainder of the alimentary canal by a sharp constriction. Behind this, the canal soon widens out into a thin-walled tube (Fig. 51, *a.*) formed of a single layer of large cells, from five to ten in cross-section, bounded internally by a fairly thick wall, apparently composed of cuticle. In side-view, the cells are pentagonal in shape, and very large. In the mid-region of the body, the alimentary canal becomes narrower, and lies dorsal to the genital organs (Fig. 55, *int.*). At the level of the ventral setae, it again widens (Fig. 52, *int.*), and its inner wall is thickened. Posteriorly, the intestine passes into a wide rectum (Fig. 56, *re.*) with thin walls of cuticle, which opens on the ventral surface by a narrow, transverse slit, the anal aperture (*a.*).

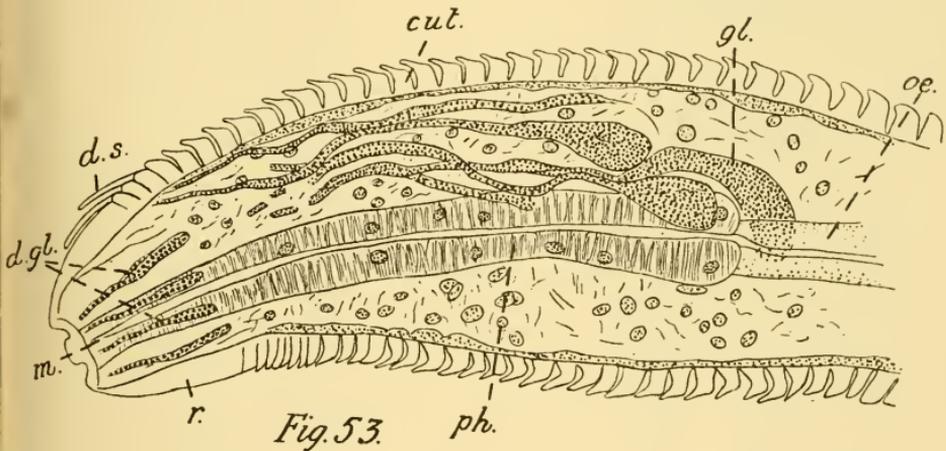


Fig. 53.—*N. tenax*.

Sagittal, longitudinal section through anterior end; ( $\times 830$ ).

A group of finely granular, somewhat pear-shaped bodies lies above the posterior end of the pharynx (Fig. 53, *gls.*). From each of these, one or more strands run forward towards the anterior end. It is not possible, in any of the specimens examined, to trace them to their termination; but it seems evident that they end in the bases of the dorsal setae, and that the bodies are glands supplying the setae with some sort of fluid.

Lying in the mesodermal tissue, just below the ventral setæ, there are, in addition, several rows of block-like, granular masses (Fig. 56, *b.m.*), similar to those described in *Ch. haswelli*. They correspond fairly closely with the posi-

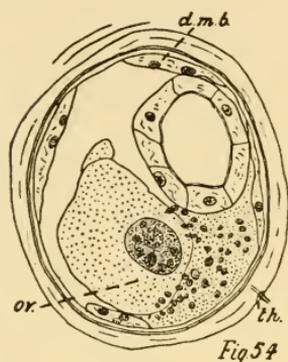


Fig. 54

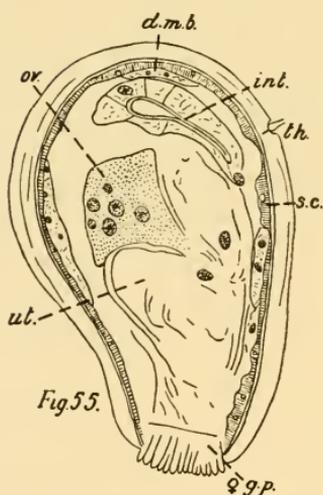


Fig. 55.

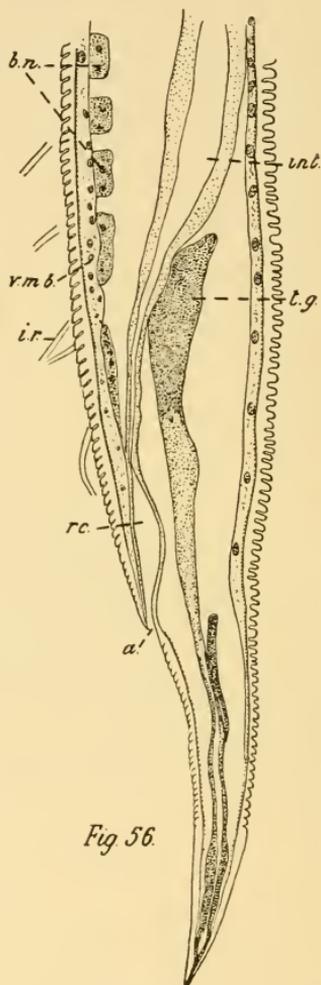


Fig. 56.

Figs. 54, 55.—*N. tenax*, ♀.\*

Fig. 56. *N. tenax*, ♀.†

tion of the setæ, though they are fewer in number, as a rule. They do not appear to communicate directly with the setæ, and

\* T.S. through genital region; ( $\times 630$ ). Fig. 54.—Section through anterior ovary. Fig. 55.—Section through ♀ genital aperture, and uterus.

† Posterior end, as seen in longitudinal section; ( $\times 550$ ).

I have not been able to determine their exact nature, but they evidently bear some relation to the setæ, and are probably glandular. A large and prominent tail-gland (*t.g.*) lies dorsal to the rectum. From this, a narrow duct, with very definite walls, runs backward to open by a prominent pore, situated at the posterior extremity of the tail. In several specimens, clear, oval drops may be seen lying in the duct.

The male genital apparatus (Pl. xlvihi., *t.*) consists of a single, straight cord running through the body-cavity ventral to the alimentary canal. It begins towards the anterior end, and appears to open posteriorly into the rectum. Its anterior end consists of a solid mass of oval sex-cells in two or three closely packed rows. Further back, the sperms become gradually differentiated, and lie free in a wide tube, which passes abruptly, about the middle of the body, into a thick-walled passage, apparently filled with a solid, granular matter. There are two, equal, penial setæ (Fig. 49, *p.s.*) lying dorsal to the rectum. Each is a long, slender, curved rod, with an expanded, flattened, proximal end.

The reproductive organs of the female (Pl. xlix.) consist of two ovaries, anterior and posterior (*a.ov.*, *p.ov.*) which are connected by a wide, median uterus. The latter opens on the ventral surface, in front of the middle of the body, by a wide, transverse slit, with thick walls. The ovaries are straight, fairly elongated, and broadly spindle-shaped. Towards the uterus, each becomes divided into a single row of long, narrow cells, cut off in a transverse direction across it. It narrows as it approaches the uterus, and is here sharply pointed, the contained cells being smaller and more numerous. The uterus forms a receptaculum seminis which, in most specimens examined, contains sperms. None of the specimens examined contained ripe ova, and the development has not been followed out.

Type-specimens, Nos. W, 456, 457; in the Australian Museum, Sydney.

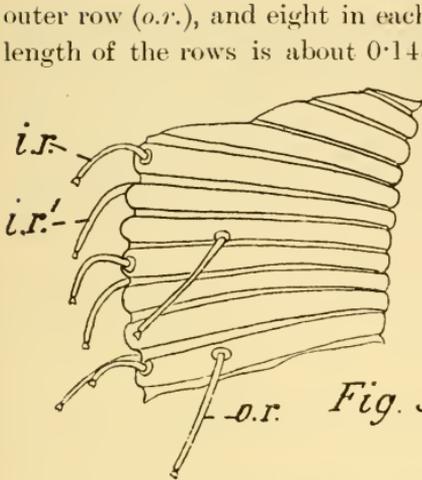
*Notochætosoma tenax*, gen. et sp.nov. Measurements in mms.

	♀	♂
Total length ... ..	1·056	1·056
Length from anterior end to neck-curve ...	0·129	0·140
Length of trunk covered by rows of setæ ...	0·188	0·133
Length from tip of tail to anterior end of rows of setæ... ..	0·328	0·284
Length from tip of tail to anus ... ..	0·092	0·103
Length of tail ... ..	0·044	0·044
Greatest width at anterior end ... ..	0·048	0·048
Width at neck-curve ... ..	0·037	0·037
Greatest width of trunk ... ..	0·074	0·070
Width at level of ventral setæ ... ..	0·040	0·051
Width at beginning of smooth tail-part ...	0·014	0·014
Length of rostrum ... ..	0·025	0·025
Length of setæ on rostrum ... ..	0·022	0·023
Length of hairs on head ... ..	0·022	0·025
Length of hairs on trunk... ..	0·011	0·018
Length of ventral setæ, outer rows ... ..	0·022	0·026
Length of ventral setæ, inner rows .. ..	0·015	0·015
Number of setæ in ventral rows, outer... ..	18	8 compound ) 7 simple )
Number of setæ in ventral rows, inner... ..	21	14 compound
Length of penial setæ ... ..	—	0·050
Length from anterior end to genital pore ...	0·432	—

NOTOCHÆTOSOMA CRYPTOCEPHALUM, gen. et sp.nov. (Plate l.).

In the same material from Vacluse, Port Jackson, in which specimens of *N. tenax* were obtained, I found a single male individual of another species, which very closely resembles it in general form. There is a total absence of a head-demarkation, and, in shape, the worm looks very like a Nematode (Plate l.). It is elongated and cylindrical, 1·056 mm. long, and tapers gradually towards each end. The anterior end is rounded; the posterior end terminates in a sharply pointed tail.

Four rows of ventral, locomotor setæ (Text-fig. 57) are present, beginning 0·048 mm. in front of the anus. The body is broken in the region of the setæ, so that the number of the setæ and the length of the body covered by them, cannot be determined with accuracy; but there are about seven compound setæ in each



outer row (*o.r.*), and eight in each inner row (*i.r.*, *i.r.'*); and the length of the rows is about 0.148 mm. The setae are short, the inner shorter than the outer. They measure respectively 0.015 mm., and 0.026 mm. They are all rather slender, and taper distally. The end-segments are small and expanded, with two, small, lateral processes.

Fig. 57.

Fig. 57.—*N. cryptocephalum*, n.sp.

Trunk in region of ventral setae, showing the character of the striations on cuticle, and the arrangement of the setae; ( $\times 650$ ).

The cuticle covering the body is much thicker than in any other species examined from New South Wales; and the transverse striae are very broad, with fairly wide intervals between them (Fig. 57). They number about 200, and are of the same character and size all over the body.

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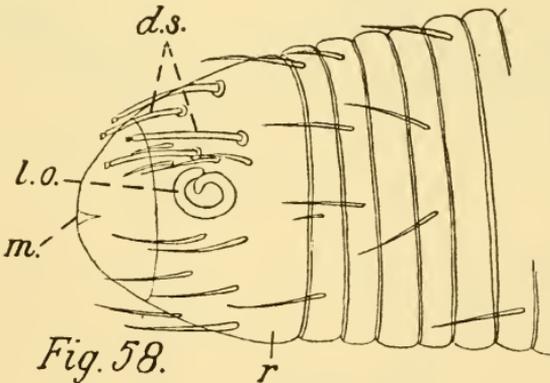


Fig. 58.

Fig. 58.—*N. cryptocephalum*.

Lateral view of anterior end; ( $\times 860$ ).

The smooth rostrum (Text-fig. 58, *r.*) at the anterior end is 0.033 mm. long. It bears two rows, each of four dorsal setae (*d.s.*), about midway between the beginning of the striated area and

the anterior extremity. They are comparatively short, 0.015 mm., and do not extend over the anterior margin of the rostrum. There are no hairs or setæ on the posterior portion of the rostrum, but several rows of hairs lying ventrad of the dorsal setæ, which are almost as stout and as long as the setæ, are on a level with them, and are arranged in an exactly similar way. The hairs on the trunk are fine and short, not more than 0.007 mm. long, and are quite inconspicuous.

The lateral grooves on the rostrum (*l.o.*) are in the shape of a strongly curved spiral, with the opening turned towards the dorsal setæ.

The anus is a transverse slit, 0.140 mm. from the tip of the tail (Text-fig.59, *a*). It is surrounded by an oval, flattened patch of cuticle, which is finely pitted. Just behind it, two stout hairs are inserted, and bend over towards it.

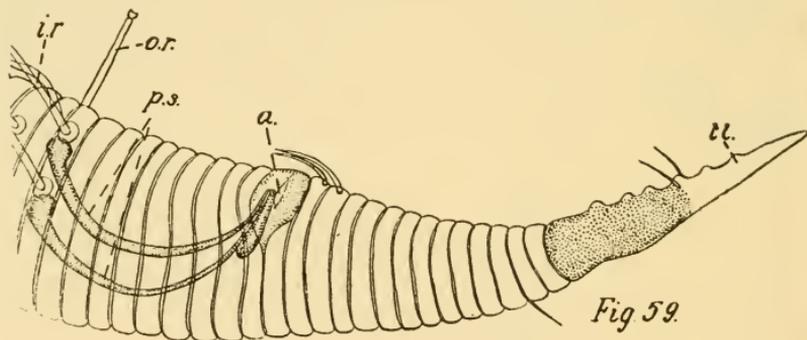


Fig.59.—*N. cryptocephalum*, ♂.

Posterior end; the penial setæ are seen through the body-wall; ( $\times 480$ ).

The shape of the tail is peculiar. It is 0.066 mm. long, slender, and sharply pointed. The cuticle covering it is finely pitted for two-thirds of the distance to the tip, and, on the ventral side, it is raised into six sharp ridges or serrations, which give it the appearance of a saw (Text-fig.59, *tl.*). About midway along its ventral surface, there are two hairs inserted close together.

The cuticle is so thick that it is almost impossible to make out any details of the internal structure.

The alimentary canal (Plate I., *a.*, *int.*) is a tube continuous

from the mouth, at the anterior, to the anus, near the posterior end, but the structure of its walls is not visible. The pharynx (*ph.*) is slender, with a slight enlargement at its posterior end.

The male genital apparatus (*t., v.s.*) is just visible in outline, and appears to be very similar in structure to that of the other species described. Two, equal, penial setae are present (Text-fig. 59, *p.s.*). They are 0.066 mm. long, and are very slender, and strongly curved, with enlarged, proximal ends.

Type-specimen, No. W, 458; in the Australian Museum, Sydney.

*Notochatosoma cryptocephalum*, gen. et sp. nov. Measurements in mms.

	♂
Total length ... ..	1.056
Length of rostrum ... ..	0.033
Width behind rostrum ... ..	0.052
Width of narrowest part behind anterior end ..	0.048
Greatest width of trunk ... ..	0.066
Width in region of ventral setae ... ..	0.048
Width at beginning of tail... ..	0.015
Length from tip of tail to anus ... ..	0.140
Length of tail ... ..	0.066
Length from anus to rows of ventral setae ...	0.048
Length of body covered by ventral setae... ..	0.148
Length of body-hairs ... ..	0.007
Length of dorsal setae ... ..	0.015
Length of hairs on rostrum ... ..	0.011
Length of penial setae ... ..	0.066
Length of ventral setae, outer rows ... ..	0.026
Length of ventral setae, inner rows ... ..	0.015

Key to the Genera of the Family *Chatosomatidae*.

- a.* Head-swelling well developed, and well marked off from the trunk by neck-constriction; pharynx enlarged in head-region to form one or more bulbs ..... *CHATOSOMA*
- aa.* No well developed head-swelling, and no definite neck-constriction; pharynx not enlarged in head to form bulbs.....  
..... *NOTOCHATOSOMA*.

Key to the Species of the Genus *Chatosoma*.

- a.* Not more than two rows of ventral setae.
  - b.* Without head-setae ..... *C. ophioccephalum*.
  - bb.* With head-setae.

- c. Setae of ventral rows compound ..... *C. claparedii*.  
 cc. Setae of ventral rows simple, and very delicate... *C. macrocephalum*.  
 aa. More than two rows of ventral setæ.  
 d. With three rows of ventral setæ.  
 e. Inadequately described species .. ..... *C. groenlandicum*.  
 ee. Recognisable species.  
 f. Rostrum short and straight; head-setæ poorly developed,  
 and not hook-like ... .. *C. tristicochæta*.  
 ff. Rostrum very long and sharply bent in a ventral direction;  
 head-setæ well-developed and hook-like .. ..... *C. longirostrum*.  
 dd. With four rows of ventral setæ.  
 g. With a conspicuous band of transverse striæ behind rostrum.  
 h. With only one row of dorsal head-setæ; rostrum provided  
 with spines. .... *C. spinosum*.  
 hh. With more than one row of dorsal head-setæ; rostrum not  
 provided with spines.  
 i. With two rows of dorsal head-setæ; trunk-hairs arranged  
 in eight longitudinal rows ..... *C. haswelli*.  
 ii. With three rows of dorsal head-setæ; trunk-hairs arranged  
 in six longitudinal rows... .. *C. hibernicum*.  
 gg. Without a conspicuous band of transverse striæ behind rostrum.  
 j. With three rows of dorsal head-setæ; rows of trunk-setæ  
 very long, extending halfway up trunk .. ..... *C. falcatum*.

Key to the Species of the Genus *Notochætosoma*.

- a. Dorsal head-setæ not inserted on rostrum; tail not serrated. . *N. tenax*.  
 aa. Dorsal head-setæ inserted on rostrum; tail serrated along ventral  
 surface ..... *N. cryptocephalum*.

All the figures, for both Plates and Text-figures, were drawn with the help of the camera lucida, the finished drawings being prepared by Mr. F. W. Aitkins, of the Technical High School, Sydney.

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## EXPLANATION OF PLATES XLIV.-L.

*Reference letters.*—*a.*, Anus—*a.o.*, Swollen anterior end of œsophagus in head—*a.or.*, Anterior ovary—*a.ph.*, Anterior pharyngeal bulb—*b.*, Banded area on head—*b.m.*, Block-masses of granular tissue below ventral setæ—*c.s.*, Compound seta of outer row of ventral setæ—*cœl.*, Cœlome—*cut.*, Cuticle—*d.s.*, Dorsal locomotor setæ—*d.m.b.*, Dorsal, longitudinal, mesodermal band—*e.h.*, End-hairs—♀, *g.p.*, Female genital aperture—*gl.*, Glands lying above pharynx—*hd.*, Head—*h.h.*, Head-hairs—*int.*, Intestine—*i.r.*, Inner row of ventral setæ—*i.r'.*, Second inner row of ventral setæ—*l.o.*, Lateral sense-organ—*m.*, Mouth—*nk.*, Neck—*n.o.*, Swollen anterior end of œsophagus in neck—*o.r.*, Outer row of ventral setæ—*œ.*, (Esophagus—*ph.*, Pharynx—*p.or.*, Posterior ovary—*p.r.*, Postanal portion of outer row of ventral setæ—*p.ph.*, Posterior pharyngeal bulb—*p.s.*, Penial setæ—*r.*, Rostrum—*rc.*, Rectum—*s.*, Simple setæ in front of inner rows of ventral setæ—*s.c.*, Subcuticular layer of body-wall—*s.s.*, Simple seta of outer row of ventral setæ—*sp.*, Sperms in uterus—*t.*, Testes—*t.h.*, Trunk-hairs—*t.h'.*, Short trunk-hairs between the long hairs—*tl.*, Tail—*t.g.*, Tail-gland—*ut.*, Uterus—*v.m.b.*, Ventral, longitudinal, mesodermal band—*v.s.*, Vesicula seminalis.

Plate xlv.—*Chaetosoma falcatum*, sp.n., ♂.

Fig. 1;—Side-view of a whole mount, (× 240), with the cuticle drawn in outline only, in order to show the position of the internal organs, the structure of which is only faintly discernible through the thick body-wall.

Fig. 1*a*.—Latero-ventral view of the posterior end of a male specimen, showing the arrangement of the four rows of ventral setæ, and the character of the striated cuticle; ( $\times 520$ ).

Plate xlv.—*Ch. falcatum*, sp.n., ♀.

Fig. 2.—Side-view of a whole mount; ( $\times 240$ ). The structure of the internal organs is not clearly visible through the thick cuticle.

Fig. 2*a*.—Posterior end of a female specimen, seen from the side; ( $\times 520$ ).

Fig. 2*b*.—A compound seta; ( $\times$  about 2000).

Plate xlvi.—*Ch. haswelli*, sp.n., ♂.

Surface-view, from the side, of a whole male specimen; ( $\times 240$ ). The very fine striations on the cuticle are only indicated in places.

Plate xlvii.—*Ch. haswelli*, sp.n., ♀; ( $\times 240$ ).

Fig. 4*a*.—Sagittal, longitudinal section through anterior and middle region of body, showing alimentary and genital systems. The body-wall has been crushed out of its normal position during the process of embedding and section-cutting, so that the cœlome is almost obliterated, and the section is narrower than the true width of the animal. The posterior end is cut out, and hairs and other delicate external structures do not show in a mount in Canada balsam.

Fig. 4*b*.—Whole mount of an immature specimen, with only a single row of dorsal setæ, and three pairs of ventral setæ.

Fig. 4*c*.—Anterior end of specimen shown in Fig. 4*b*.

Plate xlviii.—*Notochaetosoma tenax*, gen. et sp.n., ♂.

Side-view of a whole mount, ( $\times 240$ ), showing the character of the thick, striated cuticle, and other external features. The sharp curvature at the anterior end is more pronounced in this specimen than is usually the case. Plate xlix. shows the more normal curvature.

Plate xlix.—*N. tenax*, n.sp., ♀; ( $\times 240$ ).

Side-view of a whole mount, with the cuticle drawn only in outline, and the internal structure shown.

Plate l.—*N. cryptocephalum*, n.sp., ♂.

A whole mount, ( $\times 240$ ). The posterior end was broken off during mounting, and is shown separated from the rest of the body. The strong, broadly ringed cuticle does not permit of a clear view of the internal structure.

## THE VARIABILITY OF COWS' MILK.

## PART I. THE AFTERNOON-MILK.

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OF THE SOCIETY IN PHYSIOLOGY.

(From the *Physiological Laboratory of the University of Sydney.*)

(With six Text-figures.)

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## INTRODUCTION.

Although cows' milk has been used by man as a food so long, only during the last fifty years have precise methods been developed for the determination of its quality.

The keeping of herds is one of the most ancient of human industries, and the forerunner of the dairy farmer must soon have noticed that the milk obtained from different cows differed in quality. The differences first observed were, no doubt, merely differences of taste; their detection arose directly from the evidence of the senses. But later, when the arts of cheese- and butter-making were acquired, arts which are themselves older than any human record, differences in the amounts of these products obtained from different samples of milk must have made themselves apparent. Rich milk was distinguished from poor; and so

the first knowledge of the quantitative variation of the composition of cows' milk was obtained.

Towards the end of the eighteenth century, man's attention began to turn seriously to the quantitative investigation of the composition of substances; and, during that period, Parmentier and Deyeux (1790) published their quantitative analyses of cows' milk, which are among the first recorded. By that time, a fairly complete knowledge of the qualitative composition had already been acquired. As early as 1615, Bartoletti had shown that milk contains a sugar, in addition to fat and the cheese-forming substance known even then.

The first quantitative analyses of milk were, no doubt, of purely scientific interest, but, as the dairying industry has grown, and the output of its products has increased, the demand for precise methods for determining the value of these products has become more insistent. For milk is the most easily adulterated of foods. Its bulk may be increased by the mere addition of water, and the unscrupulous milk-vendor has been only too ready to avail himself of this simple method of adding to his profits. In all civilised countries, therefore, administrative bodies have been appointed to control the sale of milk and of other foods. On the recommendation of these bodies, standards are fixed by law from time to time. To these standards all milk produced for sale is required to conform. The standards vary in different countries, and in different districts of the same country. They were primarily devised for the detection of the addition of water to milk, and generally consist of statements of the percentage values of certain constituents, fat, solids not fat, and total solids, below which the values in the milk sold may not fall. The addition of substances foreign to the milk to preserve it is also forbidden, and, recently, a certain value of the freezing point has been added to the standards required in some districts.

As a result of these regulations, the chemical examination of milk has become a daily necessity, and, in the last quarter of a

century, an enormous number of analyses has been carried out, both by the authorities responsible for the control of the food-supply, and by the producers who wish to know the value of their product.

The standards set by the authorities were considered by them to be such as would be complied with by any normal, unadulterated milk. These standards, of course, were based on extensive series of investigations, and, for a time, all milk not complying with the standards was regarded as adulterated. Those engaged in the dairying industry, however, soon found that, even under normal conditions, the composition of cows' milk sometimes varied between wider limits than those prescribed by the standards. This fact has been somewhat tardily recognised, in some cases, by the authorities controlling the sale of foods, but a distinction is now made in most countries, between the sale of adulterated or pathological milk, and the sale of milk which may be simply below standard in some of its constituents.

The question whether milk has been adulterated with water, or is naturally deficient in solids, is one which is extremely difficult to settle by chemical means alone. The early investigations of Beckmann (1894), Winter (1895), Carlinfanti (1897), and others, on the freezing point of milk showed that, in the measurement of this property, lay a convenient means of detecting the addition of water. They showed that the freezing point of milk remains extremely constant, no matter how the composition may vary. The work of subsequent investigators, notably that of Brownlie Henderson and Meston (1913, 1914), in Queensland, has confirmed and extended these results. This method, however, does not detect the addition of solutions which do not alter the osmotic pressure of the milk.

The relatively large variations to which the composition of normal cows' milk is subject, and the consequent fluctuations in its butter-value, have induced dairy farmers to carry out numerous investigations with the object of determining the cause of the variations. The possession of this knowledge would be of use in

suggesting means by which the variation might be controlled and made to proceed in a desired direction, or, at least, predicted. The researches which were undertaken to acquire this information were at first confined almost entirely to determinations of the amounts of butter-fat yielded by cows.

The two outstanding factors which have been recognised to play the chief parts in determining the amount of fat which a cow yields in its milk are, firstly, the breed and individual peculiarities of the animal; and, secondly, the method of milking. The factors were recognised vaguely before any quantitative work was done on the subject, and the investigations so far carried out have done little more than confirmed and made precise the prevailing impression. Unfortunately, the investigators have concerned themselves chiefly with the output of herds, and not of single cows. For the herd is the commercial unit: it is the mixed milk from his herd that a dairyman usually sells.

Certain breeds of cow, such as the Jersey, habitually yield a milk which is rich in fat, while other breeds, such as the Holstein, give a more copious flow of a poorer milk. It has been pointed out by von Wendt (1903), that cows yielding a rich milk are, in general, small. Their calves will, therefore, lose heat more rapidly in proportion to their weight than the calves of larger animals, and will require a relatively greater supply of heat-producing material in their food. Conversely, the larger breeds of cows produce a greater quantity of milk poorer in fat, since their larger offspring require more nutritive material, but do not lose heat so rapidly, in proportion to their weight, as those of the smaller animals.

Although the breed of a cow is, for practical purposes, the most important factor determining the quality of its milk, since herds are usually dealt with, it must be remembered that the individual peculiarities of an animal are of even more fundamental importance. The formation of a breed is, after all, only a means adopted to perpetuate the qualities of certain individuals, which are superior in some way to their fellows, either in their suitability to environment, as in the natural breeds, or in their economic value, as in the breeds produced by human selection.

The breed and individuality of a cow are thus the principal factors determining its total output of milk from day to day. But the amount and quality of a sample of milk, obtained from a cow at any particular time, are subject to great variations, and these variations are dependent on the manner in which the cow is milked. It is obvious that, if a cow yields a certain amount of milk *per diem*, the more frequently it is milked, the smaller will be the quantity obtained at each milking; for it has been shown, that the number of milkings *per diem* only slightly affects the total yield (Lalim and Grande, 1913). The amount of milk which is obtained at a milking is proportional to the period which has elapsed since the last milking.

The quality of milk is also profoundly affected by the stage of milking at which the sample is collected. This fact was known to Pélégot (1836), who showed that the milk first drawn from the udder is considerably poorer in fat than the last portions of a milking. Numerous subsequent investigations have confirmed this observation, and the work on the subject was reviewed and extended by Ackermann in 1913, and again by Isaachsen, Lalim, and Grande in 1913. The first portions of a milking may contain less than 1% of fat, while the last portions contain more than 10%.

The quality of the milk obtained is affected in the opposite direction to the quantity by the periods which elapse between successive milkings. The milk obtained after a short period of rest contains a higher percentage of fat than the milk obtained after a longer period (Melander, 1892; Petersen, 1894). For this reason, the milk obtained in the morning, when cows are milked twice a day, contains a lower percentage of fat than that in the milk of the afternoon. The period elapsing between the milking in the morning and that in the afternoon is generally greater than that elapsing between the milking in the afternoon and that on the following morning.

The food which a cow eats naturally exerts a great influence on the quantity and the quality of the milk which the animal pro-

duces. But this influence seems to be nothing more than the general effect of food on the health and activity of a living organism; a well-nourished cow yields more and better milk than a poorly nourished animal. An enormous amount of work has been done to determine the relation between the food of a cow and its production of milk. The co-operative experiments which have been in progress in Denmark, since 1887, may be referred to as an instance. In these experiments, more than 4,000 cows have been examined, but the results, which have been reviewed by Annett (1915), show that the food has practically no influence on the composition or quantity of fat in the milk produced, as long as the food is sufficient to maintain the cow in vigorous health. These statements refer only to feeding on a commercial scale, and not to the administration of special substances, nor to the eating of plants which may impart distinctive odours or tastes to the milk.

Much work has also been done to determine the effect of the addition of various inorganic salts to the diet of a cow, but the investigations of von Wendt (*loc. cit.*), and of Schulte-Bäuminghaus (1903), have shown that, in this case, too, no definite effect can be produced on the quality or quantity of the milk secreted. The only effect noticed was a slight increase of calcium in the milk after the administration of calcium salts. The amount of phosphate could not be correspondingly increased.

It appears from the work of Graham (1904), however, that the amount of water, which a cow receives with its food, has a definite effect on the yield of milk. A copious water-ration appears to lead to the secretion of a larger volume of more dilute milk. "Milk may be watered through the mouth of the cow."

The effect of the injection of so-called galactogogues into the circulations of lactating animals may be mentioned here. The most effective and best known of these substances is pituitary extract. It was thought by some investigators that the injection of these substances acted as a stimulus to the secreting cells of the mammary glands, and led to an increased production of milk (Hammond, 1913; Maxwell and Rothera, 1915). The work of

Gaines (1915) demonstrated, however, that pituitary extract exerts its effect by stimulating the smooth musculature of the mammary gland, and leads to an increased extrusion, but not to an increased secretion of milk (see also Mackenzie, 1911), since the volume of milk produced under its influence is no greater than the decrease of the volume of the udder.

The effect of the seasons of the year, on the mean composition of cows' milk in Great Britain, is well shown by the monthly averages which are published, from year to year, by Droop Richmond (1887 onwards). The milk secreted during the winter-months has slightly higher percentages of fat, and of solids not fat, than the milk secreted during the summer-months. The work of Lythgoe (1914) has shown that a similar effect is exerted in North America, and that the behaviour of the percentage of solids not fat is due chiefly to variation of the percentage of protein.

Seasons, in the sense of good and bad seasons, of course, have a very marked effect on the milk-production of a cow, but this effect is an indirect one, and is due to variations in the quality and quantity of the food-supply of the animals.

The effect of the course of lactation on the composition of cows' milk has been studied in some detail. The greatest changes occur, as is well known, during the period immediately after calving. Colostrum, the milk secreted during the first day or two of lactation, contains a much larger percentage of protein than ripe milk. The extra protein, which is present in colostrum, has been shown by Crowther and Raistrick (1916) to be a globulin, identical with the globulin of the blood-plasma of the cow. In ripe milk, the amount of this globulin is very small (0.03%).

After the first week of lactation, the composition of the milk does not vary rapidly in any one direction, although it is subject to daily fluctuations. No precise generalisation can be formulated for the variation of composition during lactation. Recent work shows, however, that, on the whole, the average percentages of fat, protein and ash fall during the first three to six weeks of the post-colostral period, vary about constant values during the greater

part of the period of lactation, and increase again towards the end of the period. The yield of milk, and the percentage of sugar, on the other hand, decrease steadily during the whole period of lactation. (Crowther and Ruston, 1911; Eckels and Shaw, 1913).

The factors which determine the composition of the milk secreted by a cow thus fall into two groups: those which are due to conditions independent of the animal itself, and those which are fixed by the animal. The chief extrinsic factors are: the food, the seasons, and the methods of milking. As long as the variations of these factors are not such as to disturb the health of the cow, they have very little effect on the average output or quality of the milk. The main intrinsic factors are: the idiosyncrasy of the cow, its inherited characters or breed, and the stage of its lactation. These are the factors which determine the kind and amount of milk that a cow secretes, and, of these factors, the first two are predominant, except at the beginning and end of the period of lactation.

A cow, then, is not to be regarded as a milk-producing machine, whose output is determined by the nature of the raw material supplied, and by the conditions of working. It is a physiological mechanism whose product, though not uniform, is independent of considerable variations of the external conditions. The variations of the product are determined by the mechanism itself. This great fact is now fully recognised by the dairy-farmer, who realises that the output of his herds is to be improved by paying attention, not so much to the feed, as to the breed of the cows.

Since the composition and amount of the milk produced by a cow cannot be varied at all, by adjusting the external conditions and diet, it is important to know over what range of values the spontaneous variations of composition and properties may be expected. Such information is only to be obtained by examining specimens of milk from a large number of single cows. The paucity of complete analyses of the milk of individual cows has already been referred to. The bulk of the analyses of this kind have estimated only the volume of milk yielded by the cow and the percentage of fat; the investigators have been con-

cerned solely with the butter-producing quality of the animal. The series of more complete analyses are, in general, too short to allow any useful conclusions to be drawn from them. Among the few extensive series of analyses may be mentioned those of Collins (1904), whose object was to calculate the probabilities of deviations of different magnitude from the legal standard; and of Fresenius (1909). This work, too, however, was confined chiefly to an examination of the fat-content. Lythgoe (*loc. cit.*) carried out a very extensive investigation of a similar character, in which other constituents besides fat were determined.

The statements of the different investigators, as to the relative variabilities of the constituent of milk, are not very concordant. Nearly all agree in one conclusion, however, and that is that the most variable constituent of milk is the fat. The majority of investigators also find milk-sugar to be the least variable of the constituents, but, on this point, the agreement is not so complete.

#### *Scope of Present Work.*

The object of the work recorded in this paper is to present a series of complete analyses of samples of the evening-milk of individual cows. The cows examined were not chosen for the possession of any special qualities. They were, however, members of herds which were maintained under the most favourable conditions, and were not kept for profit. All the cows examined were supplying milk which was being used for human consumption, and all were milked under the same conditions, as regards time of milking and period since last milking. The cows had not been subjected to any change of diet for several weeks, at least, before the collection of the samples. The collection of samples extended over three seasons of the year. Over one hundred samples of milk were examined, and determinations were made of: (1) the yield of milk; (2) density; (3) freezing-point; (4) electrical conductivity; (5) total solid matter; (6) fat; (7) sugar; and (8) ash. From these results, the percentages of protein and extractives, and of solids not fat, and the total quantities of fat yielded were calculated.

The results obtained have been submitted to a simple statistical treatment, by which the most probable values of the quantities measured, and their relative variabilities, have been determined. It has been found that the quantities measured fall into three sharply defined groups, as regards their variability: the values of certain physical properties are least variable, the concentrations of the substances present are more variable, and the amounts of these substances obtained at a milking are most variable. The concentrations of the substances in solution are considerably less variable than those of the substances in suspension.

#### METHODS.

The cows examined—The cows, from which the samples of milk described were obtained, were all kept in the dairies attached to certain Government Mental Hospitals in the vicinity of Sydney. The whole output of the cows was consumed in the Institutions to which they were attached. The following data with regard to the cows were obtained from the dairy stock-books.

(1) The breed of the cow. In nearly all instances, the cows were crossbred, and the breed given is that of the predominating strain.

(2) The number of calves, to which the cow had given birth at the time of the collection of the samples of milk. In the cases of cows Nos. 23-46, records of the numbers of calves could not be obtained. Records of the ages of the cows were available, however; and to make the data for these cows uniform with those for the others, the numbers of calves have been calculated from the ages, on the assumption that the first calf was born when the cow was two years old, and that, after that age, one calf was born every year. The numbers of calves calculated in this way are only approximate.

(3) The period which had elapsed between the birth of the last calf, and the collection of the sample of milk. This period is expressed in Table ii. below, in days.

The following data were also obtained at the time of the collection of the samples:—

(4) The diet of the cows. The daily rations of the various cows were as follows:—

Cows, Nos. 1-22—Pollard, 2.5lbs.; bran, 7.0lbs.; green barley-chaff, *ad lib.*

Cows, Nos. 23-34—Bran, 8lbs.; green chaffed oats, 60lbs.

Cows, Nos. 35-46—Bran, 8lbs.; lucerne-hay, 30lbs.

Cows, Nos. 47-109—Bran, 8lbs.; cow-meal, 2lbs.; lucerne-chaff, 16lbs.

In no instance had the diet of the cows been changed immediately before the collection of the samples of milk. The shortest period, which elapsed between a change of diet and the collection of samples, was three weeks. It occurred in the case of cows, Nos. 35-46.

The period during which samples of milk were collected extended from October 31st, 1916, to April 30th, 1917. This period was one of six months, and included the latter half of Spring, the whole of Summer, and the first half of Autumn.

The cows examined were all milked twice each day. The morning-milking occurred between six and seven o'clock, the afternoon-milking between three and four o'clock. The period between the milking on one morning, and that on the following afternoon, was thus about half that between the afternoon-milking and that of the following morning. It is to be expected, from what has been stated already with regard to the proportionality between the time of rest and the yield of milk, that the amount obtained at the afternoon-milkings, from which the samples were obtained, would be about half that obtained at the corresponding morning-milkings. To test this supposition, the weights of milk obtained in the morning were measured, as well as the weights obtained in the afternoon, in the cases of a number of cows. The following Table gives the results obtained.

TABLE i.  
Weights of milk obtained at morning- and afternoon-milkings.

Cow.	Weight of milk.		Cow.	Weight of milk.	
	Afternoon.	Morning.		Afternoon.	Morning.
1	4.9	8.4	12	2.0	4.8
2	4.1	8.0	13	2.5	3.7
3	3.7	6.6	14	3.5	6.4
4	6.7	11.2	15	1.7	4.6
5	5.3	8.9	16	2.8	2.5
6	6.8	13.7	17	1.2	3.0
7	1.3	2.1	18	2.6	5.9
8	5.8	11.0	19	2.4	3.7
9	4.9	9.4	20	1.2	3.2
10	5.0	8.7	21	3.0	5.5
11	8.1	14.6	22	2.3	4.3

Mean: morning, 3.76; afternoon, 7.03; ratio, 1.87.

The average ratio of the weight of the morning-milk to the weight of the afternoon-milk is thus very nearly 2, and is practically the same as the ratio between the times between the consecutive milkings.

The cows, from which the samples of milk were obtained, were milked by hand in the ordinary way, in every case. No attempt was made to strip the udders.

The whole of the milk yielded by each cow at the milking was thoroughly mixed together by pouring from one vessel into another, six times. This is the method recommended by the Committee appointed by the London Board of Agriculture, to enquire into the methods of sampling milk (1911). In the present case, an additional precaution was taken to secure a thoroughly representative sample. The sample was not taken from one portion of the mixed milking, but, as the milk was being finally poured into the container, a portion of the stream was diverted into the sampling vessel. In this way, a sample was obtained from all portions of the mixed milk.

After what has been written above, with regard to the extreme variation of the fat-content, between the first and last portions of a milking, it is unnecessary to emphasise the importance of

thoroughly mixing the milk before taking a sample, if the sample is required to give information about the total amount of milk yielded by the cow at the particular milking under examination.

The quantity of milk obtained from each cow was determined by weighing with a spring balance. These weights are correct to about 0.1 kg.

The examination of the milk was commenced on the afternoon on which it was collected. The milk was kept in an ice-chest, at a temperature of about 5°C., during the following night. Whenever possible, all the operations, which it was necessary to carry out upon the fresh milk, were completed before the following afternoon, *i.e.*, before the milk was 24 hours old. During this period the milk was kept for about 14 hours in the ice-chest, so that it was exposed for less than 10 hours to ordinary temperatures. The temperature of the milk never rose above 20°C., and Chapman (1908) has shown that, even at temperatures as high as over 30°C., souring does not commence until about 11 hours have elapsed since milking. Whenever it became impossible, for any reason, to complete the examination of the milk in the unaltered state within the specified time, 5 drops of formalin were added to each 100 c.c. as a preservative. The addition of this small amount does not measurably alter the properties of the milk under investigation (Reiss and Sommerfeld, 1909).

**Density.**—The density of the samples of milk was determined by weighing in a pycnometer of 25 cc. capacity, provided with a thermometer. To save time, no attempt was made to fill the pycnometer at a constant temperature. The weights when filled at different temperatures were determined, and a graph was drawn, from which the corresponding weight at 25°C. could be read off. Observations were made at temperatures ranging from 10° to 30°C. Above 17°C., the points plotted lay upon, or very close to, a smooth curve, the maximum deviation being less than 2 mg. Below 17°C., the points obtained were rather scattered. This behaviour is due to the fact, which was noted as early as 1841 by Quevenne, that, at a temperature in the

vicinity of 15°C., a sudden increase of density, which has been termed the thickening of milk, takes place. Fleischmann and Wiegner (1913) have shown that this change is due to the solidification of the milk-fat, which causes a discontinuous change of density. In the present case, all the determinations of density were made at temperatures within 17°C. and 22°C. Within this range of temperature, the coefficient of expansion is practically independent of the fat-content (*cf.* Müller's Tables, 1892). The pycnometer was weighed to the nearest 25 mg., a weight which corresponds to a difference of density of 1 in 10,000. This difference is, therefore, the limit of accuracy of the determination. The pycnometer was standardised with water at 25°C., and the densities are those of milk compared with water at this temperature.

**Depression of freezing-point.**—The freezing-points of the samples of milk were determined by the method described in a previous paper (Wardlaw, 1914). The greatest variation observed between duplicate estimations was 0.002°C. The freezing point of water could be determined within the same limits. The depression of freezing point ( $\Delta$ ) is the difference between these two values; its possible range of variation is, therefore, 0.004°. As this variation is on a depression of the freezing point of water of about 0.55°C., the results obtained are comparable to 1 in 125. Milk contains substances in suspension. Its value of  $\Delta$  is, therefore, slightly lower than that corresponding to the actual amount of substances in true solution (Tezner and Roska, 1908). In the present work, only comparable values of the depression of the freezing point are required, and no attempt was made to obtain absolute values, to obtain which, special apparatus and precautions are required (Raoult, 1898). The difference from the absolute values of the present results is very unlikely to be more than 0.01°C.

**Electrical conductivity.**—The electrical conductivities of the samples of milk were determined by the usual method of Kohlrausch and Nippoldt (1869). The secondary circuit of an induction coil was used as the source of current, a

telephone was used as the null instrument, and the readings of resistance was made on a Wolff's pattern Wheatstone-bridge. The conductivity-vessel was made with vertical electrodes to hinder any settling of particles upon them. The cell-constant of the vessel was determined by measurement of the resistance of N/50 KCl solution. The determinations were all made in a thermostat at 25°C. Readings of the bridge could be made within a range of 1 in 200, and as the determination of the cell-constant was subject to about the same variation, the values obtained for the conductivities are subject to an error of 1 in 100.

**Total solids.**—The percentage of total solid matter in the samples of milk was determined by evaporating 1 cc. to dryness in a watch-glass at 102°C., in a glycerine-oven. The heating was continued for about 18 hours. The solids were cooled over  $\text{CaCl}_2$  in a desiccator before weighing. The amount of milk used was measured from a pipette which had been calibrated by weighing, the calculations being made on the weight of milk delivered. It has been shown by Poetschke (1911), that the weights of successive portions of a sample of milk, delivered from the same pipette, agree together very closely. This result was also obtained in the present case, when the extreme difference between the weights of five portions of a sample of milk, delivered from the pipette used for the analyses, was 0.0004 gm., or 0.04%. The extreme variation observed in two series of 12 test-determinations each, of the weight of total solids obtained from 1 cc. of milk, as described above, was 0.0016 gm. The results obtained in the remaining determinations are assumed to be comparable within this limit of variation (0.7%). The solids of milk are very hygroscopic, when nearly dry, and it has been shown by Leavitt (1910) and by other workers that, although closely agreeing values may be obtained for the percentage of moisture in substances like this when the estimations are carried out under uniform conditions, it is a matter of some difficulty to determine the absolute amount of water present. Further, dehydration of lactose proceeds slowly at temperatures of about 100° (Soldner, 1896).

**Fat.**—The percentage of fat in the samples of milk was determined by the method of Röse (1887), as modified by Gottlieb (1890). Milk, mixed with ammonia and an equal volume of alcohol, is shaken up with ether; petroleum spirit is then added, and the mixture shaken up again. After standing, the mixture separates into aqueous and ethereal layers. The levels of the two liquids are read off, an aliquot part of the ethereal layer is evaporated down, and the fat left is weighed. The relative accuracy of the method depends chiefly on the precision with which the levels can be read. In the present case, the volume of the ethereal layer was about 50 cc., and this volume could be determined to 0.5 cc., or about 1%. The difference between duplicate estimations might thus amount to double this value, if the errors in the two estimations were to fall in opposite directions. The actual range of variation observed in six control estimations was 2.3%, a value sufficiently close to the calculated maximum variation. The deviation from the mean is half this value, and the results are given in the Table to 1 in 100. Ten cc. of milk were used for each estimation. The milk was delivered from a pipette delivering a known weight, as described previously.

**Lactose.**—The percentage of milk-sugar was determined by the method of Salkowski (1912). In this method, milk is almost saturated with ammonium sulphate, and mixed with an equal volume of a saturated solution of ammonium sulphate. The liquid is filtered, and the specific rotation of the clear filtrate is determined,  $[\alpha]_D$  being taken as  $52.53^\circ$ . Jahnsen-Blohm (1913), and Rösemann (1914) have shown that the presence of ammonium sulphate, in the concentration used by Salkowski, lowers the optical rotation of a solution of pure lactose by about 2%. Scheibe (1901) showed, however, that, in the case of milk, optical methods in general gave higher results than gravimetric methods. This is due to the fact that, in precipitating the proteins, the volume, through which the lactose is distributed, is reduced, and its concentration is raised. Kretschmer (1913) has shown that the results obtained by Salkowski's method are about 1% higher than those obtained gravimetrically.

In the present work, the rotations could be read to  $0.02^\circ$ , or a variation of about 1%. The volume of the liquid, in the cylinders in which the precipitations were carried out, could be read to 0.5 cc. in 100. The total range of variation is thus 1.5%, which would give a possible difference between duplicate estimations of 3.0%, if all the errors in the two cases fell in opposite directions. The difference actually found between duplicate estimations was 2%. The results given are regarded as relatively accurate to 2 in 100, and are given to the nearest 0.05%. As this variation is greater than the correction proposed by Kretschmer, this correction has not been applied in the present case.

Ash.—The percentages of ash in the samples were determined on the weight of milk delivered from a 5 cc. pipette, calibrated as described. The samples were first dried at  $103^\circ\text{C}$ ., and then ashed in a muffle-furnace at a low red heat. In order to determine what loss of chlorides occurred in the ashing carried out in this way, control-determinations were made by charring the milk at a temperature below red heat, leaching out the chloride, ashing the remainder, adding the solution of chlorides, and evaporating to dryness. The results by the two methods differed by only 0.4% of the weights of ash obtained. The values in the Table are given to 0.01%, or to 1 in 70.

The substances left, after the estimation of fat, sugar and ash, consist of protein, citric acid (0.2%, Scheibe, 1891), and substances containing about 5% of the total nitrogen of the milk. Of these residual substances, the protein forms more than 90% (Munk, 1893; Camerer and Söldner, 1893). Numerous attempts were made to estimate the protein directly by precipitation with alcohol, according to the method previously described for human milk (Sikes, 1906; Wardlaw, 1914). With cows' milk, however, the precipitate obtained in this way contains a very high percentage of ash (over 10%), and in the time available for this portion of the work, it was found to be impossible to dry the precipitate to constant weight. The figures given for the percentage of protein and the remaining constituents of the milk were obtained by sub-

traction of the percentage of fat, milk-sugar, and ash, from the percentage of total solid matter. The values given for protein and the remaining substances are, therefore, subject to the variations of all the values for the percentages of fat, milk-sugar, ash, and solids; and if all these errors happened to fall in the same direction, the corresponding error of the percentage of protein, etc., might amount to as much as 5%. In practice, however, the errors will be more likely to balance one another. The figures for the percentages have been given to the nearest 0.05.

#### RESULTS.

The numerical results of the chemical and physical examination of the samples of milk discussed in this paper are given in Table ii. The Table also contains the data for the breed, age, and stage of lactation of the cows from which the samples were obtained. The entries in the several columns are as follows:—

- (1) Serial number of sample.
- (2) Date of collection.
- (3) Chief strain of breed.
- (4) Number of calf.
- (5) Age of calf in days.
- (6) Weight of sample in kg.
- (7) Density at 25°C.
- (8) Depression of freezing point ( $\Delta$ ).
- (9) Electrical conductivity in reciprocal ohms  $\times 10^{-3}$ , at 25°C.
- (10) Percentages of total solid matter.
- (11) Percentage of water.
- (12) Percentage of fat.
- (13) Percentage of solids not fat (S.n.F.).
- (14) Percentage of lactose.
- (15) Percentage of ash.
- (16) Percentage of protein and extractives.

TABLE II.  
Composition and properties of milk from individual cows at single milkings.

No.	Date.	Breed.	Calv.	Age.	Wt.	Dens.	$\Delta$	Cond.	Solids.	Water.	Fat.	S. n. F.	Lactose.	Ash.	Protein, etc.
1	31/10/16 (Spring)	Ayrshire	4	186	4.9	1.0292	0.558	4.55	13.45	86.55	4.65	8.8	4.85	0.59	3.35
2		Jersey cross	9	187	4.11	1.0306	0.552	4.65	12.1	87.9	3.3	8.8	5.3	0.57	3.25
3		Ayrshire	—	—	3.7	1.0302	0.558	4.3	13.05	86.95	4.2	8.85	5.45	0.75	2.65
4		"	8	77	6.7	1.0291	0.553	4.45	13.45	86.55	4.75	8.7	5.25	0.58	2.8
5		"	2	58	5.3	1.0313	0.553	4.25	13.95	86.05	4.55	9.4	5.15	0.64	3.6
6		Shorthorn	6	36	6.8	1.0296	0.554	4.2	13.25	86.75	4.65	8.6	5.55	0.59	2.45
7		Ayrshire	3	320	1.3	1.0314	0.552	5.2	13.15	86.85	4.05	9.2	4.55	0.70	3.95
8		"	3	17	5.8	1.0294	0.547	4.4	12.1	87.9	3.9	8.2	5.25	0.52	2.45
9		"	4	26	4.9	1.0301	0.548	4.65	12.1	87.9	3.65	8.45	5.15	0.60	2.8
10		"	3	90	5.0	1.0298	0.548	5.1	12.25	87.75	3.75	8.5	4.8	0.63	3.05
11		Holst.-J. Sh.	5	36	8.0	1.0299	0.554	3.95	13.9	86.1	5.2	8.7	5.6	0.52	2.6
12	6/11/16 (Spring)	Ayrshire	—	162	2.0	1.0272	0.559	5.1	13.25	86.75	5.2	8.05	4.85	0.53	2.65
13		Jersey cross	3	290	2.5	1.0294	0.555	4.75	13.4	86.6	4.2	9.2	4.55	0.51	4.1
14		Ayrshire	3	192	3.5	1.0268	0.565	5.05	13.45	86.55	5.35	8.1	4.5	0.47	3.15
15		Ayrs. cross	4	179	1.7	1.0296	0.560	4.4	15.05	84.95	6.3	8.75	5.0	0.58	3.15
16		Jersey	2	115	2.8	1.0306	0.568	4.55	15.0	85.0	5.0	10.0	5.05	0.56	4.4
17		Ayrshire	5	157	1.2	1.0232	0.572	7.5	10.5	89.5	3.8	6.7	2.7	0.52	3.5
18		"	—	—	2.6	1.0304	0.559	4.45	14.85	85.15	5.45	9.4	5.05	0.75	3.6
19		"	—	—	2.4	1.0291	0.568	4.75	13.0	87.0	4.5	8.5	4.75	0.69	3.05
20		"	2	287	1.2	1.0280	0.565	5.5	12.9	87.1	4.8	8.1	4.55	0.73	2.8
21		"	2	209	3.0	1.0304	0.575	4.35	14.0	86.0	5.15	8.85	4.45	0.68	3.7
22		"	3	229	2.3	1.0272	0.570	5.65	12.45	87.55	4.25	8.2	4.4	0.64	3.15

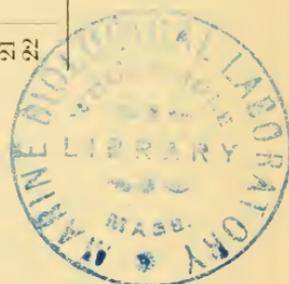


TABLE ii.—(Continued).

No.	Date.	Breed.	Calif.	Age.	Wt.	Dens.	$\Delta$	Cond.	Solids.	Water.	Fat.	S.n.F.	Lactose.	Ash.	Protein, etc.
23	13/11/16	Shorthorn.	3	164	3.2	1.0308	0.557	4.0	13.9	86.1	4.8	9.1	5.2	0.65	3.15
24	(Spring)	"	8	157	5.0	1.0291	0.558	4.4	12.7	87.3	6.2	6.5	5.05	0.61	0.85
25		"	8	262	2.5	1.0299	0.564	3.95	14.55	85.45	5.65	8.9	4.9	0.64	3.35
26		"	6	136	3.9	1.0304	0.560	4.05	13.55	86.45	4.5	9.05	5.2	0.58	3.25
27		"	6	519	2.9	1.0314	0.565	4.3	13.35	86.65	4.4	8.95	5.05	0.45	3.45
28		"	7	88	3.2	1.0295	0.563	4.6	13.0	87.0	4.45	8.55	5.0	0.63	2.9
29		"	8	113	3.0	1.0297	0.562	4.7	13.6	86.4	4.85	8.75	4.9	0.65	3.2
30		"	5	261	1.4	1.0302	0.562	4.65	13.2	86.8	4.6	8.6	4.8	0.74	3.05
31		"	6	69	2.9	1.0269	0.573	4.9	12.5	87.5	4.4	8.1	4.7	0.64	2.75
32		"	6	151	1.8	1.0300	0.580	4.55	13.9	86.1	4.9	9.0	4.85	0.71	3.45
33		"	6	36	2.5	1.0304	0.568	4.4	13.05	86.95	4.3	8.75	5.0	0.71	3.05
34		"	5	24	4.1	1.0298	0.558	4.6	13.0	87.0	4.4	8.6	4.75	0.74	3.1
35	2/1/17	"	8	72	7.1	1.0294	0.564	4.6	13.8	86.2	5.05	8.75	5.4	0.61	2.75
36	(Summer)	"	8	186	3.4	1.0296	0.577	4.6	14.3	85.7	5.0	9.3	4.9	0.59	3.8
37		"	2	142	5.9	1.0300	0.566	4.9	12.25	87.75	3.75	8.5	5.05	0.56	2.9
38		"	3	195	2.3	1.0318	0.563	3.95	15.15	84.85	5.45	9.7	5.15	0.66	3.9
39		"	8	200	5.4	1.0316	0.559	4.9	11.25	88.75	2.6	8.65	5.1	0.63	2.9
40		"	9	198	3.4	1.0288	0.565	5.2	12.8	87.2	4.0	8.8	4.75	0.60	3.45
41		"	5	151	3.2	1.0306	0.567	5.0	13.0	87.0	3.5	9.5	4.55	0.76	4.2
42		"	5	132	3.6	1.0313	0.570	4.75	12.35	87.65	4.1	8.25	5.15	0.45	2.65
43		"	8	32	4.3	1.0274	0.560	4.35	14.85	85.15	5.85	9.0	5.15	0.52	3.35
44		"	6	68	1.4	1.0294	0.563	4.7	12.15	87.85	3.85	8.3	4.05	0.76	3.5
45		"	8	244	2.7	1.0281	0.563	5.05	14.05	85.95	5.15	8.9	4.55	0.72	3.65
46		"	9	109	4.1	1.0309	0.551	4.5	13.4	86.6	4.4	9.0	5.0	0.43	3.55

TABLE ii.—(Continued).

No.	Date.	Breed.	Calf.	Age.	Wt.	Dens.	$\Delta$	Cond.	Solids.	Water.	Fat.	S.n.F.	Lactose.	Ash.	Protein,
47	15/1/17	Shth. cross	1	174	3.4	1.0332	0.554	4.05	13.6	86.4	4.2	9.4	5.2	0.74	3.45
48	(Summer)	Shorthorn	3	132	3.0	1.0319	0.565	4.85	13.3	86.7	4.35	8.95	5.0	0.74	3.2
49		Crossbred	1	46	3.9	1.0307	0.563	4.0	13.6	86.4	5.25	8.35	5.1	0.66	2.6
50		"	1	45	3.9	1.0303	0.570	4.45	13.35	86.65	4.7	8.65	5.2	0.70	2.75
51		Jersey	3	104	3.9	1.0312	0.580	4.65	11.6	88.4	3.85	7.75	5.05	0.69	2.0
52		Crossbred	5	57	5.7	1.0290	0.560	5.3	11.95	88.05	3.8	8.15	4.65	0.71	2.8
53		Ayrshire	1	77	4.3	1.0301	0.594	4.7	13.05	86.95	5.35	7.7	5.05	0.72	1.95
54		Crossbred	2	63	3.4	1.0282	0.575	4.5	15.05	84.95	6.4	8.65	5.0	0.72	2.95
55		Shth. Jersey	3	174	3.0	1.0301	0.590	4.2	14.55	85.45	5.6	8.95	4.85	0.79	3.3
56		Crossbred	3	131	3.4	1.0307	0.565	4.6	12.8	87.2	4.15	8.65	4.85	0.69	3.1
57		Ayrsh. cross	3	22	5.7	1.0308	0.570	4.15	13.95	86.05	5.0	8.95	5.3	0.70	2.95
58		Ayrshire	4	259	4.8	1.0288	0.560	4.15	15.5	84.5	6.5	8.85	5.15	0.77	3.0
59	12/3/17	Crossbred	2	221	1.8	1.0274		5.2	14.45	85.55	5.55	8.9	4.4	0.71	3.8
60	(Autumn)	Shth. cross	2	67	3.4	1.0280		4.5	16.1	83.9	7.75	8.35	4.65	0.86	2.85
61		Crossbred	3	188	3.2	1.0298		4.6	13.3	86.7	5.8	7.5	4.8	0.76	1.95
62		Shth. cross	4	8	3.2	1.0298		4.55	13.85	86.15	5.2	8.65	5.1	0.71	2.8
63		Shorthorn	4	104	4.3	1.0284		4.5	13.6	86.4	5.25	8.35	5.05	0.73	2.55
64		Ayrshire	2	35	5.5	1.0284		4.5	14.0	86.0	5.9	8.1	5.05	0.70	2.35
65		Shorthorn	3	61	4.5	1.0293		4.2	13.8	86.2	5.4	8.4	5.2	0.70	2.5
66		Crossbred	3	57	4.1	1.0292		4.0	15.0	85.0	6.25	8.75	5.25	0.71	2.8
67		"	3	67	5.0	1.0290		4.55	12.75	87.25	4.65	8.1	5.1	0.73	2.25
68		Shorthorn	5	49	3.6	1.0296		4.35	13.3	86.7	4.85	8.45	5.2	0.81	2.45
69		"	2	140	3.6	1.0302		3.95	14.6	85.4	5.85	8.75	5.25	0.79	2.7
70		Shth. cross	4	346	1.8	1.0312		4.3	16.35	83.65	6.8	9.55	5.05	0.74	3.75

TABLE II.—(Continued).

No.	Date.	Breed.	Calif.	Age.	Wt.	Dens.	$\Delta$	Cond.	Solids.	Water.	Fat.	S.n.F.	Lactose.	Ash.	Protein, etc.
71	27/3/17 (Autumn)	Shorthorn	3	125	4.1	1.0308	0.560	3.75	15.35	84.65	6.1	9.25	5.2	0.76	3.3
72		Black Jers.	5	5	4.1	1.0324	0.555	4.55	13.65	86.35	5.65	8.0	4.9	0.71	2.4
73		Ayrshire	4	12	5.8	1.0314	0.563	4.3	13.55	86.45	4.8	8.75	5.3	0.76	2.7
74	3/4/17	Crossbred	3	199	2.7	1.0300	0.547	4.55	14.7	85.3	5.75	8.95	4.4	0.77	3.8
75	(Autumn)	"	1	179	1.8	1.0290	0.564	4.7	13.55	86.45	5.15	8.4	4.75	0.71	2.95
76		Devon	2	269	1.8	1.0289	0.560	4.05	15.75	84.25	5.85	9.9	4.8	0.77	4.35
77		Shth. cross	5	134	3.9	1.0291	0.552	4.65	12.5	87.5	4.35	8.15	4.85	0.68	2.6
78		"	2	510	2.0	1.0290	0.544	5.35	13.15	86.85	4.75	8.4	4.1	0.61	3.7
79		Jersey cross	6	118	2.3	1.0308	0.581	4.75	14.55	85.45	5.1	9.45	4.7	0.80	3.95
80		Crossbred	5	223	2.3	1.0276	0.556	5.0	14.7	85.3	6.35	8.35	4.25	0.74	3.35
81		Shorthorn	3	28	4.1	1.0300	0.566	4.15	12.75	87.25	4.15	8.6	5.4	0.65	2.55
82		Devon-Shth.	4	215	1.4	1.0308	0.550	4.9	13.6	86.4	4.9	8.7	4.4	0.79	3.5
83		Crossbred	3	237	3.4	1.0307	0.553	4.15	14.15	85.85	4.8	9.35	5.05	0.72	3.6
84		"	2	197	1.4	1.0298	0.569	4.5	14.3	85.7	5.7	8.6	4.9	0.76	2.95
85		Ayr. cross	3	181	2.7	1.0308	0.558	4.5	14.45	85.55	7.65	6.8	4.85	0.68	1.25
86	16/4/17	Ayrshire	4	—	4.1	1.0305	0.572	4.3	14.75	85.25	6.2	8.55	5.15	0.76	2.65
87	(Autumn)	Shorthorn	5	73	4.1	1.0297	0.570	5.25	12.3	87.7	4.1	8.2	4.75	0.75	2.7
88		Crossbred	4	—	5.5	1.0291	0.565	4.55	14.85	85.15	5.2	9.65	5.1	0.74	3.8
89		"	5	288	2.3	1.0293	0.572	5.35	14.5	85.5	5.9	8.6	4.55	0.71	3.3
90		Shth. cross	3	241	3.2	1.0305	0.559	4.6	14.65	85.35	5.9	8.75	4.8	0.73	3.2
91		Ayrshire	3	47	5.0	1.0285	0.557	4.25	14.85	85.15	5.35	9.5	5.05	0.68	3.75
92		Shth. Jersey	4	52	7.7	1.0301	0.555	4.35	14.7	85.3	4.55	10.15	5.1	0.69	4.35
93		Jersey	3	18	6.4	1.0254	0.548	4.25	12.9	87.1	6.5	6.4	5.15	0.72	0.55
94		Ayrs. cross	5	11	6.4	1.0302	0.561	4.45	16.1	83.9	5.35	10.75	5.1	0.72	4.95

TABLE II.—(Continued).

No.	Date.	Breed.	Calv.	Age.	Wt.	Dens.	$\Delta$	Cond. Solids.	Water.	Fat.	S. n. F.	Lactose.	Ash.	Protein, etc.
95	16/4/17	Shorthorn.	5	8	3.4	1.0316	0.565	5.0	85.8	4.15	10.05	4.75	0.80	4.5
96	(Autumn)	Shth. cross	3	3	4.1	1.0361	0.593	4.55	87.35	4.35	8.3	5.3	1.06	1.95
97		Black Jersey	2	66	3.2	1.0300	0.543	4.25	85.7	5.8	8.5	5.0	0.72	2.8
98	30/4/17	Black Jersey	1	146	3.9	1.0505		4.1	86.3	5.85	7.85	5.25	0.77	1.85
99	(Autumn)	Shorthorn	4	121	2.7	1.0297		4.95	86.5	5.2	8.3	4.85	0.79	2.65
100		Ayrshire	4	155	4.5	1.0301		4.75	87.05	4.5	8.45	4.9	0.71	2.85
101		Shorthorn	6	5	5.2	1.0317		5.35	86.85	2.65	10.5	4.55	0.80	4.15
102		Shth. cross	3	86	3.6	1.0290		4.85	87.6	5.2	7.2	4.9	0.72	1.6
103		Crossbred	2	118	3.4	1.0295		4.0	84.75	6.55	8.7	5.2	0.72	2.8
104		Shorthorn	4	106	4.1	1.0294		5.55	87.4	4.45	8.15	4.45	0.75	2.95
105		"	5	109	5.7	1.0294		5.05	87.05	4.65	8.3	4.7	0.74	2.85
106		Crossbred	2	156	3.0	1.0290		4.15	83.35	8.05	8.6	5.15	0.69	2.75
107		Shorthorn	4	158	3.4	1.0298		5.0	86.25	5.4	8.35	4.45	0.75	3.3
108		Shth. cross	3	106	3.6	1.0290		4.8	86.55	5.15	8.3	4.6	0.60	3.1
109		"	1	57	1.8	1.0292		5.55	87.6	4.2	8.2	4.2	0.78	3.2

To obtain some general idea of the significance of series of figures like those in the above Table, it is usual to calculate from them certain representative or mean values. The commonest form of mean value is the arithmetic mean, and this is meant by the word, unless something else is specified. The arithmetic mean, however, is only truly representative of a series of results when all these results may be considered as equally likely to occur. A few exceptional results do not really alter a series as a whole, but such exceptional results affect the arithmetic mean value, and their effect becomes more noticeable as these results become more unlikely to occur. Such a mean value, which is unduly affected by exceptional results, has, therefore, obvious defects as the representative value of a series. Further, the arithmetic mean takes no account of the manner in which the results in a series are grouped. It gives no information whatever about the distribution of the results within the series. The arithmetic mean, on the other hand, is very simply calculated, and in series of values that have no definite grouping, it is the only form of mean value which can be obtained.

Attention may be drawn here to the fact, that the mean values of the composition and properties of a number of samples of milk are not necessarily the same as those of the milk obtained by mixing together all these samples. In calculating the arithmetic mean, no account is taken of the varying sizes of the samples. In mixing together a number of samples, however, the size of each will have an effect on the composition and properties of the mixed milk. As it is the mixed milk, and not a hypothetical mean milk which is sold, the values of the composition of the mixed milk have been calculated, and the figures for them have been inserted in brackets under the corresponding mean figures. As will be seen, the difference between the two figures in no case exceeds 2% of their value. The "mixed" values of the physical properties have not been calculated, as the variation of these is so small, that the differences from the mean values must be even less than in the case of the composition.

The sums of the percentages of the constituents in the above Table do not exactly equal the percentages of total solids. This

is due to the fact that the percentages of the constituents are not given nearer than 0.5%, except in the case of the percentage of ash.

The mean values of the results in Table ii. are given below (Table iii.). The maximal and minimal values of each quantity

TABLE iii.

Maximal, minimal, and mean values of the composition and properties of cows' milk; and actual and relative ranges of variation of the results.

	Max.	Min.	Mean.	Range.	Range Mean.
Number of calves ...	9	1	4	8	2
Age of calf ...	519	3	133	516	3.9
Weight of milk...	8.0	1.0	3.7	7.0	1.9
Weight of fat ...	416	53	182	365	2.0
Density ...	1.0332	1.0254	1.0297	0.0078	0.26
Depression of freezing point	0.594	0.547	0.563	0.047	0.084
Conductivity ...	7.5	3.75	4.55	3.75	0.66
Total solids, p.c.	16.65	10.5	13.55 (13.55)	6.15	0.45
Fat, p.c.	7.75	2.6	5.0 (4.9)	5.15	1.0
Solids not fat, p.c.	10.75	6.4	8.65 (8.6)	4.35	0.50
Lactose, p.c.	5.6	2.7	4.9 (4.95)	2.9	0.59
Ash, p.c.	1.06	0.43	0.69 (0.68)	0.63	0.91
Protein & extractives, p.c.	5.5	0.85	2.9 (2.95)	4.65	1.5
Water, p.c.	89.5	83.35	86.45	6.15	0.45

are also given, so that its range of variation may be seen. To make possible a comparison of the ranges of variation of the different quantities, the extreme range has been divided in each case by the mean value of the series. The relative ranges of variation obtained in this way may be compared together directly.

The relative variation of the density has been calculated by dividing the range, not by the mean density, but by the difference between the mean density and the density of water. The greatest range of the values of density of milk in a series of observations

is not from zero to the maximal value, but from the density of water to this value.

Similarly, the greatest range of the percentage of water in the milk is not from zero to 100%, but from the minimal percentage to this value, and the relative variation is the same as that calculated for the range of the percentage of total solid matter.

The above results show that the greatest relative variation of the values of the quantities observed is in the weight of fat obtained at a milking. This weight varies from 416 to 53 gm. The maximal weight is nearly 8 times the minimal, and the relative range is 2.

The quantity having the least relative variation is the depression of freezing point of the milk, for which the value is 0.084. For the weight of fat, the relative variation is more than 20 times as great as this.

The greatest relative variation of the percentage of a constituent of milk is that shown by the protein, in which it is 1.5. The percentage of total solid matter shows the least relative variation, the value in this case being 0.45.

Among the single constituents of the milk, the least relative variation is that shown by the lactose, in which it amounts to 0.59. The relative variation of the percentage of fat is 1.0, or nearly double that of the lactose.

In the following Table are given the mean values of the composition and properties of the samples of milk obtained at the same milkings. Each mean figure, except the first two, is for the milk of 12 cows; the first two series of mean values are for the milk of 11 cows. From these figures, comparisons may be made of the average milk obtained from small herds at single milkings. For the reasons already mentioned, these figures are not necessarily identical with the figures for the values of the composition and properties of the mixed milks of the herds.

The actual and relative variations of the different quantities are given as before. In addition, the ratios of the ranges of variation of the mean figures (Range 2) to the ranges of variation of the individual figures (Range 1) given in the previous Table are also shown.

TABLE IV.  
Mean values of composition and properties of samples of cows' milk collected at the same milking.

Samples.	1-11	12-22	23-34	35-46	47-58	59-70	74-85	86-97	98-109	Range		
										Range 1	Range 2	
	Mean											
Number of calves..	...	5	6	7	3	3	3	4	3	4	0.50	1.0
Age of calf	...	103	165	135	107	119	207	81	110	126	0.25	0.95
Weight of milk	...	5.2	3.0	3.9	4.0	3.7	2.5	4.6	3.7	2.9	0.41	0.78
Weight of fat	...	232	147	170	190	209	131	243	188	133	0.36	0.73
Density ...	...	1.0300	1.0298	1.0299	1.0304	1.0292	1.0297	1.0301	1.0297	0.002	0.26	0.067
Depression of freezing-point	...	0.553	0.565	0.565	0.571	—	0.559	0.564	—	0.018	0.38	0.032
Conductivity	...	4.5	4.45	4.7	4.45	4.45	4.6	4.6	4.85	0.65	0.21	0.14
Total solids, p.c.	...	12.95 (12.9)	13.35 (13.4)	13.3	13.55 (13.6)	14.25 (14.0)	14.0 (13.75)	14.2 (14.5)	13.55 (13.65)	1.3	0.27	0.096
Fat, p.c....	...	4.25 (4.45)	4.8 (4.9)	4.4 (4.35)	4.95 (4.75)	5.75 (5.65)	5.4 (5.25)	5.25 (5.3)	5.15 (5.1)	1.5	0.30	0.30
Solids not fat, p.c.	...	8.7 (8.6)	8.55 (8.55)	8.9 (8.85)	8.65 (8.65)	8.5 (8.4)	8.6 (8.5)	8.9 (9.1)	8.4 (8.55)	0.5	0.12	0.058
Lactose, p.c.	...	5.1 (5.15)	4.95 (5.05)	4.9 (5.0)	5.05 (5.1)	5.0 (5.0)	4.7 (4.75)	5.0 (5.05)	4.75 (4.85)	0.55	0.19	0.11
Ash, p.c....	...	0.61 (0.58)	0.65 (0.65)	0.61 (0.59)	0.72 (0.73)	0.74 (0.74)	0.72 (0.71)	0.76 (0.76)	0.74 (0.74)	0.15	0.24	0.22
Protein and extractives, p.c.	3.0	3.4 (3.45)	2.95 (2.85)	3.4 (3.25)	2.85 (2.85)	2.75 (2.6)	3.2 (3.1)	3.2 (3.15)	2.9 (2.9)	0.75	0.17	0.25
Water, p.c.	...	87.05	86.65	86.7	86.45	85.75	86.0	85.8	86.45	1.3	0.27	0.096

The ranges of variation of these mean values of the composition and properties of the milk of groups of cows are, of course, less than those of the individual samples. The extents to which the individual variations are masked by this process of averaging are widely different in the different quantities measured. The relative values of this masking-effect are shown by the figures in the column headed Range 2, Range 1. These figures show that, in the case of the values of the weight of milk obtained at a milking, the mean results have a range of variation 0.41 of the range of the individual results. The masking of the individual variation, produced by taking the mean figures, is less in the present case than in the case of the values of any of the other quantities.

The mean values of the percentages of solids not fat, on the other hand, show a range of variation which is only 0.11 of that of the individual results. The process of taking the mean values of the percentages of these substances has, therefore, a very pronounced effect of obscuring the range of variation which actually occurs, even in the case of small groups of samples, like the present.

The difference between the mean composition and the composition of the mixed milk is more pronounced in the case of these smaller numbers of samples than it was in the case of the larger number. The difference does not in any case exceed 5% of the value in question, however.

These results raise the question, whether the fact, that the samples of milk examined for commercial or legal purposes are almost invariably mixed samples, may not have given rise to an exaggerated notion of the constancy of the percentage of solids not fat. The existence of such a notion would, perhaps, lead to the establishment of a standard for the percentages of these substances, which would be too close to the mean value to make allowance for the variation met with in individual samples of milk.

The standard for the percentage of solids not fat is 8.5 in all the States of Australia, and in England. It is interesting to observe that, even among the above mean and "mixed" values of the percentage of solids not fat, one is at the standard, and one is below it. Of the individual values, nearly one-half are at or below

this standard. This fact is discussed in greater detail later.

It is interesting to notice that the group of samples (98-109), the mean value of whose percentage of solids not fat is below standard, is not the same as the group (59-70), whose "mixed" percentage is below standard. Such an instance emphasises the importance, for some purposes, of distinguishing between the mean composition and the composition of the mixed milk.

#### DISTRIBUTION OF RESULTS.

A knowledge of the distributions of the values obtained for the different properties and constituents of milk is of much greater practical importance than information about their mean values, or the extreme ranges over which they may vary. The practical question which must be answered is: Of a given number of samples of milk, how many may be expected to have values of their composition or properties below or above certain limits?

Although the limits may be chosen arbitrarily, it is useful to have some central value about which to fix them. The objection to the arithmetic mean, as already stated, is that it is unduly influenced by exceptional results. A value which is even more simply obtained than the arithmetic mean, and which is not affected by exceptional results, is the median. The median value of a series of observations is that value, above and below which 50% of the results lie. The position of the median is most easily determined graphically by plotting the values of the observations as abscissæ, and the numbers of results below each value as ordinates. The median value is that whose ordinate is equal to half the maximal ordinate. The curve drawn through the points obtained in a diagram of this kind is usually S-shaped, and was termed the ogive by Galton (1879), by whom this method of examining a series of observations was very extensively used.

The median, then, is a convenient central value about which to measure the distribution of a series of measurements. In the following Table are given the numbers of results lying between certain percentage differences from the median values of the composition and properties of the samples of milk under discussion. The numbers of results are expressed as percentages of the total and are given to the nearest whole number.



The above table shows the differences of closeness of grouping about the median of the values of the different quantities measured. The general shape of the table shows almost graphically the different ranges of variation which have been discussed above.

If a range of, say, 10% from the median be considered, the grouping of the results in the immediate vicinity of the central value may be compared. The results within this range indicate that the quantities measured may be divided into three main groups as regards their relative variability:—

(1) The total amounts of substances produced by the cows in the period which had elapsed since last milking, about 8 hours. These quantities are the most variable.

(2) The concentrations in which these substances appear in the milk. The variations of the concentrations are less, and in some cases very considerably less, than the variations of amount.

(3) The values of certain physical properties. These are the least variable of all the quantities measured.

The quantity of the milk secreted by a cow in a given time is the most variable quantity observed. As the above Table shows, only 22% of the results fall within a range of 10% from the median value.

The quantity of fat produced by the cow in the same time is rather less variable, as 33% of the results occur within 10% of the median value. This method of examining the figures, therefore, shows that there is some tendency of the amount of fat produced to be more "constant" than the total quantity of milk.

The variations of the quantities of the remaining constituents of the milk must be between that of the amount of milk, and that of the amount of fat, since, as will be seen below, the amounts of these substances produced are more dependent on the amount of milk than is the amount of fat. Their concentrations are more constant.

As regards the concentrations of the components of the milk, the substances present in milk may be divided into two classes: those which exist in suspension, and those which are in true solu-

tion. The variability of the former class of substances is much greater than that of the latter. The greatest variation is shown by the values for the percentage of fat. Within 10% of the median, 38% of the results lie. The amount of fat produced, therefore, is less influenced by the amount of milk secreted than are the amounts of the other constituents.

The percentage of protein is somewhat less variable than that of the fat, although its extreme range is the same. In this case, 42% of the results lie within 10% of the median value.

The percentage of ash is considerably less variable, as 61% of the results lie within the range specified.

The percentages of total solids, and of solids not fat are again considerably less variable. In the case of the former values, 79% of the results are within 10% of the median, and in the case of the latter values, 82% of the results are within this range.

The constituent of milk, the percentage of which is least variable, is lactose. Within 10% of the median, 84% of the values for the concentration of this substance lie.

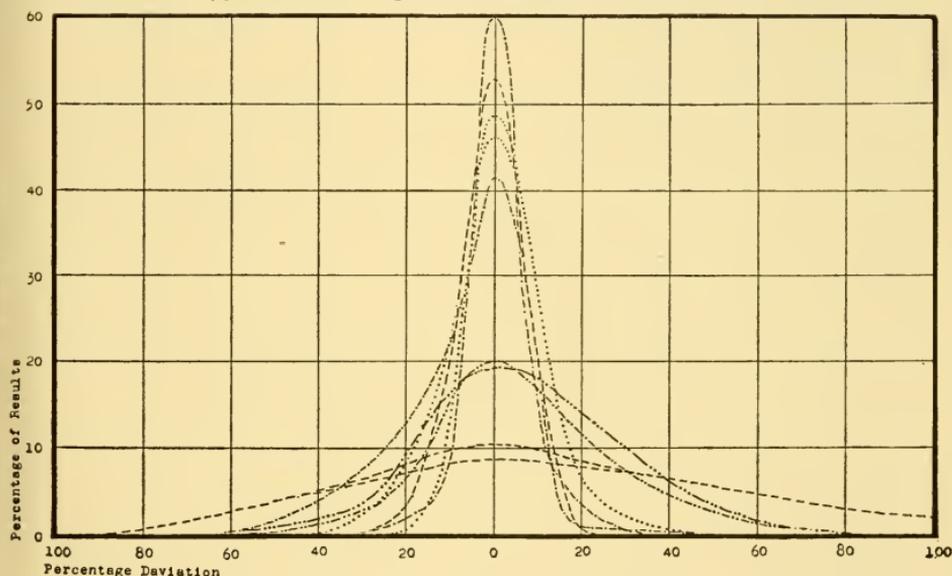
Among the physical properties estimated, the most variable is the electrical conductivity; 70% of the values of this property lie within 10% of the median. This variability is rather less than that of the ash, but considerably greater than that of the percentage of lactose.

The values of the other physical properties determined are much less variable than that of the electrical conductivity. The whole of the results for the values of the density and of the depression of the freezing-point lie within 10% of the median values. The whole of the results for the depression of the freezing point lie within 5% of the median, in fact. The molecular concentration of the dissolved substances is, therefore, the most constant property of milk.

It should be noted that the variability of the density has been calculated not on the actual values obtained, but on the differences between these values and the density of water, for the reasons stated above.

The distributions of the above series of values is shown more

clearly in the accompanying diagram (Text-fig.1). The curves are the probability or frequency curves. They show the relative closeness of grouping of the results about their most probable values. The abscissæ represent the values of the different quantities measured. To facilitate comparison, the values are expressed as percentages of the most probable values, or of the values corresponding to the maximal ordinates. Each ordinate represents the percentages of the total number of results within a certain range of values on either side of it. The range chosen is 5% of the most probable value.



Text-fig. 1.

Relative variabilities of constituents of cows' milk. Frequency or probability curves showing percentages of total number of results (ordinates) occurring within  $\pm 5\%$  of various percentage-differences from most probable values (abscissæ). The curves, taken in order from above downwards, represent: percentages of lactose, of solids not fat, and of total solids; conductivity; percentages of ash, of protein, and of fat; total weights of fat, and of milk.

These frequency curves are the derived curves of the simply obtained ogival curves of Galton. The frequency curves have been drawn by taking, as their ordinates, lengths proportional to the slopes of the corresponding points of the ogive.

The values corresponding to the maximal ordinates of these curves are the most probable values or modes, of the quantities whose variations are represented. The figures for the modes of the different quantities are given in Table vi. (p.851).

The frequency curves of the values of the depression of the freezing point, and of the density, are not shown in the diagram, as their ordinates are too high, in proportion to the range of their abscissæ, to allow them to be conveniently represented on the same scale as the other curves: all the results occur within a very small range of the most probable values.

The curves for the distribution of the other values fall obviously into three groups.

In the first group are the curves for the values of the percentages of lactose, solids not fat, total solids, and ash, and the value of the conductivity. The percentages of these values, within 5% of the most probable values, range from 53%, in the case of the concentration of lactose, to 41%, in the case of the concentration of ash.

In the second group are the curves for the values of the percentages of protein, and of fat. The percentages of results within 5% of the modes are 19% and 20% respectively.

The values of the weight of milk, and of the weight of fat, form the third group. Between these two curves, lie those for the distribution of the weights of the other constituents, as was explained above. These two curves show that, in the case of the weights of substances obtained at a milking, only 8.5 to 10% of the results lie within a range of 5% of the mode.

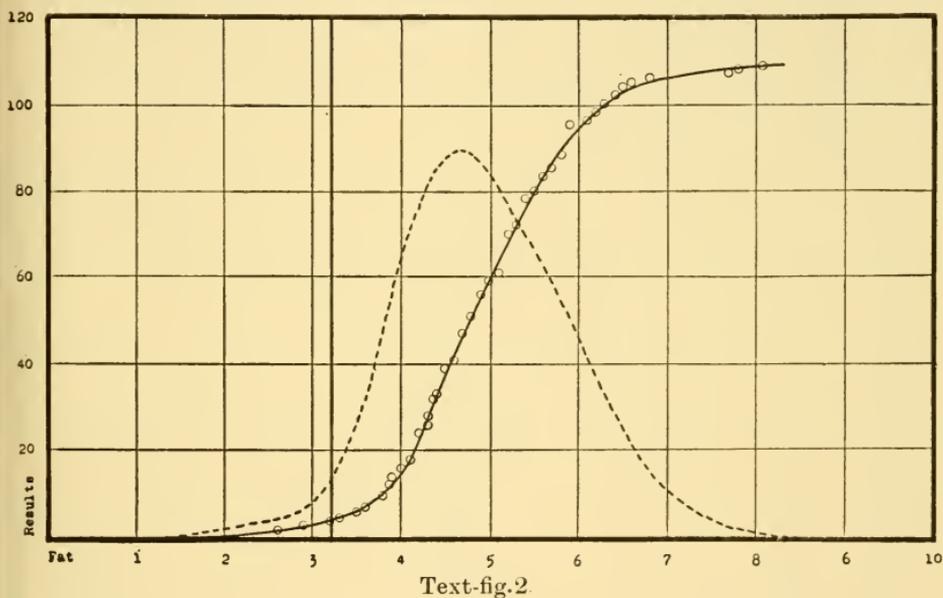
The concentrations of the substances in the first group are thus at least twice as constant as those of the substances in the second group.

The concentrations, however, even of substances in the second group, are at least twice as constant as the total weights produced, the curves for which are in the third group.

The values of the percentages of fat, and of solids not fat, are of great importance, as certain values of these quantities are chosen as standards of the quality of milk. The distribution of

these values will, therefore, be considered separately in greater detail.

In the following diagram (Text-fig.2), the ogive of the percentages of fat in the present samples of milk is given. The corresponding derived or frequency curve is shown by a discontinuous line. The percentages of fat are plotted as abscissæ. The ordinates represent the numbers of results, out of a total of 109, which occur at, or below, given values of the percentage. The circles correspond to the actual numbers of results. The ordinates refer only to the ogive, and not to the frequency



Text-fig.2.

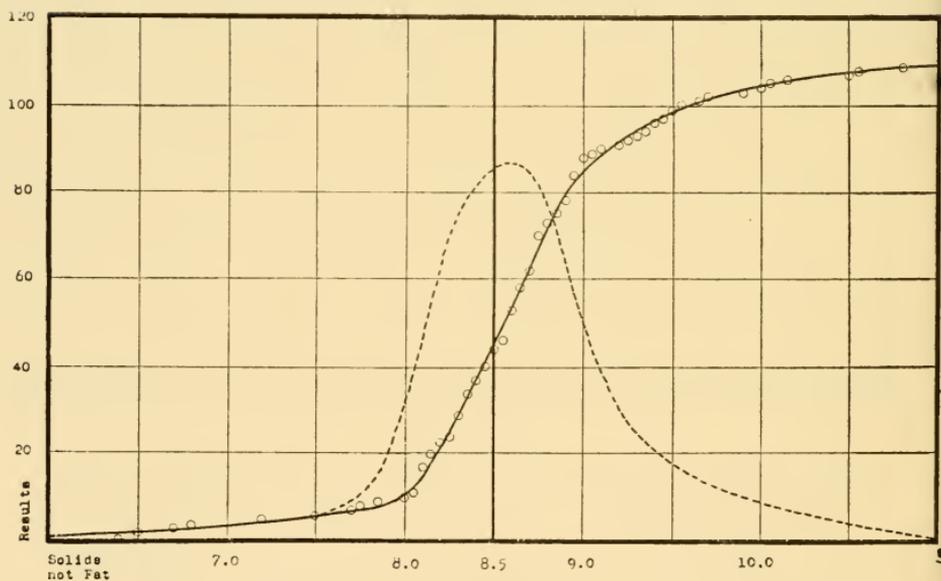
Ogive and frequency curve of concentrations of fat in cows' milk. Abscissæ: percentages of fat. Ordinates: numbers of results; ordinates refer only to ogive.

curve. The latter has been inserted only to render more obvious the information contained in the ogive. The heavy line at 3.2% indicates the position of the standard for the percentage of fat in New South Wales. The values of this standard in the other Australian States are as follows: Victoria, 3.5; Queensland, 3.3; South Australia, 3.25; Western Australia, 3.2%. The standard percentage of fat in England is 3.0.

It will be seen from the above diagram that only a small proportion of the samples examined contained percentages of fat below the legal standard of the State in which they were obtained.

The frequency curve shows that the most probable percentage of fat in these samples of milk is 4.65. It must be borne in mind, however, that the samples are of the afternoon-milk, which is richer in fat than the milk obtained in the morning.

In the next diagram (Text-fig.3), the ogive and the frequency curve of the percentages of solids not fat are given. As before, the abscissæ represent the percentage-values of the quantity measured, and the ordinates, the numbers of results at and below these values. The ordinates again refer only to the ogive. The heavy line at 8.5% indicates the position of the standard percentage of solids not fat.



Text-fig 3,

Ogive and frequency curve of concentrations of solids not fat in cows' milk. Abscissæ: percentages of solids not fat. Ordinates: numbers of results; ordinates refer only to ogive.

The above diagram shows that over 40% of the samples examined contain a percentage of solids not fat below the legal

standard. The frequency curve also shows that, in the present results, the most probable value of the percentage of solids not fat is 8.65. This value is very close to the standard, which, therefore, allows a very small margin of variation. Over 90% of these results, on the other hand, lie above a percentage of solids not fat of 7.9.

In the preceding discussion, three forms of mean value have been used, the arithmetic mean, the median, and the mode or most probable value. In series of results which are symmetrically arranged about their central values, these three means are identical. Actual series of results are rarely quite symmetrical, however, and in such cases the three means may differ. In the accompanying Table, the three forms of mean values of the results of the present series are placed together for comparison.

TABLE VI.

Arithmetic means, medians, and modes or most probable values, of the composition and properties of cows' milk.

	Mean	Median.	Mode.
Weight of milk ... ..	3.7	3.75	3.5
Weight of fat ... ..	182	173	150
Density ... ..	1.0297	1.0297	1.0297
Depression of freezing-point ...	0.563	0.562	0.562
Conductivity ... ..	4.55	4.55	4.5
Total solids, p.c. ... ..	13.65	13.45	13.24
Fat, p.c. ... ..	5.0	4.85	4.65
Solids not fat, p.c. ... ..	8.65	8.65	8.65
Lactose, p.c. ... ..	4.9	5.0	5.0
Ash, p.c. ... ..	0.69	0.70	0.72
Protein, etc., p.c. ... ..	2.9	3.0	2.9
Water, p.c. ... ..	86.45	86.55	86.75

The figures in the above Table show that the three forms of mean value are very close together for most of the quantities measured. The greatest variation is to be seen in the case of the weight of fat obtained at a milking. The mode of this weight is about 14% less than the mean. The modes of the percentage of fat and of the weight of milk obtained at a milking

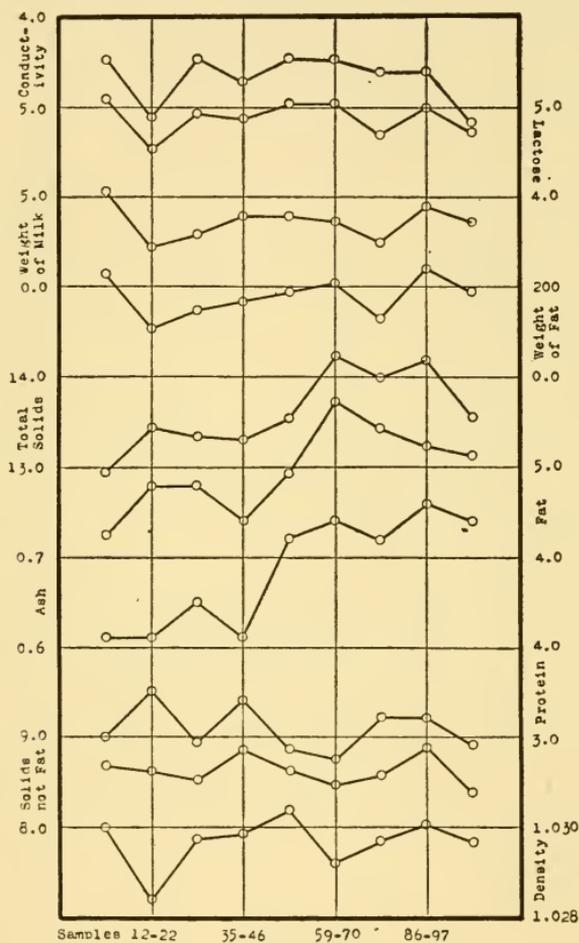
are also less than the mean values, the former to the extent of about 7%, the latter to the extent of about 6%. The mode of the percentage of total solids is also less than the mean value, as a consequence of the behaviour of the values of the percentage of fat. The three forms of mean of the percentage of solids not fat, however, are practically identical.

#### RELATIONS BETWEEN CONSTITUENTS AND PROPERTIES.

Although no general relations, between the percentages of the various constituents of the milk and the values of the various properties, are at once apparent from an examination of the figures for the individual samples, such relations do exist. So definite is the relation between the density, the percentage of fat, and the percentage of solids not fat, or total solids, for example, that Fleischmann (1885), and Helmer and Droop Richmond (1888) have devised formulæ by which the value of one of these three quantities may be calculated from the values of the other two.

From the figures for the mean values of the composition and properties of the milk collected on the same days, however, it may be seen more readily whether any relations exist between the different quantities. To display graphically any such relation, the mean values of the quantities for the several days have been plotted as ordinates in the following diagram (Text-fig.4). The different ordinates on which the values are measured are spaced equally along the abscissa, and represent the different days on which the samples were collected. The points representing the different mean values of the same quantity have been joined by straight lines. It is to be borne in mind, that the lines obtained in this way are not graphs in the ordinary sense of the word. They do not represent the values of the ordinates corresponding to given values of the abscissæ. The points on the abscissa have no "values," they simply represent different groups of samples; and the ordinates for the different values of each quantity have been joined by lines merely to connect them together, and to facilitate their comparison with the corresponding points for the values of other quantities.

The values for the electrical conductivity have been measured downwards in the above diagram to render them more easily comparable with the values of the other quantities, which have been measured upwards in the ordinary way.



Text-fig. 4.

Diagram showing relation between various properties and constituents of cows' milk. Ordinates: values of quantities measured. Points on abscissa represent corresponding values of different quantities.

The diagram shows, at once, that there is a distinct, inverse relation between the mean percentage of lactose, and the mean conductivity. Jackson and Rothera (1914) showed that a similar

definite relation between these quantities exists in the samples of milk obtained from the different quarters of a cow's udder at one milking. Their measurements of conductivity were made, however, upon milk from which the fat had been removed. These relations are also shown by the diagrams representing the effect of the number of calves or age of the cow, and the stage of lactation or age of the calf, on the mean composition and properties of the milk (Text-figs. 5 and 6).

Text-fig. 4 also indicates the existence of a direct relation between the weights of milk obtained at a milking, and the corresponding weights of fat. The mean weights of fat fluctuate in the same direction as the mean weights of milk, and do not show any tendency to remain constant and independent of the amounts of milk. The present results are for single milkings, however, and not for the daily outputs of the cows. The above Figure shows also that there is a relation between the percentages of lactose and the conductivity, and the weights of milk and of fat. Cows producing larger quantities of milk thus show no tendency to yield a secretion poorer in fat or in lactose than those producing less milk under the same circumstances.

Between the remaining mean values of the composition and properties of the milk no distinct relations are shown by the above diagram. The dependence of the density on the percentages of fat and of ash, which is to be observed in certain individual samples, is not seen when the mean values are considered. There is also no evidence that a higher percentage of fat is generally associated with a higher percentage of protein, as was thought to be the case by Hart (1906), and by Eckels and Shaw (1913).

These results do not show the direct relation between the percentage of ash and the conductivity, which was observed by F. Petersen (1904).

#### EFFECT OF BREED.

In the following Table are given the mean composition and properties of the milks of cows of different predominant strains of breed. The numbers of cows of each strain vary from 51 to

12. The calculated mean values are, therefore, not so strictly comparable as if the numbers of the cows of each strain were approximately equal.

TABLE VII.

Predominant strain.	Shorthorn.	Crossbred.	Ayrshire.	Jersey.
Number of cows ...	51	18	19	12
Number of calves ...	5	3	3	4
Age of calves... ..	4.5	4.8	4.5	3.9
Weight of milk ...	3.8	3.4	3.9	4.3
Weight of fat ...	179	186	197	213
Density ...	1.0301	1.0293	1.0294	1.0301
Freezing-point ...	0.564	0.564	0.562	0.562
Conductivity ...	4.65	4.55	4.75	4.45
Total solids, p.c. ...	13.5	14.25	13.55	13.7
Water, p.c. ...	86.5	85.75	86.45	86.3
Fat, p.c. ...	4.8	5.55	5.0	4.85
Solids not fat, p.c. ...	8.7	8.7	8.55	8.65
Sugar, p.c. ...	4.9	4.85	4.85	5.05
Ash, p.c. ...	0.69	0.72	0.66	0.67
Protein, etc., p.c. ...	3.05	3.0	3.0	3.0

The values given in this Table show that the greatest range of variation occurs in the mean percentage of fat, which is greatest in the case of Crossbred cows (5.5%), and least in the case of Shorthorn strains (4.8%). The mean yield of milk, on the other hand, is greatest in the case of the Jersey strain (4.3 kg.), and least in the case of the Crossbred (3.4 kg.). The mean total weight of fat obtained at a milking is greatest in the case of the Jersey strain (213 gm.), and least in the case of the Shorthorn (179 gm.). The mean percentage of lactose is highest in the Jersey strain (5.05%), and lowest in the Crossbred and Ayrshire (4.85%). The mean percentage of ash is greatest in the Crossbred cows (0.72%), and least in the case of the Ayrshire strains (0.66%). The mean percentage of protein and extractives is greatest in the milk of cows of Ayrshire strain (3.1%), and least in that of cows of Crossbred and Ayrshire strains (3.0%). Its range of variation is small.

Among the physical properties, the greatest variation is shown by the electrical conductivity, which ranges from  $4.75 \times 10^{-2}$  reciprocal ohms in the case of the Jersey strain, to  $4.55 \times 10^{-2}$  in the case of the Crossbred strain. The variation of the mean freezing point is within the limits of error of the determination. The density varies from 1.0301 in the milk of Jersey and Short-horn strains, to 1.0293 in that of Crossbred cows.

The average numbers of calves of the cows of the different strains range from 5 to 3. The average ages of the calves range from 3.9 to 4.8 months. The data for the effect of the number of calves or age of the cows, and of the period of lactation or age of the calves, show that these variations are not large enough to affect appreciably the present comparison of the mean values of the composition and properties of the milk of cows of different predominant strains.

#### EFFECT OF NUMBER OF CALVES OR AGE OF COW.

In the following Table are given the mean values of the composition and properties of the milk of cows which have had different numbers of calves, that is, of cows of different ages. The number of cows at each age varies from 7 to 28. In this case the means compared are those of rather widely varying numbers of results, and are not so strictly comparable as they would be, if the numbers of the cows at the different ages were approximately equal. The numbers of calves of the cows examined range from 1 to 9; the ages of the animals, therefore, range from 2 to 10 years.

TABLE VIII.

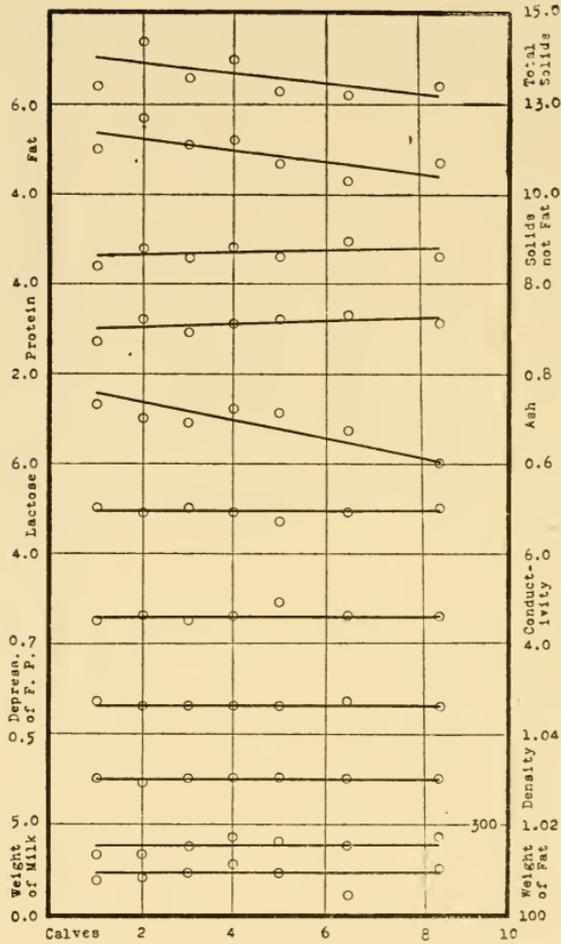
Effect of number of calves or age of cow on mean composition and properties of its milk.

Number of calves.	1	2	3	4	5	6-7	8-9
Number of cows ...	7	16	28	16	16	10	12
Age of calf ...	3.5	5.5	4.5	4.5	3.6	4.1	5.1
Weight of milk ...	3.3	3.2	3.7	4.2	3.9	3.3	4.3
Weight of fat ...	165	175	188	206	185	140	202
Density ...	1.0304	1.0293	1.0300	1.0299	1.0295	1.0300	1.0295
Depression of f.pt.	0.570	0.562	0.563	0.559	0.568	0.562	0.562
Conductivity ...	4.5	4.6	4.5	4.6	4.95	4.6	4.6
Total solids, p.c....	13.35	14.45	13.6	13.95	13.25	13.15	13.45
Water, p.c. ...	86.65	85.55	86.4	86.05	86.75	86.85	86.55
Fat, p.c. ...	4.95	5.7	5.1	5.15	4.65	4.3	4.75
Solids not fat, p.c.	8.4	8.75	8.58	8.8	8.6	8.85	8.7
Lactose, p.c. ...	4.95	4.85	4.95	4.95	4.7	4.85	5.0
Ash, p.c. ...	0.73	0.70	0.69	0.72	0.71	0.67	0.60
Protein & ext., p.c.	2.7	3.2	2.85	3.1	3.2	3.3	3.1

The mean ages of the calves of the cows of different ages are shown by the above Table to range from 5.5 to 3.5 months. The data given for the variation of composition and properties, with the period of lactation, show that this range is small enough to be without noticeable effect on the present comparisons.

In the accompanying diagram (Text-fig.5), the mean values of the composition and properties of the milk of cows of different ages, or which have had different numbers of calves, have been plotted as ordinates, against the numbers of calves as abscissæ.

This diagram shows at once that the mean values of the physical properties of milk, density, freezing point, and electrical conductivity are unaffected by the age of the cow. The weight of milk yielded, and the percentage of sugar present are also unaffected. The mean percentage of protein increases from 3.0 to 3.3 during the period examined. The mean percentage of fat, on the other hand, decreases from 5.4 to 4.3, and the mean percentage of ash decreases from 0.75 to 0.62, during the same period. The percentage of total solids falls, as the age of the cow increases, from 14.0 to 13.2.



Text. fig. 5.

Effect of number of calves, or age of cow, on composition and properties of milk. Ordinates: values of composition and properties. Abscissae: numbers of calves.

EFFECT OF PERIOD OF LACTATION.

The effect of the period of lactation on the composition and properties of cows' milk is shown by the figures in the following Table. The periods have been expressed as months of thirty days each. The number of cows at each stage of lactation is also given. The values of the composition and properties are the mean values for the number of cows at each stage.

TABLE IX.  
Variation of mean composition and properties of cows' milk with stage of lactation.

Month of lactation.	1	2	3	4	5	6	7	8	9	10-11	Over 10-11
Number of cows ...	13	14	14	10	10	13	12	5	6	5	2
Number of calves ...	4.1	3.7	4.2	4.5	3.4	3.8	4.8	3.4	5.0	3.4	4.0
Weight of milk, kg. ...	4.9	4.9	4.1	3.7	3.8	2.9	3.3	2.2	2.2	1.8	2.5
Density ...	1.0306	1.0295	1.0291	1.0299	1.0305	1.0294	1.0299	1.0287	1.0294	1.0299	1.0302
Depression of f. point	0.561	0.560	0.566	0.568	0.563	0.566	0.563	0.557	0.561	0.561	0.560
Conductivity ...	4.5	4.4	4.7	4.7	4.45	4.75	4.7	5.0	4.4	5.0	4.8
Total solids, p.c. ...	13.4	13.7	13.35	13.6	13.4	13.65	13.6	13.85	14.6	14.05	13.25
Fat, p.c. ...	4.6	4.95	5.05	4.95	4.8	5.3	5.05	5.15	5.6	5.1	4.6
Solids not fat, p.c. ...	8.8	8.75	8.3	8.7	8.6	8.35	8.55	8.7	9.0	8.95	8.65
Lactose, p.c. ...	5.1	5.1	4.9	4.85	5.05	4.75	4.8	4.5	4.85	4.65	4.6
Ash, p.c. ...	0.73	0.67	0.70	0.67	0.68	0.67	0.65	0.72	0.73	0.68	0.53
Protein & extr'ves, p.c.	2.9	3.0	2.65	3.15	2.8	3.1	3.1	3.5	3.45	3.65	3.6
Water, p.c. ...	86.6	86.3	86.65	86.4	86.6	86.35	86.4	86.15	85.4	85.95	86.75
Weight of fat, gm. ...	227	243	208	182	182	150	159	115	15.8	88	112

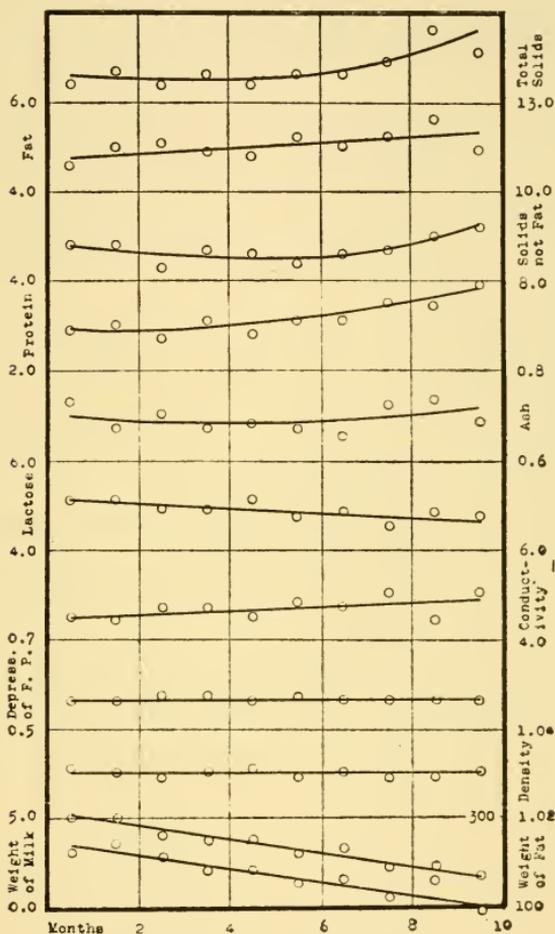
The mean number of cows at each month of lactation up to and including the seventh, is 12, and the maximum deviation from this mean is 2. After the seventh month, the number of cows in milk shows a sudden falling off.

The average number of calves to which the cows at each month of lactation had given birth is given in the third column, to the nearest whole number. This number, for the first seven months, varies between 4 and 5. The average values of the composition and properties are thus quite comparable for these months, both as regards the numbers of samples examined at each month, and as regards the mean age or number of calves of the cows. After the seventh month, the variation of the number of calves is from 3 to 5, and as the numbers of results examined at each month are only half those examined in the earlier period, the mean values of the composition and properties of the milk secreted during the eighth and later months are, perhaps, not so strictly comparable with those of the milk secreted during the earlier periods of lactation. Of the total number of samples examined, 86 were of the milk obtained during the first seven months of lactation, 16 were of the milk of the eighth to eleventh months, and 2 were of the milk of later periods.

In the accompanying diagram (Text-fig. 6) the values of the properties and composition given in Table ix., have been plotted as ordinates, while the corresponding periods of lactation have been plotted as abscissæ. Smooth curves have been drawn through the points obtained in this way.

The scales of the ordinates are not the same for each curve. In the case of the curves for percentages of total solids, fat, solids not fat, protein, and sugar, the distance between adjacent horizontal lines represents a variation of 2%. In the case of curve for the percentages of ash, this distance represents a variation of 0.2%. For the other curves, this distance corresponds to the following variations: density, 0.02; freezing-point, 0.02°C.; conductivity,  $2.0 \times 10^{-2}$  reciprocal ohms; weight of milk, 5.0 kg., weight of fat, 200 gm. The scales of the ordinates are the same as in Text-figure 5.

The curves show that the percentage of fat in cows' milk tends to rise slowly during the whole period of lactation.



Text-fig. 6.

Effect of age of calf or stage of lactation on composition and properties of cows' milk. Ordinates: values of composition and properties. Abscissæ: ages of calves (months).

The percentage of protein falls slightly during the first three months, and then rises till the end of lactation, when its value is nearly 30% higher than it was in the first month.

The percentage of ash falls slightly during the first five months,

and during the remainder of the period rises again, finally reaching its initial value.

The percentage of lactose falls steadily during the whole period of lactation, the final value being about 10% lower than the initial value.

The variation of the percentage of total solids is dependent on that of the separate constituents. This value, like that of the percentage of protein and of ash, falls at first, and then rises again.

The value of the conductivity increases steadily during the whole period of lactation; the final value being about 10% higher than the initial.

The values of the density and of the freezing-point remain constant through the whole course of lactation.

The weight of milk obtained at a milking falls steadily and rapidly as lactation proceeds, the yield towards the end being less than half of what it was at the beginning.

The weight of fat obtained at a milking also tends to fall steadily during the whole period of lactation, and to about the same extent as the weight of milk, in spite of the fact that the percentage of fat tends to increase.

As was stated above, the weights of the remaining constituents are more dependent on the total amount of the milk than is the weight of fat. The weights of these constituents will, therefore, fall steadily during lactation with the weight of the milk.

In conclusion, I wish to express my indebtedness to Professor Sir Thomas Anderson Stuart, in whose laboratory this work was done; and to Dr. E. Sinclair, Inspector General of the Insane, by whose courtesy the samples of milk discussed in this paper were obtained. I also wish to thank Dr. H. G. Chapman for his helpful criticism during the progress of the work.

#### SUMMARY.

The variations of the composition and properties of samples of afternoon-milk from 109 normal cows are discussed.

The following were the most probable values of the quantities measured: depression of freezing-point, 0.562°C.; density, 1.0297;

electrical conductivity,  $4.5 \times 10^{-3}$  reciprocal ohms; total solids, 13.24%; fat, 4.65%; solids not fat, 8.65%; lactose, 5.0%; ash, 0.72%; protein and extractives, 2.9%.

The depression of the freezing-point, and the density were the least variable quantities measured.

The concentrations of the constituents were much less variable than the total quantities obtained at a milking.

The concentrations of the soluble constituents were much less variable than those of the constituents in suspension. The concentration of lactose was the least variable, that of fat the most variable.

About 3% of the samples contained a percentage of fat below 3.2; about 40% of the samples contained a percentage of solids not fat below 8.5.

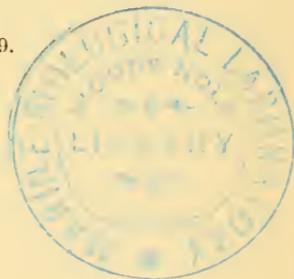
The mean percentages of fat and of ash were greater in the milk of older cows; the mean percentage of protein was less. The percentages of the other constituents, and the properties, were not affected by the age of the cow.

The mean yields of milk became lower in the later stages of lactation; the mean percentages of lactose was also slightly lower in the milk of the later stages of lactation, but the electrical conductivity, and the percentages of fat and of protein became higher as the stage of lactation became later.

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## THE COMPOSITION OF EXPIRED ALVEOLAR AIR.

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In a previous communication(1) dealing with the percentage of carbon dioxide in expired alveolar air, it has been shown that the amount of carbon dioxide in the last 625 c.c. of expired air does not vary beyond the error of the analytical method, when the expulsion has been performed within two seconds, for quantities of air of two litres or over, and within one second for quantities of one to two litres of expired air. The investigation of the composition of expired alveolar air has now been extended to the estimation of oxygen as well as carbon dioxide, and more accurate analytical methods have been employed for the determinations of the amounts of the gases.

*Methods.*—The samples of air have been obtained in the same way as in the previous research(1) by collecting them through small capillary pipes set in a long brass tube, into which the subject of the experiment breathed. The tube is the same as used previously, and the capillary tubes have been fixed 250, 500, 1750 and 2000 mm. from the mouthpiece. The analyses of the gases of the samples have been performed in a large Haldane apparatus, the carbon dioxide being absorbed by potash, and the oxygen by alkaline pyrogallie acid. The carbon dioxide can be estimated to 0.01 c.c. in a measurement of 1 c.c., giving an analytical error of 1 in 100. The percentages of carbon dioxide in the samples are calculated, therefore, to the second decimal place. The error in the determinations of oxygen has been ascertained in a series of

estimations of the amount of oxygen in the air of the room. The results of these analyses are given in Table i.

TABLE i.

Date.	Bar. Press. mm. Hg.	Temp. °C.	Percentage of oxygen obtained.	Average per-centage.	Range.
11. vii. 17	764.8	16.1	20.90, 20.87, 20.87, 20.88, 20.84, 20.81	20.86	± 0.05
12. vii. 17	758.0	14.7	20.93, 20.94, 20.97	20.95	± 0.02
16. vii. 17	755.6	16.5	20.87, 20.86, 20.89	20.87	± 0.02
18. vii. 17	747.0	14.3	20.89, 20.87, 20.88, 20.85, 20.90, 20.91	20.88	± 0.03

The figures in this Table for the oxygen in the air of the room have varied from 20.81 to 20.97%. These figures are all somewhat lower than 20.96, but the differences between the analyses on different days do not exceed 0.16 parts in 20.89 parts of air, and the duplicates do not vary by more than 0.09 parts in the same quantity. In the first series of experiments, a plug of glass-wool was placed in the brass tube, as proposed by Henderson, Chillingworth and Whitney(2), but this was omitted in a second series. The omission of the plug of cotton-wool has made no difference to the ease with which the breath displaced the air in the brass tube. It would appear that the expired air is driven into the tube at such a rate, that the air already in the tube is displaced by the advancing wave of the entering air. I am indebted to Mr. F. W. Carpenter for the suggestion, that the air respired into the brass tube behaves like an "explosive wave," driving the contents of the tube before it.

*Results.*—As it felt easier to breathe quickly into the empty brass tube, and as the results of the analyses show the least change along the tube, the results of the second series of experiments, in which no glass-wool was placed in the brass tube, will be described first. The figures are given in Table ii.

TABLE II.

No. of expt.	Volume of ex- pired air in c.c.	Percentage of O <sub>2</sub> in samples of expired alveolar air, col- lected at distances from the mouthpiece of				Percentage of CO <sub>2</sub> in samples of expired alveolar air, col- lected at distances from the mouthpiece of			
		25 cm.	50 cm.	175 cm.	200 cm.	25 cm.	50 cm.	175 cm.	200 cm.
1	1150	15·73	15·58	15·77	15·77	4·80	4·83	4·72	4·72
2	1250	16·40	16·42	16·47	16·46	4·52	4·54	4·45	4·41
3	1250	16·35	16·41	16·44	16·45	4·44	4·47	4·41	4·37
4	1275	15·94	16·02	16·04	16·01	4·71	4·71	4·68	4·64
5	1300	16·17	16·22	16·19	16·21	4·65	4·66	4·59	4·57
6	1300	16·32	16·40	16·58	16·61	4·42	4·43	4·32	4·32
7	1450	16·44	16·39	16·69	16·54	4·41	4·44	4·29	4·26
8	1475	15·74	15·71	15·89	15·91	5·02	5·00	4·87	4·89
9	1550	16·92	16·93	16·97	17·03	4·34	4·26	4·27	4·26

The results are arranged in the order of the volume of the expired air. The expired air has been expelled as quickly as possible from the lungs. The brass tube contains 625 c.c. between the mouthpiece and the outlet, 200 cm. from the mouthpiece. The results show that the air, filling the brass tube, in the neighbourhood of the outlets at 25 cm., and 50 cm., invariably contains slightly less oxygen, and slightly more carbon dioxide than that in the neighbourhood of the outlets at 175 cm., and 200 cm., from the mouthpiece. The difference in experiment 9 amounts to less than 0·07% oxygen, and to less than 0·04% carbon dioxide; while, in experiment 7, the difference amounts to 0·02% oxygen, and to 0·15% carbon dioxide, the greatest difference observed in this series. It would thus appear that, in a respiration of about 1300 c.c., the last 625 c.c. show a change in composition of not more than 0·15% carbon dioxide, and 0·20% oxygen. The range of variation in the composition of the last 625 c.c. to be expelled from the lungs depends on the speed with which the air is driven into the brass tube. The quicker the air enters, the more uniform is the composition of the last portion. As the period of expiration becomes prolonged, the percentage of carbon dioxide in the last part of the expired alveolar air rises, while the percentage of oxygen falls (*vide* experiment 4 of Table iii.).

A more extensive series of figures has been obtained in the first set of experiments, in which glass-wool was placed in the brass tube. The results, arranged according to the depth of the expiration, which varied from 900 c.c. to 1625 c.c., are recorded in Table iii.

TABLE iii.

No. of expt.	Volume of expired air in c.c.	Percentage of O <sub>2</sub> in samples of expired alveolar air, collected at distances from the mouthpiece of				Percentage of CO <sub>2</sub> in samples of expired alveolar air, collected at distances from the mouthpiece of			
		25 cm.	50 cm.	175cm.	200cm.	25 cm.	50 cm.	175cm.	200cm.
1	900 (duplicates)	16.62	16.58	16.69	16.68	4.24	4.21	4.11	4.08
		16.54	16.57	16.70	16.70	4.20	4.23	4.12	3.99
2	1000	16.62	16.69	16.98	17.06	3.98	3.97	3.77	3.74
3	1025	17.05	16.99	17.24	17.30	4.02	4.08	3.91	3.84
4	1050	16.68	16.86	16.92	16.95	4.37	4.14	4.24	4.17
5	1100	16.12	16.30	16.36	16.52	4.62	4.51	4.46	4.35
6	1125	15.99	16.05	16.07	16.13	4.48	4.43	4.53	4.37
7	1175	16.77	16.76	16.77	16.71	4.03	4.12	4.09	4.03
8	1175	16.56	16.57	16.75	16.74	4.28	4.27	4.22	4.15
9	1200	15.70	15.76	15.93	15.98	4.70	4.71	4.54	4.54
10	1290	16.81	16.71	16.84	16.87	4.04	4.03	3.94	3.88
11	1200	16.99	16.88	16.98	17.08	4.26	4.28	4.13	4.08
12	1300	16.62	16.70	16.83	16.90	4.17	4.13	4.05	3.99
13	1325	16.82	16.94	17.03	17.08	4.25	4.18	4.12	4.08
14	1325	16.73	16.77	16.97	17.00	4.23	4.22	4.10	4.04
15	1325	16.27	16.28	16.30	16.39	4.39	4.40	4.35	4.30
16	1325	16.61	16.70	16.80	16.81	4.04	4.06	3.88	3.88
17	1350	16.37	16.42	16.39	16.48	4.20	4.17	4.13	4.10
18	1400	15.90	15.96	16.08	16.10	4.76	4.70	4.71	4.63
19	1425	16.26	16.35	16.54	16.60	4.31	4.31	4.19	4.13
20	1425	16.23	16.30	16.47	16.56	4.29	4.30	4.18	4.13
21	1450	16.47	16.54	16.64	16.66	4.57	4.58	4.53	4.51
22	1450	16.64	16.56	16.63	16.66	4.22	4.31	4.27	4.24
23	1625	16.40	16.35	16.51	16.56	4.41	4.32	4.18	4.22

The figures show that the expired air, which is collected at a distance of 200 cm. from the mouthpiece, contains more oxygen and less carbon dioxide than that leaving the lungs at the end of expiration. In two experiments, viz., Nos. 7 and 22, there appears to be no evidence of any change in composition in the portion of expired alveolar air examined. In experiment 2, the difference amounts to 0.36% oxygen, and to 0.22% carbon dioxide, the

greatest difference observed in this investigation. The total volume expired in experiment 2 is 1000 c.c., so that the composition of the last 600 c.c. does not vary by more than 0.36% oxygen, and 0.22% carbon dioxide. In experiment 9, which is typical of many, the oxygen has decreased 0.22%, and the carbon dioxide diminished 0.16%.

From these two series of experiments, it appears that the carbon dioxide increases slowly in the expired air during a rapid expiration. The rate of increase varies, but, in the last 600 c.c. expelled from the lungs, the increase is not more than 0.22% carbon dioxide, while it is not more than half this figure in 17 out of 32 experiments.

*Discussion of Results.*—Two methods have been used recently to examine the composition of successive portions of the air expired. Haldane(3) has measured the percentage of carbon dioxide in the final portion of the air breathed out during a series of expirations of increasing depth. He has concluded, from his results, that the deeper part of an expiration contains no more carbon dioxide than the middle part. Krogh and Lindhard(4) have examined successive portions of the air breathed out during work, the samples being collected at intervals of some hundredths of a second. They have found that each successive portion of the expired air contains more carbon dioxide than that which precedes it, and less than that which follows it. They have been unable to apply this method to the examination of the breath expired when the body is at rest, but they have employed a modification, whence they conclude that the percentage of carbon dioxide rises slowly at the end of a normal respiration, the curve of the concentration of carbon dioxide in the breath showing a marked tendency to become asymptotic.

The method used in this paper makes it possible to examine the composition of a part of the air of the same breath to a certain degree of accuracy. When the analysis is made sufficiently delicate, it is possible to detect a change in the composition of the final 600 c.c. expelled from the air-passages. The concentration

of carbon dioxide increases by a variable amount in different experiments, but never more than 0.22%, while the increase can hardly be detected in some experiments. The percentage of oxygen diminishes by not more than 0.36%.

In considering which of these methods yields the most correct information as to the composition of the expired air, it may be noted that Haldane's experiments have been made on different respirations. His method of estimating carbon dioxide has been sufficiently accurate to detect the change of composition, but the fact that he obtained his samples at various depths of respiration from different breaths has not permitted him to recognise small differences in concentration. Krogh observed the change of concentration during work by analysis of successive samples collected at consecutive intervals of time, but, for conditions of rest, his curve was mainly the result of interpolation. In the method employed in this investigation, the analyses are made on successive portions of the same respiration. When the gaseous analysis has only been capable of recognising the change of concentration of 0.2% in the amount of carbon dioxide, the composition of the final 600 cc. expired has appeared uniform; but, with greater delicacy, a change in concentration of not more than 0.22% of carbon dioxide, and 0.36% oxygen has been observed.

These results are in accord with the results of Krogh and Lindhard. They support Lindhard's contention (4), that the final portion of the expired air has not the same composition as the air in the alveoli of the lungs, since the portion of the alveolar air in the trachea and bronchioles will contain more carbon dioxide, than the air expelled from the mouth, as it leaves the alveoli later.

#### *Conclusions.*

(1) Estimations of the percentage of oxygen and carbon dioxide in the successive portions of air rapidly expelled from the lungs, show that the concentration of carbon dioxide diminishes by not more than 0.22% in the final 600 c.c., respired, and that the concentration of oxygen augments by not more than 0.36% in the same portion of the breath.

(2) The change in concentration is independent of the depth of respiration, but depends on the speed with which the air is expired. The more slowly the air is breathed out, the greater the change in concentration of the gases of the final portion.

(3) These results lead to the inference, that the alveolar air in the pulmonary atria at the end of an expiration contains a lower concentration of oxygen and a higher concentration of carbon dioxide, than the air last expelled from the mouth in the rapid expiration.

I beg to express my indebtedness to Professor Sir Thomas Anderson Stuart, in whose Department this research has been conducted, and to thank Dr. H. G. Chapman for his encouragement and assistance in this work.

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*Addendum.*—Seven sets of analyses with Haldane's apparatus were made on the contents of the brass tube after washing it out with gaseous mixtures containing known amounts of carbon dioxide. The results demonstrated the completeness of the replacement.

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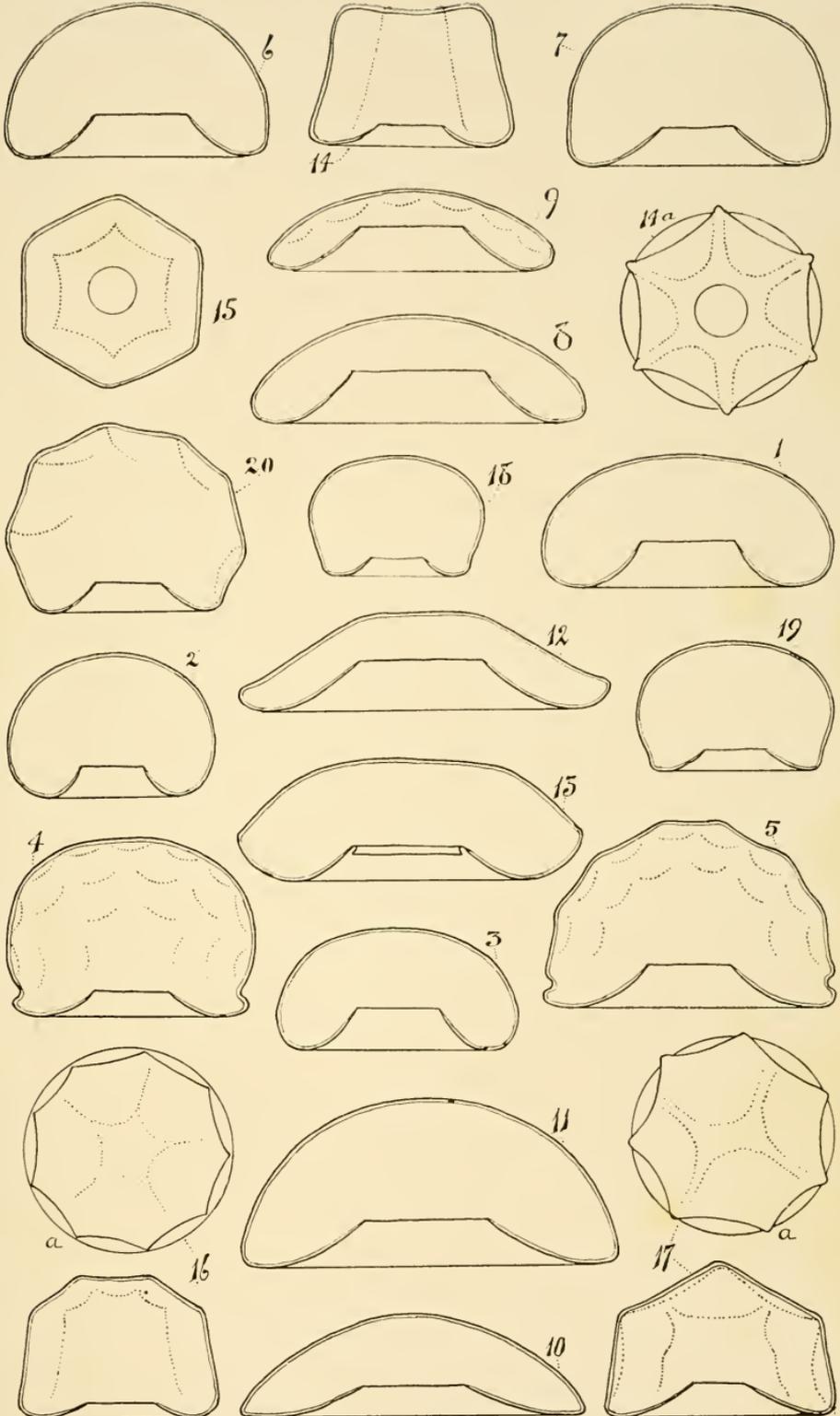
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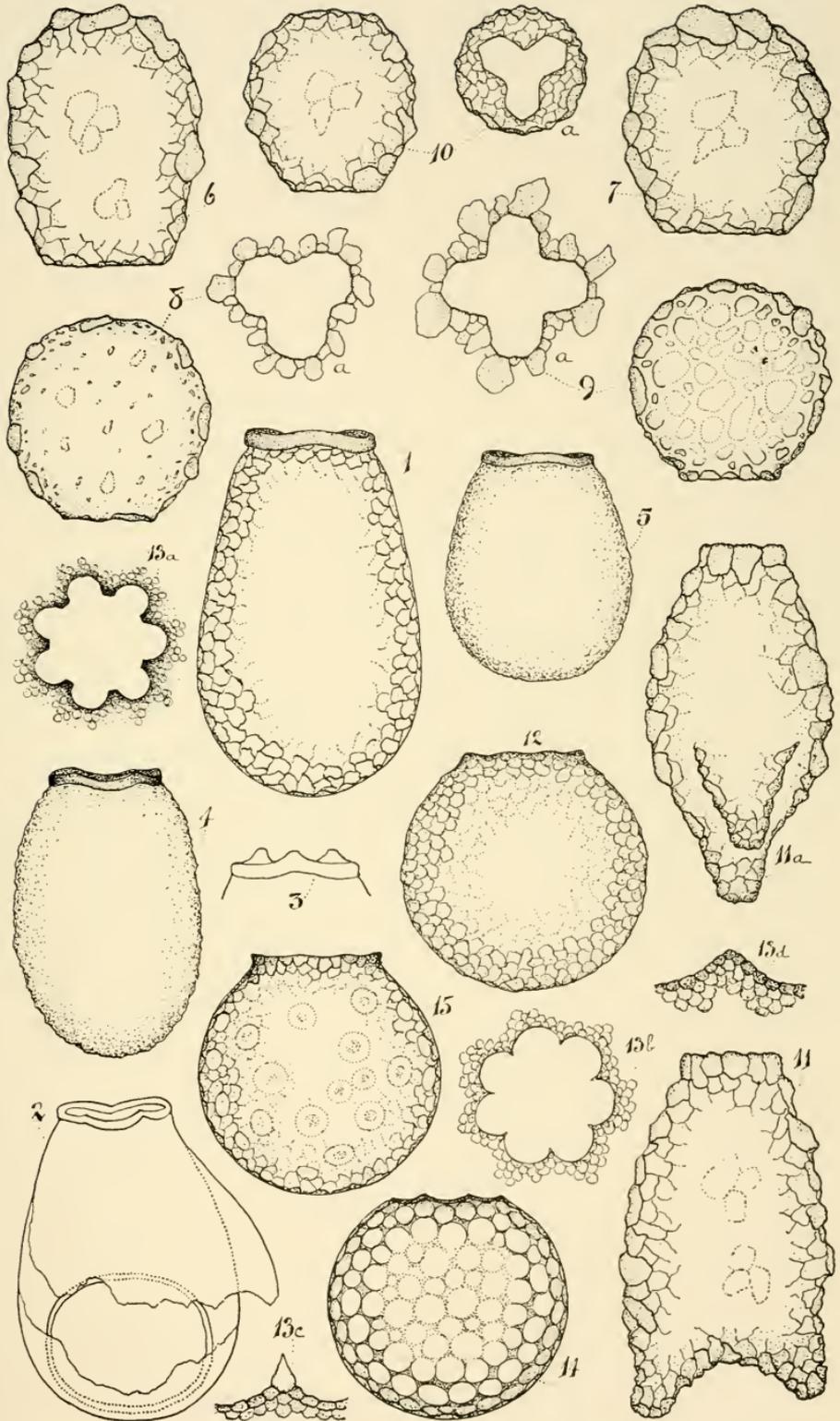






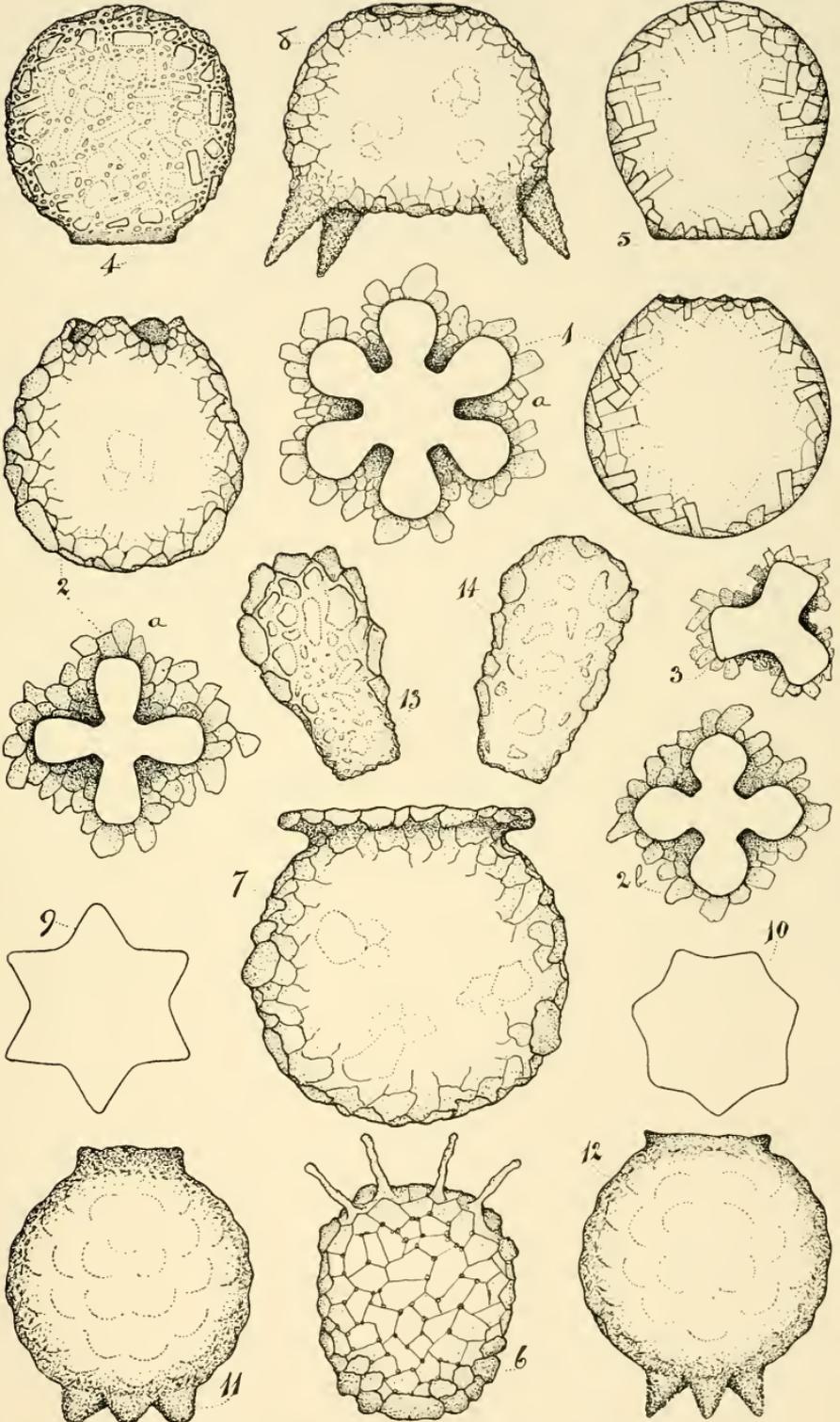
Rhizopods of Sydney and Lismore, N.S.W., (*Arcella*).





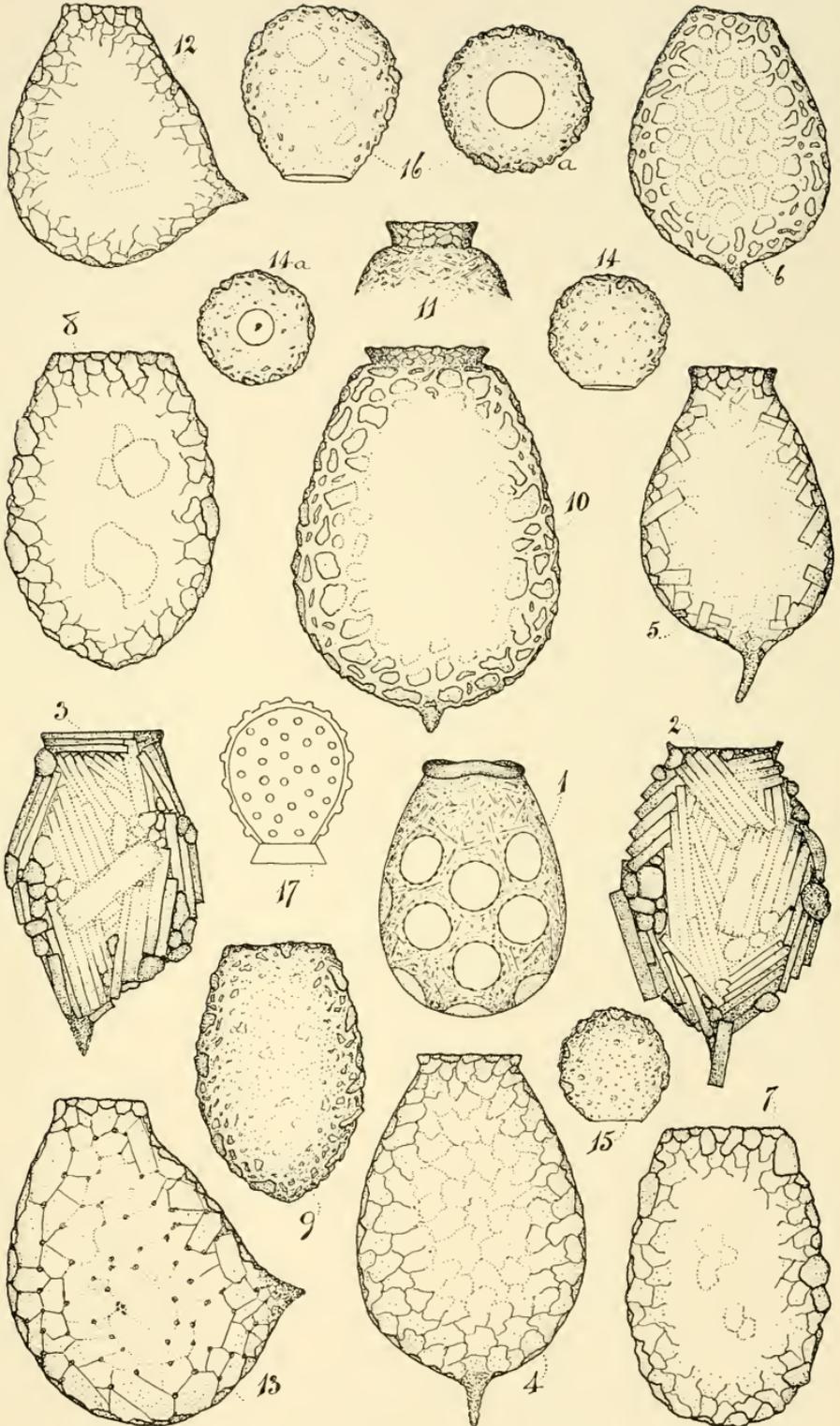
Rhizopods of Sydney and Lismore, N S.W., (*Difflugia*).





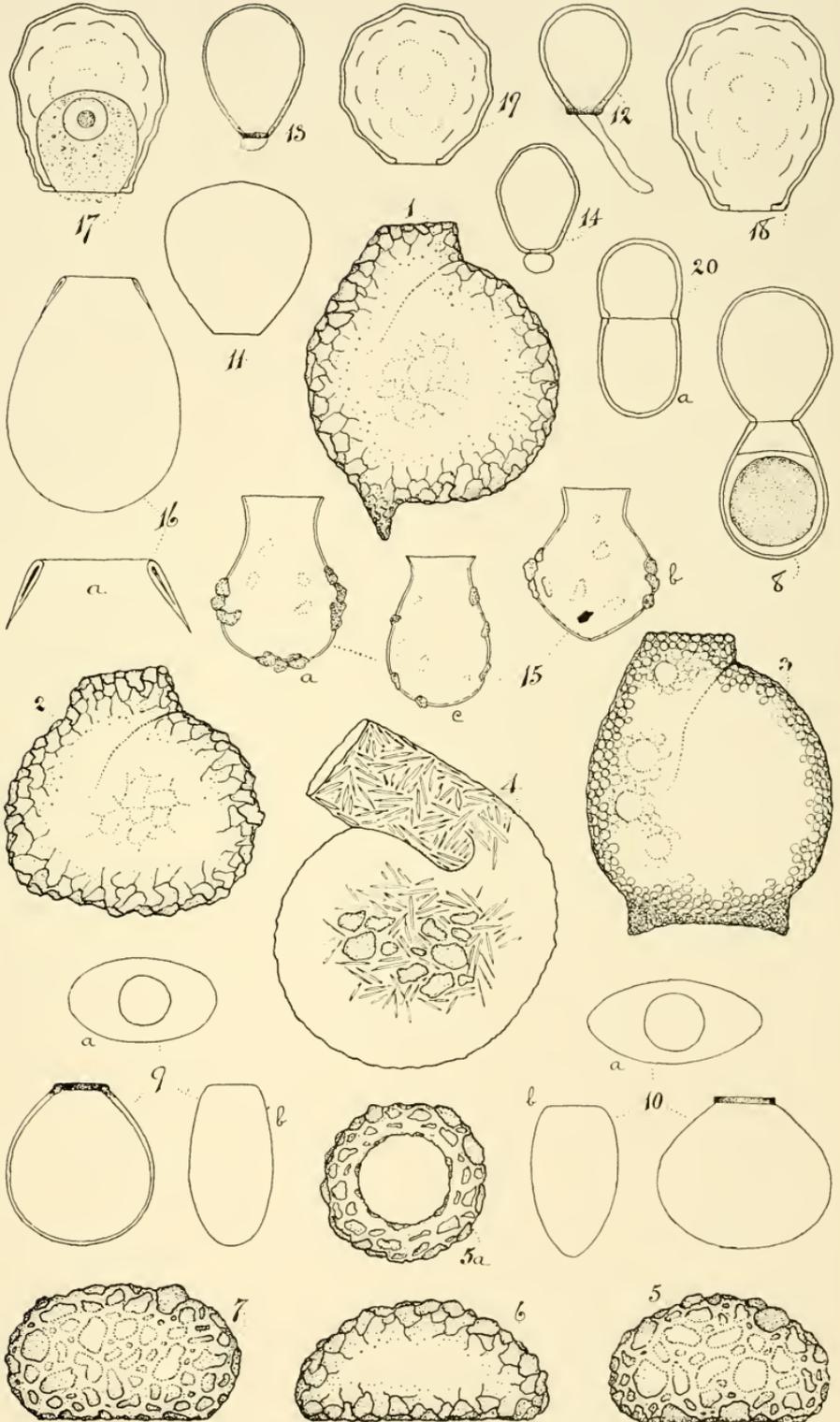
Rhizopods of Sydney and Lismore, N.S.W., (*Difflugia*).





Rhizopods of Sydney and Lismore, N.S.W., (*Difflugia*, *Cucurbitella*).



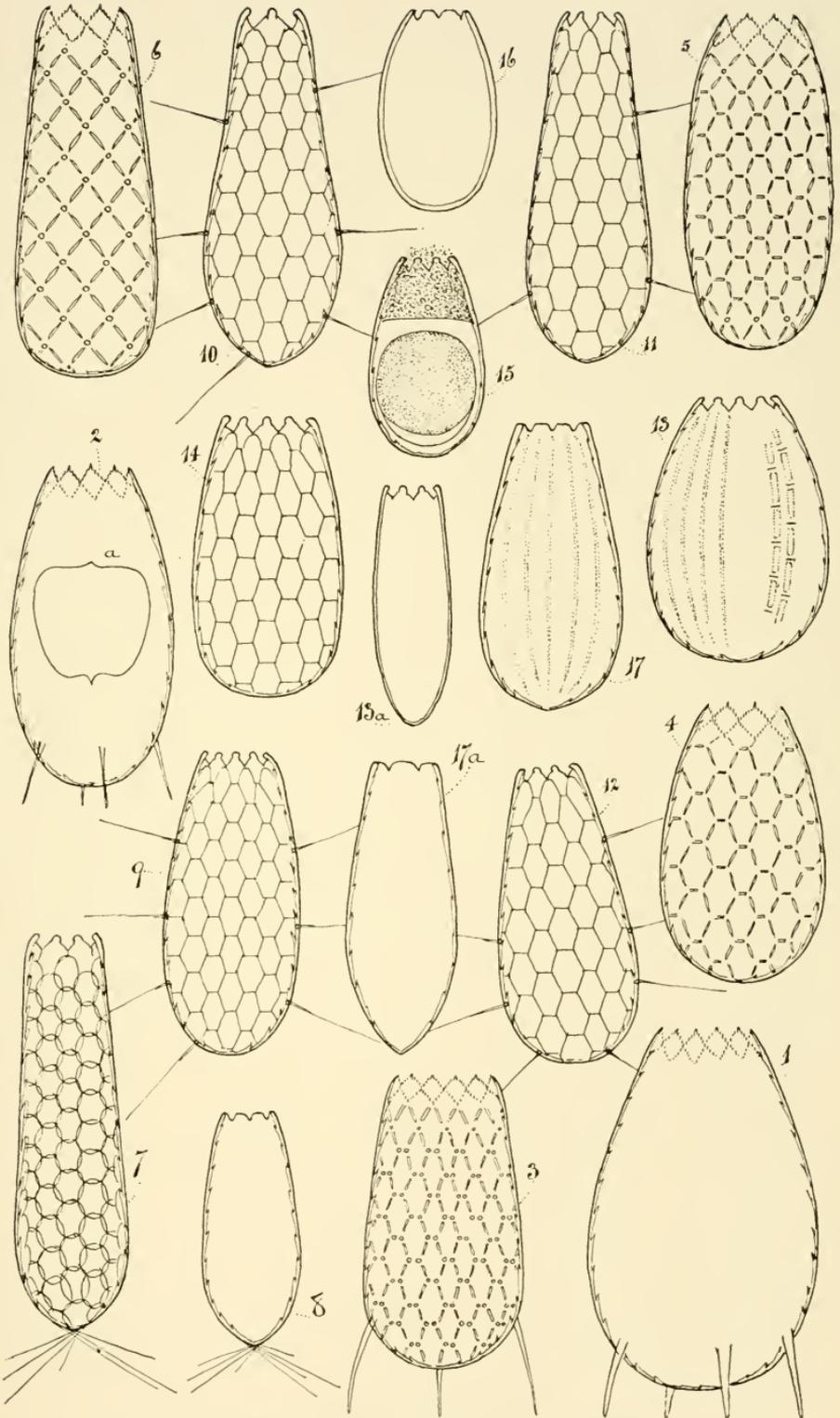


Rhizopods of Sydney and Lismore, N.S.W., (*Lesquerusia*, *Phryanella*, *Cryptodifflugia*).



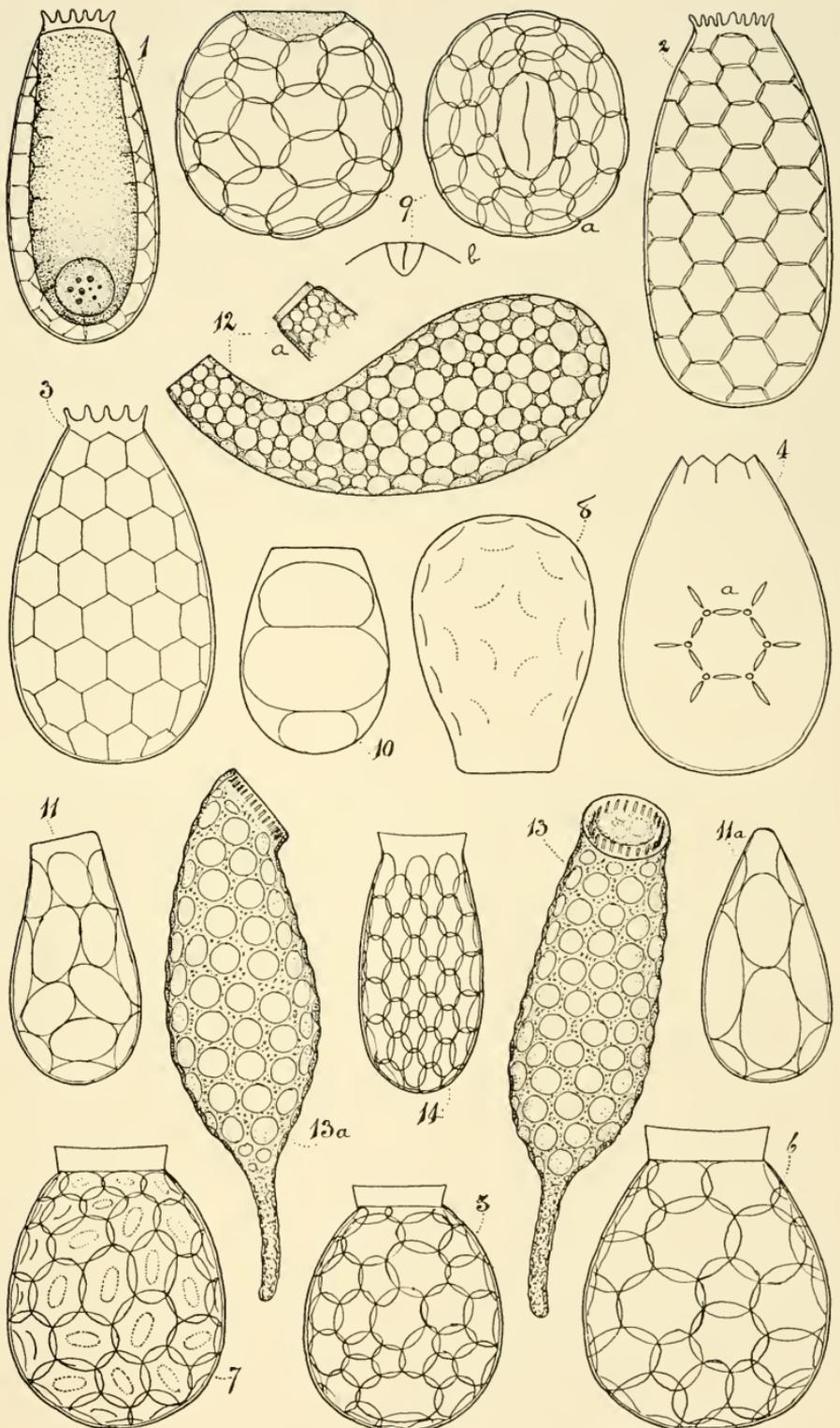






Rhizopods of Sydney and Lismore, N.S.W., (*Euglypha*).





Rhizopods of Sydney and Lismore, N.S.W., (*Sphenoderia*, *Trinema*, *Cyphoderiopsis*).



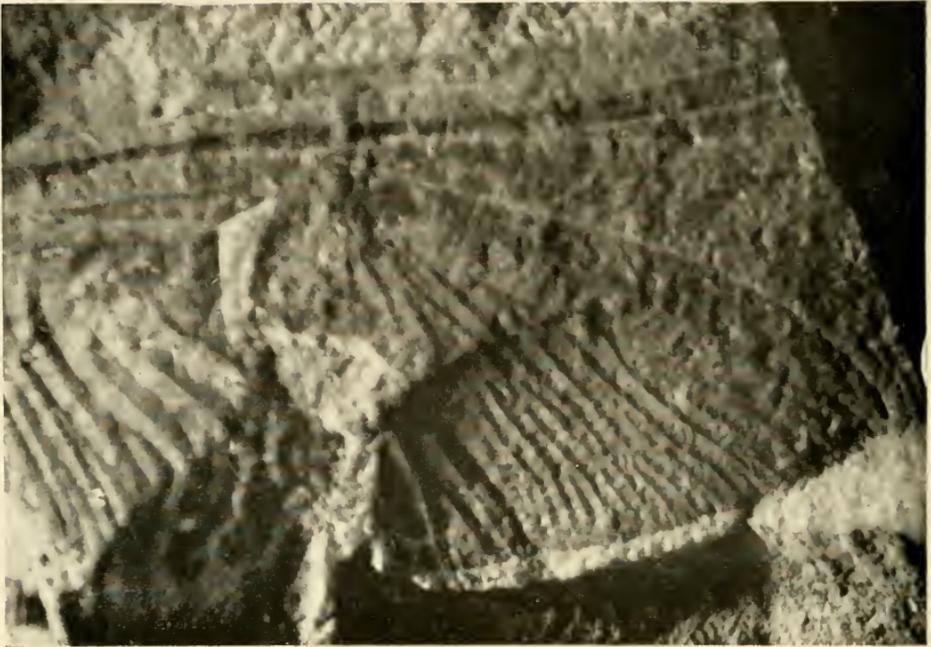


Fig. 9. *Eschuidiopsis hindersiensis* (Woodward); hindwing; ( $\times 4$ ).



Fig. 10. *Eschuidiopsis hindersiensis* (Woodward); hindwing; ( $\times 4$ ).



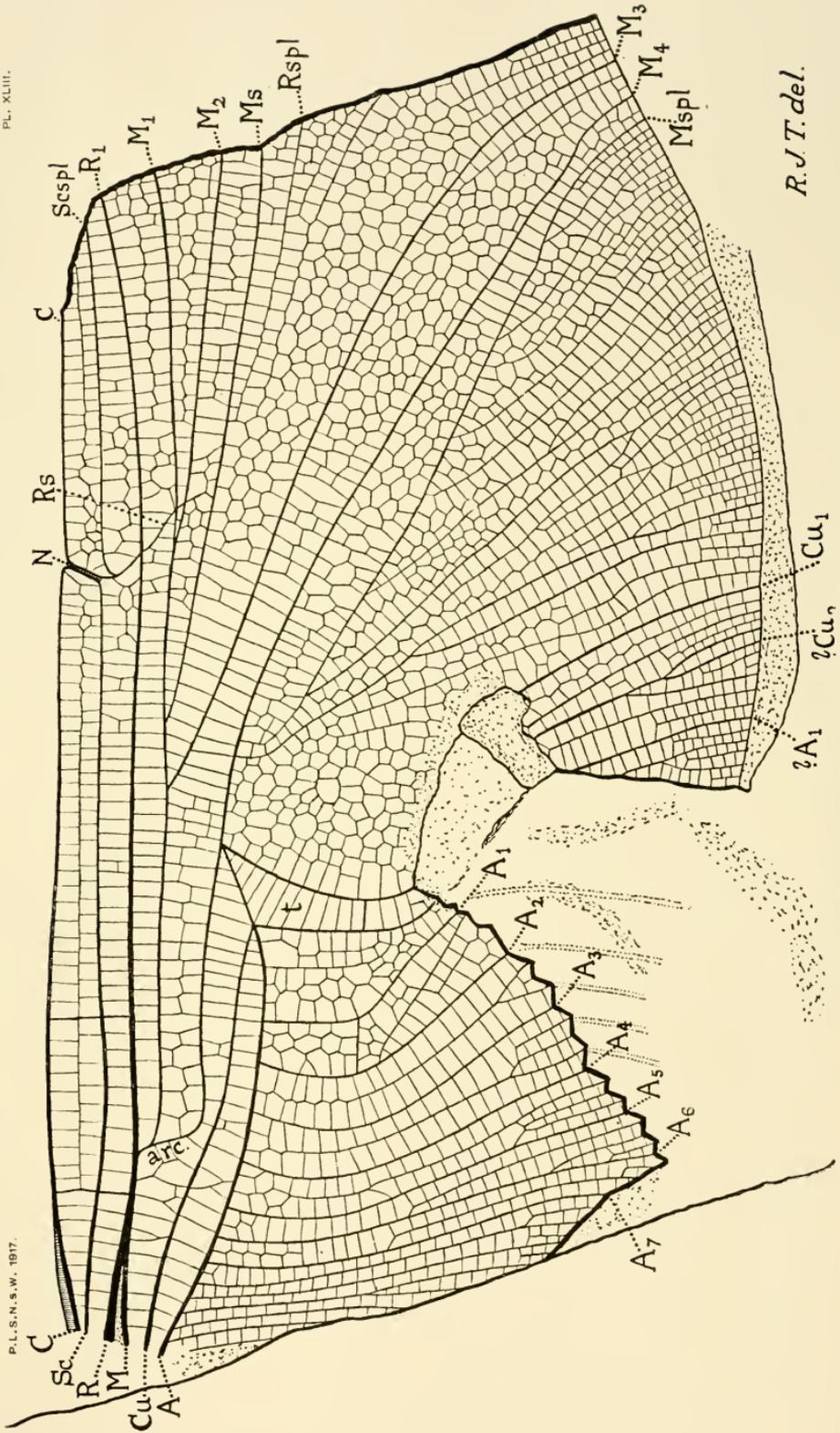
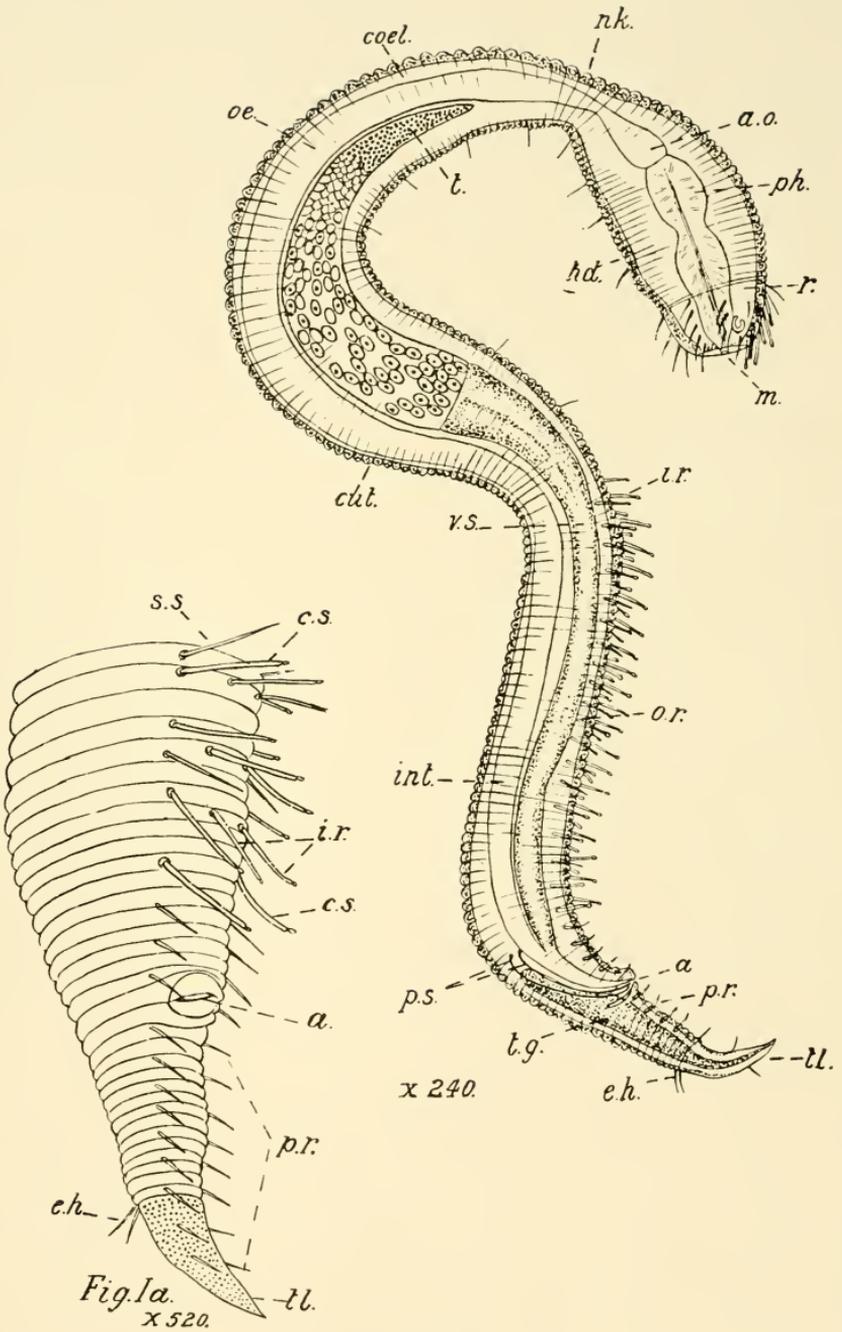


Fig. II. *Eschscholopsis flindersiensis* (Woodward); hindwing; ( $\times 6$ ).





*Chatosoma falcatum*, sp. n.



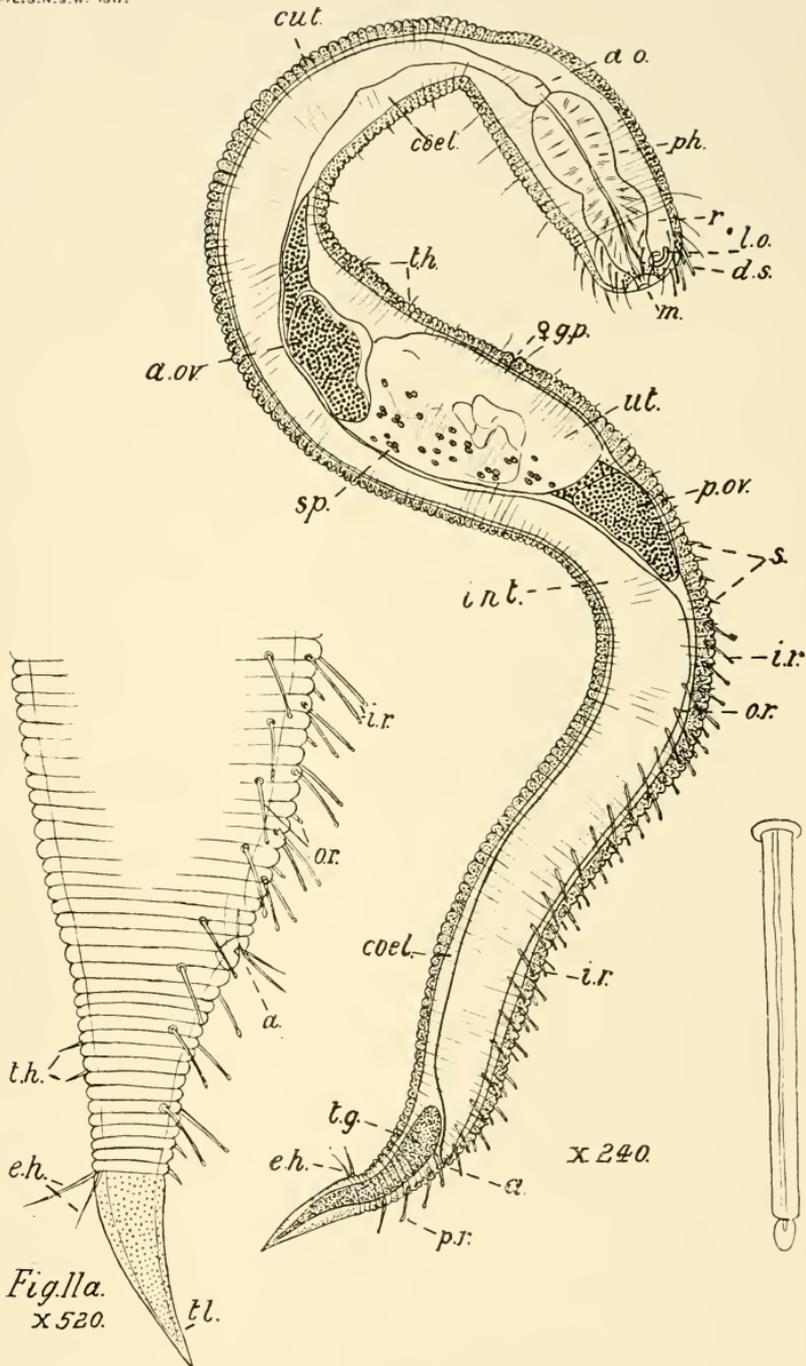
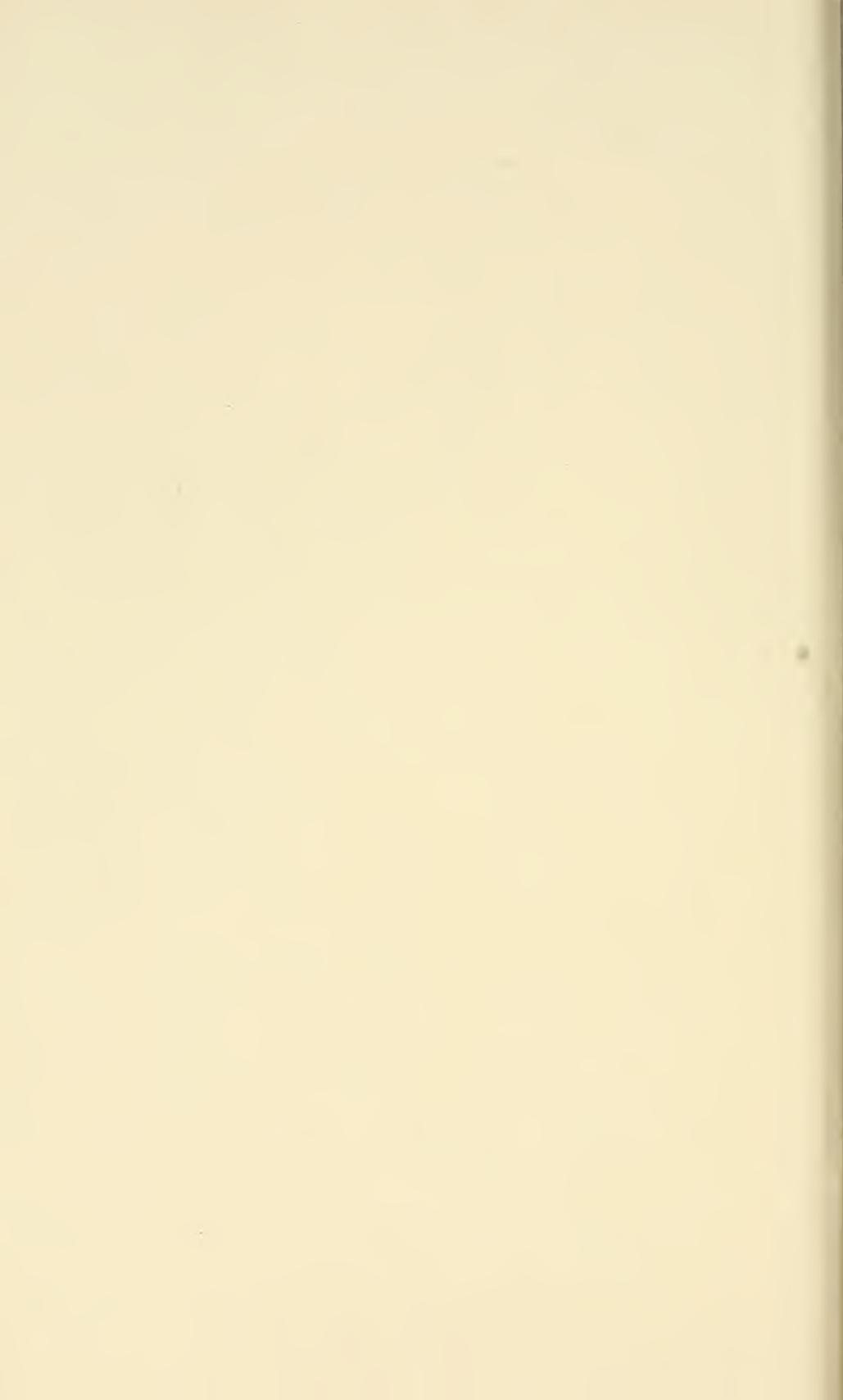
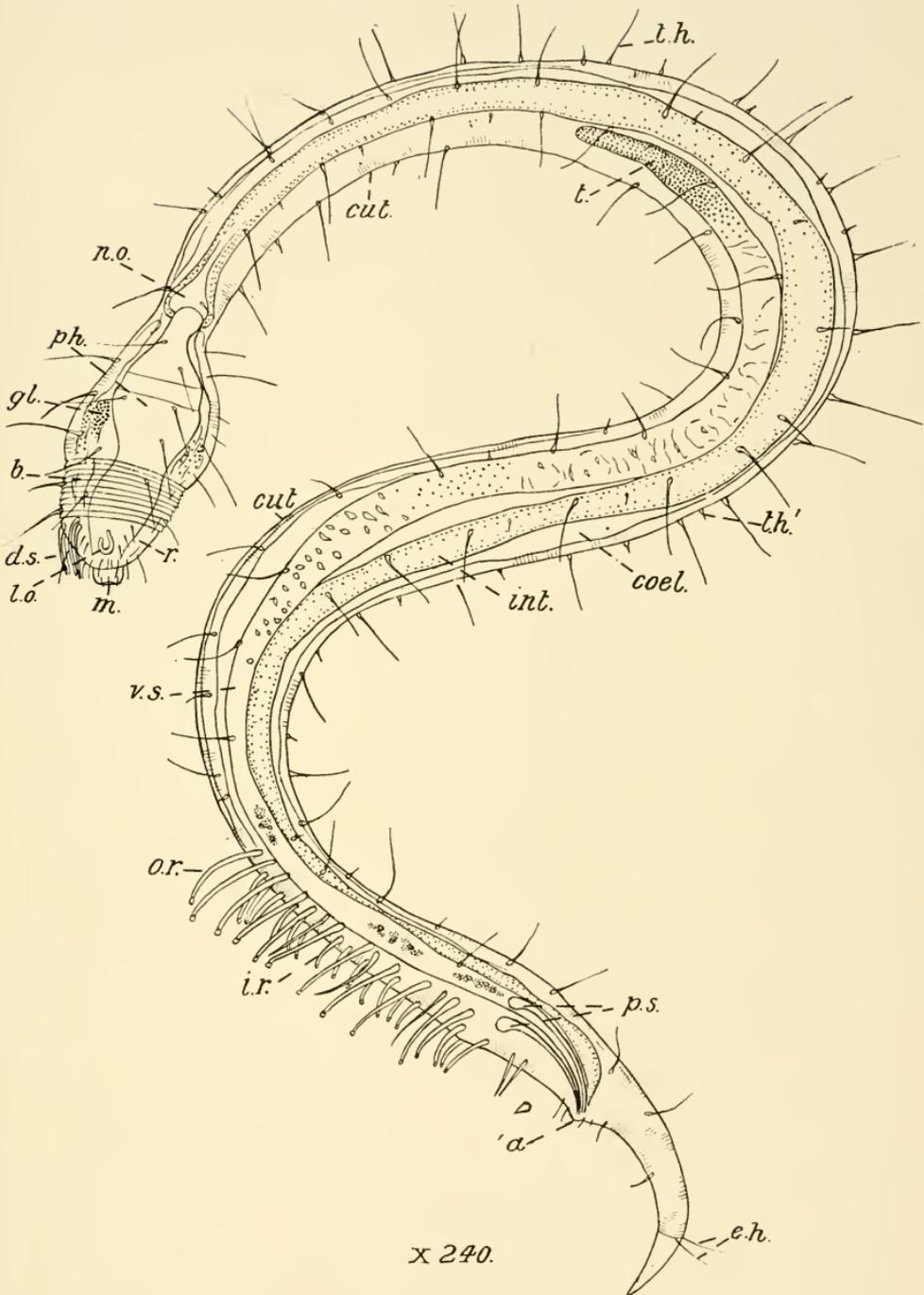


Fig. 11a.  
x 520.

Fig. 11b.

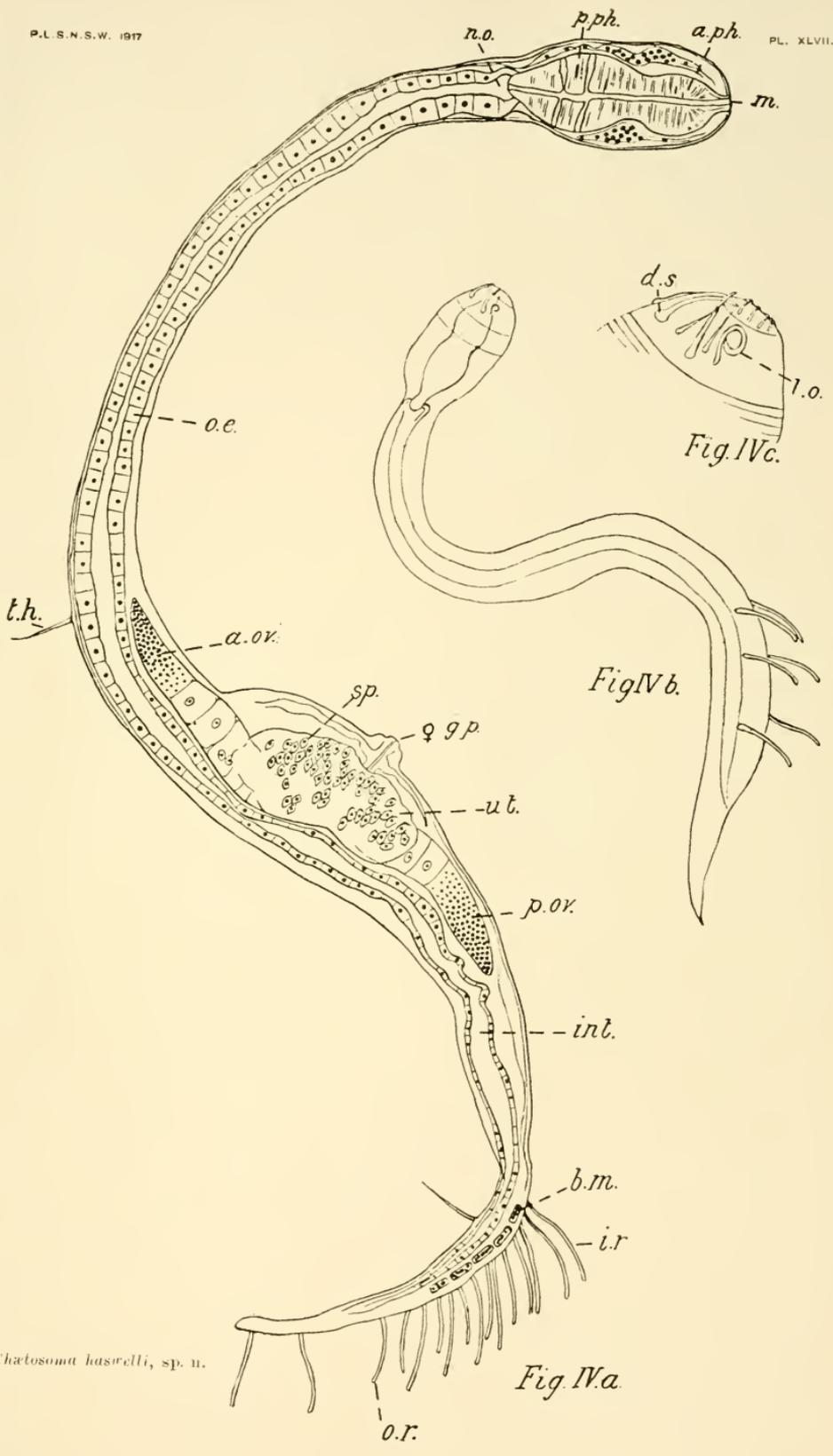
*Chelosoma falcatum*, sp. n.





*Chatosoma haswelli*, sp. n





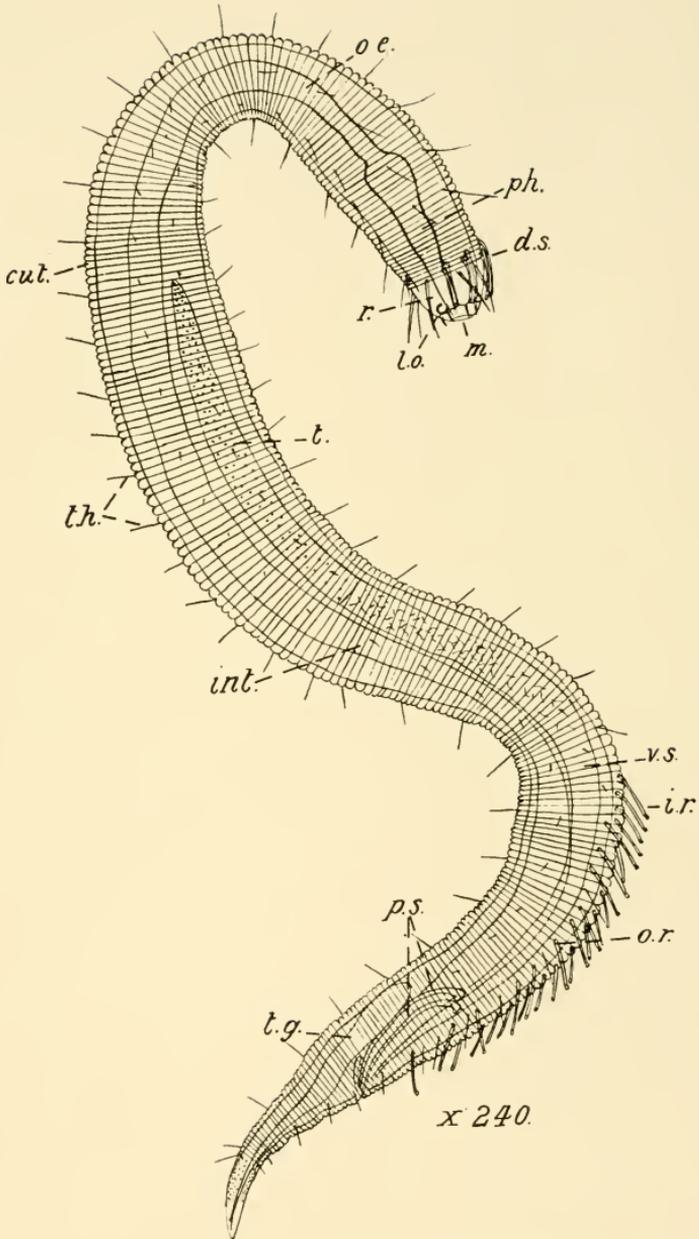
*Chatosoma haswelli*, sp. n.

Fig. IVa

Fig. IVc

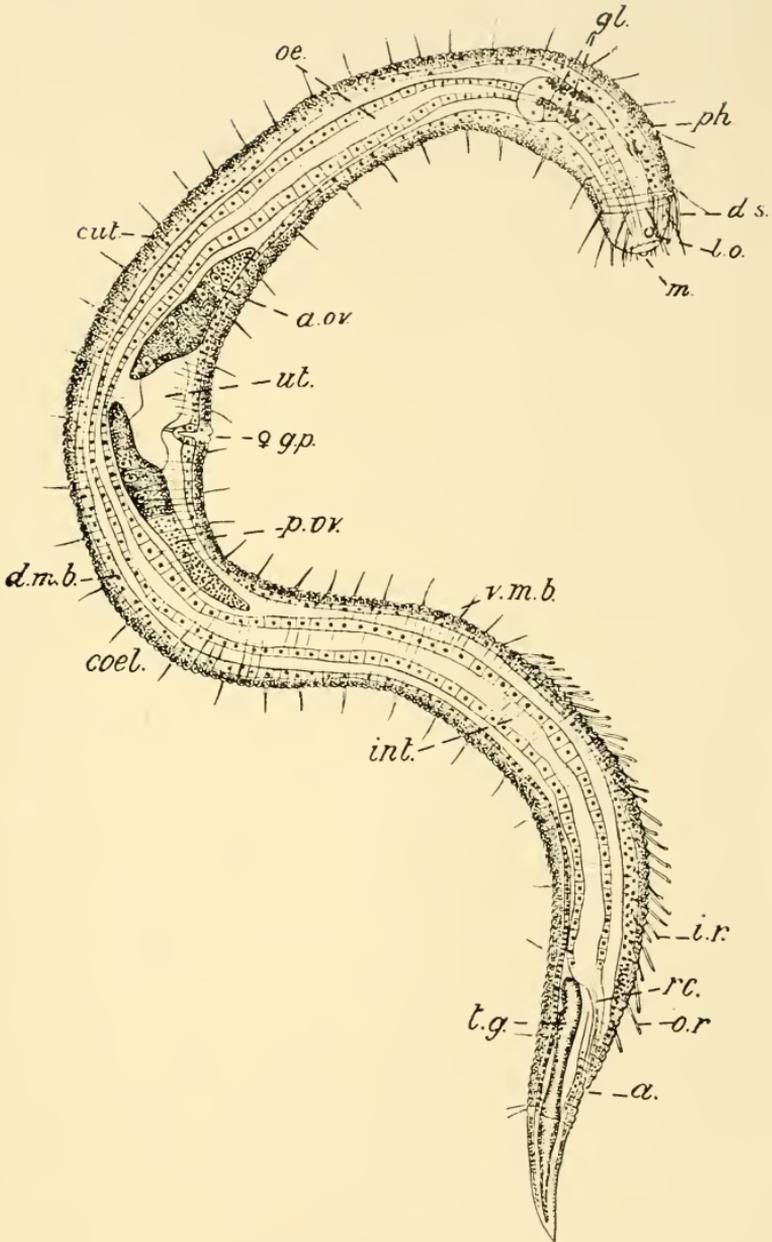
Fig. IVb





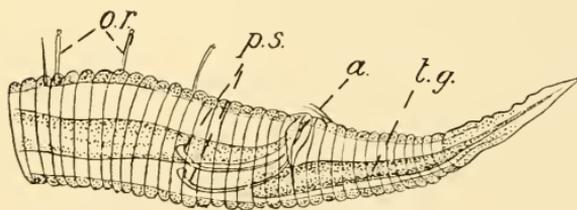
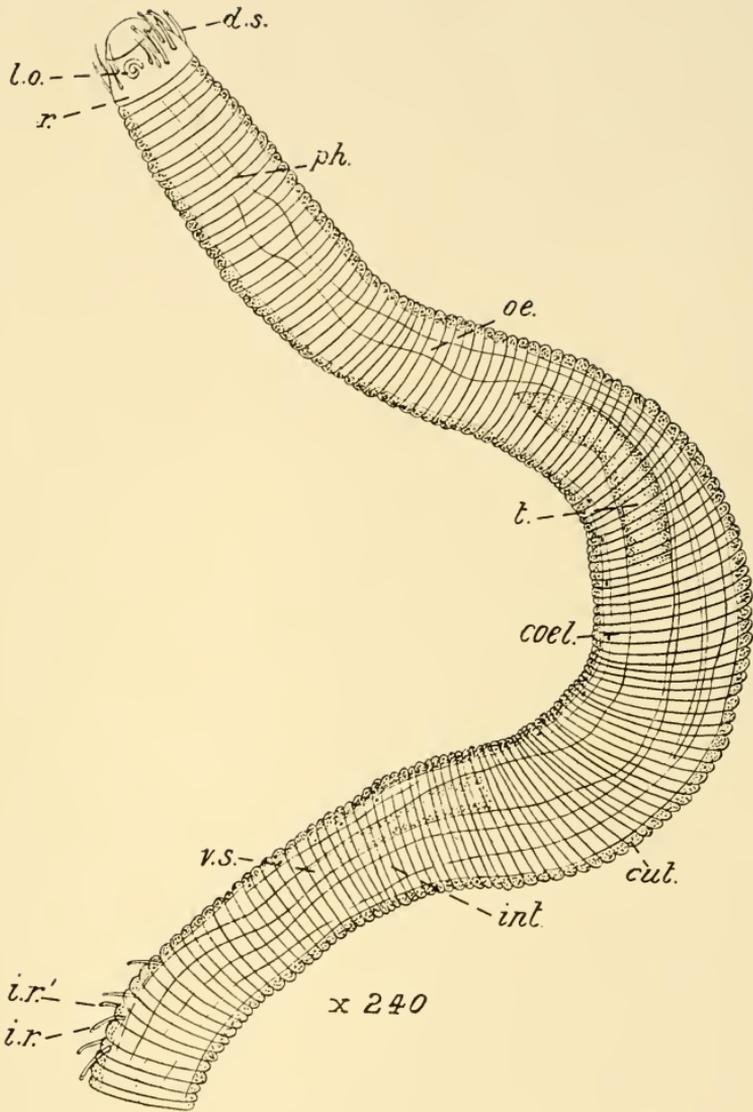
*Notochatosoma tenax*, g. et sp. n.





*Notochatosoma tenax*, g. et n. sp.





*Notochatosoma cryptocephalum*, sp. n.



Issued 11th July, 1917.

Vol XLII.

Part 1,

No. 165

THE  
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1917

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Vol XLII.

Part 2,

No. 166

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NEW SOUTH WALES

FOR THE YEAR

1917

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[Plates xvi.-xxii.]

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## THE SINGLE CELL CULTIVATION OF YEAST.

BY R. GREIG-SMITH, D.Sc., MACLEAY BACTERIOLOGIST TO THE SOCIETY.

The method of isolating single cells of yeast by means of the pen, and growing them in tiny drops of nutrient fluid on cover-glasses in a moist chamber, as recommended by Pául Lindner, was a great advance upon the older gelatine process as practised by Hansen. The Lindner-method is in general use at the present time. It has some disadvantages, however, as will be recognised when the method, which I am about to describe, has been tried.

The pen acts by the capillary nature of its split, and it is a simple step to adopt a glass capillary, such as may be obtained by drawing out a heated piece of glass tubing until the tube is of the necessary bore. A four-inch piece of glass tubing of 4 mm. bore, heated in the bunsen flame until soft, and drawn out to about thirty inches, will furnish several suitable capillaries. The heating sterilises the glass, and the capillary is ready for use when broken or cut into short lengths of, say, five inches. It is better to cut the capillary with a fine file to ensure a clean cut. A broken end will not make a good contact with the cover-glass, when the yeast-suspension is spotted. If the hand is used to cut or break the tube, the capillary can be sterilised by passing it rapidly through the flame before using.

The capillary is dipped into the suspension of yeast-cells, and inclined at an angle. The liquid rushes up the capillary but soon stops. The capillary is withdrawn, and 16 to 20 spots are dotted upon a sterile cover-glass, just as in the Lindner-method. The size of the spot can be regulated by inclining the capillary more or less to the vertical, and by the duration of contact with the cover-glass. The aim is to have the spot of such a size as can be included in the field of the microscope.

6. TOPSENT, E.—“Contributions à l'Etude des Spongiaires de l'Atlantique Nord.” Resultats des Camp. Scient. du Pr. de Monaco, 1892, Fasc. ii., p.116, Pl. xi., fig.7.
7. —————“Diagnoses d'Eponges Nouvelles de la Méditerranée et plus particulièrement de Banyuls.” Arch. Zool. Exp. et Gen., 1892, (2), x., Notes et Revue, p. xxv.
8. —————“Materiaux pour servir à l'Etude de la Faune des Spongiaires de France.” Mém. Soc. Zool. France, 1896, ix., p.121.
9. —————“Spongiaires des Açores.” Resultats des Camp. Scient. du Pr. de Monaco, 1904, Fasc. xxv.; (a) p.152, Pl. i., fig.10; Pl. xiii., fig.13; (b) p.138.
10. —————“Spongiaires de l'Expédition Antarctique Nationale Ecossaise.” Trans. Roy. Soc. Edinburgh, 1913, xlv., Part 3, p.616, Pl. i., fig.4; Pl. vi., fig.15.
11. WHITELEGGE, T.—“Scientific Results of the Trawling Expedition of H.M.C.S. ‘Thetis’.—Sponges, Part ii.” Mem. Austr. Mus., iv., Part 10, 1907, pp.509, 512.

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EXPLANATION OF PLATES XXI.-XXII.

Plate xxi.

*Rhabdosigma mammillata* Whitelegge.

Fig.1.—Longitudinal (desarcodised) section of the skeleton (of a digitiform lobe), showing extra-axial region thereof and also portion of the axial region; ( $\times 15$ ).

Fig.2.—Entire specimen; ( $\times \frac{2}{3}$ ).

*Echinaxia frondula* Whitelegge.

Figs.3, 4.—Pattern of the skeleton as shown in longitudinal section parallel to and in the mid-plane of the (lamelliform) sponge. Fig.3 shows more distinctly the longitudinal spicule-strands; in Fig.4, which is from a thinner section, many long interstitial megascleres are also to be seen; ( $\times 50$ ).

Plate xxii.

*Echinaxia frondula* Whitelegge.

Figs.1, 2.—Skeleton as seen in longitudinal section perpendicular to the surface of the (lamelliform) sponge. The desarcodised section (shown in Fig.1) is from near the upper margin of the sponge; the other (undesarcodised) is from near its base; ( $\times 50$ ).

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## CORRIGENDA.

Page 47, line 7, and p.48, line 17—*for* Text-fig.1, *read* Text-fig.3.

Page 60, line 7—*for* formation, *read* function.

Page 70, last line of note—*for* nucleus of *end.*, *read* nucleus of alveolar tissue.

Page 98, line 5 }  
 Page 99, line 29 } *for* *Argiocyemis*, *read* *Argyocyemis*.

Issued 22nd December, 1917.

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[Plates xxiii.-xxxiii.]

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