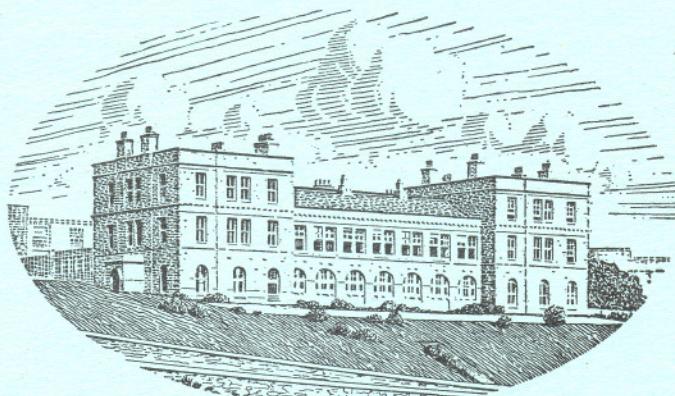


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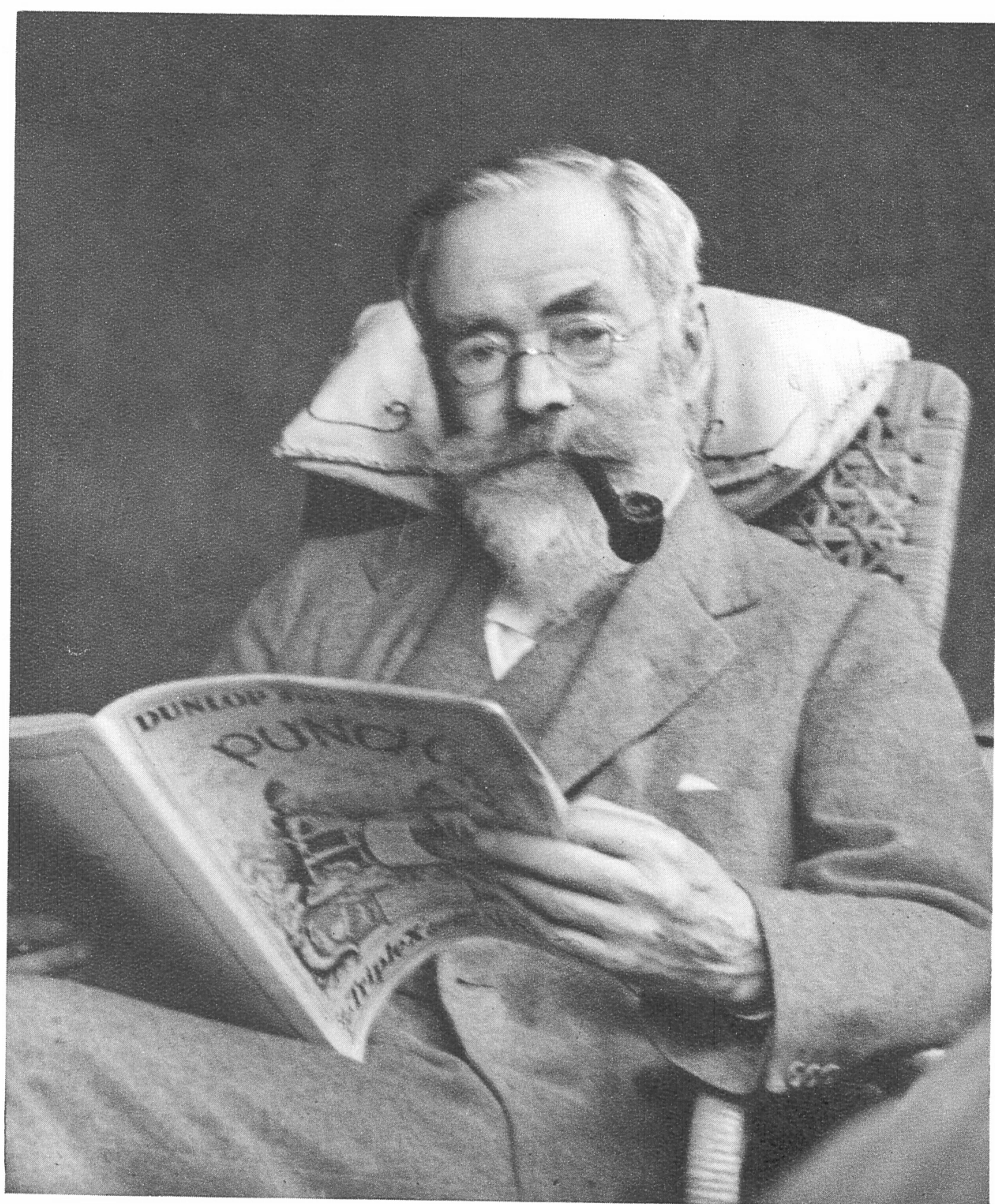
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Geoff Biddle

## GEORGE PARKER BIDDER

1863-1953

The death of George Parker Bidder in his 91st year on 31 December 1953 has removed our last personal link with the days of the early development of the Marine Biological Association and its struggle for existence between the end of last century and a few years before the First World War. After the death of E. J. Allen in 1942 the only other survivor who had played a major part in the story besides Bidder was Walter Garstang, who died in 1949. Apart from those like Allen and Garstang, who were directly responsible for carrying out the work for which the Association was founded, no one contributed more vitally to its final success than Bidder, whose shrewd counsel and wise benefactions were undoubtedly factors in the survival of the Plymouth laboratory. In these days of recognition by the Government of the needs of science it is not easy for us to appreciate what the difficulties must have been for those who had to prove to an unresponsive audience that marine biological research was worth while. Those men were remarkable in their generation, and one of the most remarkable of them was G. P. Bidder.

George Parker Bidder was born in London on 21 May 1863. His life fulfilled the expectations aroused by the endowment of unusual gifts and characteristics passed to him through his parents. His father, George Parker Bidder, Q.C., was the son of George Parker Bidder, C.E., the 'calculating boy'. His mother was Anna McClean; her father was the engineer J. R. McClean, F.R.S., and her brother was Frank McClean, F.R.S. From his father's side George Parker Bidder the third inherited, among many gifts, especially his power of calculation and his unusual memory; from his mother's side came in part, at least, that remarkable vision and judgement which led him to do so many things at the right time, and his ability to handle people with ease and harmony.

His grandfather, George Parker Bidder the first, was born at Moreton-hampstead on the north-eastern borders of Dartmoor in 1806. The son of a stone-mason, he had an amazing natural ability for calculation, and was taken round the countryside for profit as the 'calculating boy'. Thanks to the intervention of certain gentlemen he was enabled to have University training and eventually became a leading railway engineer, collaborating with the Stephensons. In later years he owned an estate at Mitcham, south of London, known as Ravensbury Manor, where his grandson often stayed as a child. Later, when

The frontispiece is from a photograph taken in 1934 by Ramsey and Muspratt, of Cambridge, who have kindly given permission for its reproduction.

owing to ill-health he moved to Ravensbury, Dartmouth in Devon, his son occupied Ravensbury Manor, Mitcham, and it became the grandson's home.

George Parker Bidder, the third, was educated at King's Preparatory School, Brighton, and at Harrow, where he was in the sixth under Dr Butler. While at Harrow he won the Prize Poem in 1881. On leaving Harrow Bidder spent one year at University College, London, on the advice of F. M. Balfour, attending the lectures of Ray Lankester, virtually the founder of the Marine Biological Association, its first Honorary Secretary, and its President from 1890 to 1929. After completion of this year he went as an Exhibitioner in Mathematics and Science to Trinity College, Cambridge, where he took the Natural Science Tripos in 1884 and 1886. On his arrival in Cambridge, Balfour having recently died, the lecturers in zoology were W. F. R. Weldon and S. F. Harmer. On one occasion these two took young Bidder shore-collecting in Guernsey and it is possible that this strengthened an interest in marine biological research apparently first aroused by reading Jules Verne's *Twenty Thousand Leagues Under the Sea*; although as a boy he had not been without experience of the sea, having cruised from Dartmouth in his grandfather's yacht *Mayfly*. He was later to own his own yachts the *Chanceit* and *Myrtle* in which he used to sail at Plymouth.

On the completion of his degree Bidder went to the Zoological Station at Naples where he held the Cambridge University table in 1887, '88 and '89, and was the guest of Anton Dohrn in 1890, '91 and '93. It was during these years that he did much of his experimental work on sponges which was not to see publication until some thirty years had passed.

While in Naples Bidder, whose appreciation of visual beauty was always acute, became much interested in sculpture and archaeology; this interest led later to a small publication entitled *Arcus*, giving convincing arguments that this word was used for bow-drills. During this period, also, he purchased a hotel in Naples which he called 'Parkers' and ran successfully for many years, an act which has now become legendary.

Bidder also became extremely proficient in long-distance and underwater swimming; he would no doubt have been fascinated by the possibilities for underwater research opened up by modern free-diving appliances.

Bidder made his first visit to the Plymouth laboratory in 1890, but he did not work there until 1893, being recorded in the Reports of Council for that and the succeeding two years as working on 'sponges'. In 1893 E. J. Bles was Director and E. J. Allen was a visiting research worker. It was then that Allen and Bidder met for the first time, and it was no doubt an appreciation of Allen's greatness, coupled with the deep friendship that developed between them, that moved Bidder to devote so much loving thought to the affairs of the Association. Allen became Director on 12 January 1894. One of his first publications after taking up his duties was a report on the sponge fishery of Florida and the artificial culture of sponges, prepared at the request of the

Colonial Office, with a view to the introduction of sponge culture in the Bahamas. Bidder was by then an authority on sponges, and it was natural that Allen should have called on him for assistance. Allen's report was followed in the *Journal* by a note by Bidder on projects for the improvement of sponge fisheries. Perusal of this paper shows how far ahead of his time Bidder was in his functional outlook. A reminder of his experience as an underwater swimmer also is to be found in his suggestion that naked divers should be encouraged to try the use of water spectacles: 'while every student knows that the imperfection of the submerged human eye can be corrected by convex lenses, there is a wide gulf of ignorance separating the student from the pearl-diver of the Indian Ocean'.

From 1893 to 1896 Bidder was a regular visitor at Plymouth, but in 1896 his father died and a great amount of business fell on his shoulders. Amongst other things he inherited interests in a dock, a dry dock, a colliery, a Danish gas company, a derelict Cornish lead mine, and a farm at Mitcham. He is not recorded among the list of visiting research workers at Plymouth again until the year 1899 when he married Marion Greenwood, a physiologist of some distinction, and they came to live in Plymouth. The Bidders remained in Plymouth until 1902 after the birth of their elder daughter Caroline, now Mrs Barclay Russell and the mother of two girls and a boy of whom their grandfather was very proud. They then moved to Cambridge and shortly into Cavendish Corner, which was to remain the home until he died. In 1903 was born their younger daughter Anna, who is now a Founder of the Association.

During the period since his father's death, in addition to his zoological and other cultural activities, Bidder had been attending monthly meetings of the Board of Directors of Cannock Chase Colliery (he was managing director from 1897 to 1908, and chairman from 1915 to 1919), making occasional visits to Denmark, and going annually to his hotel in Naples. In 1903 he began to complain of a tiredness from which he had been suffering for many years and by 1905 he was definitely shown to have tuberculosis. This illness, during which he was forbidden to use a microscope, struck at a time of life when he should have been in his prime. He made winter visits to Davos and to Assouan, and indeed was at one time given only a few months to live. Yet after 10 or 12 years of this semi-invalidism and care he made a complete recovery.

During those years, however, Bidder was in fact anything but inactive. He had the time to do an immense amount of wide reading, thus gaining that great breadth of knowledge from which he so evidently drew in his conversation and publications. For several years, from 1909 to 1913, he went with his family each autumn to Mundesley on the Norfolk Coast. Here he became very much interested in the local geology and coastal erosion. At home also he was known to spend much time in his bathroom experimenting with his bottom-trailers designed for the study of currents in the North Sea. The knowledge so derived led to a demand by the Admiralty for his services during the 1914-18

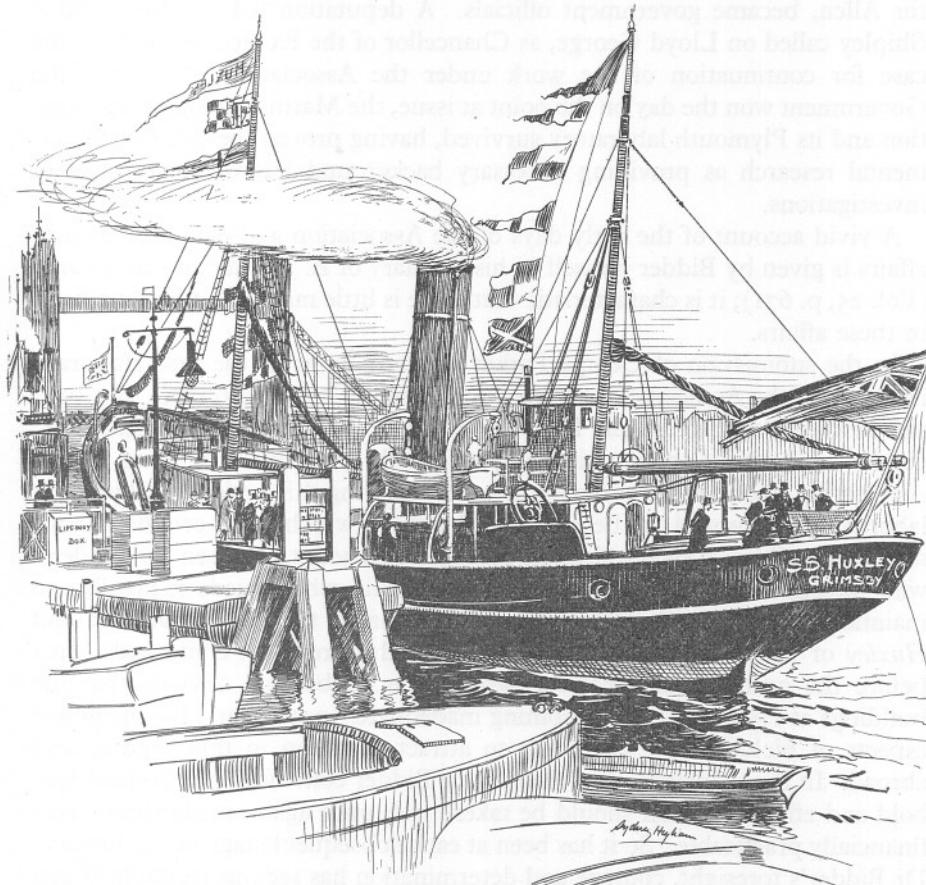
war when he was attached to H.M.S. *Vernon* for research. By 1909 he had already adopted those habits of unusual hours for which he became so well known and which persisted until the end of his life; in fact he was often said to be like the Snark in that 'he would frequently breakfast with afternoon tea and dine on the following day'.

It was during the earlier period of his illness that Bidder was also especially active in the affairs of the Marine Biological Association. By 1909 he had played a part which must have been vital to the future of all marine biological and fisheries research in this country. In 1902, under the auspices of the International Council for the Exploration of the Sea, the southern North Sea and English Channel were allotted to England for investigation. There being no government department in England concerned with Fishery Research, H.M. Treasury requested that the Association should act as agents to the Government for the equipment and working of a vessel to do research in this southern British area, the Scottish Fishery Board having consented to act in a similar capacity for the northern area. The Hon. Treasurer (J. A. Travers), the Hon. Secretary (E. J. Allen) and G. P. Bidder, together with W. Garstang, were appointed by the Council a committee to inquire as to the best means of providing a suitable vessel for carrying out the investigations and to draw up a scheme of organization for the work. For the investigations in the English Channel it was decided to use the *Oithona*, the acquisition of which vessel by the Association had been made possible by Bidder in 1901. As regards the North Sea investigations the Government were unable to consider the purchase of a ship, and it was necessary to charter. In August 1902 the Secretary reported to the Council that 'Mr Bidder had made an offer to purchase a steam trawler which he would let to the Association upon favourable terms for use in the International Investigations'. The vessel he purchased was a 115 ft. steam trawler, the *Khedive*, which was renamed *Huxley*, in honour of the first President of the Association.

In the minutes of the Association is to be found the following: 'Council wishes to place on record its sense of the value of Mr Bidder's generous assistance in obtaining a vessel for the purposes of the N. Sea exploration.' For this North Sea work the Association rented a house in Lowestoft, and it is to be noted that in each of the years 1903 to 1906 Bidder was appointed to the Committee to visit and report on the work of the Lowestoft laboratory.

In 1905 the Council of the Association were asked to consider whether the work on which they had hitherto been engaged could not be carried out on a reduced scale in future, so as to admit of a substantial sum being allocated to the Board of Agriculture and Fisheries for the collection of statistics. On 28 June 1905 the Treasury sanctioned a grant of £5000 a year for two years' further work on the international investigations, saying 'This sum is less by £500 than the grant hitherto allocated to the Association from votes of Parliament; but my Lords understand that Mr G. P. Bidder has generously

offered to return to the Association a sum of £500 a year for two years in respect of the money paid to him for the hire of the Steam Trawler *Huxley*, so that the Association will be enabled through his liberality to carry on its scientific work on the same scale as in the past three years.' The Council considered 'that this offer has been the means of a satisfactory arrangement being made by the Treasury for the continuation of the English scientific work'.



S.S. *Huxley* lying off Billingsgate for the inaugural inspection before starting the International Investigations in the North Sea. (From a drawing reproduced in the *Daily Graphic* of 3 December 1902.)

In 1907 Bidder sold the *Huxley* to the Association on easy yearly terms of payment on condition that, when the Association finally sold the vessel, any profits on the original cost might be kept by him to found a trust for research. In 1909 the *Huxley* was sold with a profit of £750 with which Bidder founded the Ray Lankester Fund to enable selected investigators to work at the Plymouth laboratory.

The *Huxley* did an immense amount of pioneer research, and I have outlined its history at some length because there can be no doubt that this, made possible by Bidder, must have weighed heavily in the Association's favour in its most critical year, 1909. In that year it was proposed that the Board of Agriculture and Fisheries should take over responsibility for English marine research; and eventually the whole of the Association's scientific staff, except for Allen, became government officials. A deputation led by (Sir) Arthur Shipley called on Lloyd George, as Chancellor of the Exchequer, to state the case for continuation of the work under the Association. Although the Government won the day on the point at issue, the Marine Biological Association and its Plymouth laboratory survived, having proved the value of fundamental research as providing necessary background knowledge for fishery investigations.

A vivid account of the early days of the Association and of some financial affairs is given by Bidder himself in his obituary of E. J. Allen in this *Journal* (Vol. 25, p. 671); it is characteristic that there is little mention of his own hand in these affairs.

In the submission of George Parker Bidder's name as the first Honorary Member of the Association at the Annual General Meeting on 4th July 1945 the newly elected President Prof. (Sir) James Gray said: 'We recall with particular gratitude the decisive support which Dr Bidder has given to this Association since he first became a member. During this period the Plymouth laboratory has moved far beyond the objectives which must originally have seemed possible; as each milestone has been reached it has been Dr Bidder's wise foresight, courageous faith and financial aid which made it possible to maintain the advance.' Here followed allusion to the vessels *Oithona* and *Huxley* of which I have already told. Then the President continued: 'Just before the outbreak of the First World War, it became obvious that the buildings at Plymouth were becoming inadequate—particularly for the newer aspects of biology then beginning to attract attention in this country and abroad. In the doldrums after the war Dr Bidder convinced the Council that bold and effective action should be taken, and, once again, made these plans financially practicable. So it has been at each subsequent stage in our history; Dr Bidder's foresight, courage and determination has seen us through. When we survey our present-day activities and gratefully acknowledge the extent to which we owe these to Government support, we must not forget the debt which we owe to Dr Bidder. The early years of this century were a very critical period in our life history and had it not been for his efforts, and his unflinching determination, no amount of Government aid would afterwards have enabled us to avoid disaster. The financial contributions which Dr Bidder has made from time to time amount to a munificent sum, but it has been the time at which they were given and the encouragement which they inspired which have been decisive in the development of the Plymouth Laboratory. Bis dat qui cito dat.'

It was this latter characteristic of right timing which enabled Bidder to play so decisive a part, not only in the affairs of the Marine Biological Association, but in the general development of zoology in this country. Such occasions were, his purchase of the *Quarterly Journal of Microscopical Science*; his formation of the Company of Biologists and resulting saving of the *Journal of Experimental Biology*; and his quick action at the end of the Second World War in persuading the Royal Society to come to the assistance of the Stazione Zoologica at Naples.

Bidder's love for Naples was, perhaps, second only to that for the Marine Biological Association. Reinhardt Dohrn says in a letter to me:

'Among the younger generation of British friends—young as compared to the generation of T. H. Huxley, Francis Balfour, and Ray Lankester—it was G. P. Bidder who had most probably the closest contact and personal friendship with Anton and Marie Dohrn. He must have been frequently at our house during the eighties and the nineties because, young as I was then, I have a distinct recollection of him and his vivid conversation and his characteristic laugh. My parents mentioned him often, him and also the famous, almost classic story, of how he came to take over the bankrupt hotel on the Corso Vittorio Emanuele, which later became such a success as Parker's Hotel.'

Dohrn speaks of Bidder's help to the Stazione between the wars and says:

'It was Bidder's strong wish to keep alive the personal contact between the British biologists and the Zoological Station, and equally alive was his tendency to act as a catalyser of these contacts. Of this, I too have also had proof again and again. But especially so after the last war when a critical situation threatened the Zoological Station and when it seemed doubtful whether the tradition of the Stazione could be continued. Bidder then intervened decisively in more than one case. I am glad to have an opportunity to state this fact here: on 14 October, 1943, scarcely two weeks after the allies had occupied Naples, Bidder—as Chairman of the Marine Biological Association—wrote a letter to the Editor of *The Times* in which he expressed his joy about the news that the Zoological Station had been spared. Many of his fellow countrymen would share his joy because many of them had worked at Naples. And of special value to us here was the fact that he gave vent to his joy "that the Director (friend of us all) was still there in charge in August" (and that this "gave grounds for hope that we may yet see in the Stazione again men of many tongues and more nations working side by side in the fraternity of a common endeavour to understand the living world that surrounds us") which meant that also in the future the Station would go on in the old tradition.'

This letter written by Bidder has been of great significance for the fate of the Zoological Station. There was, at that time, a kind of "Reader's Digest", edited in Italian by the Military Authorities, called *il Mese* (the Month). In no. 3, December 1943, Bidder's letter to *The Times* was published in Italian. What that meant for the position of the Zoological Station then, and to myself

and my collaborators, need not be emphasized to you. But more was to come: two months later the Allied Military Government informed us that the Royal Society had decided on an emergency grant of £1000.'

Bidder, who was awarded the Sc.D. of Cambridge in 1916, was a prominent figure in biological circles. He was on the Council of the Marine Biological Association from 1899 until his death, a Governor of the Association from 1905, and its President for the years 1939 to 1945. When he resigned from his Presidency the Council instituted the election of Honorary Members of the Association so that they might show their appreciation to Bidder by bringing forward his name as the first to be elected. His last visit to Plymouth was in 1941 during the air raids when the laboratory was badly damaged. The destruction of Plymouth and the haunts he knew so well as a young man caused him great distress.

His colliery interests and knowledge of coal mines made him the obvious secretary for the British Association Committee which reported in 1904 on the probability of *Ankylostoma* (Miner's Worm) becoming a permanent inhabitant of British coal mines in the event of its introduction.

He was President of the Zoological Section of the British Association in 1927. During his year of office he thought that it would be valuable to have intermediate meetings of the section. The Recorder of that day, Prof. Frank Balfour-Browne, being in favour of the suggestion, Bidder and he started to arrange a meeting for January. The Council of the British Association did not, however, approve, so these two started the Association of British Zoologists, of which Bidder was the first Chairman, a position in which he excelled. He was Vice-President of the Linnean Society in 1924 and 1931, and its Zoological Secretary from 1928 to 1931; and an Honorary Vice-President of the Ray Society. He was President of the Devonshire Association in 1929, and of the South Western Union of Naturalists in 1928; to both he gave Presidential addresses proving his wide knowledge and the originality of his outlook. From 1920 to 1927 Bidder gave the course on sponges for Part II students at Cambridge and it was my privilege to be one of the first to listen to these fascinating and stimulating lectures. Bidder loved microscopy, and in his practical teaching he insisted upon the right use of the microscope. It was only by making the best possible use of the instrument that fine details of the cell could be seen, for throughout his work it was the living cell and its complexity that fascinated him. He edited Vosmaer's posthumous *Bibliography of Sponges* which was published in 1928.

As regards his own scientific research, Bidder's two major contributions to knowledge were on the hydraulics of the sponge, and the invention of the bottom-trailer, a bottle so weighted that it was only just heavier than sea water and would drift in bottom currents.

The work on sponges was started at Naples during the years 1887 to 1892 and continued at Plymouth from 1893 to 1896, but the first full report was not

made until the British Association Meeting in Hull in 1922, when E. J. Allen was President of Section D. It was published in the *Quart. J. micr. Sci.* the following year. 'For a long time I proposed to myself to make a further series of experiments to clear up doubtful points, but recognizing that I shall not now do so, I have reconsidered all the experiments this year (1922) and recalculated all results and formulae.' He did, in fact, later work for short periods at Plymouth and at Naples, on the currents of sponges, on which he read a paper at the British Association at Leicester in 1933.

It is generally recognized that Bidder was well in advance of his time when he did the research last century which led to his two papers 'The relation of the form of a sponge to its currents' and 'The perfection of sponges'. Their delayed publication until 1923 and 1937 covered the period of the general rise in this country of functional morphology and experimental biology. The papers were models of lucidity; mathematical calculations were relegated to appendices, and the results were available in simple language for all to understand. One of their characteristics was the illustration of details by comparison with graphic everyday phenomena. 'I worked with two calcareous species of sponges, having oscula at the end of tubular prolongations, which reach the size and shape of a child's thumb in the case of *Leucaltis*, and of a child's finger in the case of *Leucandra aspera*'.

While remarking that under the microscope flagella appear to move with the speed of an express train, in fact, he says 'no part of the most rapidly moving flagellum ever attains the rapidity of motion of a snail'. To illustrate that there is no co-ordination in the movement of the flagella in a sponge he says 'A collar cell flagellate surface is comparable mechanically to a seine-net with a number of fishes fixed by their gills in the meshes.'

Again 'The remarkable achievement of the perfected hydraulic organ in sponges is that from this waving of hairs  $\frac{1}{10000}$  of an inch in thickness at a mean speed of 7 feet an hour, there is produced an oscular jet with an axial velocity of over half a foot a second (280 times the speed of the flagellum), which in *Leucandra* throws to the distance of 9 inches five gallons a day or a ton in six weeks.' This is a good example of how he stressed the comparison of time and distance in small organisms.

For those species of deep-water Hexactinellida set in a vertical plane with no evolution of pressure chambers: 'Food is brought to them, waste is taken away. For them in their eternal abyss, with its time-like stream, there is no hurry, there is no return. Such an organism becomes a mere living screen between the used half of the universe and the unused half—a moment of active metabolism between the unknown future and the exhausted past.'

The following is very typical: 'A pretty method was arrived at accidentally, when I found the coloured jet marked by dark beads or nodes, caused by my pulse shaking the pipette; the length between any two nodes, divided by three-quarters of a second, gives the core-velocity of that part of the jet.' Bidder

once told me how valuable he found a *musca volitans* in his eye for microscopic work: he had calibrated it for different oculars and objectives of his microscope and could use it as a quick approximate eye-piece micrometer for measurements.

His sense of humour was keenly developed, and all his discussions were liable to be punctuated at intervals by that characteristic laughter when he had suddenly noted a humorous aspect. This sense could not be withheld even from his writings. Discussing the ancient history of sponges and climates in his Presidential Address to the British Association Zoology Section at Leeds he said: '...so that 10 or 20 million years would give them as many steps in evolution, to make a flagellate from nothing, as it has taken us to build up a flagellate into that highest of all living creatures, a member of the British Association (section D)'. Or, when writing on flagellar motion 'at higher frequencies the stroke is the shorter, as in a school-master's cane; at lower frequencies the stroke is the longer, as in a fisherman's trout-rod'.

Many of his remarks have deep significance. In an article in the *British Medical Journal* on Senescence he says: 'I suggest that we were not born with infinitely senescent brains and rejuvenated epithelium; rapidity of division in our skin has been evolved to keep it clean and whole; undivided brain cells have been evolved because their function is to remember.' On a visit to his house in 1945 (he was then 82) he took me across his lawn for his daily reading of the maximum and minimum thermometer. On being asked why he kept the thermometer so far from the house he said that it was so that he could daily exercise his memory, as he did not write the readings down until he had returned indoors.

Bidder wrote a number of other papers on sponges besides the two I have specially mentioned. Of these remaining papers Dr M. Burton writes: 'Although fairly numerous, they were all short, but he contrived to include the occasional idea that was of fundamental importance, the most outstanding being his emphasis on the distinctness of the Hexactinellida from the rest of the Porifera, epitomized in his use of the phyla Nuda and Gelatinosa.'

Bidder's invention of the bottom-trailer, in the words of Dr J. N. Carruthers '...represented a powerful means of attack in the study of bottom water movements. Bidder found the important result that the main drift in all his series, when generally summarized, seemed to be in the opposite direction to the migration of the plaice at the same time of year. "So far therefore" he concluded "the experiments confirm the view of those naturalists who suppose that bottom feeders, like trout, tend to move against the current".

Moreover, Bidder expressed the opinion that his evidence favoured the supposition that the percentage of bottles in a given area recovered by the trawl did not differ largely from the percentage of plaice of 21–26 cm in the same area caught by the trawl in the same time. Hence the bottles serve as an instrument for assessing the intensity of trawling because they cannot migrate.

Bidder, in a manuscript given to me, stated that his bottles were trawled up by the fishermen at the rate of 55% per annum.

He deduced the time of onset of the winter drift from south to north in the southern North Sea and of the draught getting into the Skagerrak with the onset of winter. This latter he found makes itself felt by degrees further and further south without drawing on the English Channel water until the end of the year.

Of course, the most significant thing was Bidder's finding that many of his bottom-trailers got cast on the English shore, whereas surface bottles would, for the most part, go across the North Sea. He deduced, regarding the bottom flow, that the isochrones (his word) of the stream-front were shaped on the shoreline; and such a formation of the bottom current at once suggested to him the creeping-in of heavy water. This is of course now well known—that river outflow induces an ingoing bottom set of salt water.'

I have been privileged to see extracts from many of the tributes sent to his family by his friends young and old. While many recalled his culture, his original and fascinating conversation, his child-like curiosity, or his ingenuity, all stressed his courteous and kindly nature. No young student was beneath his consideration, and the numbers, in all stations, whom he helped by word or deed, will never be known. Truly his left hand knew not what his right hand gave.

In conclusion, I cannot improve upon the words of the obituary notice in *The Times* of 1 January 1954: 'But perhaps he brought to the service of humanity and of science qualities rarer and more important than those which have given other men greater reputations. For the timely help and wise advice which he gave to many, both scientists and non-scientists, were guided by unusually understanding sympathy, and were completely unselfish. These qualities had as their natural symbol his unfailing and distinguished courtesy; and that also helped to give its characteristic style to that sturdy bearded figure with white tie, Inverness cape, and quite unconsciously patrician bearing, whose departure many men and women in all branches of study and in all ranks of society will now most sorely regret.'

F. S. RUSSELL

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## EXPERIMENTS ON THE HUMANE KILLING OF CRABS

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(Plates I and II)

The main purpose of the work described in this paper was to find a humane way of killing crabs for eating, but the method that resulted from the investigation is applicable also in the biological laboratory, unless it is necessary that the nerve ganglia should be intact.

### MATERIAL

The whole of the work was done on the edible crab, *Cancer pagurus* L.

In order to get reliable results, capable of repetition by others, it was necessary to work with fully active crabs, and this could only be done near the sea. The whole of the work reported in this paper was done at the Marine Biological Laboratory, Plymouth, at the end of August 1953.

Crabs brought in by fishermen were placed in a large outdoor aquarium, plentifully supplied with running sea water. No crab was used unless it gave evidence of full normal activity. The first piece of evidence required was that it should make vigorous attempts to avoid capture by a net.

Each crab, when so captured, was brought from the aquarium into the laboratory and subjected to further tests. It was found best to place the crab the right way up in a large basin of sea water. Care was taken to devise tests that would be practical and reliable. A normally vigorous crab gives these responses to stimuli:

*Ocular reaction.* The crab retracts the 1st and/or 2nd antennae when the hand is passed from one side of the animal to the other above the eyes. (The hand must not touch the animal.)

*Antennal reaction.* The crab retracts the 1st antennae when the distal (pigmented) end of the middle joint of the protopodite of this appendage is gently touched with a seeker.

*Maxilliped reaction.* The crab folds the 3rd maxillipeds towards the body and/or brings the chelae towards one another when the 3rd maxilliped is gently pressed away from the body with a seeker.

*Pincer reaction.* The crab grasps a hard object placed so as to touch the tubercles on the propodite of the chela.

The first two tests provide evidence that the cerebral ganglion is intact, while the third and fourth do the same for the posterior ganglion. These were

the tests usually applied, though others were sometimes used. No crab was used in the experiments unless it showed normal responses.

The twenty-five crabs subjected to the main control and experimental procedures are distinguished in this paper by the serial letters A to Y. Details of the experiments with these crabs are given in Table I on p. 18. Two crabs which were treated in such a way that the experiments could not conveniently be summarized in the same table are designated AA and BB.

#### METHODS

Fishmongers boil crabs in a solution of common salt approximating in concentration to sea water. At the seaside, sea water is sometimes used. In the experiments described in this paper, sufficient tap water was added to 288 g of culinary bar-salt to make 8 l. of solution. This solution (approximately 3·6% sodium chloride solution) was heated in a metal vessel by a large gas burner.

All the crabs used in this investigation, except one, were boiled in this solution, most of them after preliminary treatment intended to render them insensible. It was thought advisable to watch carefully for movements of the appendages during the process of boiling, and the temperature was therefore kept somewhat lower than the actual boiling-point. The mean temperature at the moment of immersion was 96° C. Although the gas-burner was left on, the temperature was usually somewhat lower (mean, 92° C) after the lapse of 6 min, when in every case except one all movement was at an end. The immersion of crabs in this very hot water is called 'boiling' for short throughout this paper.

It was found convenient to hold the animal in tongs, nearly horizontally, the right way up, during immersion. Crab A was placed upside down.

The special procedures intended to render the crabs insensible are described in the appropriate places below.

#### RESULTS

##### *Controls (no Treatment before Boiling)*

Seven crabs, A to G, were boiled alive without previous treatment.

In all cases the time elapsing between the moment of immersion and the occurrence of the last movement was carefully noted.

When an untreated crab is placed in the hot water, the autotomy-reaction occurs immediately in the chelae and walking legs. All these ten appendages are instantly raised and pressed against the edges of the carapace. This movement is complete in about 10 sec. If it is powerful enough, the appendage breaks off at the fracture-plane in the basi-ischium. The greater part of the appendage falls away, either at once or after a short lapse of time. While the appendage is held firmly against the carapace, it cannot move until it breaks. When it breaks, the stump is free to move, and it often does so. There is,

however, no continuous movement. On the contrary, all that one observes is an occasional twitch. In general, the reaction to the heat is simply a powerful, continuous contraction of the extensor muscles of the appendages that have fracture-planes; that is to say, the chelae and walking legs.

The following are the periods (in minutes and seconds, to the nearest 5 sec) that elapsed between immersion and the last movement: 0·15, 4·15, 6·50, 2·45, 2·35, 2·45, 0·10 (mean, 2·50).

Although these periods were always measured, they seem to have little value. It seems probable that apart from the contraction of the extensors, movement is not initiated by the nervous system. It seems much more likely that it is caused by the direct effect of the heat on the substance of the muscles. The following experiment was done to test this hypothesis. A crab (AA) was exposed to the vapour of chloroform until it appeared to be dead. On removal from the vapour it made no spontaneous movement, not even a flicker of the 1st antennae or of the flagella of the maxillipeds. No response was given to any of the tests. This crab was boiled in the usual way. No appendage was thrown off by autotomy, but the last movement occurred 2 min 5 sec after immersion. It seems certain that this was merely the result of the shortening of a muscle of a dead crab under the influence of heat.

Movement of an appendage was also occasionally observed to be caused by the expansion and escape of air contained in the branchial chambers.

For these reasons it was concluded that the timing of the last movement was useless, and that it would be best to concentrate attention on the autotomy-reaction, which is governed by the nervous system.

The total number of appendages thrown off by each crab is shown in Table I (p. 18). There is not much difference between one appendage and another in the tendency to be thrown off on boiling.

Among the seven control crabs, only one appendage (a 4th walking leg) was absent by previous injury when boiling started. Of the sixty-nine appendages present, forty-two (61%) were thrown off on boiling.

#### *Immersion in Strong Salt Solution before Boiling*

It was found by Aaser (n.d.) that if lobsters are placed in a strong solution of common salt (350 g to each litre of water), they may subsequently be boiled without showing any other reaction than a single flip of the abdomen. Quite short periods in the strong salt solution sufficed—a minute or even less.

In the present investigation the same method was tried on crabs. Exactly the same salt solution as that used by Aaser was employed. The temperature of the solution was 18–19° C.

Five crabs, BB and H to K, were each placed in strong salt solution for 10 min. The experiments were done separately, so that full attention could be concentrated on the reactions of each animal.

When the crabs were placed in the strong salt solution, the first antennae

were at once strongly retracted and could therefore give no response. In general, the animals then at first remained still. After a short lapse of time (half a minute or so) some movement of the walking legs started, with feeble locomotion in some cases. No response was given to stimulation of the maxillipeds, and the pincer reaction was very weak. Spontaneous movement had ceased before the 10 min had elapsed.

TABLE I. DEGREE OF AUTOTOMY AFTER DIFFERENT TREATMENTS

Crab	Sex	Width of carapace (cm.)	Treatment before boiling	No. of autotomy appendages	
				Absent before boiling	Lost by autotomy on boiling
A	♀	12	None (control)	0	2
B	♀	16	None (control)	0	8
C	♂	18	None (control)	0	9
D	♂	14	None (control)	0	7
E	♂	14	None (control)	0	7
F	♀	16	None (control)	0	3
G	♂	15	None (control)	1	6
H	♂	16	Immersion in strong salt solution	1	4
I	♂	16	Immersion in strong salt solution	0	10
J	♂	18	Immersion in strong salt solution	0	10
K	♂	16	Immersion in strong salt solution	0	9
L	♂	19	Immersion in tap water	0	6
M	♂	11	Gradual increase of temperature to boiling	0	3
N	♂	16	Piercing of brain at 85°	0	5
O	♂	17	Piercing of brain at 85° and v.n.-m. at 110°	1	1
P	♂	12	Piercing of brain at 85° and v.n.-m. at 110°	0	0
Q	♂	14	Piercing of brain at 85° and v.n.-m. at 110°	0	0
R	♂	14	Piercing of brain at 85° and v.n.-m. at 110°	1	0
S	♂	12	Piercing of brain at 85° and v.n.-m. at 110°	0	2
T	♂	15	Piercing of brain at 85° and v.n.-m. at 110°	0	1
U	♂	14	Piercing of v.n.-m. at 85°	0	0
V	♂	16	Piercing of v.n.-m. at 85°	1	0
W	♂	16	Piercing of brain at 60° and v.n.-m. at 85°	0	0
X	♂	15	Piercing of brain at 60° and v.n.-m. at 85°	0	0
Y	♂	17	Piercing of brain at 60° and v.n.-m. at 85°	0	0

The piercing angles given are only approximate. v.n.-m.: ventral nerve-mass.

The crab BB was returned to sea water. It soon gave the ocular and antennal reactions again, but irreparable damage had been done, and it was found to be dead about 6½ h later.

The other four crabs (H to K) were boiled immediately after they had been taken from the strong salt solution. Before the experiment they had thirty-nine appendages capable of autotomy, only one walking limb of one animal having been absent; thirty-three appendages were thrown off during boiling. The percentage of autotomy, 85%, was even higher than in the controls.

It is clear that short immersion in strong salt solution is not a suitable method for rendering crabs insensible. The difference from lobsters is no doubt caused by the far greater protection of the gills in the crab. The effect

of the strong salt is only slowly felt if the respiratory current is weak or intermittent, or if there is much air in the branchial chambers.

The American crab, *C. magister*, which lives below low-tide mark in Pacific seas just as *C. pagurus* does in ours, and is thus not subjected in nature to any but very small changes in the osmotic pressure of the surrounding medium, is unable to regulate the osmotic pressure of its blood against water of higher salinity than the sea (Jones, 1941). If *C. pagurus* were left for a sufficient period in strong salt solution, it would eventually die from increase of the osmotic pressure of its blood, as indeed crab BB died; but there is no likelihood that this slow death would be painless.

#### *Immersion in Cool Tap Water before Boiling*

It was thought worth while to find whether a crab could be rendered insensible quickly by immersion in tap water.

The water was at 17° C. The crab (L) was much more active than the ones placed in strong salt solution. The respiratory current was strong. Some unco-ordinated movement occurred (the animal grasped a maxilliped with the chela of the opposite side, and later it moved its maxillipeds from side to side). The animal moved about from time to time.

At the end of 10 min the crab was boiled. Of the ten autotomy-appendages, six were thrown off.

From a study of *C. pagurus* placed in water of lower salinity than that of the sea, Hukuda (1932) concluded that the gills are permeable to salts in these circumstances, so that the saline concentration of the blood drops. The placing of the animal in tap water must result in eventual death from loss of salts from the blood, but the experiment recorded in this paper shows that the process is not a quick one.

#### *Gradual Increase of Temperature to Boiling*

According to Sinel (n.d.), if a lobster is placed in weak salt solution and the temperature is gradually raised, the animal becomes lethargic and dies at about 27° C. The heating to this temperature took about 3 min in Sinel's experiments. Sinel recommends this as a humane way of killing both lobsters and crabs, though he appears not to have made experiments with crabs. This result was supported by an experiment carried out on lobsters under the auspices of the Trondhjem Society for the Protection of Animals (Anon., n.d.).

Aaser (n.d.) put lobsters in a weak solution of common salt at 9° C and gradually heated the water. They moved about at first, but their activity gradually decreased and they died between 38° and 45° C. Aaser found that in general a lobster immersed in water at 26° C continues to respond to stimuli for about  $\frac{3}{4}$  h.

In the present investigation, a crab (M) was placed in a 3·6% solution of common salt at 19° C. The solution was gradually warmed. The temperature

of  $27^{\circ}$  C was reached in about 7 min. The crab was still active. The temperature was increased gradually till boiling point was reached in 28 min. The crab was active at  $26^{\circ}$  C and continued to move about as the temperature rose higher. It attempted to escape by pushing backwards against the sides of the vessel. At  $32^{\circ}$  C the movements began to become unco-ordinated. At  $34^{\circ}$  C it was much less active and there was no locomotion; both chelae were thrown off. The 1st walking limb of the left side was subsequently lost. At  $40^{\circ}$  C, reached in  $8\frac{1}{2}$  min, the last movement was seen (very slight change of position of the last walking limb); no response was given to stimuli.

Crabs only perform autotomy when they are vigorous, and it is probable that if the experiment had been performed on a specimen obtained from a fishmonger, no limb would have been thrown off. However, this method of killing crabs does not commend itself on humanitarian grounds.

#### *Piercing the Nerve Ganglia before Boiling*

Some years ago I visited Billingsgate fishmarket and witnessed the 'sticking' of two crabs before boiling (Baker, 1949). A steel awl mounted in a wooden handle was used. The animal was laid on its back and the point of the awl was pushed through the exoskeleton in two places, referred to by the operator as the 'mouth' and 'back passage'. I noted where these points were. The anterior one was the very small depression in the middle line at the posterior end of the sternum of 1st antennal segment, immediately in front of the epistoma (Pl. 1, fig. 1). The posterior one was the large depression in the middle line at the posterior end of the sternum of the segment of the chelae (Pl. 1, fig. 2). It was evident that the awl would necessarily damage the two main nerve centres of the body, the brain (fused cerebral ganglia) and ventral nerve mass. What I saw encouraged me to believe that it would be useful to investigate this method.

I made a special dissection to find out in what direction the awl should be pointed in order to reach the two ganglia from the depressions mentioned.

The animal must be placed as horizontally as possible on its back. It would be best to have a bench with shaped hollows in it, suitable for holding crabs of various sizes horizontally. However, it suffices in the laboratory to twist a duster into a roll and to shape this in a ring, against which the edges of the carapace can rest.

The awl must of course be kept in the sagittal plane of the animal. The angle at which it is held will be described as follows. If it is held vertically, the angle is  $90^{\circ}$ . If the handle of the awl is moved forwards, a lower angle is recorded, till  $0^{\circ}$  would be reached if the point of the awl were directed horizontally backwards.

I have had a special awl made for sticking crabs. The steel point is fine, so as to make as small a hole as possible and thus allow little blood to escape. It is 25 mm long. This length is designed to reach easily the ventral nerve-

mass of the largest crab. The wooden handle is 30 cm long. This length is designed to make it possible to pierce the largest crab without the animal being able to reach one's fingers with its pincers. With a crab of average size, a push of about  $4\frac{1}{2}$  mm would suffice to penetrate the brain, while about 9 mm would be required for the ventral nerve-mass. A push of 13 mm should suffice for the ventral nerve-mass of the largest crab. The awl cannot be inserted up to the hilt, because the latter will not enter the depression in the sternum of the segment of the chela; that is why the steel point is made longer than 13 mm.

It is not necessary (nor indeed possible) to observe the piercing angles very exactly, for the brain is close to the point of the awl when one starts to push, and the ventral nerve-mass, though deeper, is much larger.

When the piercing has been done, the handle of the awl should be moved about slightly in all directions, to complete the damage to the ganglia.

In the first experiment (crab N), the brain only was pierced, at about  $85^\circ$ . The antennal response was now no longer given, but the animal naturally still gave the maxilliped and pincer responses. The crab was at once boiled. It lost five of its ten autotomy limbs. Destruction of the brain cannot prevent autotomy, for this process is mediated by the ventral nerve-mass, as Frederic showed long ago (1882).

Both ganglia of six crabs (O to T) were pierced in the next series of experiments. The brain was pierced at about  $85^\circ$ , the ventral nerve-mass at about  $110^\circ$ . No maxilliped or pincer responses were observed in any of these crabs after piercing, but there was some slight spontaneous flickering of the flagella of the maxillipeds of some of them. There was also at first some spontaneous twitching of the distal parts of the 1st antennae. One of the crabs (P) gave a very slight antennal response, another (S) an ocular response.

Each of these six crabs was boiled directly its responses had been observed. Two of them each lacked one chela before boiling; there were thus fifty-eight autotomy limbs among the six crabs. Of these, four (7%) were thrown off during boiling. Three of the six crabs lost no appendage.

These results were encouraging, but both the responses and the autotomy showed that the direction of the awl was not quite accurate. Another dissection was made, and it was decided to try about  $60^\circ$  for the brain and about  $85^\circ$  for the ventral nerve-mass. In piercing the latter at  $85^\circ$ , it is important not to try to insert the point of the awl into the very bottom of the hole in the sternum, for the depression turns posteriorly, and the awl will have to be held at a lower angle than  $85^\circ$  if its point is to touch the bottom. One should hold the awl at about  $85^\circ$  and place it as far back in the hole as is consistent with the maintenance of this angle.

In two crabs (U and V), the posterior ganglion only was pierced. No maxilliped or pincer reaction was given, but V gave the ocular reaction and at first showed some spontaneous twitching of the distal parts of the 1st antennae.

This was only to be expected, since the brain was intact. On boiling, neither crab lost any appendage.

In a final series of experiments, both the brain and the ventral nerve-mass of three crabs (W to Y) were pierced in the new directions (Pl. II, figs. 1 and 2). These new directions appeared to be correct, for none of these three crabs gave any response to stimuli. There was, however, some slight spontaneous movement of small parts, such as has already been mentioned: namely, a twitching of the distal extremity of the 1st antennae, and a flickering to and fro of the exopodites of the maxillipeds. In addition, crab Y slightly moved its mandibles and the basal parts of its maxillipeds. It will be recalled that the mandibles receive some innervation from the little paraoesophageal ganglia, which cannot be pierced from outside the animal.

Each of the crabs W to Y was boiled directly its responses and spontaneous movements had been noted. No appendage was absent before boiling. Of the thirty available appendages, none was lost on boiling.

The five crabs U to Y possessed forty-nine autotomy limbs before boiling. The ventral nerve-mass of each of these crabs was pierced at about  $85^{\circ}$ . It is to be noted that not a single limb was lost on boiling.

From a culinary point of view, it is desirable to prevent the escape of blood when the crab is pierced, partly because the blood coagulates on the surface of the animal when it is boiled and thus disfigures it, and partly because it is thought that the taste of the crab would be affected if blood were lost.

Care must be taken when piercing the brain of a small crab, or the point of the awl will pass through the dorsal part of the carapace and thus allow the escape of blood.

If the piercing is done carefully, with a fine-pointed awl, little blood should be lost. It is a good plan to fold the abdomen back into its normal position after piercing the ventral nerve-mass, and to fix it there by tying a string round the animal. The posterior awl-hole will thus be closed, and since air cannot enter it, blood cannot easily escape from the anterior hole.

#### *Note on Accidental Autotomy*

Two of the crabs (O and R) threw off a chela each at or immediately after the piercing of the ventral nerve-mass at  $110^{\circ}$  (before boiling). Autotomy did not occur with any of the five crabs in which this ganglion was pierced at  $85^{\circ}$ . Frederic (1882) remarks that on one occasion autotomy occurred when he applied an electric shock to the ventral nerve-mass of *Carcinus*.

#### DISCUSSION

The evidence from responses to stimuli, and especially from autotomy, suggests that the best method of rendering a crab insensible is to pierce the brain and ventral nerve-mass with an awl in the way directed.

The experiments of Bethe (1898) showed that the brain of *Carcinus maenas* is a reflex-inhibiting organ, which to a large extent controls the adaptive responses of the animal. Those who associate cephalic dominance with cephalic consciousness will be inclined to pierce the brain before the ventral nerve-mass. If so, the appendages that are innervated by the ventral nerve-mass will move actively until the latter has been pierced.

It is fortunate that the method that seems best from the point of view of animal welfare should also commend itself to fishmongers and canners, because it enables crabs to be boiled without losing appendages, however vigorous they may be, and without the waste of time involved in other methods that have been suggested.

The technique of piercing could be taught in 15 min to any person of ordinary intelligence who was accustomed to handling crabs. Preliminary demonstrations could conveniently be given on a preserved specimen.

It is particularly to be noted that a method that is suitable for the crab may be quite unsuitable for the lobster, and conversely. This is because the crab has only a single ventral nerve-mass instead of a chain of twelve separate ganglia, while the gills of the lobster are much more directly exposed to the environment than those of the crab.

The work was carried out under the auspices of The Universities' Federation for Animal Welfare, and with the Federation's financial support. I thank the Director and Staff of the Marine Biological Laboratory, Plymouth, for helping me in every possible way, especially by ensuring a regular supply of very vigorous crabs. Mr P. L. Small took the photographs (Plates I and II).

#### SUMMARY

The following methods intended to render crabs (*Cancer pagurus*) insensible before boiling were tried: immersion in strong salt solution, immersion in tap water, gradual increase of the temperature to boiling, and piercing of the brain and ventral nerve-mass with a steel-pointed awl. The responses of the crabs to stimuli, and especially the autotomy reaction on boiling, were carefully observed. The experiments showed that the best method is to pierce the brain and ventral nerve-mass. It is important to hold the awl at the proper angles when inserting it in the two positions.

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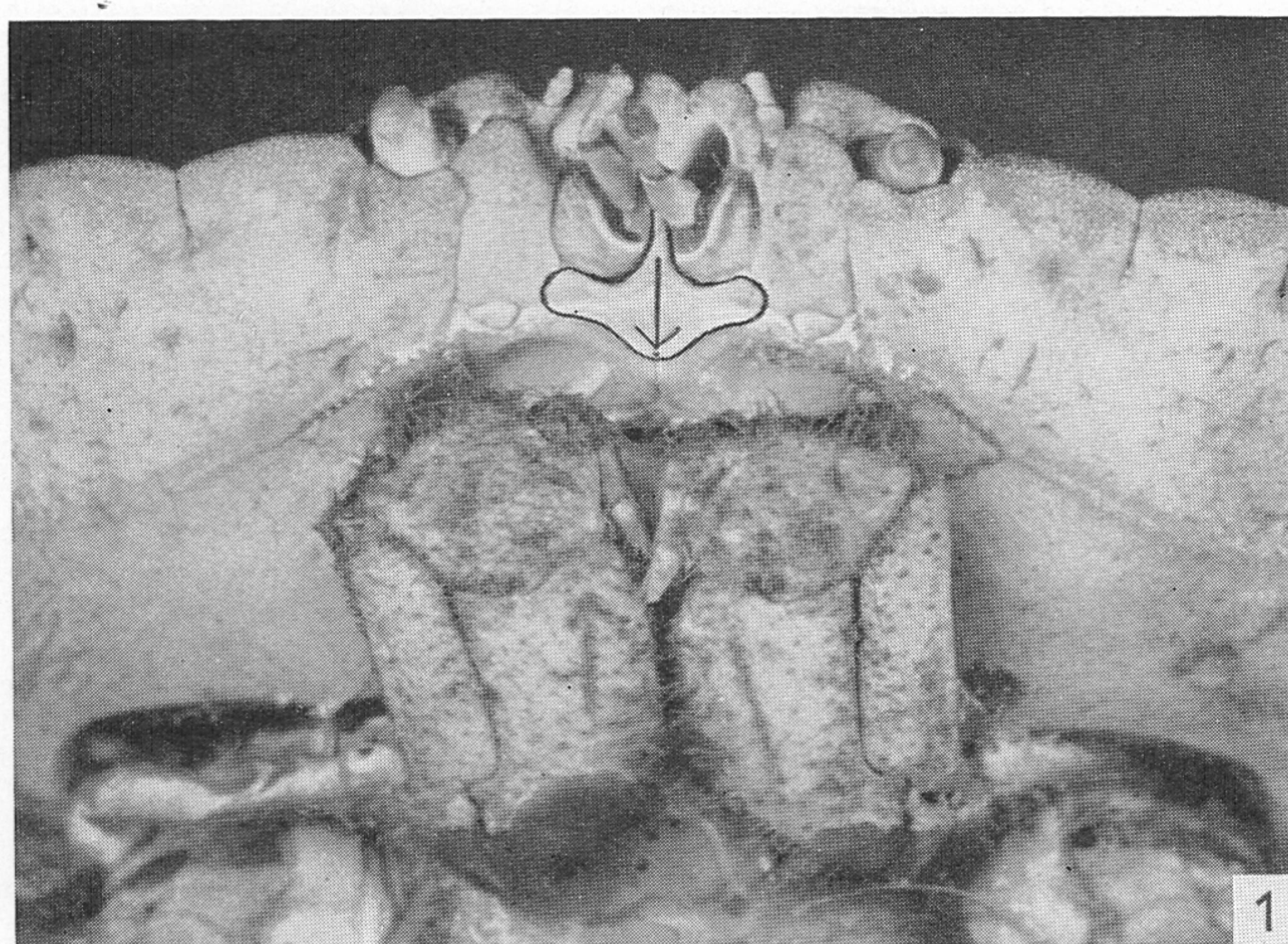
#### EXPLANATION OF PLATES

##### PLATE I

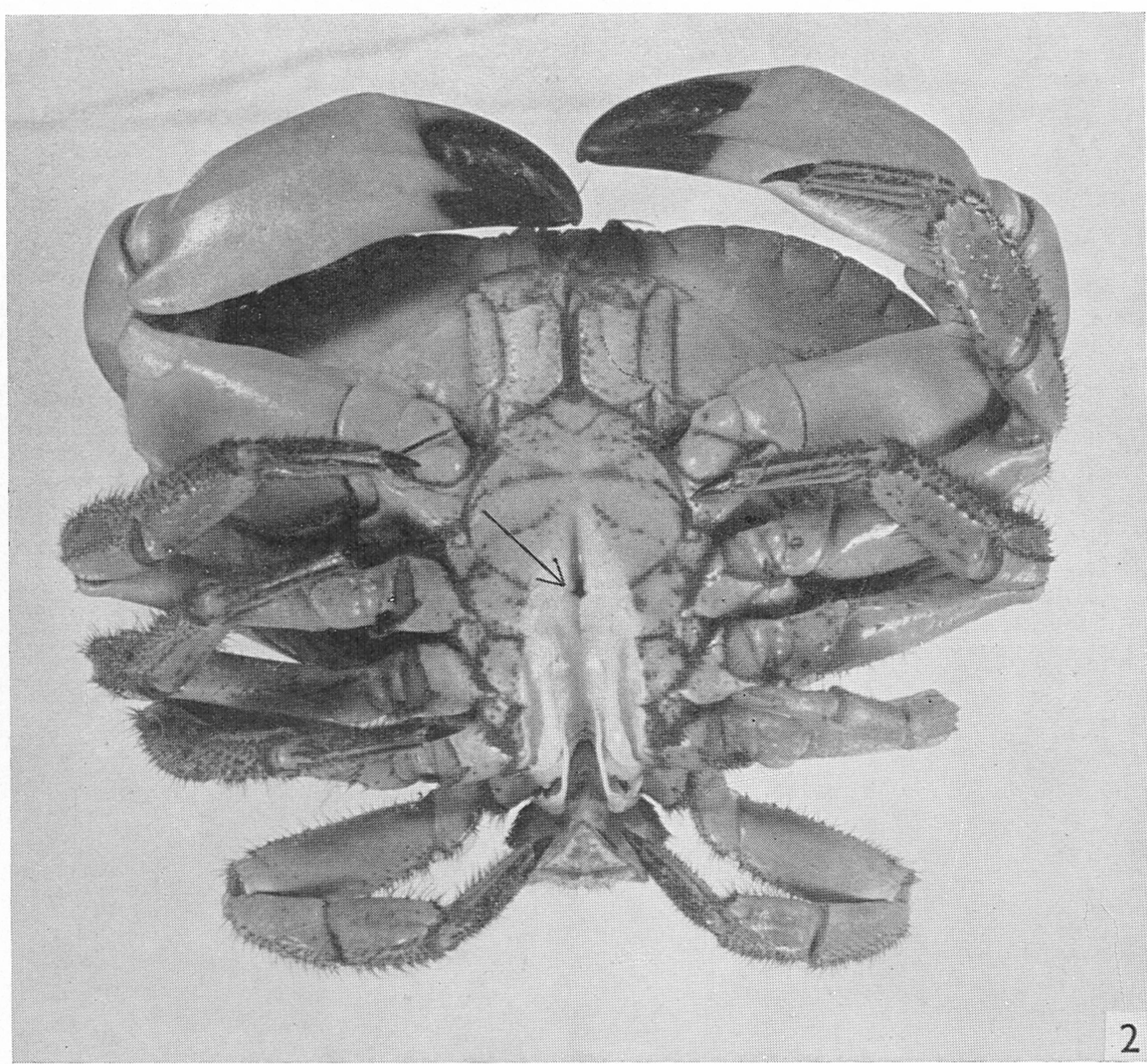
- Fig. 1. The anterior end of a crab, in ventral view. The sternum of the segment of the 1st antennae has been outlined in ink in the photograph. The arrow points to the small depression in which the point of the awl must be inserted to pierce the brain.
- Fig. 2. Ventral view of a crab. At the posterior end of the sternum of the segment of the chelae is the large depression (marked by an arrow) in which the point of the awl must be inserted to pierce the ventral nerve-mass.

##### PLATE II

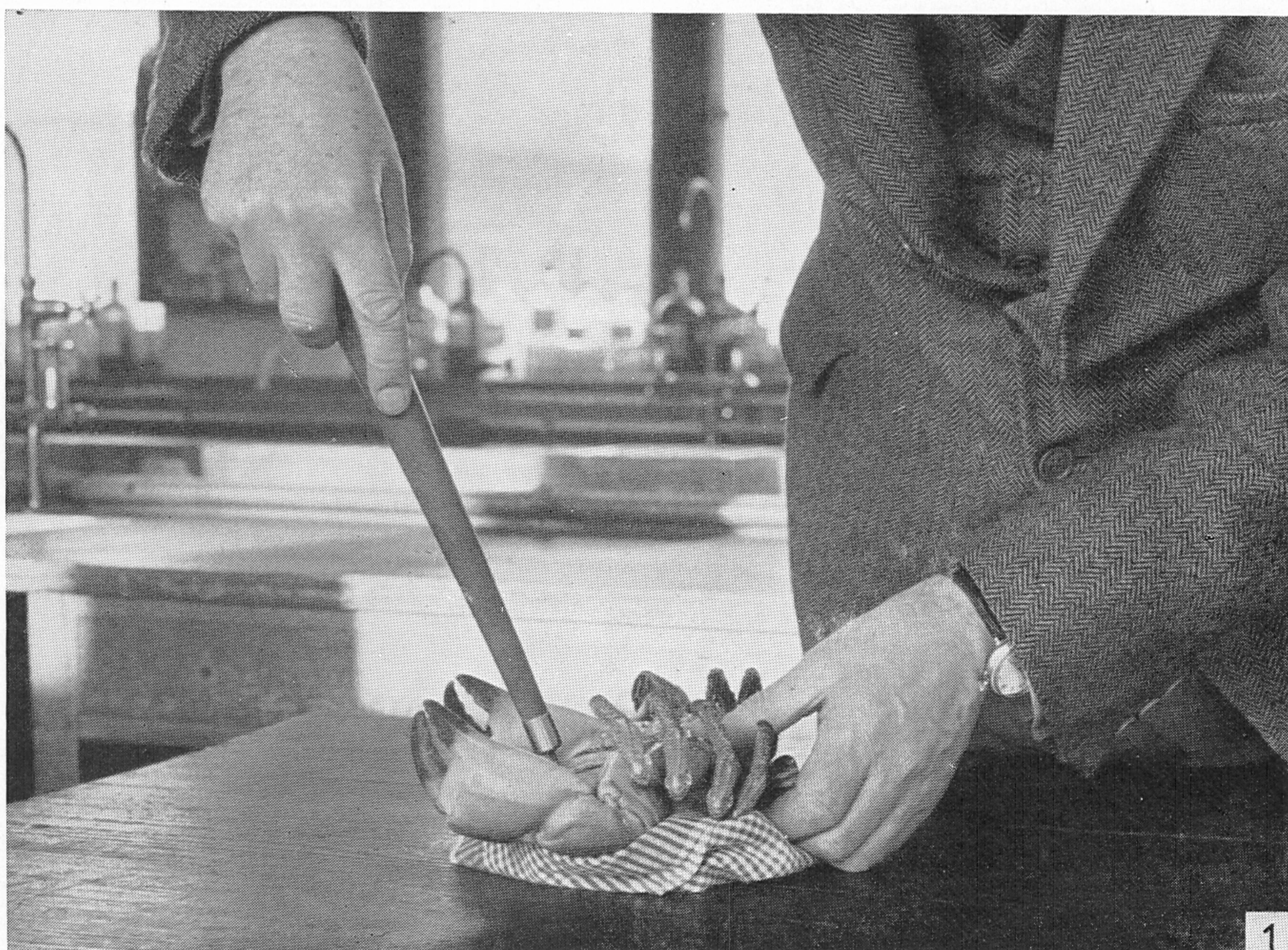
- Fig. 1. Piercing the brain of a crab with the specially designed awl.  
 Fig. 2. Piercing the ventral nerve-mass.



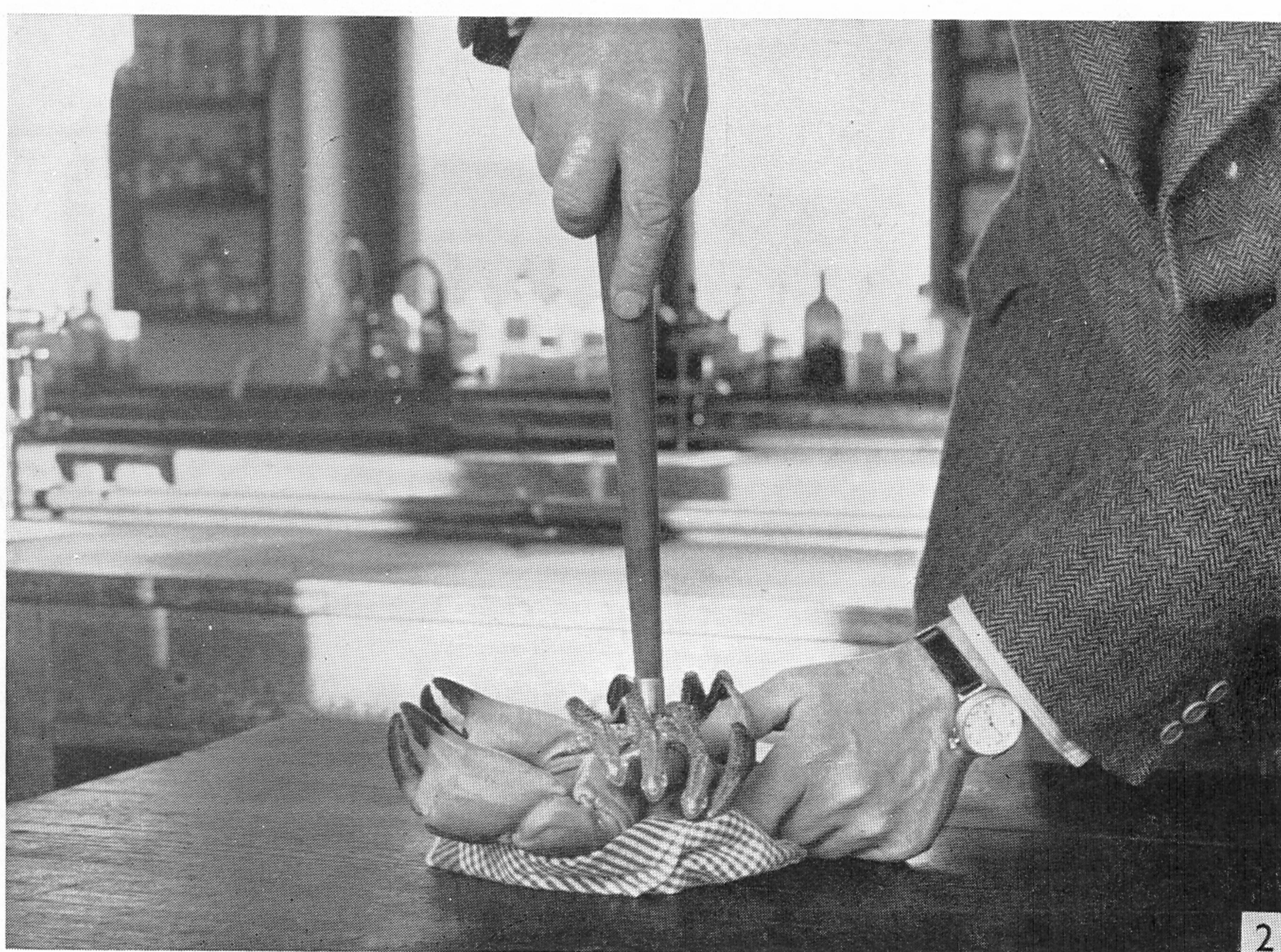
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## ODOSTOMIA AS A PEST OF OYSTERS AND MUSSELS

By H. A. Cole and D. A. Hancock

Fisheries Laboratory, Burnham-on-Crouch, Essex

(Plates I and II and Text-fig. 1)

A recent study by Fretter & Graham (1949) has shown that members of the family Pyramidellidae are ectoparasites, each species feeding on a particular host, usually a tubicolous polychaete or a lamellibranch, by piercing the body wall with the buccal stylet and sucking blood, and perhaps tissue debris, by means of the buccal pump. A list of hosts, each with its specific parasite, was given.

Subsequently, Cole (1951) recorded the presence in oysters of a species, *Odostomia eulimoides* Hanley, which had been observed by Fretter & Graham, and recorded previously by Jeffreys (1867), as feeding on *Pecten maximus* (Linné) and *Chlamys opercularis* (Linné). The *Odostomia* were lodged in small pockets inside the ventral margin of the shell of 2-year-old native brood oysters dredged from near Paglesham in the River Roach, Essex. The pockets were formed as a consequence of the withdrawal of the mantle in response to the irritation caused by the proboscis of the parasite during feeding.

It was suggested that, although *Odostomia* could not be regarded as a major parasite of oysters, its attack must result in considerable irritation, some loss of condition, and possibly small permanent malformations of the shell.

In December 1953, Mr J. E. Francis, foreman of the Colne Fishery Board, brought to the Burnham Laboratory some oysters of marketable size, i.e. 5-6 years of age, taken from the Pyefleet Channel, River Colne, which appeared to be suffering from the cumulative effects of attacks by *Odostomia*. Many of the oysters were malformed and some were dying. Oysters in a similar condition had been noted about 7 years previously. Further researches into the incidence of the parasite and its effects on oysters form the basis of this paper.

We are much indebted to the Colne Fishery Board, and in particular to Mr Francis, for material and information. We are also indebted to Dr Vera Fretter for the identification of the species of *Odostomia* found during the investigations.

## OBSERVATIONS

*Odostomia in Oysters*

A sample of forty oysters was received from the Pyefleet Channel in January 1954. They had been noticed and removed by oystermen during normal dredging operations from among several thousand oysters of marketable size. They were 5–6 years of age, and 76 mm in average diameter. These oysters were specially selected as having some malformation of the edge of the shell, and it was estimated by Mr Francis that they occurred once or twice in every 2000 oysters. Of the forty oysters received, all showed symptoms similar to, but far more serious than, those recorded by Cole (1951) as resulting from an attack by *O. eulimoides*. The presence of a small gastropod was noted, and this was found to be the same species of *Odostomia*. In one dying oyster no less than seven *Odostomia* were found. In others one, two or three were present. In some no parasites were found, but, since the oysters had been collected several days before and kept alive in baskets in the oyster pits, and had been handled frequently during dredging and sorting, it is not surprising that they had been lost.

In less severe cases the *Odostomia* were observed in small characteristic pockets usually on the ventral or anterior margin of the oyster (Pl. I, fig. 1). These were similar to those described by Cole (1951). Prolonged irritation by a larger number of *Odostomia*, however, causes a much more violent response by the oyster. The pockets become merged to affect almost the whole edge of the shell, and penetrate farther towards the adductor muscle. The edges of the pocket are marked by numerous thin, approximately parallel, laminae of shell substance, marking the successive positions of the withdrawn mantle edge as the oyster had attempted to evade the probing of the parasite. The margin of the shell becomes thickened, in the worst cases being approximately 1 cm wide with double or triple lips (Pl. I, fig. 2).

In the most severe cases, the constant irritation appears to interfere with the normal metabolism of the oyster. Great ridges of shell substance are laid down following the path of the withdrawn mantle. Finally the point of attachment of the adductor muscle is reached, but the process continues, with the result that the adductor muscle is almost completely covered by a thorn-like ridge of brownish deposited shell material (Pl. I, fig. 3). Attack from the ventral margin, as normally occurs, will first affect the catch component of the adductor muscle and will cause the oyster to gape. In this condition, sand and silt will be driven into the mantle cavity with resultant suffocation and death. Such a condition was, in fact, commonly observed among the severely affected oysters. In one severe instance, only a narrow band of one-tenth of the adductor muscle remained fully functional. This displacement of the adductor muscle may occur inside either valve of the oyster (Pl. I, fig. 3; Pl. II, fig. 4),

but is most usually found in the flat valve. Sometimes, the two valves are affected simultaneously, resulting in a condition in which it is virtually impossible for the oyster to close. In most of the severe cases it was possible to follow the development of the attack by the presence of successive layers and ridges of shell which led back to the damaged adductor muscle. Occasionally, however, although the margin of the oyster shell showed severe pockets, a thorn-like growth was present intruding into the adductor muscle with no connecting layers of similar material between it and the pockets (Pl. II, fig. 5). The impression received was one of severe irritation of the oyster, with consequent strain on the adductor muscle, which had manifested itself in a violent disturbance of its metabolism.

In several of the oysters examined, retraction of the mantle, accompanied by thickening of the edges of the shell, had taken place to such an extent that there had been room for settlement of mussel spat inside the margins of the two valves. The mussels had grown, causing the valves to part even more, finally producing the effect shown in Pl. II, fig. 6.

Of the forty oysters examined in this sample, twenty were so severely affected that the adductor muscle was damaged, and of these, eleven were dying or already dead from the effects of *Odostomia*. It should be mentioned that none of these oysters showed evidence of shell disease, which occurs very rarely in the River Colne, but which when present produces in its final stages effects of a somewhat similar nature in oysters. Occasionally severe attack by *Polydora* may produce blisters on the shell which resemble the pockets caused by light attack by *Odostomia*. With practice, a substantial proportion of oysters affected by *Odostomia* may be detected in the field by the thickening and distortion of the shell edge. However, the superficial resemblance between the condition it causes, in the early stages of attack, and damage by other enemies, such as *Polydora*, has delayed the recognition of the parasite on beds where it is now known to occur.

The appearance of such badly affected oysters on the Colne Fishery amongst those intended for market caused some concern. It was decided to investigate the frequency of the occurrence of *Odostomia* in younger oysters on the grounds in the River Colne from which oysters intended for market are taken for final fattening in the Pyefleet Channel. A random sample of 3-year-old brood oysters was dredged, isolated carefully, and examined for the presence of *Odostomia*. In ten, of fifty-four oysters, *Odostomia* was actually present, each of the oysters showing retraction of the mantle and typical pocketing of the shell margin. In six others there was definite evidence, and in four slight evidence, that *Odostomia* had been present. A maximum of three *Odostomia* was found in one oyster, and a total of fifteen in the ten oysters. Three additional *Odostomia* were found in the container. This represents a minimum of 30% incidence of *O. eulimoides* in 3-year-old brood oysters at the lower end of the oyster grounds in the River Colne. Samples from higher upstream

indicate that *Odostomia* occurs less abundantly there, but the full pattern of its distribution has not yet been determined.

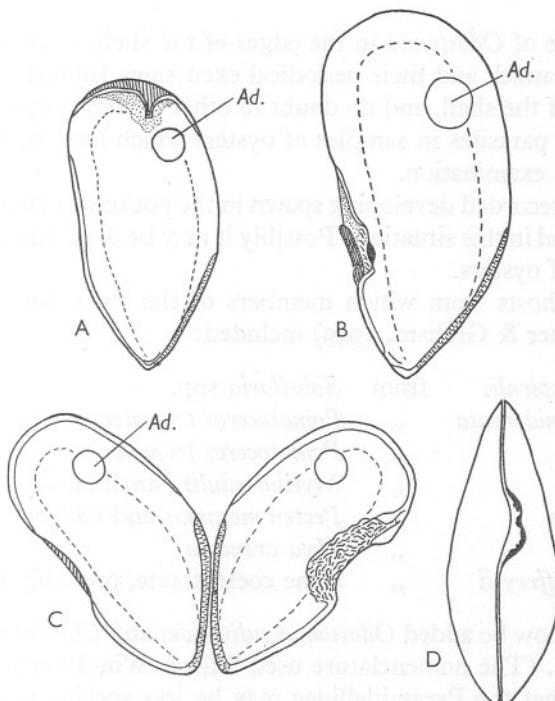
The evidence that *Odostomia* attack can cause the death of some oysters, and may render others unfit for consumption, led to a search for the parasite when examining oysters from other areas. A feature of its occurrence is its appearance in unexpected circumstances, which has led to the belief that the general incidence of *Odostomia* in oysters must be high. For example, the first oyster opened in the River Colne for a condition measurement contained an *Odostomia*. A small brood oyster from Creeksea, River Crouch, examined for drilling by *Urosalpinx*, contained *Odostomia*. The first oysters opened at Fambridge, River Crouch, and in Salcombe harbour, for other reasons, each contained *Odostomia*. In the River Crouch, 17% of the brood oysters from Brickfield Bight, Bridgemarsh Island, a ground approximately mid-way between the head-waters and the mouth, were infected, while 8% of the brood oysters taken from the Shop Laying, Paglesham, River Roach, also contained *Odostomia*.

*O. eulimoides* has now been recorded in oysters from Creeksea, Brickfield Bight and Fambridge in the River Crouch, and Shop Laying and Common Shore in the River Roach. An oyster from Salcombe harbour contained a pyramidellid which proved to be a different species, *Chrysallida obtusa* (Brown) which is here recorded for the first time in oysters.

#### *Odostomia in Mussels*

Information had been received from Dr E. W. Knight-Jones that *Odostomia scalaris* (Macgillivray) was present in large numbers on *Mytilus edulis* (Linné) from the pontoon of Menai Bridge pier. It was thought that an investigation of these mussels might provide a useful comparison with the effects of *Odostomia* on oysters. A sample of 200 mussels, kindly despatched by rail by Dr Knight-Jones, was examined for the presence of *Odostomia* and for any retraction of the mantle comparable with the condition found in oysters. Retraction of the mantle to form a pocket had occurred in eight of the mussels, and, although the *Odostomia* were not present inside the shell, there is little doubt that they had been the cause. The pockets were strictly similar in form to those described in oysters (see Text-fig. 1). Each occurred usually on one valve, while the other valve had overgrown slightly in an effort to complete the gap. The pocket was then just visible externally as a slight twist in the shell margin. Forty-two *O. scalaris* were found on the outer surfaces of the mussels and in the container. Several of these were kept alive in an aquarium tank with small *Mytilus*, and after a short time were found to have taken up a feeding position. Each *Odostomia* was attached to the outer edge of one of the valves with its proboscis protruding into the siphonal aperture of the mussel, and they remained in this position for several days. The length of the proboscis was such that it could have produced the retrac-

tion of the mantle without actual entry of the *Odostomia* between the valves as occurs in oysters. This, combined with the fact that the mussels had undergone a long rail journey, may explain why no *Odostomia* were found inside the shells of the mussels.



Text-fig. 1. A, B, Inner surfaces of the left valves of two mussels showing 'pockets' caused by *Odostomia*; A, in posterior margin, B, in ventral margin. C, inner surface of mussel attacked by *Odostomia* at ventral margin. D, ventral margin of mussel C. *Ad.*, adductor muscle.

#### DISCUSSION

In the present investigation up to seven *O. eulimoides* were found in one oyster, but, since many parasites are lost during dredging and handling, it is likely that still more may occur in large oysters. Cole (1951) found two pairs occupying separate pockets in small 2-year-old oysters from the River Roach, Essex, but the damage caused was not severe. It may be conjectured, therefore, that the very serious damage here described is either produced by substantially more than the maximum number so far recorded, or by attack by smaller numbers over a period of years.

It is probable that in the first stages of attack the *Odostomia* attach themselves to the lips of the shell and probe the edges of the mantle with their long probosces. Later, as a pocket is formed by the deposition of new shell along the line of the retracted mantle edge, near the site of attack, the *Odostomia* are able

to move inside the shell and live more or less permanently within the oyster. It is clear, however, that they must leave the pockets at times, e.g. when copulation takes place, and in an aquarium tank *Odostomia* have been noticed moving over the outer surface of oysters which had obviously suffered from attack.

The presence of *Odostomia* in the edges of the shells of oysters during the early stages of attack and their periodical excursions from the pockets to the outer surface of the shell, and no doubt to other oysters, explains the relative infrequency of parasites in samples of oysters which have been dredged and handled before examination.

Cole (1951) recorded developing spawn in the pockets in June, but none has since been found in this situation. Possibly it may be deposited also in crevices on the shells of oysters.

The list of hosts from which members of the Pyramidellidae had been recorded (Fretter & Graham, 1949) included:

<i>Chrysallida spiralis</i>	from	<i>Sabellaria</i> spp.
<i>Odostomia unidentata</i>	"	<i>Pomatoceros triqueter</i>
<i>O. lukisii</i>	"	<i>Pomatoceros triqueter</i>
<i>O. scalaris</i>	"	<i>Mytilus edulis</i> (small only)
<i>O. eulimoides</i>	"	<i>Pecten maximus</i> and <i>Chlamys opercularis</i>
<i>O. trifida</i>	"	<i>Mya arenaria</i>
<i>Turbonilla jeffreysii</i>	"	some coelenterate, probably <i>Haleciun</i> sp.

To these may now be added *Odostomia eulimoides* and *Chrysallida obtusa* from *Ostrea edulis* L. (The nomenclature used follows Winckworth, 1932.)

It appears that the Pyramidellidae may be less specific in their choice of host than has been suggested.

*Odostomia*, as a parasite of oysters, has some practical importance, not only because of its frequency in young oysters whose growth is likely to be retarded. It has been shown that full-grown oysters may be seriously weakened and killed and it would be inadvisable to include obviously infected oysters in a market consignment, since the discovery of a number in the condition illustrated might prejudice future sales. No measure of control can so far be suggested other than the destruction of large oysters which show the multiple-lipped condition and are light in weight, suggesting low flesh content.

#### SUMMARY

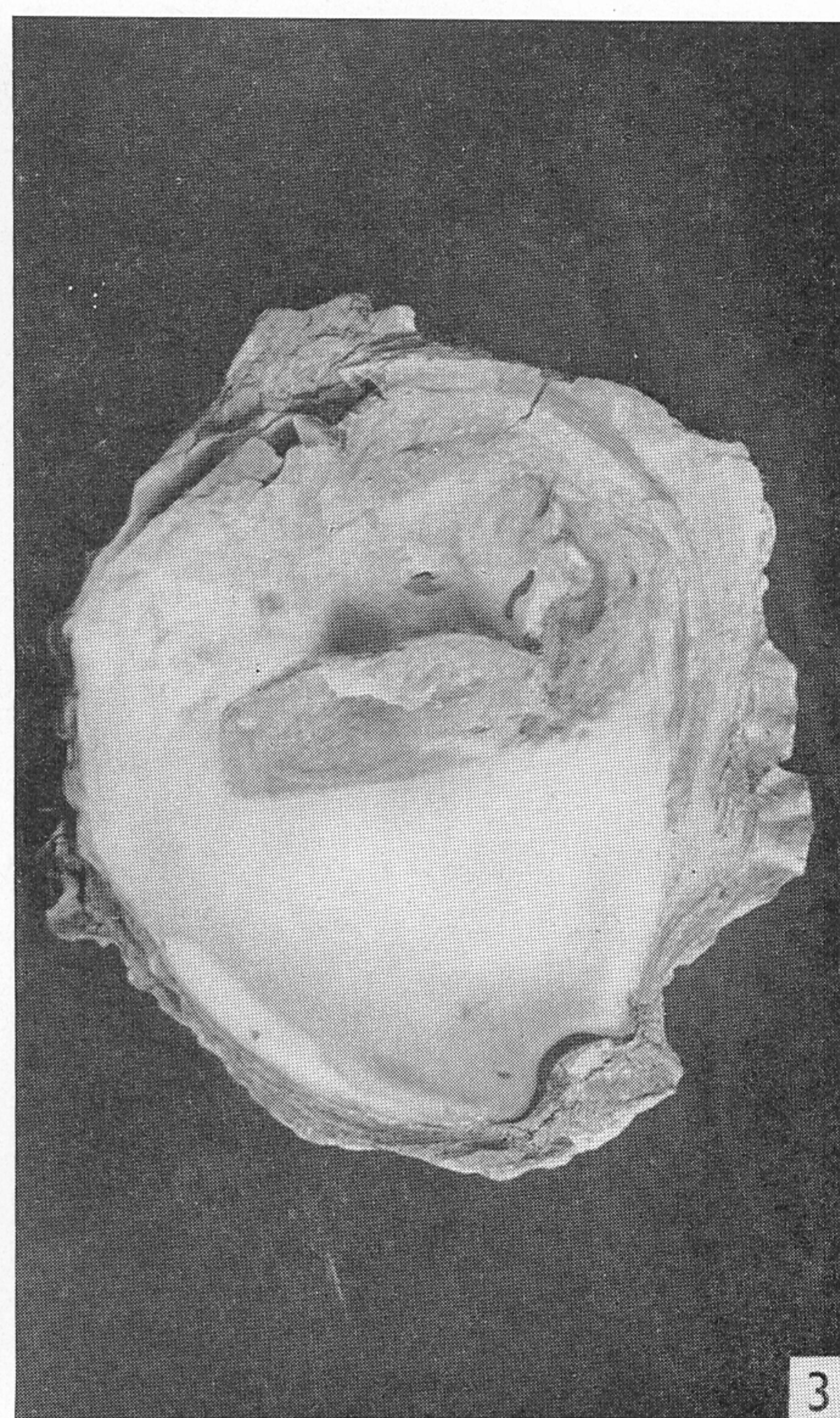
A description is given of serious damage to oysters of marketable size taken from the River Colne, Essex, caused by *Odostomia eulimoides*. An incidence of 30% infection was found in 3-year-old oysters.

*Chrysallida obtusa* is recorded for the first time as a parasite of oysters.

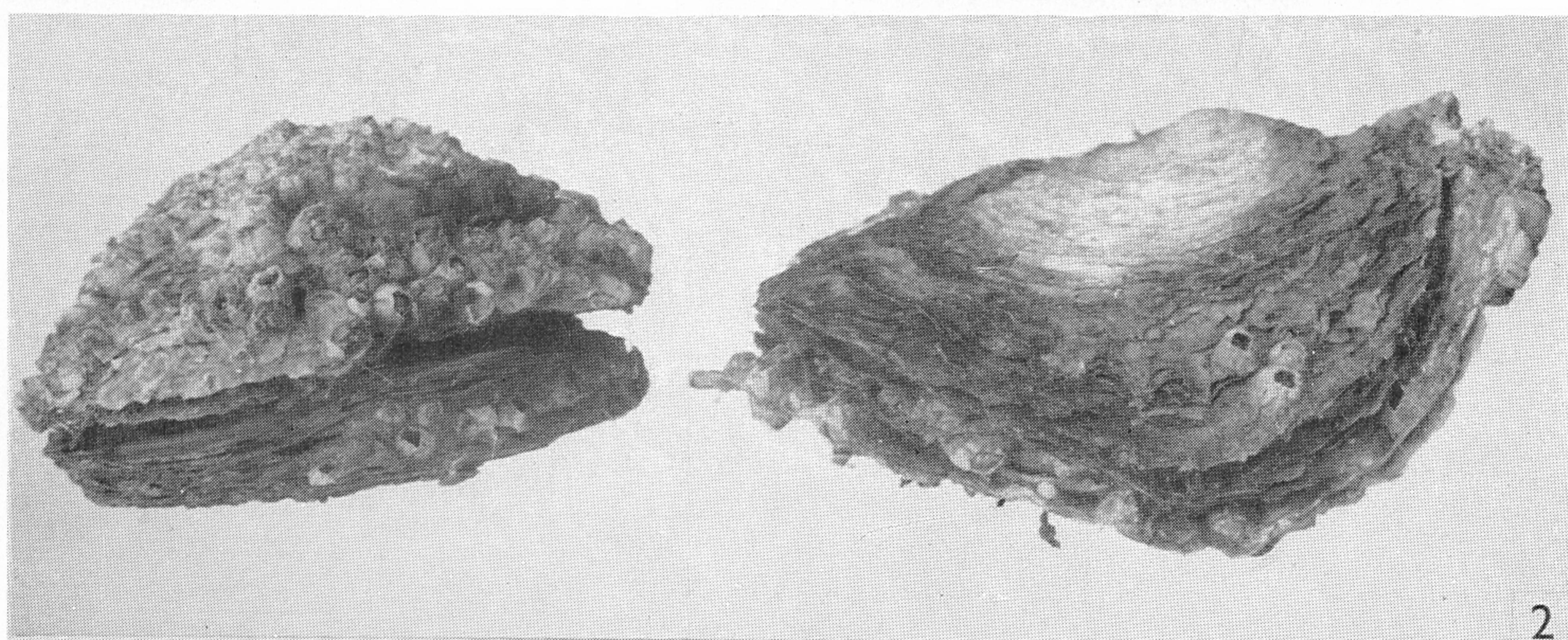
The effects of *Odostomia scalaris* on *Mytilus edulis* are similar to those described in oysters resulting from attack by *Odostomia eulimoides*.



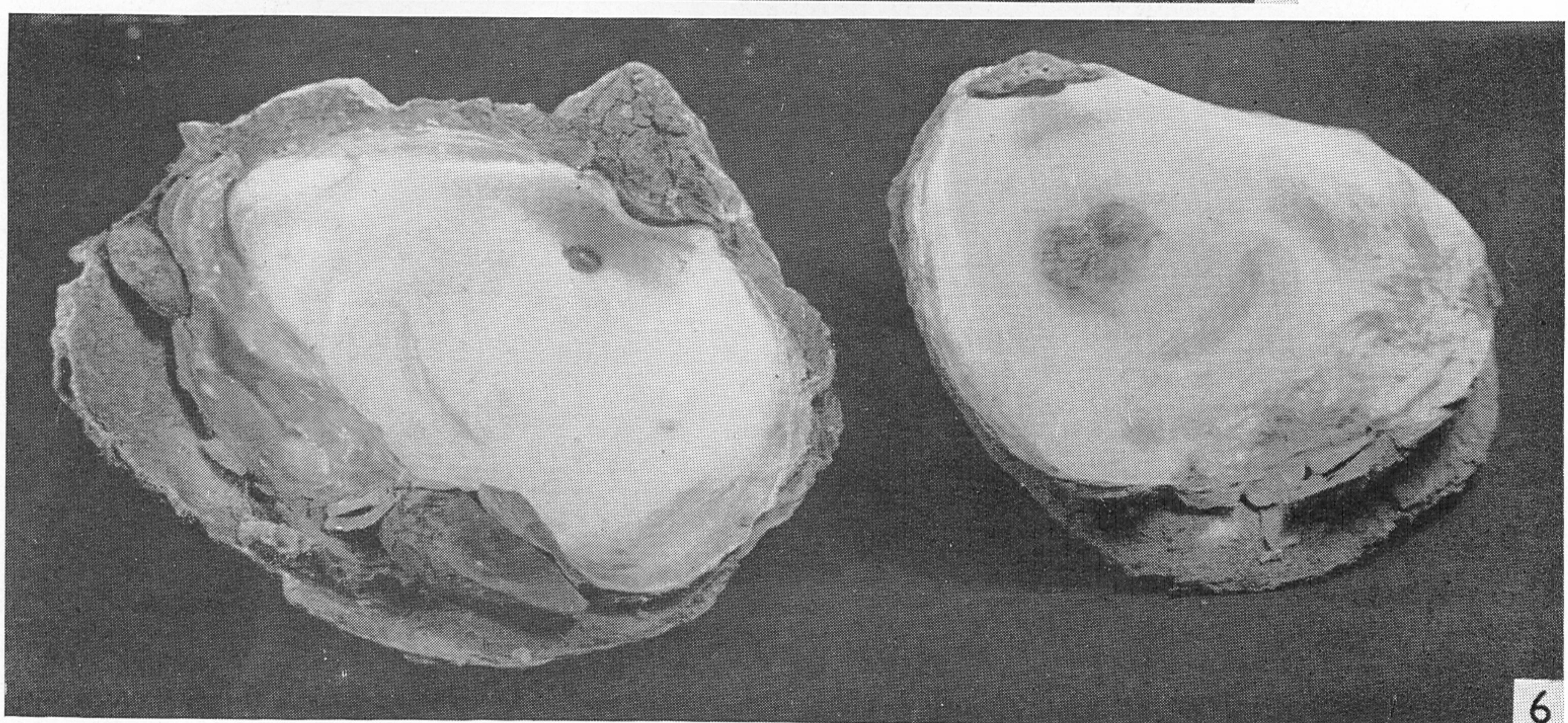
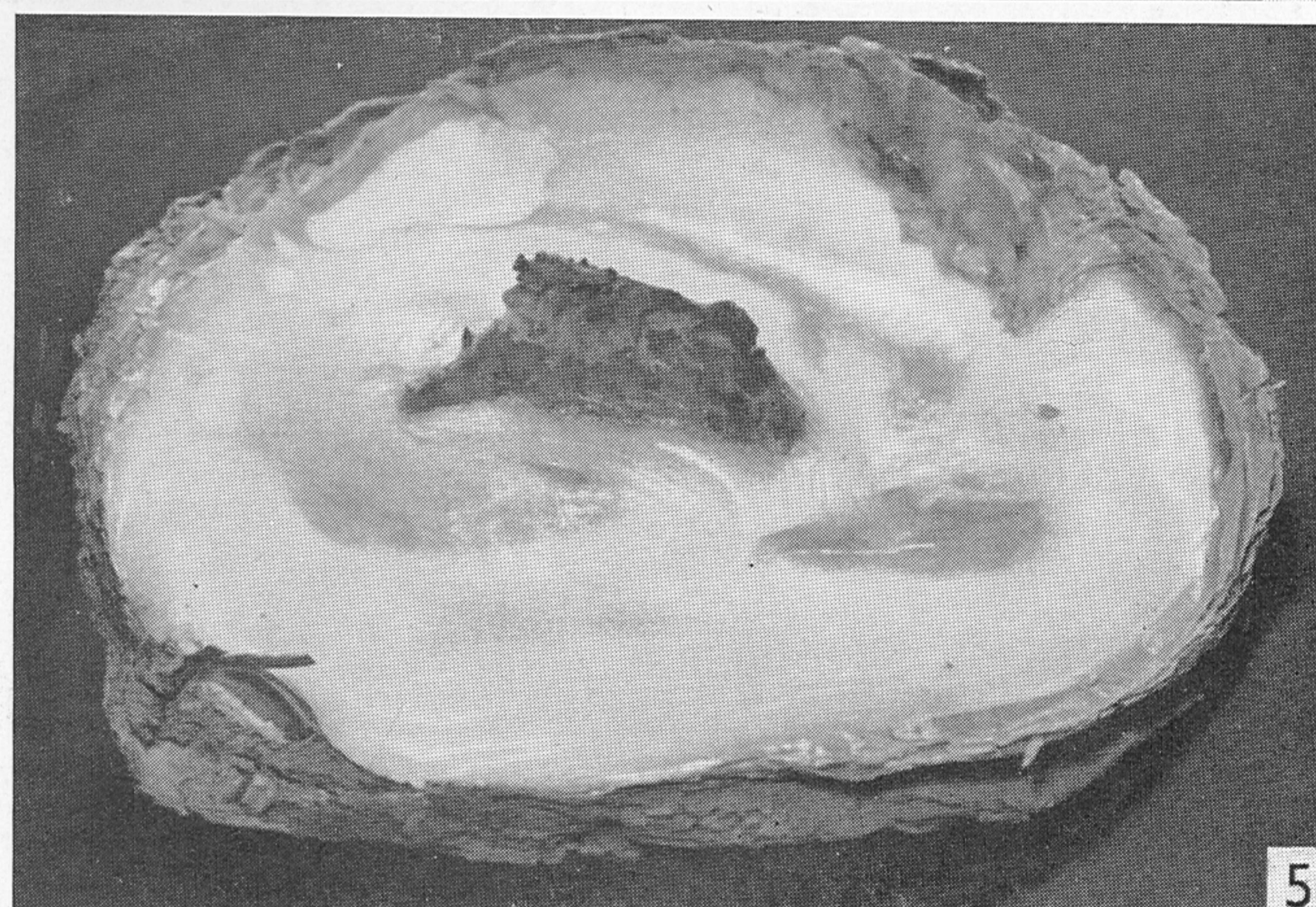
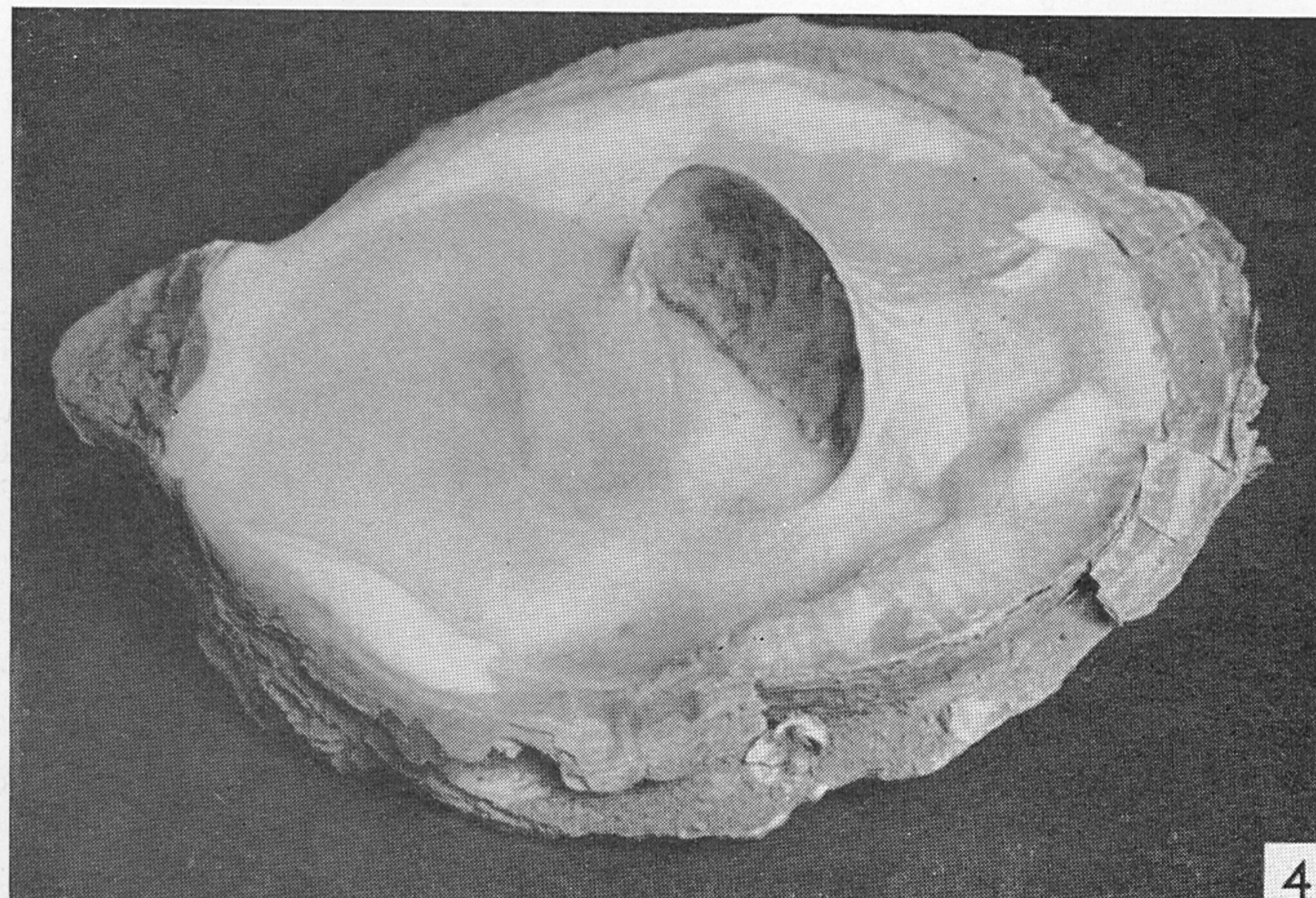
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WINCKWORTH, R., 1932. The British marine Mollusca. *J. Conch.*, Vol. 19, pp. 211-52.

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Shells of *Ostrea edulis* showing *Odostomia* *in situ* in characteristic marginal 'pockets'.  
Fig. 2. Ventral margins of two oysters thickened as a result of *Odostomia* attack.  
Fig. 3. Inner surface of the left valve of an oyster with adductor muscle attachment affected by severe attack by *Odostomia*.

PLATE II

- Fig. 4. Right valve of an oyster attacked by *Odostomia*.  
Fig. 5. Right valve of an oyster showing typical *Odostomia* 'pocket' and affected adductor muscle attachment.  
Fig. 6. Oyster shells showing effects of severe *Odostomia* attack and settlement of mussels between the valves.

## SALINITY VARIATION IN INTERSTITIAL WATER OF SAND AT KAMES BAY, MILLPORT, WITH REFERENCE TO THE DISTRIBUTION OF *NEREIS DIVERSICOLOR*

By Ralph I. Smith

Department of Zoology, University of California, Berkeley, California

(Text-figs. 1-5)

The sheltered beach of Kames Bay, Millport, Isle of Cumbrae, Scotland has been the scene of several important studies on intertidal zonation (Stephen, 1929; Elmhirst, 1931; Watkin, 1942). The last paper is most comprehensive and records in detail the zonation of over twenty species of animals. In the course of the present study I have found Watkin's account very accurate, but incomplete in respect to salinity variation in the interstitial water of the sands. My attention was initially directed to Kames Bay in a search for a population of *Nereis diversicolor* O. F. Müller inhabitating an essentially 'marine' environment in respect to salinity. The suitability of Kames Bay was suggested by a statement of Watkin (1942, p. 558):

The salinity of the tidal water may be expected to agree with that of the waters of the Firth and to show little or no variation from high to low water, and to be about 32 parts per thousand.... The extent of the fresh-water influence in Kames Bay is very slight. A small stream crosses the beach on its western edge, but the line of sampling is some distance from it.

And on p. 544:

the volume of water of this stream is small and the effect over the whole bay cannot be considered as seriously affecting the type of fauna.

The present report will give evidence that other sources of fresh water in Kames Bay, apart from the small stream mentioned above, are not insignificant, and that the distribution of *N. diversicolor* on the beach may be correlated with the occurrence of brackish water.

As shown in Fig. 1, the western third of the beach is subject to the run-off of a smaller and a larger freshwater stream issuing from culverts in the sea wall. In October 1953, samples of interstitial water were taken along a transect (A) running seaward across the slope affected by these freshwater rivulets. *N. diversicolor* was found to occur in rather uniform numbers along this line, from a point 35 m from the sea wall (i.e. below the high-water neap-tide level) out to 90-100 m. The chloride of the interstitial water varied from

5 parts per mille at 35 m to 16‰ at 100 m (that is, salinities of 9 and 29‰ respectively). These values were maintained beneath nearly fresh over-flowing stream water during low tide (at 100 m the flowing surface stream had a chloride of 2·4‰). Such a situation has been repeatedly observed in estuarine environments (Reid, 1930, 1932; Alexander, Southgate & Bassindale, 1932). The broad distribution of *N. diversicolor* contrasted, however, with the narrow zonal grouping reported by Watkin, and seemed possibly related to the wide zone of freshwater influence. As a check of this supposition a second transect (B) was established near the centre of that two-thirds of the beach presumed to be free of freshwater influence. The results of preliminary sampling on transect B showed the expected sharper zonal grouping of *N. diversicolor*, but also an unexpected band of low interstitial salinity extending along the beach in the zone of greatest abundance of the worm. This finding prompted a more careful examination of this part of the beach and of the salinity profile of the interstitial water.

#### GENERAL DESCRIPTION OF THE BEACH

In October 1953, the beach presented a distinct 'upper slope' of loose sand with windrows of debris and cast wrack, commencing close to the sea wall, approximately at the high-water mark of the highest spring tides (EHWS), and ending some 35 m to seaward, 2 ft. or so vertically below the high-water mark of neap tides. From the foot of the upper slope there extended seaward a 'middle flat' of smooth, usually ripple-marked, sand which at about 80–90 m from EHWS began to slope more noticeably to seaward. Roughly, the start of this 'outer slope' coincided with the seaward margin of the zone of *Arenicola marina* and the beginning of the zone of *Nephthys* sp. Along the inner margin of the middle flat part of the beach, a series of shallow pools retained a layer of water even at low tide. These depressions probably resulted from the churning effect of mild surf action at high water, and the steeper upper slope to wave wash at such times. The outer slope may be the result of comparable periods of wave action at low tide. Without going into detail, it is clear from the generally sinusoidal form of the tidal curve at Millport that the upper and lower slopes must experience relatively longer periods of breaking waves than does any similar area of the middle flat, over which the tide advances and retreats more rapidly, and of course the sigmoid configuration of the beach profile, once established, further accentuates the differences in duration of wave action resulting from the character of the tidal curve. It is in the relatively stable middle flat that *Nereis* and *Arenicola* are established in large numbers. The presence of these forms has been stated by Dahl (1952) to be characteristic of mud flats rather than of sand beaches of the sort whose zonation he has recently described, and it might not be unfair to characterize this part of the Kames Bay sands as a mud flat lacking mud. In stormy weather and throughout the winter months the long wash and sweep of waves

tend to fill in the angle at the foot of the upper slope. By November 1953, the foot of the upper slope had advanced about 10 m seaward from its earlier level, and by February 1954, the beach presented an uninterrupted, slightly concave, slope from the centre of the middle flat nearly to EHWS mark. The morphology of the beach is obviously not static, but represents a dynamic equilibrium of complex forces.

The substratum varies somewhat in different parts of the beach, the upper slope being of noticeably coarser sand than the middle or outer zones. However, the populated parts of the beach are, on the whole, of remarkably uniform particle size (according to Watkin, chiefly 0·2–0·5 m) with a very small fraction of silt or clay, and no black deposits in the areas studied. The most striking discrepancy in substrate occurs at the foot of the upper slope where the sand is cut away in summer to a thin layer about 3 in. deep, overlying some 3 in. of stony gravel, and this in turn covering a hard layer of mixed stones embedded in fine reddish sand.

A most noticeable feature of that section of the beach which receives no surface streams of fresh water is the fact that the lower half of the upper slope and the middle flat to seaward of its foot remain continuously wet and glistening at low tide. The appearance suggests that subsurface seepage reaches the surface of the sands above the line where the upper slope terminates, and that it permeates the sands of the middle flat for a considerable distance. This band of wetness occupies the greater part of the width of the beach. A small well-drained band occurs near the ledges bounding the beach on the east, and a wider 'dry' band is found in approximately the centre 15–20% of the beach just to the east of the main stream. The characteristic wetness of the sands in the rest of the beach seems to be present regardless of immediate weather conditions. It has been observed in the course of this study from October to April, and is evident in an aerial photograph taken in August 1947 (Fig. 2). Since this drainage appears to be of fresh water, the sands of the inner edge of the middle flat must be to some extent exposed to brackish conditions during the period of low tide. It might be assumed that any intrusion of brackish water would be replaced by sea water at high tide, but this is not necessarily so, and it appears from the literature that few workers have considered in detail the possible variations of salinity in the interstitial water that may occur on sand beaches during the tidal cycle. Bruce (1928) discusses surface and subterranean intrusion of fresh water on the beach at Port Erin and concludes from a single series of observations that '...there is a progressive fall in salinity [of the interstitial water] from the time the beach is uncovered until the advancing tide has nearly reached the point of observation, when the salinity rapidly rises to the full sea-value'. No interstitial salinities were recorded from beneath sea water, and it seems probable that Bruce was working in an area of surface drainage of fresh water. Pirrie, Bruce & Moore (1932) did not attach much importance to lowered interstitial

salinity at Port Erin, for they conclude that 'no very close correlation must be looked for between the distribution of the fauna and the salinity of the beach'. Emery & Foster (1948) have shown that the salt-water table in the sands of exposed Californian beaches rises and falls with the tides, but lagging

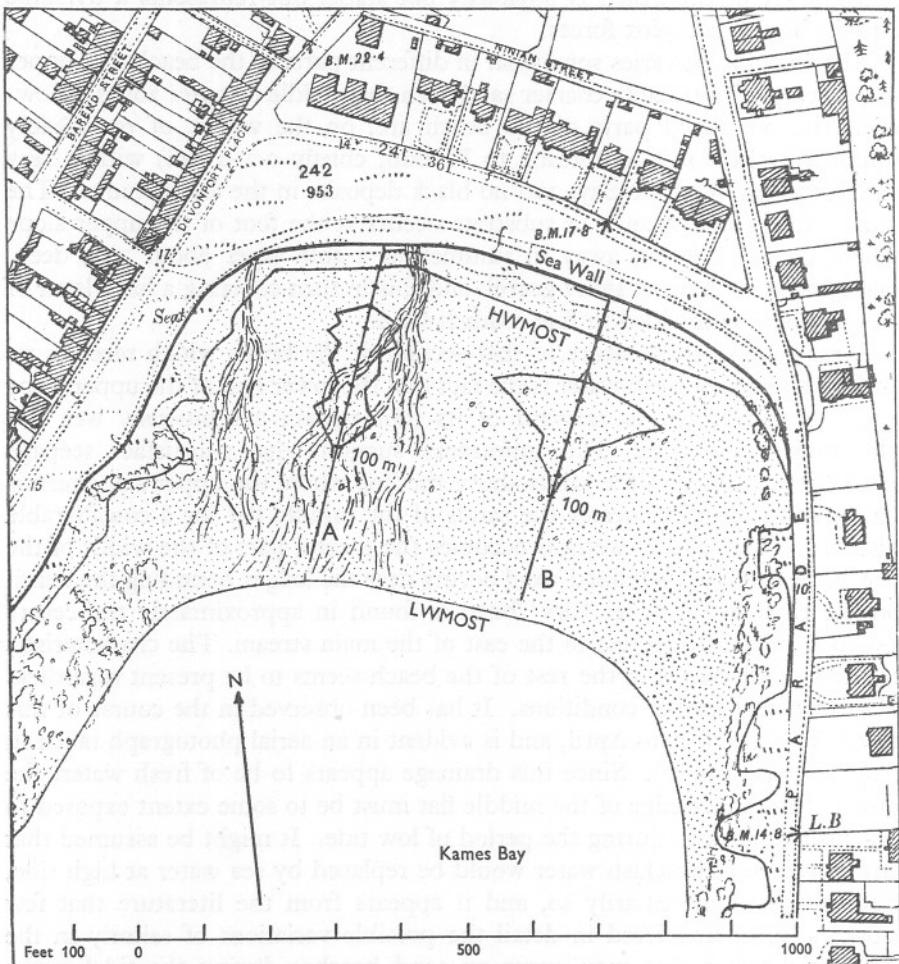


Fig. 1. Map of Kames Bay, a composite from Ordnance Survey sheets, Buteshire, CCXVI-10 & 11 and -14, 1896 edition, 1:2500; reproduced by permission of Ordnance Survey Office. Sea wall, streams, and transects A and B added. Polygons on transects show relative density of *N. diversicolor*.

by 1–3 h and with decreasing amplitude as the land is approached. Such a fluctuating salt-water table might be of considerable importance to animals of the upper mid-tidal sands. In attempting to assess the importance of the apparent freshwater intrusion into the *Nereis* zone at Kames Bay, two possi-

bilities were considered: (1) If the interstitial drainage from the land were super-imposed upon a fluctuating salt-water base, the freshwater layer might



Fig. 2. Unretouched aerial photograph of Kames Bay, apparently at low tide, August 1947, showing as a darker area the position of the wet band in the upper slope of beach. (R.A.F. photo no. 4103, CPE/SCOT/UK/261, 13 August 47, F/20'/16,600', 82 Sqdn. Crown Copyright Reserved. Published by permission of the Air Ministry.)

be elevated at high tide so that the nereid zone would receive fully saline sea water twice daily. But if (2) the level of freshwater drainage were determined by geological factors which precluded oscillation of the salt-water table, then

it would be possible to conceive of a situation where fresh water flowed at a fixed level regardless of the tide, and so penetrated the nereid zone beneath the incoming tide, subject to mixing with the overlying sea water. The latter possibility would be of obvious interest when one considers the occurrence of such a typically estuarine form as *N. diversicolor* on an apparently marine beach.

#### METHODS

Transects A and B are indicated in Fig. 1 and may be readily located by reference to houses near the bay. Transect B, on which most work was done, started at the lower eastern corner of the sloping concrete apron at approximately EHWS mark and ran directly seaward. Distances were measured with a 25 m length of trawl line marked off in 1 and 5 m intervals. Although subject to error by stretching, it gave sufficiently reproducible results for the purpose. Water samples were sucked up from a depth of 6 in. beneath the sand surface by means of a rubber tube and a stiff plunger, originally of Pyrex tubing (of 5 mm bore, flamed down at tip to 1 mm), later replaced by a brass tube of comparable dimensions. It was found best to make the orifice large enough to admit sand grains freely; these tended to settle and did not seriously interfere with sampling. For use in coarse sand the orifice is better placed at the side of the tip rather than terminally. In use, the plunger was pushed to the desired depth (a large cork on the tube formed a stop), a sample sucked up and discarded, the tube re-inserted while blowing to exclude water, a second sample brought up, the tube wiped off, a small quantity of water discharged, and the remainder collected in a vial. When subsurface samples are taken in this simple way from beneath overlying sea water there is likelihood of contamination, but as such would only tend to lessen the differences observed, the general conclusions reached would not be invalidated. Aliquots of 1 ml. were titrated from a 5 ml. burette with  $\text{AgNO}_3$ , 19.16 g/l., equivalent to 4 g chloride per litre, standardized against a carefully prepared solution of NaCl (equivalent to 10 g chloride per litre), diluting each aliquot with 10 ml. of distilled water and using 5% potassium chromate indicator. Since this procedure is not that of standard hydrographic practice, the results have been expressed in grams of chloride per litre at room temperature (a value approximating 'chlorosity') rather than as 'chlorinity'. The differences, for our purpose, are inconsequential. For obtaining the density of worms at each station a square box of galvanized sheet-iron without bottom, of  $\frac{1}{10} \text{ m}^2$  area, was pressed into the sand to a depth of 6 in., the enclosed sand dug out, and washed through a sieve of 16 meshes to the inch. Only *N. diversicolor* were collected; counts are expressed in numbers of heads obtained regardless of size, although it was noted that the larger worms were more abundant at about the 50-60 m level. Transect B was studied on 31 October-2 November 1953, 12-13 February, and 18 March 1954, with special attention paid to obtaining

interstitial water samples from beneath overlying sea water at high tide or during the ebb, when salinities in the sand might be expected to be maximal. Unpublished rainfall and salinity data recorded at the Millport Laboratory were kindly made available by Dr H. Barnes.

#### OBSERVATIONS (TRANSECT B)

31 October–2 November 1953. Two series of water samples were taken on falling tides and one on a rising tide; one series of collections of *N. diversicolor* was taken at corresponding stations. On 1 November the transect was sampled successively 5 times, from 1 h past the start of the ebb until the turn of the tide. On this day the foot of the upper slope lay at 50 m from EHWS mark, having advanced somewhat from the mid-October position, but the break at its foot, where the stony layer lay close beneath the surface, was still distinct. It was raining intermittently, following a fortnight of heavy rains (the Marine Station had recorded 5·39 in. of rain in the previous 15 days). The interstitial chloride profiles (Fig. 3 A–F) indicated a brackish zone which tended to move seaward as the tide fell, and which was distinct even beneath the shallow wash of the retreating sea water. As the tide fell, the surface water became progressively less saline, probably as the combined result of dilution by subsurface drainage and of lateral mixing with fresh water from the streams to the west. Under the conditions of high freshwater discharge prevailing that day the chloride of the bay water was lowered from 17 to 12·8 g/l., but at the turn of the tide the inflooding sea water had a nearly normal chloride value of 17 g/l. (= salinity of 30·5%).

On 2 November, two series of interstitial water samples taken beneath sea water up to 20 in. deep on the falling tide confirmed the results of the previous day and showed more clearly the low salinity of the interstitial water beneath ebbing sea water (Fig. 3 G, H). The lowering of subterranean chloride was apparent out to 50–60 m from EHWS mark. The possibility was considered that these results reflected abnormal conditions caused by heavy rainfall in the previous 2 weeks.

12–13 February 1954. The earlier work was repeated following a period of relatively dry weather. There had been rains on 5–6 and 9–10 February, but the total rainfall for the previous 17 days had been only 1·57 in. By this time winter storms had so smoothed out the upper half of the beach that no break was apparent between upper slope and middle flat. Several inches of sand had been deposited above old burrows of *N. diversicolor*, but with little or no displacement of the population. The zone of *Arenicola* was unaltered. One salinity profile was taken during rain on 12 February on a rising tide and a second series of three in light rain on the ebbing tide, 13 February. The chlorides obtained on the falling tide (Fig. 4) agreed with the previous findings, brackish interstitial water being evident beneath sea water and detectable out to 60–70 m, about to the inner edge of the zone of *Arenicola*.

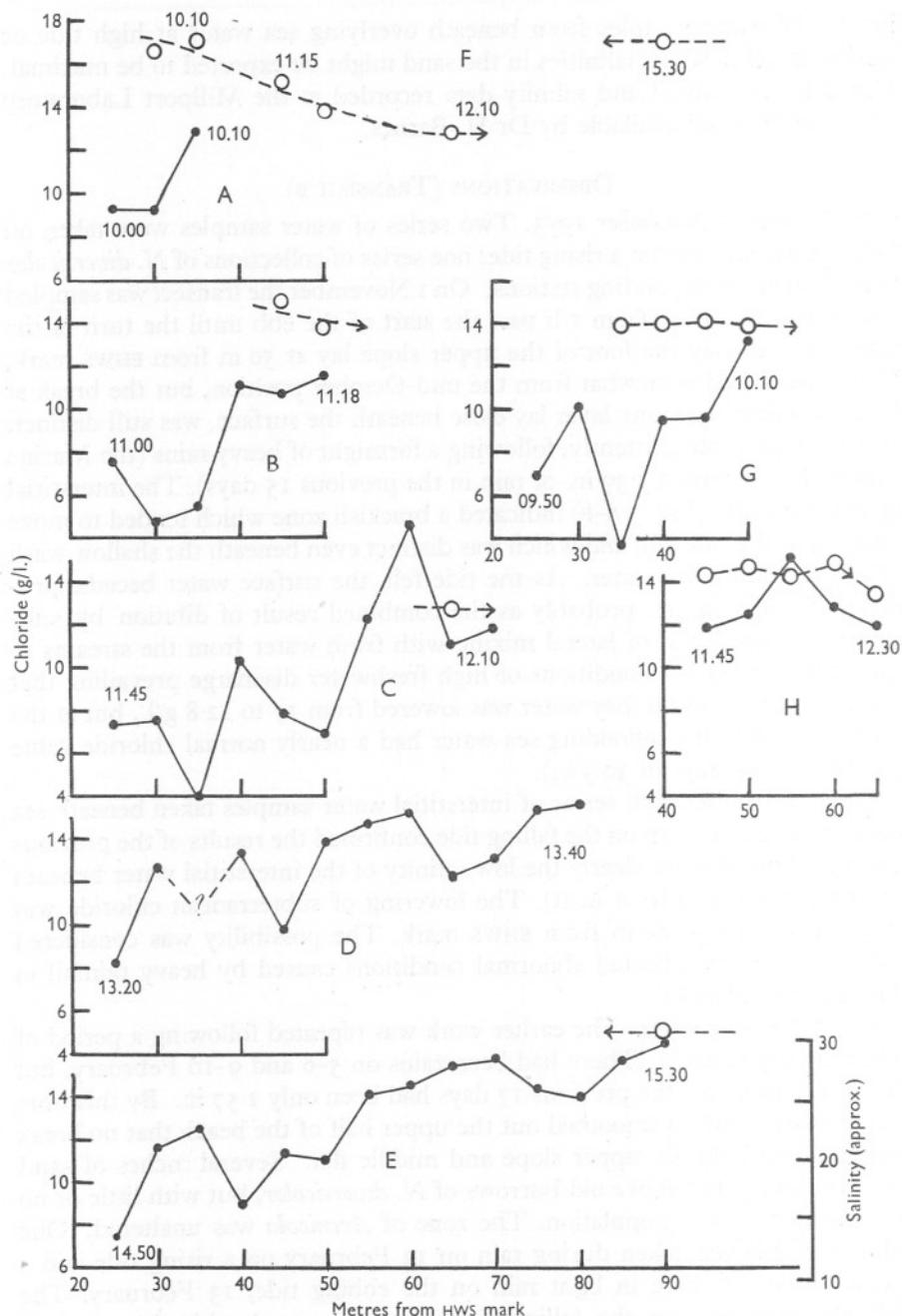


Fig. 3. 1-2 November 1953. Solid curves A-E, chloride of interstitial water on falling tide 1 November, taken successively at times indicated; dashed curves, chloride of overlying sea water, plotted collectively as curve F, the point at 15.30 being at start of rising tide. Insets G and H, similar, for falling tide on 2 November.

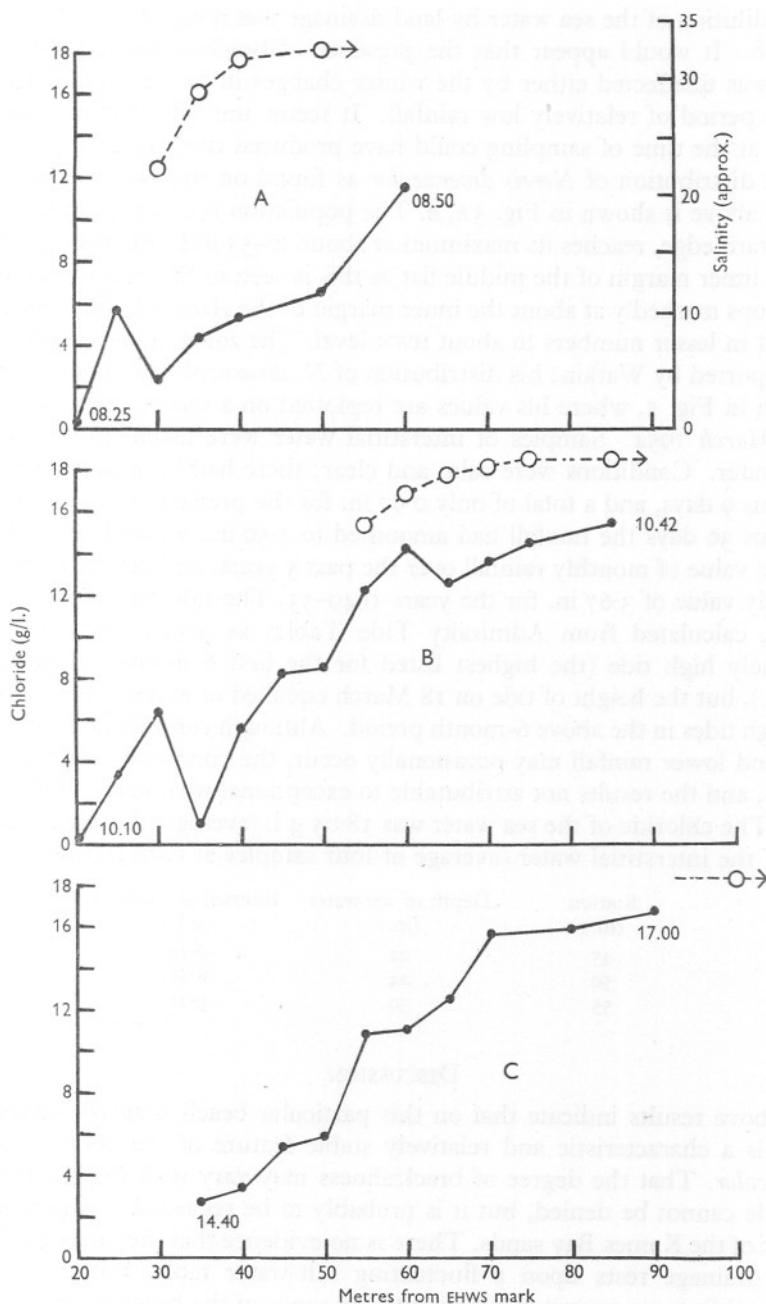


Fig. 4. 13 February 1954, falling tide. Solid curves, interstitial chloride; dashed curves, chloride of overlying sea water. Times of sampling as indicated.

Some dilution of the sea water by land drainage was noticeable at the start of the ebb. It would appear that the presence of brackish water beneath sea water was unaffected either by the winter changes in beach configuration or by the period of relatively low rainfall. It seems unlikely that rain actually falling at the time of sampling could have produced these results.

The distribution of *Nereis diversicolor* as found on the two occasions discussed above is shown in Fig. 5A, B. The population is sharply limited on its shoreward edge, reaches its maximum at about 50–55 m from EHWS mark (i.e. on the inner margin of the middle flat as this is seen in the summer months), and drops markedly at about the inner margin of the *Arenicola* zone, although present in lesser numbers to about HWN level. The zonation agrees well with that reported by Watkin; his distribution of *N. diversicolor* is shown for comparison in Fig. 5, where his values are replotted on a metric scale.

18 March 1954. Samples of interstitial water were taken from a boat at high water. Conditions were calm and clear; there had been no rain for the previous 9 days, and a total of only 0·97 in. for the previous 16 days. For the previous 30 days the rainfall had amounted to 3·50 in., which is close to the median value of monthly rainfall over the past 5 years, and below the average monthly value of 3·67 in. for the years 1949–53. The tide was above average height, calculated from Admiralty Tide Tables as 9·7 ft. This is not an extremely high tide (the highest listed for the first 6 months of 1954 was 11·5 ft.), but the height of tide on 18 March equalled or exceeded 71% of the 303 high tides in the above 6-month period. Although combinations of higher tides and lower rainfall may occasionally occur, the conditions seemed quite typical, and the results not attributable to exceptional weather or tidal conditions. The chloride of the sea water was 18·05 g/l. (average of three samples); that of the interstitial water (average of four samples at each station):

Station (m.)	Depth of sea water (in.)	Interstitial chloride (g/l.)
45	22	9·14
50	24	6·85
55	30	9·26

#### DISCUSSION

The above results indicate that on this particular beach brackish interstitial water is a characteristic and relatively stable feature of the zone of *Nereis diversicolor*. That the degree of brackishness may vary with season, rainfall, and tide cannot be denied, but it is probably to be regarded as a permanent feature of the Kames Bay sands. There is no evidence that the intrusive fresh-water drainage rests upon a fluctuating salt-water table, but rather that geological features permit it to flow into the sands of the beach with sufficient head to maintain brackish conditions in the sand even at high tide. Such conditions may be peculiar to this beach and are not necessarily to be found

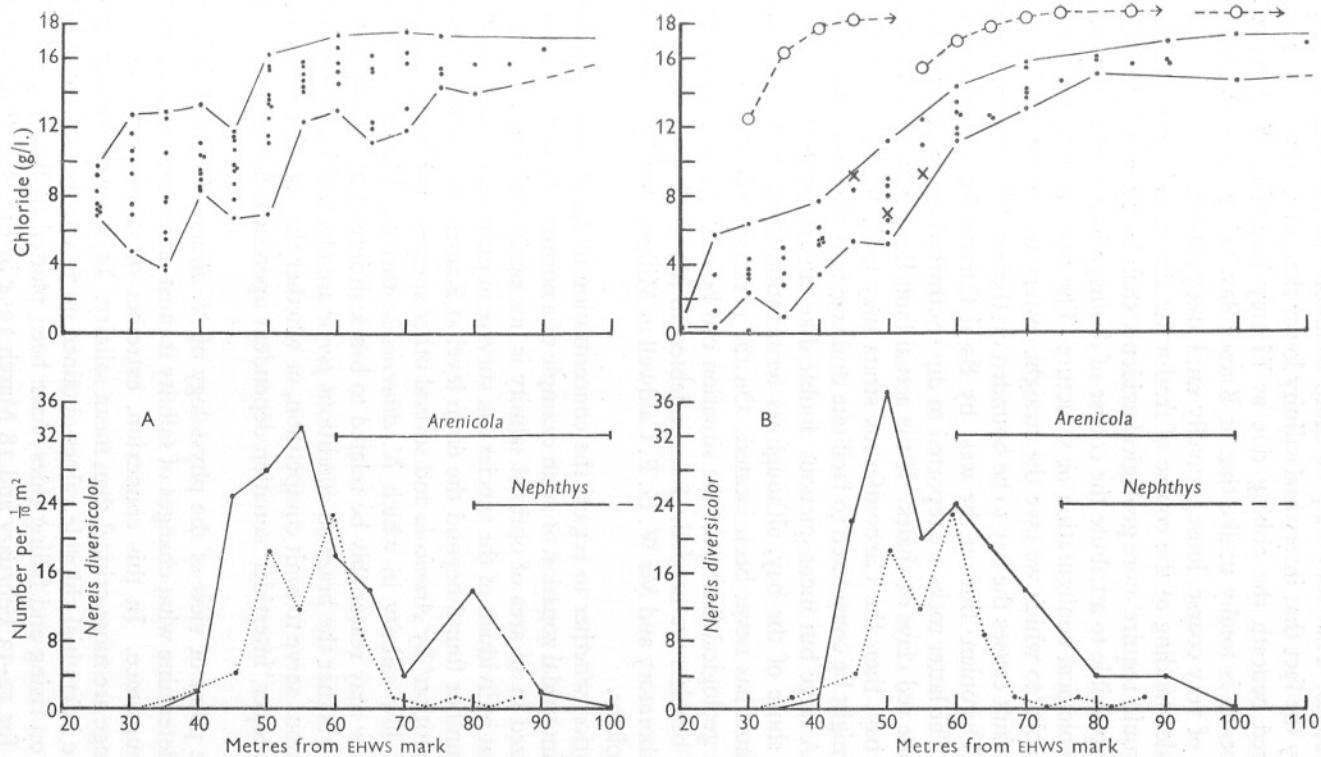


Fig. 5. A (upper), distribution of interstitial chlorides on one rising and two falling tides, 31 October–2 November 1953; B (upper), same for one rising and one falling tide, 12–13 February 1954, plus chlorides of overlying seawater during ebb 13 February and interstitial chloride ( $\times$ ) at high tide 18 March. A, B (lower), distribution of *N. diversicolor* in present studies (solid curves) in worms per  $0.1\text{ m}^2$ . Dotted curves show distribution of *N. diversicolor* as reported by Watkin (1942), replotted on metric scale. Horizontal bars indicate range only of certain other polychaetes as noted in present study.

on other beaches. That they may be more common than hitherto noted is suggested by the fact that interstitial salinity lower than that of sea water has been observed beneath the ebbing tide at Fintray Bay, Isle of Cumbrae, where the beach is totally unlike that at Kames Bay, being exposed, steeply sloped, and of very coarse, loose, gravelly sand and pebbles.

A full understanding of the course of freshwater drainage into the Kames Bay sands would require more geological evidence than is at present available, and it is not possible to attribute the course of drainage into the beach to any particular geological configuration or structure. The bay includes the Great Cumbrae Fault 'to which we owe the straight eastern boundary...' (Gunn, 1903). This fault causes the bay to be bounded on the east by Upper Old Red Sandstone (Devonian) but on the west by Basal Carboniferous Calciferous Sandstone. The latter rocks are reported to dip southward toward Kames Bay and to include red clays or shales. If the actual fault lies beneath the eastern side of the bay, then the Carboniferous strata may underly the site of this study, and might be considered to facilitate drainage into the sands, but proof is lacking. A large but inconspicuous double dike cuts through the rocks at the eastern shore of the bay, although its termination beneath the sands at the actual fault has never been located. On the present evidence no assessment of the geological factors in the situation can be made; more studies on a variety of beaches are needed. I am indebted to Mr T. B. Bagenal of the Millport Laboratory and Mr W. G. E. Caldwell of Millport for discussions of the local geology.

The question whether to regard the concentration of *N. diversicolor* as an instance of intertidal zonation or as an example of a normally estuarine species being localized in an area of optimal salinity is not easily answered. There is no doubt that individuals of the species can survive in pure sea water. Possibly the lesser number found beyond the 60 m level at Kames Bay simply results from 'competition' by *Arenicola* and several other species unable to populate the zone of low salinity in which *N. diversicolor* thrives. The zonation of *N. diversicolor* may reasonably be related to brackish interstitial water, but it is not clear whether the brackish conditions *per se* are favourable, or whether these conditions serve to limit competition, or whether the zonation observed is in part a proper 'intertidal' zonation dependent upon tidal factors exclusive of salinity.

From the point of view of the physiology of *N. diversicolor* itself, it is of interest to determine what changes of salinity it must encounter in its zone of maximal abundance. In this connexion, extremes of salinity and rates of salinity change are more critical than mean salinity. In order to examine this point, all the interstitial chloride values obtained on 31 October–2 November 1953 (both on rising and falling tides) have been plotted in Fig. 5A, and all such values for 12–13 February and 18 March 1954 in Fig. 5B. From these charts, in which the extremes of chlorinity are outlined, it would appear that

whereas *Arenicola marina* must seldom encounter chlorinities below 12 parts per mille in Kames Bay, the bulk of the nereid population may regularly in the course of a day have to endure chlorinities between 12 and 5–6‰, and some must experience chlorinities as low as 4‰. That this is well within the tolerance of the population is suggested by laboratory experiments in which worms from Kames Bay have been adapted by steps to chlorinities as low as 0·25‰. This is about 1·4% of Millport sea water, which has a mean chlorinity of 17·76‰ (= salinity 32·90‰), with monthly chlorinity means varying from 18·43 to 16·55‰ (based upon 5-year period June 1948 to May 1953).

If the field observations reported here may be regarded as of typical conditions, *Nereis diversicolor* is not exposed at Kames Bay to salinities which in themselves approach the limit of its osmotic tolerance. It is likely that at high tide the worms experience salinities above the values observed for the interstitial water, since these worms probably resume irrigation (and may emerge to feed) as soon as the rising sea water covers them (Wells & Dales, 1951). Hence, while the actual salinity endured at low tide may be that of the interstitial water, the salinities experienced at high tide must be the resultant of circulated sea water being diluted by brackish water in the sand about the burrows. The fact that brackish water remains available may well protect the worms against too abrupt rises of salinity at each high tide, and thus lessen the impact of salinity changes, somewhat as does the salt-water content of estuarine flats exposed to over-flowing fresh water at low tide. The actual rate of change of salinity experienced by *N. diversicolor* at Kames Bay cannot be determined from the present data, but an inspection of Fig. 5 suggests that in the zone of maximal abundance the worms experience a change in chlorinity of not less than 6 parts per mille per tidal rise or fall, and probably within 2 h.

These studies were carried out while the author held a Fulbright Exchange Lectureship in the Zoology Department, University of Glasgow. Field work and analyses were done at the Scottish Marine Biological Association Laboratory at Millport, to whose Director and staff I am grateful for much kindness and assistance.

#### SUMMARY

An hitherto undescribed, relatively stable, zone of brackish interstitial water is reported from the upper mid-tidal sands of Kames Bay, Millport.

Lowered interstitial salinity persists even beneath overlying sea water at high tide. Present evidence does not permit evaluation of the geological factors involved.

The zonation of *Nereis diversicolor* on this beach seems correlated with the belt of lowered salinity, but it remains unclear whether the brackish conditions *per se* are favourable, or if they serve to limit competition from other species.

It would appear that the observed zonation is not purely dependent upon tidal factors exclusive of salinity.

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## INNERVATION OF THE HEART OF *PRAUNUS FLEXUOSUS* (MYSIDACEA)

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(With Plate I and Text-fig. 1)

The heart nerves of *Praunus flexuosus* Leach (= *Macromysis flexuosa* White) have been stained with methylene blue applied in various ways. As a rule the animals were injected with a solution prepared immediately before use by mixing 1 part of 0·5% solution of methylene blue in distilled water or of rongalit white with two parts of sea water. The injection was made into one of the posterior segments of the abdomen with the needle pointing forwards. When three or four animals are treated at the same time, usually in at least one of them the staining reaction takes a satisfactory course and its progress can be observed under the microscope, owing to the transparency of the tissues. In a few minutes after the injection some of the nerves are already visible. Later the coloration becomes more general and more intensive, but soon begins to fade out and can disappear completely.

For staining the heart nerves by a second procedure, i.e. by immersion in a weak solution of the dye, the animals should be sectioned along the ventral side and their digestive organs and gonads taken out. It is, moreover, necessary to remove the large chromatophores situated on the ventral side of the pericardial diaphragm, and this membrane has also to be pulled out. The heart thus becomes exposed with its ventral wall uppermost, which can be cut along the middle line to give better access to the main trunks of the heart nerves situated on its dorsal side. The same way of exposing the heart has to be followed when the preparations obtained by the method of injection are to be fixed, as the ammonium molybdate solution does not penetrate through the chitin.

The fixation of the staining caused the same difficulties as experienced with amphipods (Alexandrowicz, 1954). It is obvious that in these animals the bond between the dye and the stainable substance of nerves is of a very unstable nature, and only at the moment when the coloration attains the peak of its intensity the fixation may give somewhat more satisfactory results. It is difficult to estimate this right moment, especially as various nerve elements may take up the dye at different times. Thus, for instance, the nerve cells in the heart of *Praunus* stain but rarely and always later than the fibres. By waiting too long for their possible appearance many otherwise good preparations are lost.

### OBSERVATIONS

The heart of Mysidacea situated in the thoracic region has a shape of a fusiform tube continuing forwards and backwards into the anterior and posterior aorta respectively, the only vessels which can be seen from the dorsal side (Text-fig. 1A). As stated by Delage (1883) the limits of the heart tube, which at its ends does not differ in width from these vessels, can be determined by the presence of the valves. It may be added that the muscle fibres of the heart do not pass on to the arteries and therefore the places where they stop mark the boundaries of the heart. The muscle fibres, of finely cross-striated type, are arranged in a single layer. They turn in right-handed spirals as in the Isopoda, whereas in the Amphipoda the direction is reversed.

From the ventral side of the heart arise several arteries. The largest of them, the sternal artery, originates at a certain distance from the posterior aorta and a little to the left of the middle line. The two hepatic arteries arise close to each other and to the anterior aorta. According to Delage there are, in addition, two unpaired arteries originating between the hepatic and the sternal arteries: the anterior of them, which as this writer remarks is very slender, could not be recognized in methylene-blue preparations.

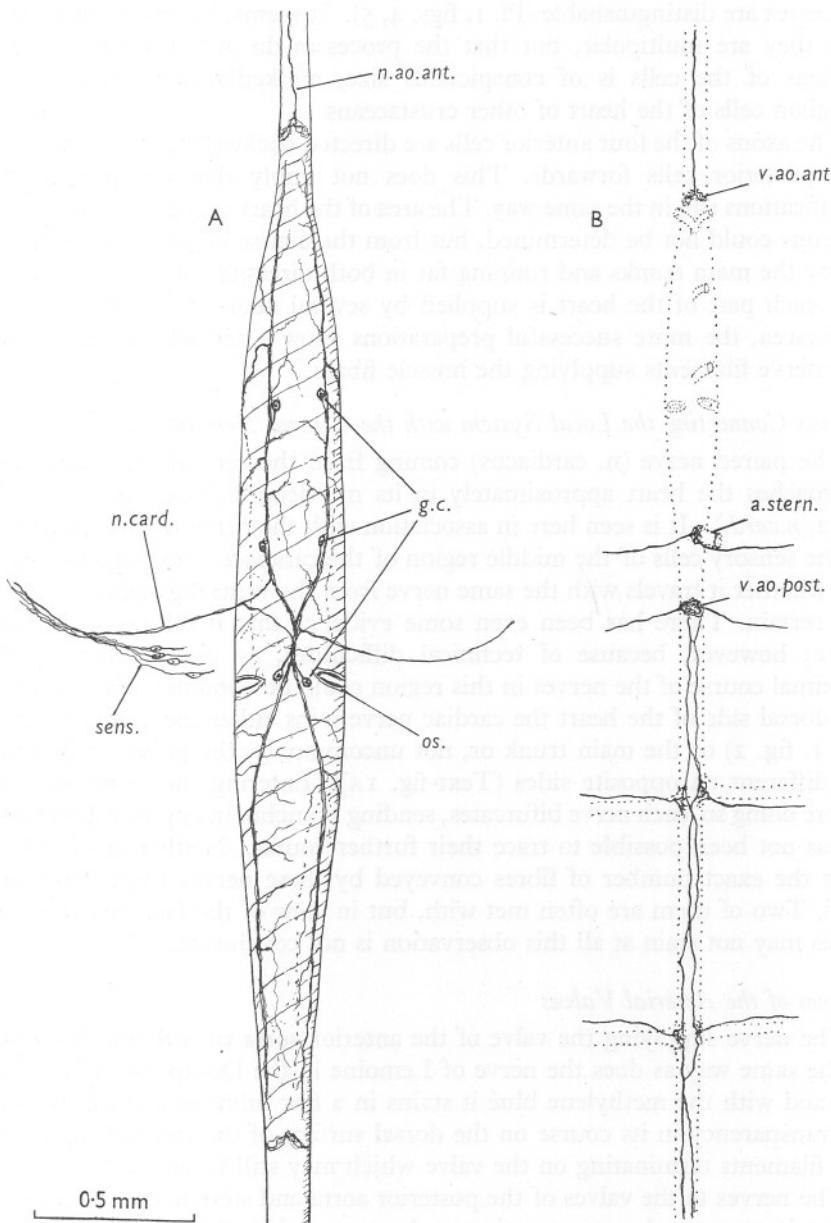
#### *Nerves of the Heart*

The three systems of nerves observed in the heart of all Malacostraca investigated up to now have been also found in the Mysidacea. There are: (1) a local system made up of neurons situated in the heart itself, (2) nerves connecting the local system with the central nervous system, and (3) nerves of the arterial valves.

#### *Local System*

The main elements of the local system are situated on the outside of the dorsal heart wall. A short trunk, lying just in front of the ostia and bifurcating at each end, forms a characteristic figure with the branches resulting from these divisions (Text-fig. 1A; Pl. 1, fig. 1). The two anterior and the two posterior trunks, running in opposite directions, and traceable up to the ends of the heart, give off many branches, the largest of them passing on to the ventral side of the heart.

The ganglion cells, the axons of which make up these trunks, number no less than six. Two of them are situated near the posterior end of the unpaired main trunk, and four on the anterior trunks, two on each of them (Text-fig. 1A, g.c.; cf. the photograph, Pl. 1, fig. 3, on which four of the cells are seen). Their situation can vary; the middle pair can lie nearer or farther from the main trunk, but the most anterior ones have always been found far forwards approximately midway between the ostia and the anterior end of the heart. The cells usually look as if they were unipolar; only rarely some dendritic



Text-fig. 1, A: *Praunus flexuosus*. View of the heart from the dorsal side showing the main nerve trunks and their branches. *g.c.*, ganglion cells; *n.card.*, cardiac nerve; *sens.*, sensory nerve cells; *n.ao.ant.*, nerve of the valve of the anterior aorta; *os.*, ostium. B: nervous system of the arterial valves. The heart is seen from the ventral side. *v.ao.ant.*, valve of the anterior aorta; *v.ao.post.*, valve of the posterior aorta; *a.stern.*, sternal artery.

processes are distinguishable (Pl. I, figs. 4, 5). It seems, however, more likely that they are multipolar, but that the processes do not stain clearly. The nucleus of the cells is of conspicuous size, markedly larger than in the ganglion cells of the heart of other crustaceans.

The axons of the four anterior cells are directed backwards, and those of the two posterior cells forwards. This does not imply that all their further ramifications run in the same way. The area of the heart supplied by individual neurons could not be determined, but from the course of the branches given off by the main trunks and running far in both directions it may be inferred that each part of the heart is supplied by several neurons. As usual in the Crustacea, the more successful preparations show a remarkable richness of fine nerve filaments supplying the muscle fibres.

#### *Nerves Connecting the Local System with the Central Nervous System*

The paired nerve (*n. cardiacus*) coming from the central nervous system approaches the heart approximately in its mid-length (Text-fig. IA; Pl. I, fig. 2, *n.card.*). It is seen here in association with the nerve that carries fibres of the sensory cells of the middle region of the carapace (Text-fig. IA, *sens.*), but whether it travels with the same nerve from the ventral ganglionic cord is not certain. There has been even some evidence that it follows a different route; however, because of technical difficulties, no clear picture of the proximal course of the nerves in this region could be obtained. Passing on to the dorsal side of the heart the cardiac nerve joins either the anterior trunks (Pl. I, fig. 2) or the main trunk or, not uncommonly, the points of junction are different on opposite sides (Text-fig. IA). Entering the trunk, or even before doing so, each nerve bifurcates, sending branches in opposite directions. It has not been possible to trace their further course. Neither am I able to state the exact number of fibres conveyed by these nerves from the neural cord. Two of them are often met with, but in view of the fact that the finer fibres may not stain at all this observation is not conclusive.

#### *Nerves of the Arterial Valves*

The nerve supplying the valve of the anterior aorta runs down this vessel in the same way as does the nerve of Lemoine in the Decapoda. In animals injected with the methylene blu  it stains in a few minutes and can be seen by transparency in its course on the dorsal surface of the stomach up to the fine filaments terminating on the valve which may still be active.

The nerves to the valves of the posterior aorta and sternal artery are given off by the segmental nerves running on the ventral side of the extensor muscles and therefore can be visible only after dissection of the animals. They do not stain so readily as in the anterior aorta and can be but rarely noticed, the more so that during various manipulations described above the fine nerves can be easily torn away. Maybe for the same reason I was unable to trace the nerves

to the remaining four arteries arising on the ventral side of the heart. On the other hand, it has been easy to observe the innervation of the posterior aorta in its course down the abdomen. This vessel, called also abdominal artery, is supplied in each segment by fine nerves running along its segmental branches. These nerves give off two sorts of fibres: some, very short, end at the origin of the segmental paired vessels; others run alongside the abdominal artery establishing connexions of the whole system including the valve at the posterior end of the heart. The arrangement is strikingly similar to that found in decapods (Alexandrowicz, 1929, 1932), and it seems highly probable that this system of nerves in mysids is also destined to supply the valves at the exit points of the segmental arteries in the abdomen, although the latter could not be clearly seen in *Praunus*.

#### DISCUSSION

The recorded results provide another proof, that, in the Crustacea, various components of the heart innervation follow one fundamental pattern. The differences observed appear to be rather of minor character. As regards the local system in mysids its peculiar feature, compared with amphipods and isopods, is the arrangement in pairs of the ganglion cells and their situation at a certain distance from the middle line, whereas in the two other groups all cells lie in the unpaired median trunk. The number of cells (six) found in *Praunus* is the same as stated for *Ligia oceanica* (Alexandrowicz, 1952) and assumed to be the most probable in *Marinogammarus marinus* (Alexandrowicz, 1954). However, rather than to admit that this coincidence be not a fortuitous one, I am bound to express doubts whether the number found in *Praunus* is correct. For one reason, it is surprising that the posterior part of the heart, about half of its total length, should be deprived of nerve cells. In various crustaceans with an elongated heart such as the Amphipoda, Isopoda and Stomatopoda, ganglion cells are present along the whole of its length, and in the Decapoda it is in the posterior half that the ganglionic trunk, or the major part of it, is located. Also, in view of the symmetrical disposition of the main trunks in mysids, the presence of the cells in the posterior trunks could be expected. The negative results may be due to the insufficient staining of cells in this region. Moreover, owing to the deeper situation of the posterior part of the heart, it is much less accessible to observation by transparency which has been otherwise very helpful.

As regards the nerves connecting the local system with the central nervous system it must be pointed out that only one pair of them has been noticed in mysids, whereas in amphipods two such paired nerves and in stomatopods even three are present. Perhaps in mysids as in decapods the cardiac nerve conveys fibres of different kind and origin, but, as mentioned before, its proximal course could not be determined. On the other hand, it is not certain

whether there is really only one pair of the cardiac nerves. With animals of such a small size nerves made up of very tiny fibres may not show at all.

It is possible for this reason that I could not detect the nerves on the pericardial diaphragm which, as in other crustaceans, might be expected to supply the muscle fibres of the pericardium. It is true that such muscle fibres were not observed, but this again may be due to the short-comings of the technical methods.

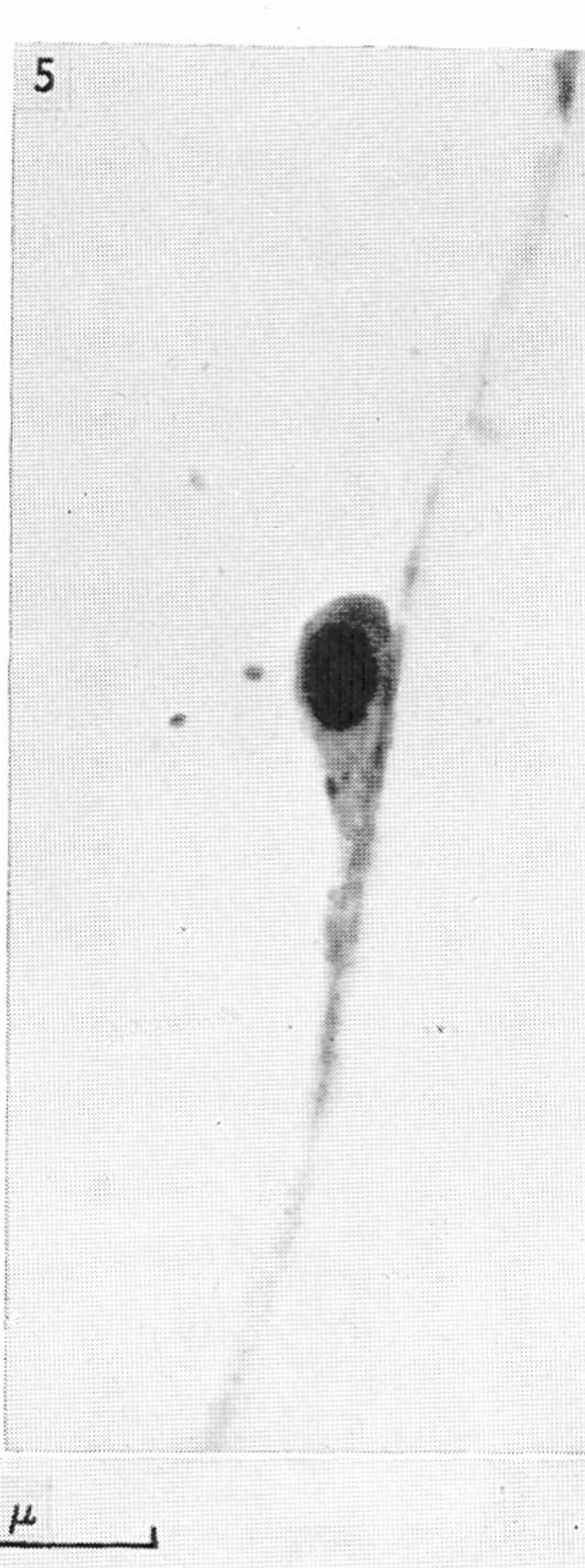
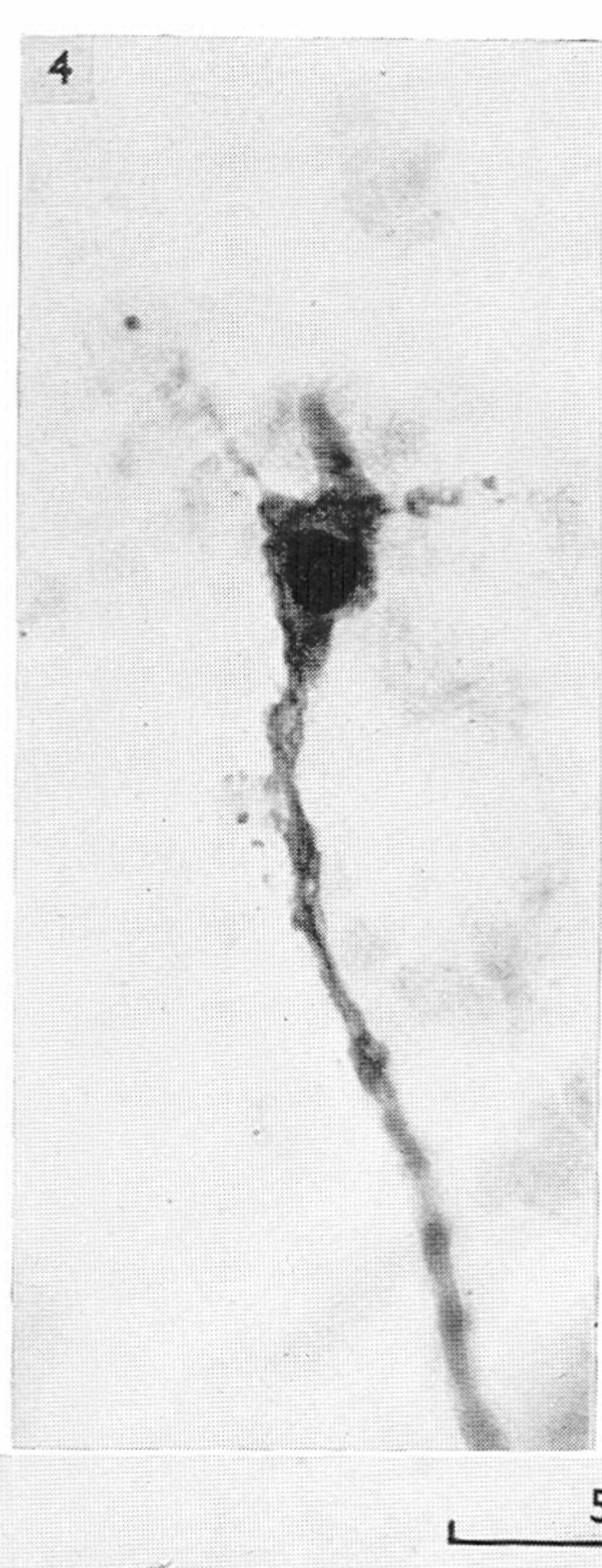
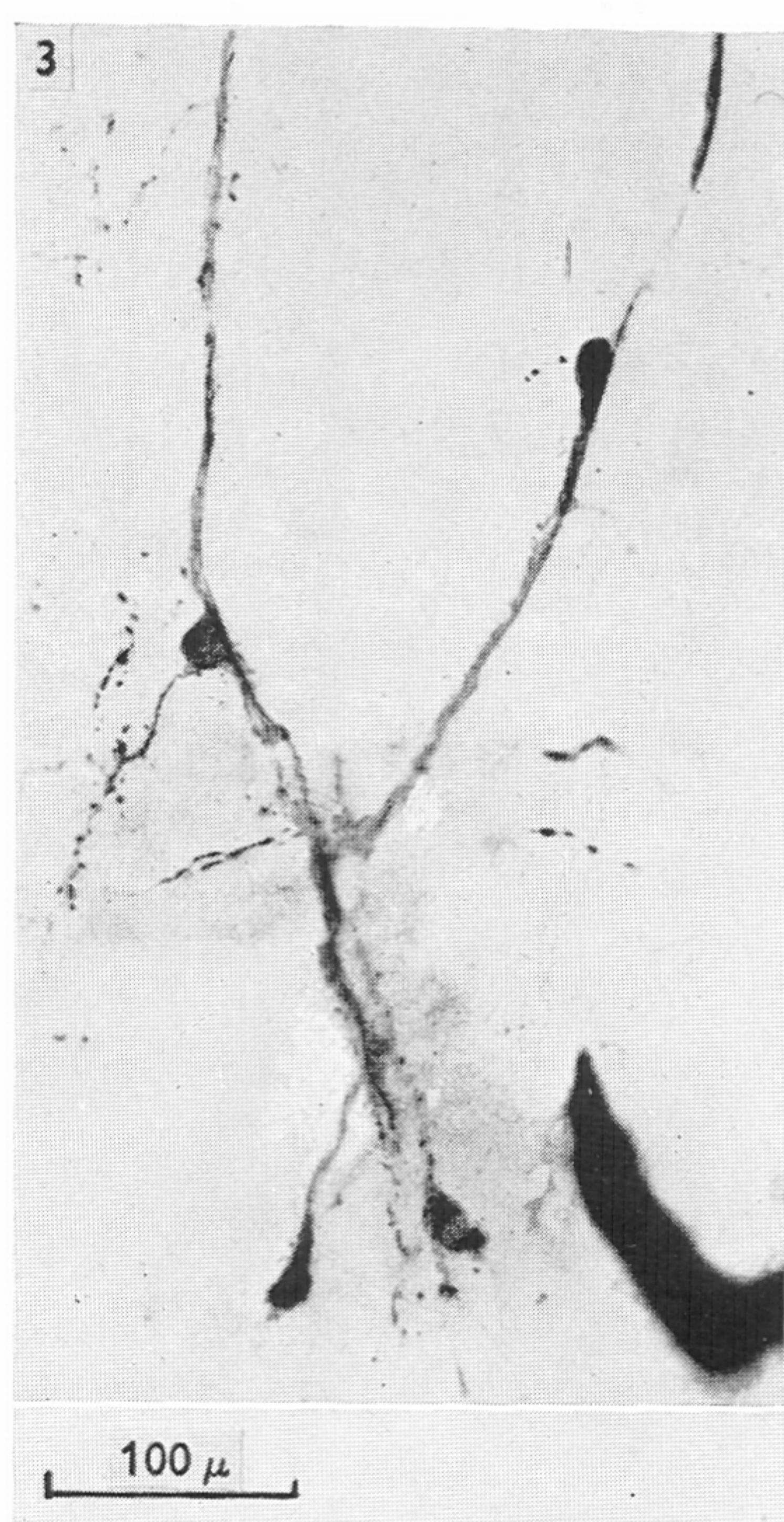
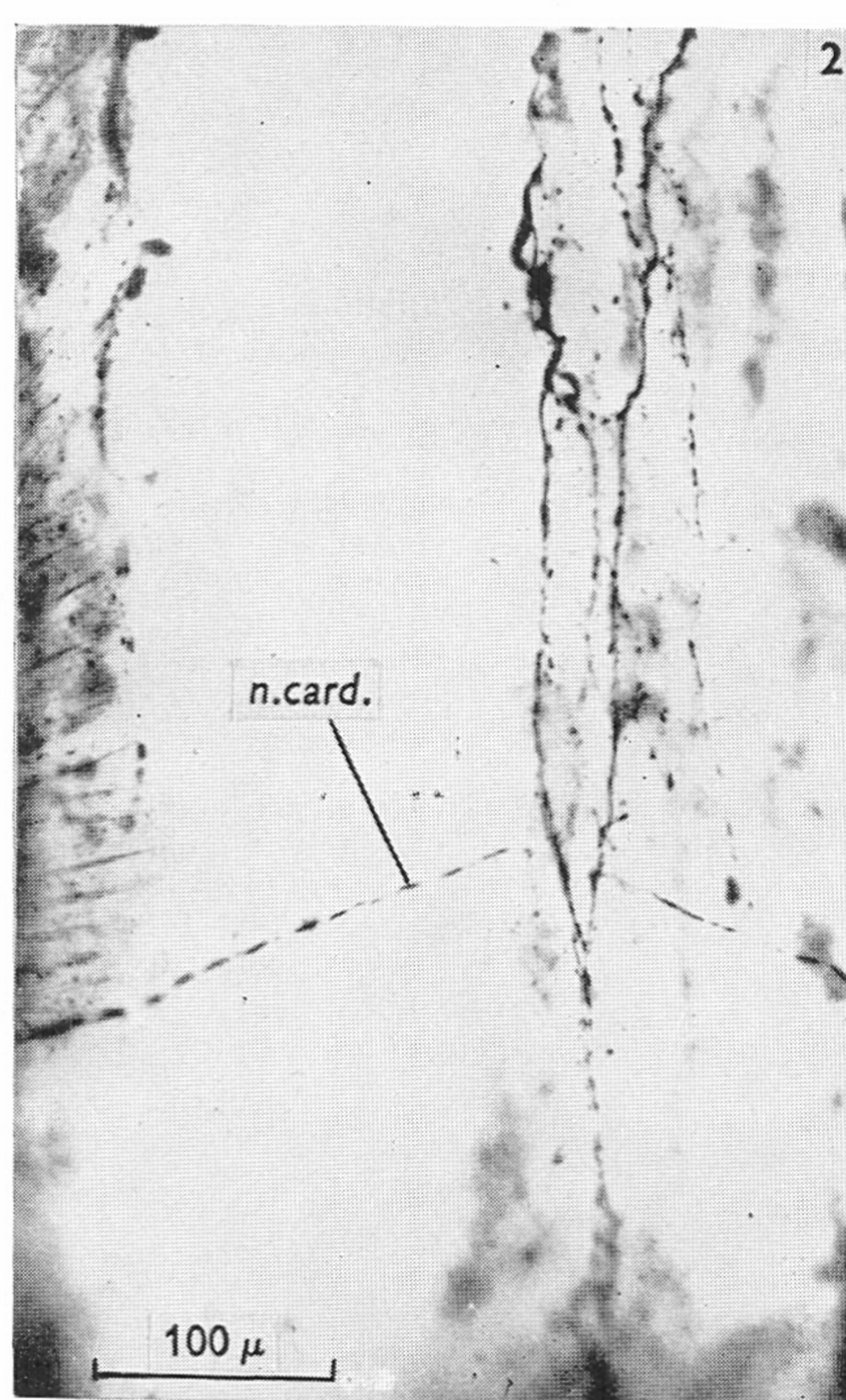
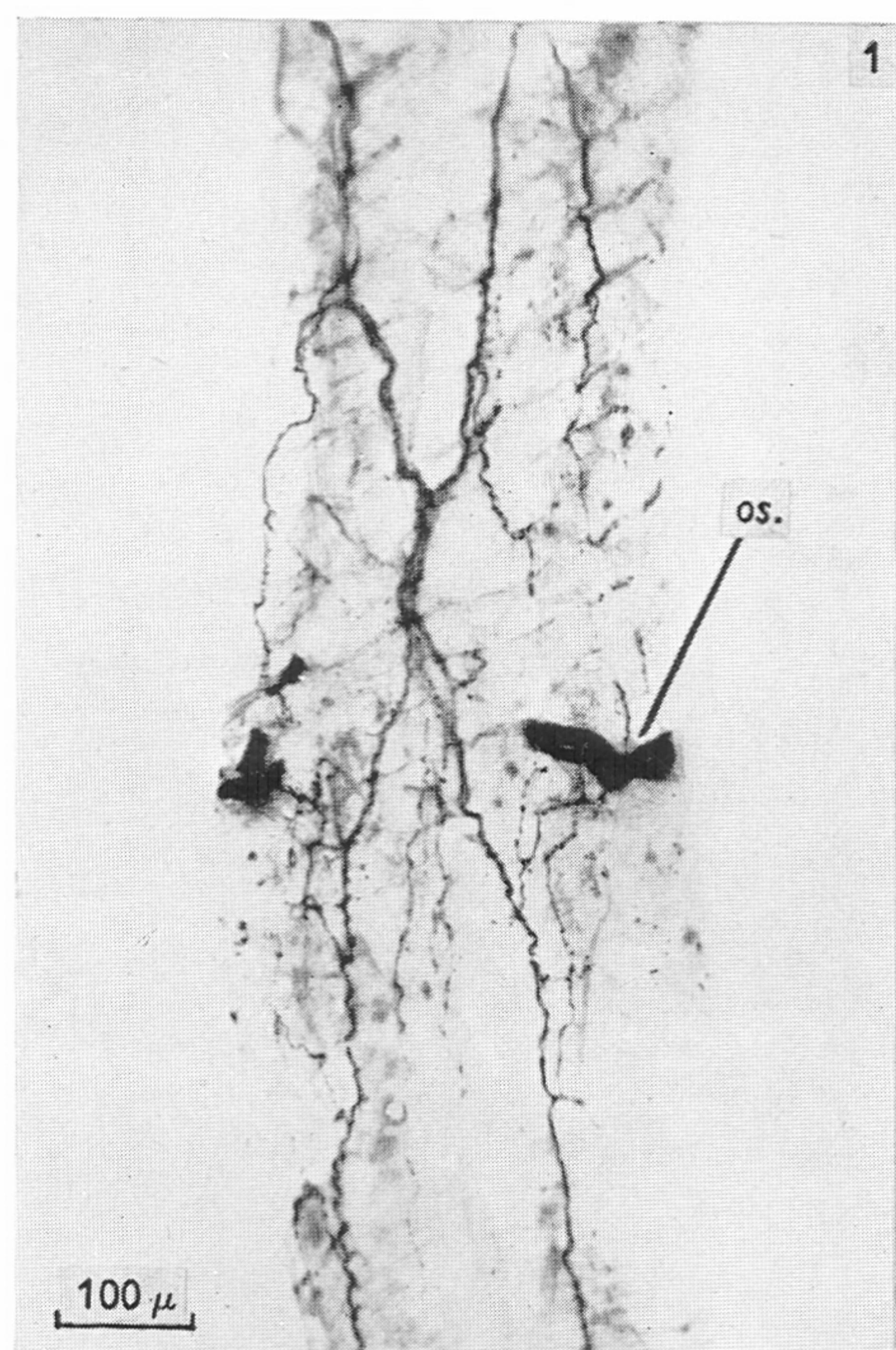
Uncertainty also remains regarding the occurrence of the pericardial organs, i.e. nerves of special structure which have been found in the Decapoda, Stomatopoda and Amphipoda. In *Praunus* there are nerve elements which in their arch-like arrangement resemble the pericardial organs in *Leander serratus* (see Alexandrowicz, 1953). However, because of their insufficient staining and even more because of their small dimensions, their nature could not be established. It is to be hoped that with species of larger size this point, as well as others mentioned above, might be satisfactorily elucidated.

#### SUMMARY

In the heart of *Praunus flexuosus* three systems of nerve elements have been found. The local system consists of no less than six neurons. Their cell-bodies are situated on the dorsal heart wall near the short unpaired trunk and its two anterior branches. The axons of the nerve cells innervate the muscle fibres of the heart. A pair of cardiac nerves joining the trunks of the local system establish the connexion with the central nervous system. A separate set of nerves supplies the valves of the vessels arising from the heart. The nerves of the valve of the posterior aorta are connected with the nerves running alongside this vessel which receive segmental branches coming from the abdominal ganglia.

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## EXPLANATION OF PLATE I

All photomicrographs have been made from preparations of the heart of *Praunus flexuosus* stained with methylene blue, fixed in ammonium molybdate and mounted in dammar-xylol.

Fig. 1. Middle part of the heart with main nerve trunks and some of their branches. Ganglion cells and cardiac nerves are not visible. *os.*, ostium.

Fig. 2. Middle part of the heart with main trunks and cardiac nerves (*n.card*) entering the anterior trunks. The position of the anterior trunks close to each other is due to elongation of the heart produced artificially.

Fig. 3. Middle and posterior pair of ganglion cells.

Fig. 4. Anterior ganglion cell.

Fig. 5. Middle ganglion cell. Note the size of the nucleus.

## FEEDING AND DIGESTION IN TEREBELLID POLYCHAETES

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(Text-figs. 1-9)

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### INTRODUCTION

Most of the naturalists of the nineteenth century were attracted by the tube-building abilities of the terebellid polychaetes rather than by their feeding behaviour. As Dalyell (1853) says in his *Powers of the Creator*, referring to terebellids: 'All are architects, nor of contemptible skill, as we shall find on contemplating their mechanical labours.' Perhaps the best known English work dealing with this subject is the paper by Arnold Watson (1890) on the tube-building habits of the sand mason (*Lanice conchilega*), and while concerned mainly with tube building, the paper does include some observations on the feeding habits. Earlier, Gosse (1854) had recorded his observations on the behaviour of terebellids, and some additional information may be found in Quatrefages's Treatise of 1865. The first serious attempt to find out more about the morphology and histology of the gut in terebellids was made by Claparède (1873), and this work was published posthumously in his monograph on the Polychaeta Sedentaria. Steen (1883) soon after recognized the division of the terebellid stomach into glandular and muscular portions, and this morphological work was further extended by Meyer (1887) in his 'Studien über den Körperbau der Anneliden', and, more especially, by the Swedish morphologist Axel Wirén (1885). This latter paper forms the most substantial

contribution to our knowledge of the anatomy of terebellids. Work on this group at Uppsala was extended further by Hessle (1917, 1925). Some information may also be found in McIntosh's (1922) Monograph, and in the Memoir on *Amphitrite* by Thomas (1940), but few details are given about feeding and digestion in the latter work, and most of the descriptions in the former are misleading.

Feeding and digestion have been studied in only a few polychaetes. The most important paper is that of Brasil (1904) on *Pectinaria koreni*, and this includes a useful résumé of the previous work on feeding and digestion in polychaetes. The paper by Nicol (1931) on *Sabella* is perhaps the best-known work published since then, and additional references to gut morphology in polychaetes will be found also in the recent paper by Ullman & Bookhout (1949) on the histology of the gut of the maldanid, *Clymenella*.

An attempt has been made in the present paper to give a comparative account of the feeding mechanism and the functional morphology of the gut in the principal genera of the Terebellidae. Most of this work has been done at Plymouth, but observations have been made elsewhere, both in this country and in America, on the feeding habits and the structure of the organs concerned. Although Thomas (1940) has given a useful account of some aspects of the anatomy of *Amphitrite johnstoni*, the description of the gut is incomplete and may well be amplified. Owing to its large size *A. johnstoni* is the most suitable species available in this country for such studies. A description of the anatomy and feeding behaviour in this species will be presented first. Feeding and morphology of the gut will then be compared in other terebellids that have been studied.

I am indebted to the Director and Staff of the Plymouth Laboratory for their kindness and help, and to Mr Norman Tebble of the British Museum (Natural History) for supplying me with specimens of some genera not represented in our fauna, and which I have not yet had the opportunity of studying alive.

#### FEEDING AND FUNCTIONAL MORPHOLOGY OF THE GUT IN *AMPHITRITE JOHNSTONI*

*A. johnstoni* Malmgren is one of the largest of the British terebellids, often attaining a length of 20–25 cm and a breadth of 2 cm or more in the anterior region of the body. It is a pink or brown worm living in mud or very muddy sand, and in spite of Dalyell's eloquence, it does not construct a tube. The mainly vertical burrow may be 2 or 3 times the length of the worm and fundamentally is always U-shaped. There is no distinction between the two openings of the burrow as the worm can, and frequently does, reverse within the burrow. Near Plymouth this species can be obtained in numbers only at low-water mark of spring tides in the River Yealm and at Salcombe.

As in all terebellids the prostomium is represented externally by a large number of extensible tentacles and a cowl-like flap forming an upper lip to the mouth (Fig. 1, *Ul*). The first segment is reduced, save for the ventral part which contributes to the series of ventral lips to be described (Fig. 1, *Ol*). The anterior part of the body is much swollen, and the ventral part of each segment in this region contains well-developed mucous glands (Fig. 1, *Mg*). In tube-building species these glands are important in secreting the lining of the tube. This anterior region containing these mucous glands which open ventrally on conspicuous 'ventral shields' is often referred to as the 'thorax'. In *A. johnstoni* there is, just posterior to the thorax, a smooth, slightly swollen region of the body, and it is this region which contains the coiled fore intestine (Fig. 1, *F.int*). This is exceptional; in most terebellids the body tapers uniformly towards the posterior end, and in many there is no real division of the body into an anterior thoracic region and a posterior abdominal region. Anteriorly there are three pairs of branched gills (Fig. 1, *Br*). While respiratory exchange takes place over the whole surface of the body, these gills are obviously concerned solely with respiration. The prostomial tentacles, on the other hand, are concerned only with the collection of particles of detritus or other matter for food.

#### *Behaviour*

The feeding behaviour of *A. johnstoni* was studied in the laboratory. Worms will accept glass U-tubes of the right calibre, and will live in them for at least 6 months when placed on the bottom of a tank through which a flow of sea water is maintained. Without an artificial tube, and without adequate circulation, this species will not survive long in laboratory conditions. Worms were also studied in artificial sand and mud burrows made between sheets of glass separated by a loop of plasticine and clamped together vertically in a deep tank. Though such an arrangement simulates the natural conditions more closely than glass U-tubes lying horizontally on the bottom, it was found that the behaviour of the worms in either apparatus was not noticeably different, and most of the observations have been made on worms housed in glass tubes. Mud sandwiches have the disadvantage, not only of the difficulty of maintaining the mud in a healthy condition, but also in that the surface over which the tentacles can extend for feeding is more restricted than when the mouth of the tube lies on the bottom of a tank. The activity of the worms in both types of apparatus has been followed, and the activity recorded kymographically for continuous periods of up to a week for any one worm, with adaptations of the apparatus developed by Wells (Fig. 2; see also Wells, 1949; Wells & Dales, 1951). These studies were made to determine how much time was occupied with feeding, and how feeding was related to other activities of the animal.

Irrigation of the burrow is almost incessant, provided that the openings of the burrow remain submerged. Both in glass tubes (Fig. 2), and in glass/mud/

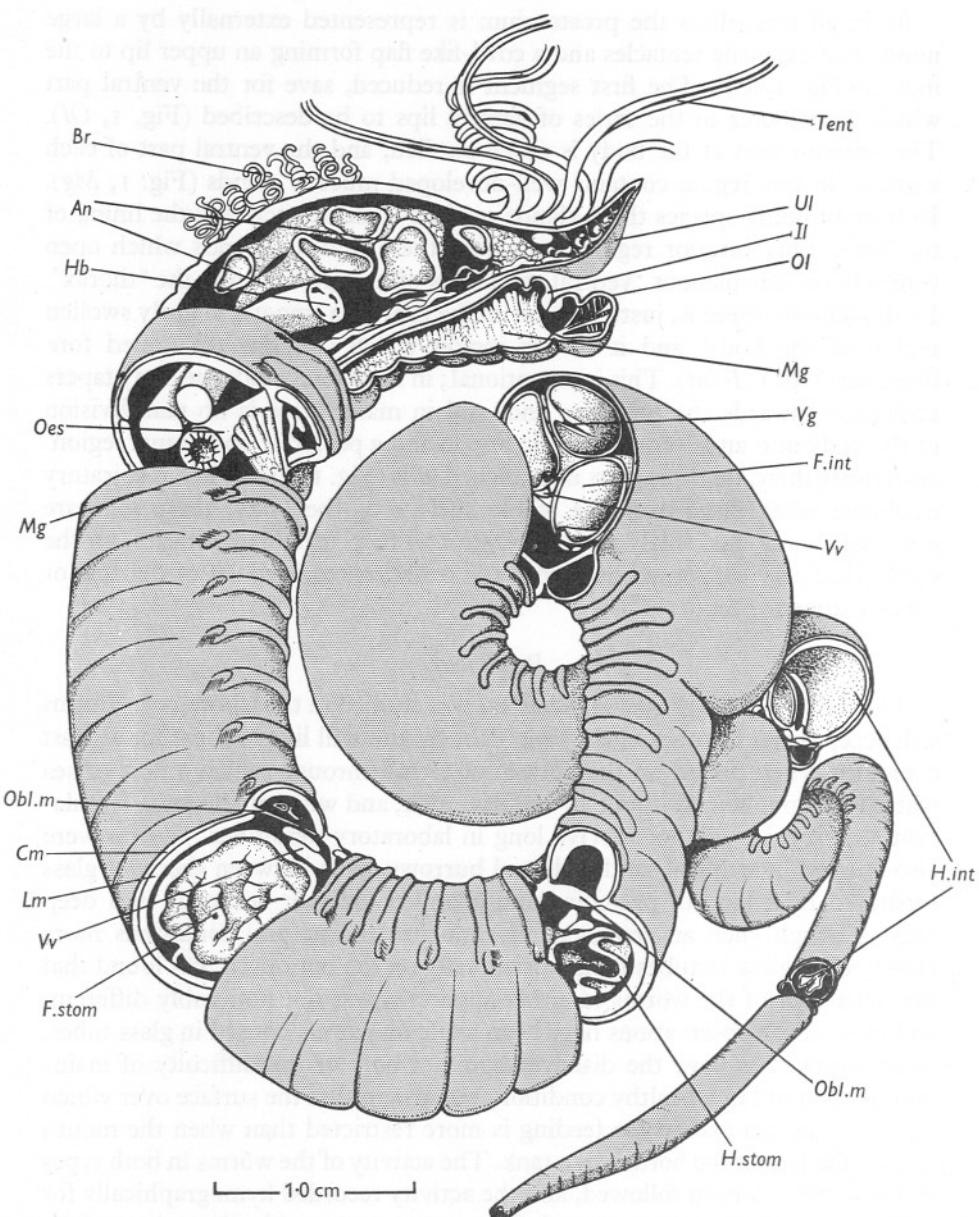


Fig. 1. *Amphitrite johnstoni*, illustrating the main regions of the gut in relation to the rest of the body. An, anterior nephridia; Br, branchiae; Cm, circular muscles; F.int., fore-intestine; F.stom, fore-stomach; Hb, heart body; H.int., hind-intestine; H.stom, hind-stomach; II, inner lips; Lm, longitudinal muscle; Mg, mucous glands; Obl.m., oblique muscles; Oes, oesophagus; Ol, outer lips; Tent, tentacles; Ul, upper lip; Vg, ventral gutter; Vv, ventral vessels.

glass sandwiches the worm will irrigate the tube or burrow by peristaltic contractions of the body-wall initiated at the hinder end of the thorax and travelling forwards. At about  $17^{\circ}\text{C}$  each wave reaches, or almost reaches, the anterior end before the next wave is initiated, about 15 sec occurring between each wave. Such irrigation behaviour may continue for many hours

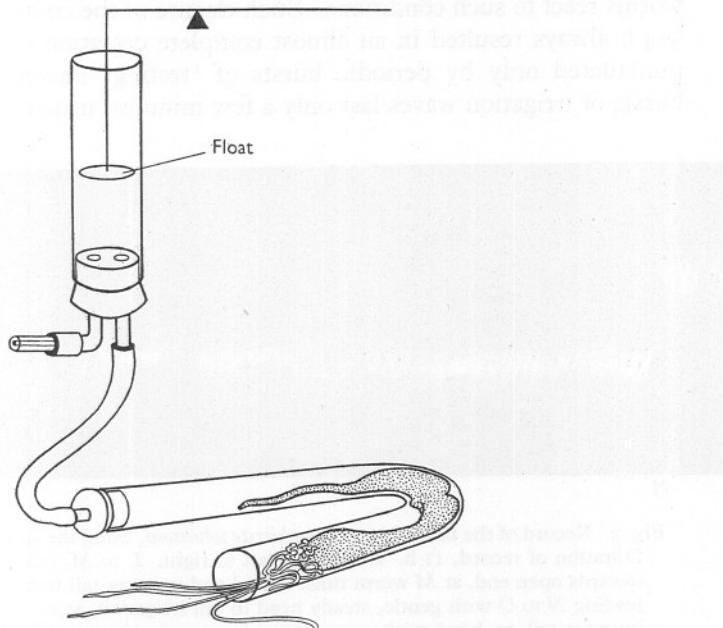


Fig. 2. Apparatus used for recording the behaviour of *Amphitrite johnstoni*. The whole apparatus is immersed in a tank, through which a flow of sea water is maintained at a constant level to that of the float.

with little other activity apart from slight stretching, or occasional forward extension of the body owing to a gradual slipping back during irrigation. Occasionally the direction of irrigation is reversed, the waves travelling back from the head, this usually occurring when active feeding is taking place. All the worms which were kept in the laboratory fed for about 50% of the time, weakly irrigating in a head to tail direction while doing so, and alternating such feeding periods with periods of much more vigorous and sustained irrigation from tail to head, during which the body is usually allowed to slip back in the tube until the hinder end of the body is almost exposed from the burrow opening. Defaecation usually occurs when the worm is in this position, the hinder end of the body being everted as much as 3–5 cm from the burrow opening; a faecal pellet is then ejected, and the body withdrawn immediately afterwards. This kind of behaviour was seen in all the terebellids studied. The type of record obtained with a worm lying in a horizontal tube is shown in Fig. 3.

The irrigation of the burrow can be made to cease immediately by connecting the two openings of the tube (see the apparatus used for *Nereis*, Wells & Dales, 1951). Such a constraint may resemble the conditions in nature when the burrows are uncovered by the tide. Actually this would happen only once or twice a month, but it is of interest to know how the worms react to such conditions. Such closure of the circulation for periods of 3-4 h always resulted in an almost complete cessation of irrigation activity, punctuated only by periodic bursts of 'testing' movements. These short bursts of irrigation waves last only a few minutes, usually in one, then in the

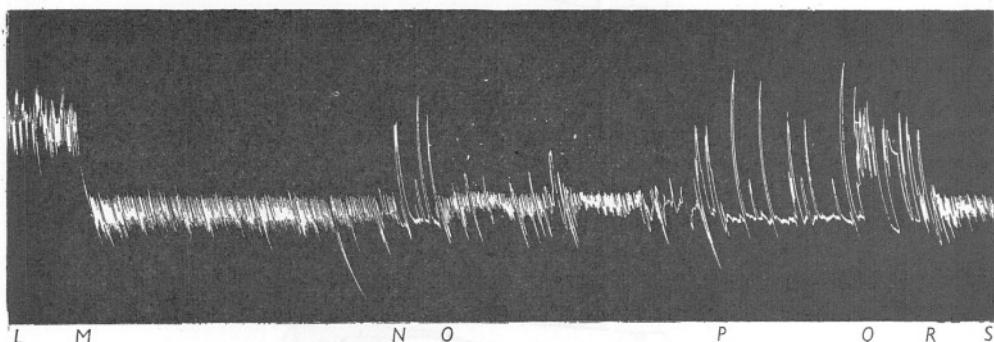


Fig. 3. Record of the behaviour of *Amphitrite johnstoni*, using the apparatus shown in Fig. 2. Duration of record, 11 h. Read from left to right. *L* to *M*, tail to head irrigation, head towards open end, at *M* worm turns round and irrigates tail to head, turning again at *N*; feeding *N* to *O* with gentle, steady head to tail irrigation, at *O* turns tail to opening and irrigates tail to head with some excursions up tube, turning again at *P* to feed; big spikes are due to excursions at mouth of tube during feeding, otherwise trace shows constant head to tail irrigation; at *Q* feeding ends and tail to head irrigation resumed (head still towards opening), turning at *R*. The frequent reversals are due to one end of the tube being connected to the recording apparatus.

other direction, followed by complete quiescence for] some minutes or only by slight stretching movements. Worms never reversed themselves in the tube under these conditions. If a worm is placed in a U-tube only partly filled with water, irrigation activity is completely replaced by occasional exploratory movements. Immediately on opening the circulation or on immersion of the tube, vigorous irrigation results.

In nature, *Amphitrite johnstoni* is commonly accompanied by a commensal scale-worm, *Gattyana cirrosa* (Pallas). The *Gattyana* is usually able to run forward in the burrow to rob the *Amphitrite* of part of its food since irrigation movements are reduced when ingestion occurs. When the terebellid is lying in the tube and irrigating, the *Gattyana* is usually quiescent, and commonly lies against the body of the terebellid just behind the thorax. Presence or absence of the *Gattyana* makes no discernible difference to the behaviour of the *Amphitrite*.

*Operation of the Tentacles**Feeding*

The tentacles are extremely extensible hollow structures with a complicated musculature enabling them to be moved in a great variety of ways. The adoral surface, comprising rather less than one-half of the total area, is ciliated, and this part usually forms a ciliated groove. All the cilia beat towards the mouth. The interior of the tentacle communicates with the anterior coelom, and movement of fluid within the cavity can easily be followed by observation of the coelomic corpuscles. Fig. 4 gives some idea of the structure and operation of the terebellid tentacle. While these illustrations refer to *Terebella lapidaria* L., the structure of the tentacle is in general features the same in all terebellids. Fig. 4 C is a stereogrammatic cross-section to show the main features of the musculature; Fig. 4 D illustrates some of the histological details.

The thick epidermal layer contains mucous cells, not only in the ciliated part, but throughout the epidermis. Longitudinal muscles extend the length of the tentacle but are not present where most lateral movements occur, that is, along the edges of the groove (*Lm*). Vertical or transverse muscles (*Tm*) link the middle of the ciliated area with the opposite side of the tentacle. In addition, there are oblique muscles (*Obl. m*) running in the direction of the axis of the tentacle, with their proximal insertions always on the side remote from the ciliated groove, and also individual muscle fibres (*Tmf*) spanning the inner and outer edges of the groove. The main longitudinal muscles, which enclose five longitudinal nerve strands (*Tn*) the median of which is the largest, are clearly for the retraction of the whole tentacle. The transverse and oblique muscles are responsible for the curling and rolling movements of the tentacle, while the individual fibres towards the edges of the groove cause the rippling and squeezing movements of the edges.

The great extensibility of the tentacles has been the subject of much comment. The comparison has been made between the terebellids with their highly extensible tentacles, and the ampharetids which possess rather similar tentacles which are not, apparently, very extensible. One of the differences between the two families to which attention has been directed in this connexion, is the presence in the terebellids of four conical pouches on the first diaphragm—this diaphragm cutting off an anterior coelom from the general body cavity. It was first suggested by Meyer (1887) that it is the contraction of these sacs and the forcing of fluid into the tentacles that causes their extension. The sacs are not principally muscular, however, and it seems unlikely that pressure of the fluid is itself responsible for the extension of the tentacles. By the dilation and relaxation of these sacs it may be that during the continual movements of the tentacles and the anterior part of the body, the coelomic pressure is maintained at a constant level ensuring continued normal working of the muscles in every position of the body. If a living worm is pinned down in a dish, and the anterior coelom opened, the worm will, after

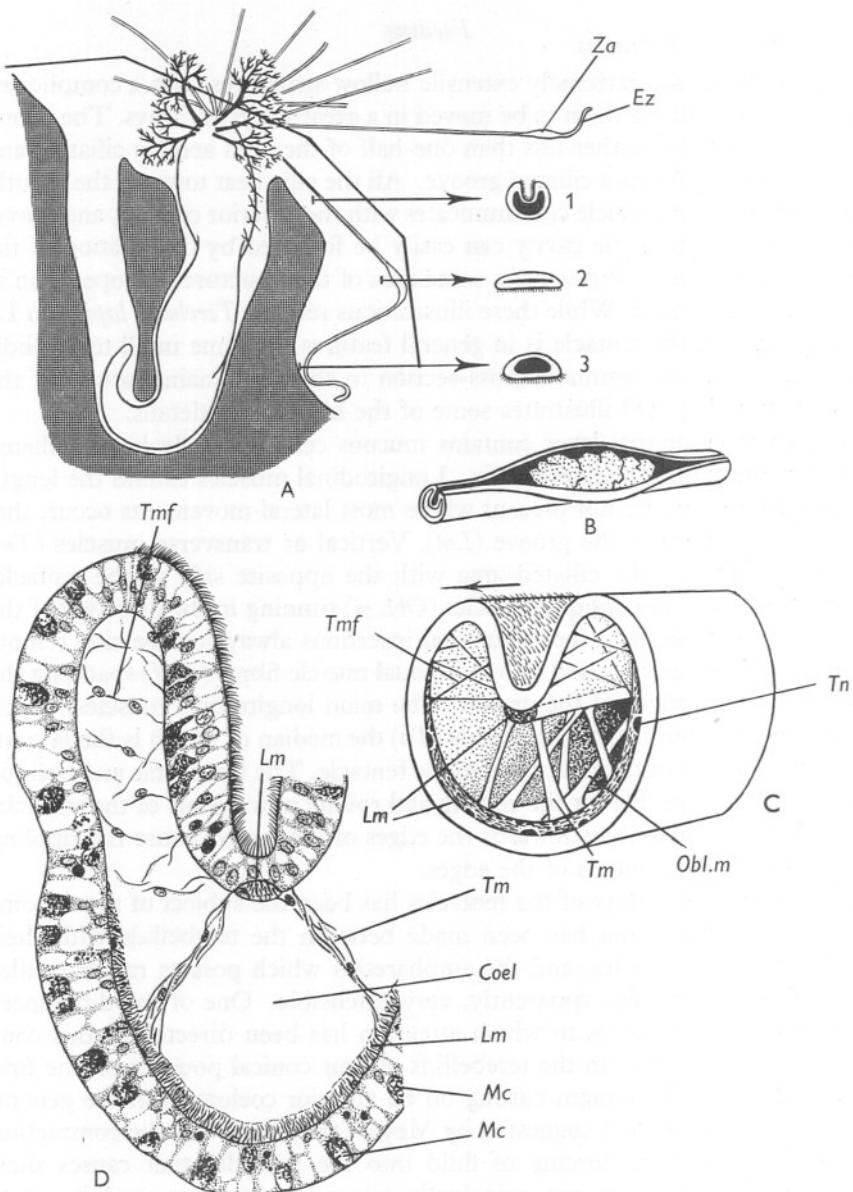


Fig. 4. Operation and structure of the tentacles in *Terebella lapidaria*. A, animal in feeding posture within its tube in the silt beneath a rock slab; Za, zone of attachment; Ez, exploratory zone; 1, appearance in cross-section in extended part of tentacle, 2, at zone of attachment, 3, in exploratory part. B, conveyance of particles along groove by ciliary action and squeezing by the transverse muscle fibres. C, stereogram of a tentacle to show muscle system. D, transverse section of tentacle to show histological details. Coel, coelomic space; Lm, longitudinal muscle; Mc, mucous cells; Obl.m, oblique muscles; Tm, transverse muscles; Tmf, transverse muscle fibres; Tn, tentacular nerves.

the initial shock period has passed, extend its tentacles almost as much as before, even though no pressure can be exerted by the coelomic fluid.

Most of the extension of the tentacle is due to ciliary creeping; by rolling over and opening out the ciliary groove in order to present a flat ciliated surface to the substratum, the tentacle can crawl along rather like a planarian. After a certain amount of extension the tentacle attaches itself to the substratum just behind the tip, by mucus, and perhaps also by suction caused by contraction of some of the transverse muscles. The tentacle is always flattened at this point (Fig. 4A, Za) and the cavity must be virtually occluded. This 'segmentation' of the tentacular cavity may be an important factor enabling the further extension of the tentacle, which is continually releasing and re-attaching to the substratum a short distance behind the tip. In this way the tentacle follows a somewhat circuitous path thereby efficiently exploring the surroundings for food or debris. By the continual release and reformation of the 'point d'appui' the tentacle is being straightened and tautened, enabling the tentacle to be extended each time to its maximum extent. Small particles may be conveyed back along the ciliary groove when the tentacle is in this position, but sooner or later the tentacle is pulled in and re-extended in a new direction. In this way a large area can be gleaned of detritus.

In *Amphitrite johnstoni*, and in all the other terebellids that have been studied, the tentacles have been found to be insensitive to light. This is not so in all terebellids, as Welsh (1934) found in *Terebella gigantea* that the distal third of the tentacle was sensitive to light. All terebellids, on the other hand, are very sensitive to tactile stimuli, and the touching of one tentacle may cause a prompt retraction of all the tentacles.

#### *Lip-mechanism*

No particle can be conveyed direct to the mouth as the cilia do not extend to the base of the tentacle, and all particles are received first by the lips.

All particles collected by the tentacles are conveyed to the lips by one or more of the following methods.

- (1) Very small particles such as diatoms, single sand grains and so forth may be conveyed along the groove of the tentacle by the action of the cilia. The groove may be partly open or completely closed so as to form a tube (Welsh, 1934, has also observed this in *T. gigantea*).
- (2) Larger particles are usually conveyed along the groove partly by action of the cilia and partly by a squeezing action of the sides of the groove (Fig. 4B).
- (3) Particles too large to be transported by either of these methods are usually secured by the sides of the groove and the tentacle then pulled in.

As already mentioned, the upper lip is formed from the prostomium. It is provided with muscle fibres running in different directions from the ventral to the dorsal side giving the lip a variety of movements (Fig. 5C-E). The oral

surface is completely ciliated, and slightly grooved, the grooves running down towards the mouth. Beneath the mouth are two pairs of lips, the outer pair of which may be further subdivided so that a series of ridges is formed. The inner pair of lips is formed from the stomodaeum; the outer pair of lips is derived from the ventral part of the first segment. The two pairs of lips

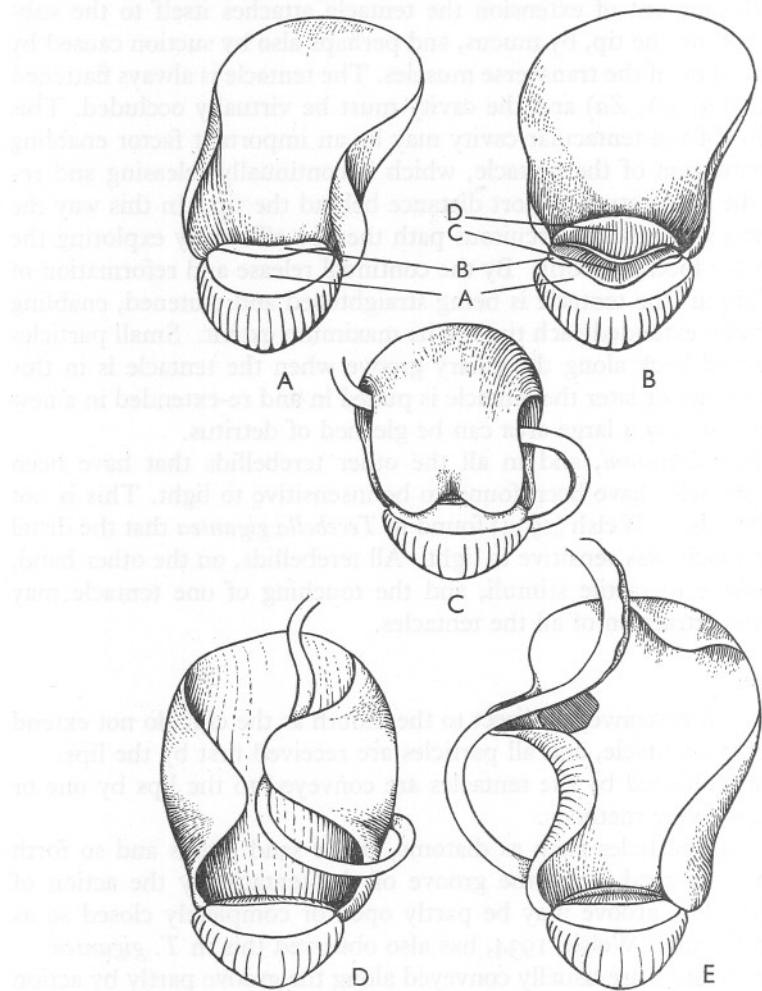


Fig. 5. Lip movement in *Amphitrite gracilis*. A and B show the rolling action of the inner lips; C-E, typical movements of the upper lip. A, B, C and D lips (see text).

usually differ in appearance; the outer pair is ciliated and has mucous gland-cells in the epithelium, and in most terebellids is consequently yellowish or whitish in appearance. The inner lip-pair is usually reddish, and often grooved; these lips are never ciliated nor are there any mucous gland-cells in the epithelium (Fig. 5A, B). This series of lips can be recognized in all

terebellids, though they are variably developed. For convenience, the outer fold of the outer lip-pair is referred to here as 'lip A', the inner fold 'lip B'; the outer fold of the inner lip-pair, 'lip C', and the corresponding inner fold as 'lip D'. In some terebellids only the inner part of lip A functions as a lip, in the sense that it is directly concerned with the food, the outer part often being developed into a platform or flange concerned with tube-construction. A fifth lip fold, 'lip E', can usually be recognized inside lip D.

Some idea of the musculature of the lower lips can be had from Figs. 6 and 7C. The musculature of the outer lip-pair (A and B) is derived from the circular and longitudinal segmental muscles of the ventral side. Those on the dorsal side are not recognizable. The transverse or circular muscle coat is not

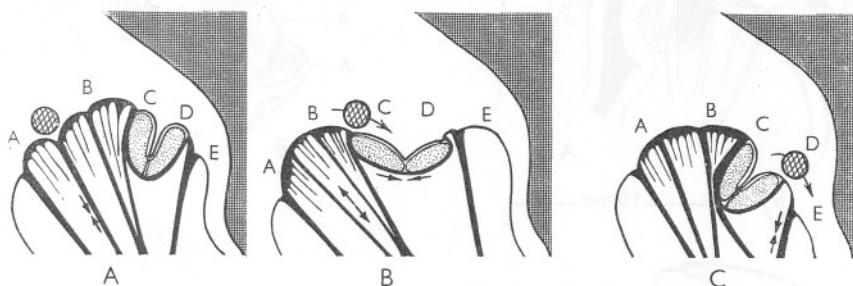


Fig. 6. Diagram illustrating the lip movements in *Amphitrite johnstoni*.  
For explanation see text.

well developed, and in *Amphitrite johnstoni* buckling movements of the outer lips in the transverse plane are not important in feeding. The longitudinal muscles, on the other hand, are very important, and these are attached in two main transverse fascicles externally visible as lips A and B, and these muscles may be further divided each into two groups so that a groove can be formed in each lip. It is the serial contraction of these longitudinal muscles that gives the outer lip-pair their characteristic rippling action. The lips forming the inner pair (C and D), on the contrary, are provided each with a large band of transverse muscle backed on the inside by a thinner band of longitudinal muscle running from the outer edge of lip C to the inner edge of lip D. The contraction of the longitudinal muscle makes these inner lips pout outwards, and when this is accompanied by a relaxation of the transverse bands, and is combined with the rippling action of the outer lip-pair, the inner pair of lips make a scooping movement into the hollow temporarily formed by the outer pair at the moment when lip B is somewhat contracted. This is illustrated in the diagram (Fig. 6). Here (Fig. 6A) both lip A and lip B are shown divided by the longitudinal muscles; though the lower part of lip A forms a parapet to the lip and is not really concerned with feeding. The circular or transverse muscles of the outer lip-pair are not shown here. The food-bolus arrives in the groove between A and B, the groove being deepened by contraction of the

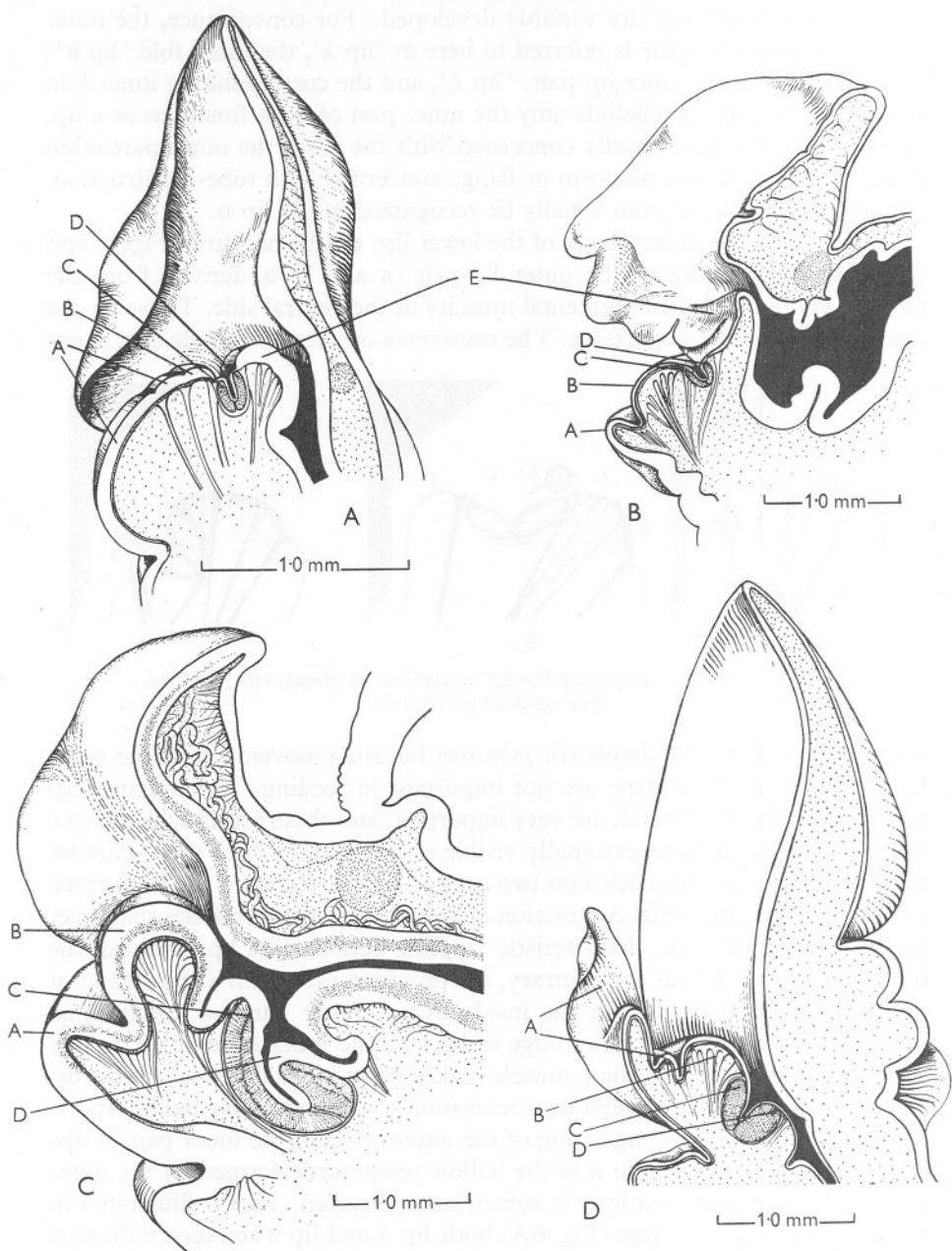


Fig. 7. Comparison of lip structure in four terebellids: A, *Polycirrus aurantiacus*; B, *Terebellides stroemi*; C, *Amphitrite johnstoni*; D, *Lanice conchilega*. A, B, C, D and E, lips.

longitudinal muscles as shown. By a serial contraction of these muscles (from left to right in the diagram) the bolus is rolled forwards on to the outer rim of lip c (Fig. 6B). The inner lips receive it, opening out by contraction of the longitudinal muscle, close on to it, and roll forwards to the opening of the mouth by contraction of the longitudinal muscle between d and e, and also by some action of the transverse muscles of c and d which run round the sides of the mouth (Fig. 6C). This cannot be adequately shown in the diagram, and this explanation is much simplified. In practice the food may be in the form of a string running the length of the lip, or a rounded particle to one side. In consequence there is a great variety of curling movements to convey the food to the mouth.

It has been mentioned already that the outer lips, A and B, are ciliated. These cilia beat towards each other so that small particles alighting on either lip are swept down into the main groove between the two. In *A. johnstoni*, though not generally in other terebellids that have been studied, the tentacles tend to be wiped across these outer lips so that all the food particles are collected there. When a certain quantity has been collected, the inner lips scoop up the contents in the way already described. There seems to be little selection of the particles to be ingested. Obnoxious substances reaching the lips cause the complete retraction of all the lip surfaces. When these are again extruded, the unrolling action of the outer lips effectively pushes away the offending matter on to the ventral side of the body. Apart from this, virtually any particle of small enough size will be ingested, while particles which are too large cannot be scooped up by the inner lip-pair and are merely pushed off the outer lip groove. In most terebellids these are the particles which, having become covered with mucus from the lips A and B, are incorporated into the substance of the tube.

In practice the feeding movements of *A. johnstoni* do not always correspond exactly to the idealized behaviour described above, and in none of the terebellids studied are these movements very precise. Some of the particles arriving in the neighbourhood of the mouth may be wiped directly on to the inner lips, some on to the upper lip. Some of the movements regularly performed by the upper lip in *A. gracilis* are shown in Fig. 5. Small particles arriving on the upper lip will be conveyed directly down into the mouth by ciliary means. Larger particles will find their way down on to the lower lips, where they will be rolled either into the mouth, or off on to the outer lip parapet according to their size.

#### *Morphology and Histology of the Gut*

The mouth leads into a long narrow oesophagus which opens into a wide thin-walled fore-stomach. A pharyngeal region cannot strictly be distinguished. The fore-stomach opens into a hind-stomach, easily recognized by the thick layer of muscle giving it a glistening appearance, and passes to an

intestinal region which leads to the anus. The anterior part of the intestinal region is coiled, and lies in the swollen part of the body immediately posterior to the thorax (Fig. 1, *F.int.*); the hind intestine is straight and supported by septa. All the septa in the thoracic region, and also in the part of the body occupied by the fore-intestine, have been reduced save for the single anterior septum mentioned previously. It is the reduction of the septa in this region that enables the fore-intestine to become coiled, the length of this part of the gut thereby being increased to 4 times the length of the part of the body in which it is contained. Although the septa are lacking in this region, the intestine and stomach are suspended by muscles attached to the mid-dorsal line of the body-wall (Fig. 8 C, *Dsm*). The muscles are well developed and may cause the slight, but possibly important, writhing movements of the intestine.

Internally the oesophagus is ciliated, and the epithelium contains many mucous cells closely resembling those described and illustrated by Brasil (1904) in *Pectinaria* (Fig. 8 D). Mucous cells are present in varying proportions throughout the gut with the exception of the muscular hind-stomach, but mucous cells are most numerous in the oesophagus and towards the hinder end of the posterior intestine which again is entirely ciliated. The epithelium of the oesophagus is thrown into folds. Near the mouth these form transverse ridges at right-angles to the axis of the oesophagus; farther back they form sinuous ridges parallel to the axis of the gut. The oesophagus has a well-developed layer of inner circular muscle and outer longitudinal muscle.

The fore-stomach is much less muscular, and the external layer of longitudinal muscle in this region and in the hind-stomach and fore-intestine is poorly developed but for one large strip running along the mid-dorsal line and related to the dorsal suspensory muscles. The circular muscles are also reduced in the fore-stomach but are, nevertheless, capable of causing constricting movements. Externally, as well as internally, the wall of this part of the gut is thrown into folds, and presents a yellowish appearance. Internally there are no ciliated cells, but the epithelium contains secretory cells of various kinds, and replacement cells towards the base. All the cells in this region have a brush-border (Fig. 8 E). Apart from the mucous cells most of the secretory cells present a vacuolated appearance the cytoplasm between the vacuoles being very granular (Fig. 8 E, *Sc. 1*). There are also a few cells containing very large granules which stain red with azan and blue-black with iron haematoxylin, and presumably also of a secretory nature (Fig. 8 G, H, *Sc. 2*).

The hind-stomach contrasts strongly with neighbouring regions by virtue of the well-developed muscles in the gut wall. This muscle all belongs to the circular muscle layer, and in spite of contrary assertions in the literature, all the fibres run in a circular direction and may be separated into thin washer-like lamellae. The longitudinal muscle layer is mainly represented by the mid-dorsal strip already mentioned, but this is well developed and capable of strong contractions. Internally the epithelium is uniform, and consists of

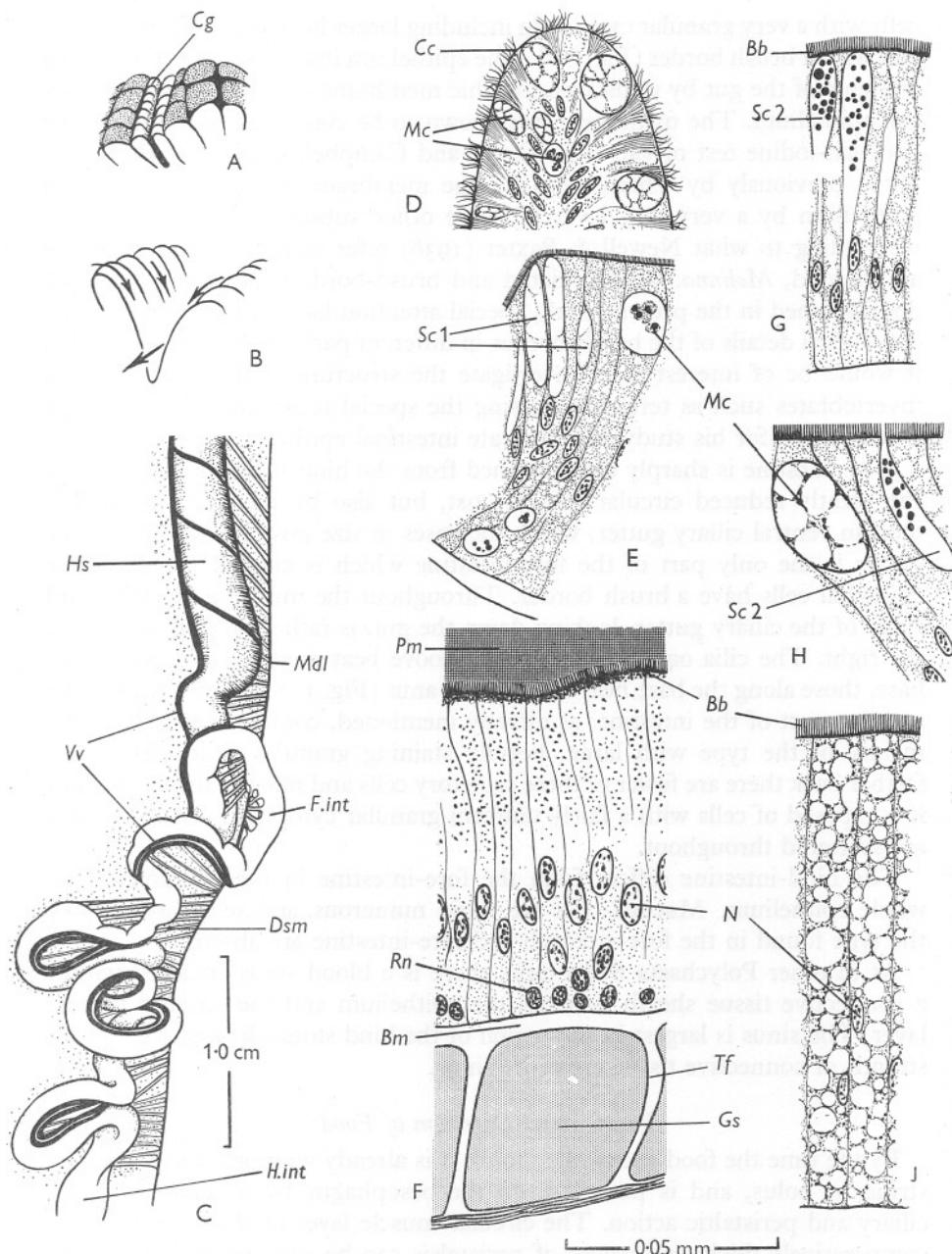


Fig. 8. A, appearance of ciliary groove in life; B, ciliary currents within the groove; C, hind-stomach and fore-intestine in *Amphitrite johnstoni* to show coiling and dorsal suspensory muscles; D–H histology of the gut in *A. johnstoni*: D, oesophagus; E, fore-stomach; F, hind-stomach; G, H, fore-intestine; J, fore-stomach in *Polycirrus*. Bb, brush-border; Bm, basement membrane; Cc, ciliated cell; Cp, ciliary gutter; Dsm, dorsal suspensory muscles; F.int, fore-intestine; Gs, gut sinus; Hs, hind-stomach; Mc, mucous cell; Mdl, mid-dorsal line; N, nucleus; H.int, hind intestine; Pm, peritrophic membrane; Rn, nucleus of replacement cell; Sc 1, Sc 2, 1st and 2nd types of secretory cell; Tf, transverse fibre; Vv, ventral vessels.

cells with a very granular cytoplasm including larger heavily staining granules, and with a brush border (Fig. 8 F). The epithelium itself is separated from the contents of the gut by a thick peritrophic membrane presumably secreted by the epithelium. The membrane was shown to be composed of chitin by the chitosan-iodine test of van Wisselringh and Campbell (Richards, 1951). As noted previously by Thomas (1940), the membrane is separated from the epithelium by a very thin layer of some other substance, presumably corresponding to what Newell & Baxter (1936) refer to as 'coagulum' in the ampharetid, *Melinna*. While ciliated and brush-borders have been carefully distinguished in the present work, special attention has not been given to the cytological details of the brush-border in different parts of the gut. However, it would be of interest to re-investigate the structure of the cell border in invertebrates such as terebellids, using the special techniques developed by Baker (1942) for his study of vertebrate intestinal epithelia.

The intestine is sharply distinguished from the hind-stomach, not only by the greatly reduced circular muscle coat, but also by the initiation of the median ventral ciliary gutter, which increases in size posteriorly. The ciliary gutter is the only part of the fore-intestine which is ciliated, all the other epithelial cells have a brush border. Throughout the intestine the left-hand ridge of the ciliary gutter, looking down the gut, is rather taller than that on the right. The cilia on the sides of the groove beat downwards towards the base, those along the base beat towards the anus (Fig. 1, *Vg*; Fig. 8 A, B). The anterior part of the intestine, as already mentioned, contains secretory cells, mainly of the type with large, heavily staining granules (Fig. 8 H, *Sc 2*); farther back there are fewer of these secretory cells and most of the epithelium is composed of cells with a fairly uniform granular cytoplasm. Mucous cells are scattered throughout.

The hind-intestine differs from the fore-intestine in the ciliation of the whole epithelium. Mucous cells are more numerous, and secretory cells of the type found in the fore-stomach and fore-intestine are absent.

As in other Polychaeta Sedentaria, there is a blood sinus enclosed within a connective tissue sheath between the epithelium and the circular muscle layer. The sinus is largest in the region of the hind stomach (Fig. 8 F) where strands of connective tissue cross the sinus.

#### *Passage and Digestion of Food*

By the time the food enters the mouth it is already wrapped into a mucous string or bolus, and is passed down the oesophagus by a combination of ciliary and peristaltic action. The circular muscle layer of the oesophagus is comparatively thick, and waves of peristalsis can be seen passing down the oesophagus in dissected living specimens.

Though food may be transported mainly by ciliary means in ciliary feeders such as serpulids and sabellids, the importance of the cilia in transporting food in the gut of other polychaetes has probably been overstressed.

Hanson (1948) has drawn attention to the direction of peristalsis in the gut of serpulids and sabellids. In small transparent individuals in which movements of the gut may be watched under the microscope, the direction of peristalsis is apparently always towards the mouth. This was reported earlier by Claparède (1873) and Stephenson (1913). In terebellids it is difficult to make direct observations on gut movements, but as the whole part of the stomach region is unciliated, transport of the contents in this region at least must be due to muscular action of some kind. Peristalsis in the oesophagus is clearly in a posterior direction; in the fore-intestine, only antiperistalsis has been seen, quite strong waves of contraction passing anteriorly, notwithstanding the relatively thin muscle layer in this region. These movements of the fore-intestine can be seen by transparency of the body-wall, and are quite distinct from the writhing movements of the whole intestine. In the fore- and hind-stomachs, strong constricting and segmenting movements may be seen on opening a worm, but these movements soon cease, and no definite peristalsis was ever seen in either region. However, the movements of the body-wall, and of the dorsal suspensory muscles, may have an important effect on the transport of the gut contents. The change in direction of the irrigation contractions of the body-wall from anterior to posterior during feeding, may be significant in this connexion. Certainly defaecation is accompanied by violent contractions of the hinder part of the body-wall, which presumably exerts a squeezing action on the hind-intestine. The slight movements of the body in the region of the fore-intestine which may be seen in a normal worm, are related to the writhing movements of the gut itself, presumably through the intermediary of the dorsal suspensory muscles, and these movements may also be important in transport of the gut contents. Although Hanson (1948) may be right in assuming that the ventral ciliated groove is responsible for the transport of the gut contents posteriorly against the antiperistaltic action of the gut itself, the importance of the body-wall movements should not be underestimated. The ciliary gutter may be of use in rapidly removing indigestible material from the site of absorption. The excised hind-stomach, when suspended under slight tension in a bath of sea water, shows periodical longitudinal contractions which may be recorded kymographically. These contractions are probably mainly caused by the dorsal longitudinal muscle strip, and occurred at intervals of about 5 min in a fresh preparation. These movements may help in pumping the food from the fore-stomach to the intestine. The irrigation movements of the body-wall seem to be spontaneous, as Wells has found in many other polychaetes, a body-wall strip attached to the nerve cord showing very regular bursts of activity. No clear connexion between this activity and the gut movements could be detected in preparations in which a strip of body-wall and the different parts of the gut were linked to levers recording traces on the same drum. Transport of the gut contents is probably mainly muscular, and due to a variety of movements of the gut itself, and to the body-wall.

The pH of the oesophageal contents is distinctly lower than that of the outside medium, and it may be that some secretion of enzymes takes place towards the posterior end of the oesophagus, as a few secretory cells similar to those in the stomach are found there. The pH of the oesophageal contents is usually about 7·0; that of the fore-stomach is about 6·0 in a feeding worm, and this distinctly acid medium is maintained throughout the following regions of the gut, only rising significantly on entering the hind-intestine, where the pH rises gradually from 6·2 at the beginning of the septate region to about 7·2 at the rectal end. In starved worms the pH throughout the gut is much higher, and little different from that of the coelomic fluid (7·2–7·4).

Simple spot-tests for enzymes in the gut contents showed that a lipase was present in the fore-stomach and fore-intestine; strong amylase activity and rather weak protease activity in the fore-stomach, and rather stronger protease activity with some amylase activity in the fore-intestine. Tests on extracts of the washed tissues of the fore-stomach and fore-intestine taken from starving worms confirmed the impression that protease activity was stronger in the fore-intestine, and amylase activity stronger in the fore-stomach. Brasil (1904) found amylase activity only in the most anterior part of the mid-gut of *Pectinaria koreni* (corresponding to the fore-stomach in terebellids) and associated this with the 'claviform glands' he described (similar to Fig. 8 E, Sc 1). Protease activity was found in the second part of the mid-gut (corresponding with the fore-intestine of terebellids), and this Brasil associated with the 'cellules à ferment' (similar to Fig. 8 G, H, Sc 2) which are most numerous in this region. Lipase activity seemed to be weak in *Pectinaria*. He found also that the pH throughout the gut was slightly alkaline, but this may easily have been due to admixture with the strongly buffered body fluid, for, as Nicol (1931) points out, sampling the contents of the gut without admixture of the body fluid is difficult, and would be particularly so in a worm as small as *Pectinaria*. Brasil's tests were all done on extracts of the tissues. His findings are confirmed by these observations on terebellids, as the distribution of the two main kinds of secretory cell is much the same as he describes, and also corresponds with the site of amylase and protease activity. This distribution of secretory cells is similar in all the other terebellids studied; Fig. 8 J shows the appearance of the fore-stomach of *Polycirrus aurantiacus* Grube in which all the cells are of the vacuolated kind. Digestion is probably entirely extracellular, as the epithelial cells throughout the stomach and fore-intestine have a brush-border, and no amoebocytes were found in the gut.

Absorption, as shown by uptake of iron from iron saccharate ingested by normally feeding worms, takes place also in the fore-intestine and in the anterior part of the hind-intestine. None of the iron was ever found in the cells of the fore-stomach. This was confirmed with *Terebella lapidaria* which was found to feed more readily on iron saccharate.

## EVOLUTION OF FEEDING MECHANISMS IN TEREBELLIDS

*Feeding and Lip-Structure*

All terebellids have the basic lip-structure which has been described, consisting of an inner and outer pair of lips below the mouth, and a single lip above. While the two pairs of lower lips may be recognized in all the genera which have been studied, the relative proportions and the development of each of these lips vary considerably, and these variations are related to different habits of feeding and of tube-building.

There is no doubt that the tentacles and the upper lip are derived from the prostomium, and not from the first segment as stated by McIntosh (1922). This is clear from the studies of Salensky (1883) and Wilson (1928) on the larval development, and from Nilsson's (1912) study of the nervous system. There is also no disagreement among morphologists that the first segment (peristomial segment, 'segment buccal'), while chaetigerous and possessing a nephridium in the larva (Wilson, 1928), is greatly reduced in the adult, where all that remains are the parts forming the outer lips A and B, and the ventral musculature. In some forms, the lateral lips or collar which extend round the dorsal side of the body and which are usually adaptations for tube-building, are also derived from the first segment.

The inner lips (c and d), which have been referred to as the 'Schlundsaec' by Meyer (1887) and the 'buccal organ' by Elrington (1909) and Wilson (1928), correspond to the 'muscle bulb' described in various archiannelids by Jägersten (1947). This organ is a very distinct structure and is usually red in colour in adult animals. It is outside the scope of the present paper to discuss the relations of this structure in the various families of Polychaeta Sedentaria, and its relationship with the eversible proboscis of the Errantia, but it may be noted that the structure is found throughout the Sedentaria occurring in families whose members have feeding habits very different from those of terebellids.

Four examples to illustrate the variation in lip structure are shown in Fig. 7. Of these, *Amphitrite johnstoni* (Fig. 7C) and *Terebellides stroemi* Sars (Fig. 7B) are found in mud or muddy sand; *Polycirrus aurantiacus* (Fig. 7A) is found under stones and in rock crevices, and *Lanice conchilega* (Fig. 7D) occurs in sand. Of these, *Lanice* is the best tube-builder, *Terebellides* does not really build tubes, although particles rejected in feeding and those in contact with the body become agglutinated to form a sleeve in which the worm lies. Neither *Polycirrus* nor *Amphitrite johnstoni* lives in tubes, the latter living in an unlined burrow in mud as described above. *Polycirrus* is more mobile and pulls itself about under stones by the tentacles, which are extremely numerous and completely hide the whole of the anterior part of the body; it is only by the amputation of the majority of the tentacles that feeding may be observed in this species. *Terebellides*, on the other hand, has extremely short tentacles, which are used much less for feeding than in most terebellids.

In *Lanice*, the upper lip is chiefly employed for building the tube—the lip is extremely flexible, and as described by Arnold Watson (1890) is the organ which is mainly responsible for laying in place the grains of sand and shell which constitute the tube, after having been plastered with mucus by the lower lips. The lower lip A is also expanded into a kind of collar, and this is used in applying more mucus to the inside of the tube as the rim is extended or repaired. As will be seen, it is the outer part of lip A which is thus modified, and rolling movements can still be executed during feeding by the inner part of A, by B, and the inner lip-pair, as in *Amphitrite johnstoni*. In *Lanice*, particles which are small enough to be ingested find their way down into the 'gullet' formed between the upper and lower lips and are accepted by the inner pair of lips and swallowed. Large particles will come to rest between the upper lip and the outer part of lip A, and these will be either incorporated into the tube or rejected according to suitability or need. It will be seen that C and D are quite well developed, though perhaps not so well as in *Amphitrite johnstoni*. Contrast, on the other hand, the size of the upper lip in these two species, one of which does, the other which does not, build a tube.

Unlike *Amphitrite johnstoni* and *Lanice*, *Polycirrus*, living as it does under rocks, is usually in the prone position while feeding. The very large upper lip is commonly curled into a partly closed tube or scroll, through which the tentacles are drawn. Particles collected under the upper lip are periodically pushed into the mouth by the movements of the lower lips, of which the outer pair A and B are the better developed, A forming a long mucus-producing tongue. The much greater use of the upper lip and of lip A, as compared with *Amphitrite johnstoni*, for example, is clearly reflected in the proportions of the parts concerned. Particles which may be gathered by the tentacles but which are too large to be ingested are simply pushed away by the rolling movements of A and B.

In *Terebellides* the lip structure has been profoundly modified (Fig. 7B). The upper lip is extended into a complete funnel joining laterally with the lip E. Both pairs of lower lips are almost excluded from the mouth, but the funnel in *T. stroemi* is much lower in the mid-ventral line, and the rolling movements of lips A-C may still help to push food collected in the funnel into the mouth. In the Antarctic species *T. minutus* the funnel is even more complete. As mentioned already, the tentacles are short in this genus, and *Terebellides* has been described as scooping up the mud on which it feeds with the ventral side of the funnel. Unfortunately *Terebellides* is not found intertidally on our shores, and I have not had the opportunity of studying the feeding movements, or those of *Artacama*, another aberrant genus to which reference will be made later. Both genera are cold water forms found in the Arctic and Antarctic regions, but Thorson (1946) states that they are the commonest bottom polychaetes in the Øresund. Hesse (1925), working in Sweden, was apparently familiar with *Terebellides*, and he writes: 'Terebellides... gräbt mit

der grossen zweilobierten Platte unterhalb des Mundes'. The 'zweilobierten Platte' clearly refers (from his fig. 3) to the ventral parts of the funnel, which in his figure is shown buckled up into a kind of scoop. If this movement is accompanied by the usual rolling of the lower lips, mud may be pushed into the mouth. Later, in the same context, he writes: 'Bei *Terebellides* werden sie aber an dem zweilobierten Hautkamm unterhalb des Mundes aufgenommen. Da dieser Kamm ein wenig vom Munde entfernt ist, gelangen die Partikeln nach dem Kamm durch eine Rinne, die dadurch zu Stande kommt dass der vordere Teil des Mundbodens sich eine Tülle bildend ausstülppt. Von der Spitze dieser Tülle fallen die Partikeln auf den Kamm.'

*Artacama* seems to have taken digging even further. In this genus, lip A has been enormously expanded into a papillated 'proboscis' which is presumably used for digging (Fig. 9). Lip B is fairly well developed, but the inner lip pair (C and D) are relatively small, and this suggests that these inner lips are not as important as in *Amphitrite johnstoni*, for instance. Like *Terebellides*, *Artacama* is a mud-dweller, and while the tentacles are not as short as in the former genus, they are certainly not as long as in some other terebellids. Genera such as *Terebellides* and *Artacama* are relatively deep water forms, and it may be that these rather different feeding habits are correlated with the different kind of deposit from that occurring around low-water mark.

*Terebellides*, and its near relatives, does, in its lip structure, come close to the ampharetids, which though differing in other ways, also have a funnel-shaped upper lip excluding both pairs of lower lips (see Fauvel, 1897). Hessle (1917) postulated a separate family for these genera, which he called the Trichobranchidae, and from which he derived not only the terebellids, but also the amphictenids and ampharetids. In lip structure *Terebellides* is more primitive than Hessle's type-genus *Trichobranchus* which has the upper lip cleft ventrally to allow the lower lips to roll into the mouth. For many anatomical reasons it is unlikely that the terebellids arose directly from the ampharetids, and Hessle is probably correct in regarding *Terebellides* and its near relatives as closely approaching the stem form. The conclusion is therefore reached that the most advanced terebellids are those with highly developed tentacles and tube-building habits, and that the group has been derived from tubeless forms, living and feeding on mud.

#### *Variations in Gut Morphology*

Regarding the gut itself there is little variation in morphology or in the proportions of the parts, and no significant differences in histology. The oesophagus in all species is remarkable for its length. This is probably related at least in part to the very different morphology of the anterior region of the body; the large ventral mucous glands, the nephridia and heart-body take up much space in the coelom, and an expanded gut could not well be accommodated.

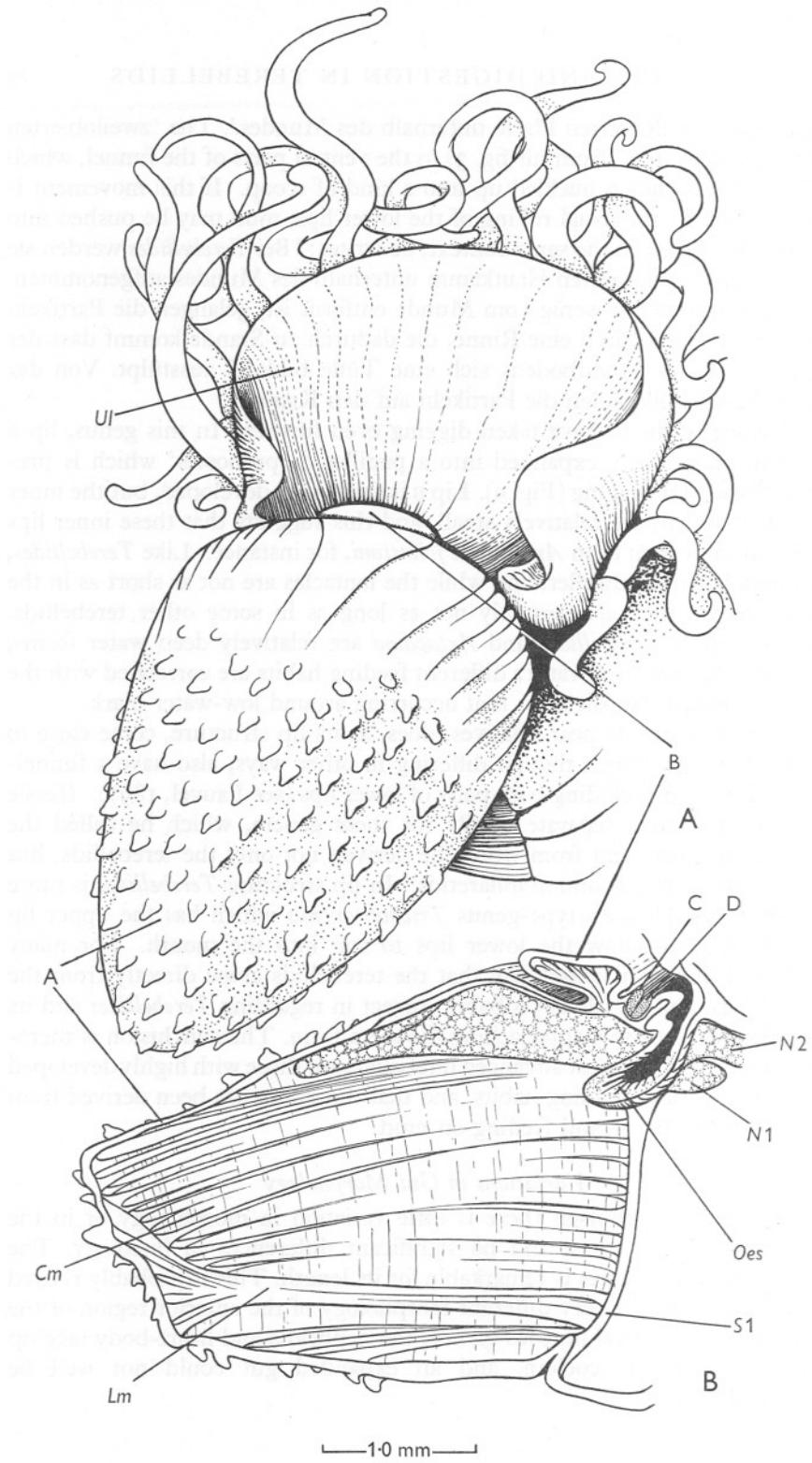


Fig. 9. *Artacama proboscidea*. A, external appearance of the 'proboscis' and lips; B, stereogram of a sagittal section through the proboscis. A, B, C and D, lips; Cm, circular muscle; Lm, longitudinal muscle; Ul, upper lip; Oes, oesophagus; S1, 1st septum; N1, N2, 1st and 2nd anterior nephridia.

Although the length of the muscular hind-stomach seems to be very variable, these differences are more apparent than real. Thus in all the species examined from the smallest, such as young *Polycirrus*, to the very large fully grown *Amphitrite edwardsi*, the fore-stomach represents 10–15% of the total length of the gut, the muscular hind-stomach 5–12%, and the intestine 65–75%. On the other hand, the epithelial area of the fore-stomach in *Terebellides* (and also of its near relative, *Octobranchus* (McIntosh, 1922)), is much increased by being thrown internally into folds and extended forwards as slight lateral lobes partly enveloping the oesophagus. In this, the gut resembles that of ampharetids, but there is no muscular stomach in the latter group (Fauvel, 1897), though there is a peritrophic membrane (Newell & Baxter, 1936). Wirén (1885) illustrates the gut of *Artacama* as being much coiled, with the oesophagus reflexed into the 'proboscis'. This may be an effect of fixation, as the animals which have been dissected during the present investigation did not have the oesophagus reflexed in this way.

There are no diverticula or oesophageal pouches in terebellids, as there are in many errant polychaetes. The 'salivary glands' mentioned by Quatrefages (1865) were probably nephridia, and the 'glandular organ entering the alimentary canal dorsally at the base of the oesophagus' in *Lanice* referred to by McIntosh (1922) is presumably the heart-body.

Bearing in mind the different feeding habits and the probable ancestry of the group it is interesting to note that larvae of terebellids have a simpler gut, the muscular hind-stomach being recognizable only when the young worm has adopted the adult mode of life (Salensky, 1883; Elrington, 1909; Wilson, 1928; and Thorson, 1946).

#### SUMMARY

The feeding and the functional morphology of the gut of *Amphitrite johnstoni* are described, and later compared with that of other terebellids. Feeding is effected by the tentacles bringing particles to the lips, where some sorting occurs, and the control of movement of the tentacles and the lip mechanism are described. The gut in all terebellids consists of an oesophagus, fore-stomach (comprising 10–15% of the total gut-length), muscular hind-stomach (5–12%) and intestine (65–75%). The muscular hind-stomach acts as a mixer and contains a peritrophic membrane. Enzymes are secreted by the fore-stomach and the fore-intestine. Strong amylase activity, weak protease activity and lipase activity is found in the fore-stomach; strong protease activity, amylase and lipase activity is found in the fore-intestine, and these differences are correlated with differences in histology. The gut has a pH of 6·0 in the digestive regions in feeding worms. Absorption occurs in the fore-intestine and the anterior part of the hind-intestine. Transmission of food throughout the gut is caused mainly by muscular action. The lip structure of *Amphitrite*, *Polycirrus*, *Lanice*, *Terebellides* and *Artacama* is briefly described in relation to the different habits of these genera.

## APPENDIX

## Notes on the Methods Used

This study has been based on an examination of the following species in particular: *Amphitrite johnstoni* Malmgren, *A. edwardsi* (Quatrefages), *A. gracilis* (Grube), *Terebella lapidaria* (Kahler), *T. californica* Moore, *Lanice conchilega* (Pallas), *Loimia medusa* Sav., *Polynnia nesidensis* (Delle Chiaje), *Nicolea venustula* (Montagu), *Pista cristata* (Müller), *Thelepus cincinnatus* (Fabricius), *T. crispus* Johnson, *Polycirrus aurantiacus* Grube, *P. calidrum* Clap., *Trichobranchus glacialis* Malmgren, *Terebellides stroemi* Sars, *T. minutus* Hessle, *Artacama proboscidea* Malmgren.

Material for sectioning was fixed mainly with Bouin's and Gilson's fluids, cut at  $7.5\ \mu$  and stained with Heidenhain's azan, or iron haematoxylin with mucicarmine, alum carmine, or Orange-G. Mann's methyl-blue-eosin was also used. 10% neutral sea-water formalin was used for fixing tissues prior to testing for absorbed iron after feeding with saccharated iron carbonate as recommended by Glick (1949). These sections were counterstained with Orange-G (in absolute alcohol) as this stain forms a good contrast with the blue of absorbed iron.

The B.D.H. capillitor method was used to measure the acidity of the gut contents, bromothymol-blue (pH 6.0-7.6) and bromocresol purple (pH 5.2-6.8) covering the pH range found. This method gives a value to the nearest 0.2 pH on very small quantities. When measurements were made, the different parts of the gut were ligatured, the whole gut dissected out, dried on filter-paper to avoid contamination with coelomic fluid, and the contents withdrawn from each region with a fine glass pipette.

The presence of proteases in the gut contents was demonstrated by the ability to digest gelatin in the form of pieces of photographic film; amylases by testing for remaining starch after incubation of the sample in starch solution, and lipases by action on olive oil stained with Nile blue.

Details of the apparatus used for 'closing the circulation' are to be found in Wells & Dales (1951), Fig. 1. (*Nereis*), and the arrangement of the glass/mud/glass sandwich apparatus from Wells (1949), Fig. 2.

Drawings of histological and anatomical details have been prepared with the aid of a camera lucida.

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## VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

### III. EUPHAUSIACEA

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(Text-figs. 1 and 2)

#### INTRODUCTION

Our published work has shown that the northern euphausiids, *Meganyctiphanes norvegica*, *Thysanoessa raschii* and *T. inermis*, contain much higher concentrations of vitamin A than we have found in any other marine Crustacea (Kon & Thompson, 1949a; Batham, Fisher, Henry, Kon & Thompson, 1951; Fisher, Kon & Thompson, 1952, 1953, 1954). In the antarctic species, *Euphausia superba*, the concentration of vitamin A in samples taken from the alimentary canals of baleen whales (Thompson, Ganguly & Kon, 1949; Kon & Thompson, 1949b) was similar to that found in *Meganyctiphanes norvegica* from the gut of arctic baleen whales (Fisher *et al.*, 1952), but both were very much lower than in free-swimming *M. norvegica*. No corresponding free-swimming specimens of *Euphausia superba* had been analysed.

This evidence indicated that the Euphausiacea, as a group, might be richer in vitamin A than other Crustacea. We have, therefore, attempted to obtain as many other euphausiid species as possible for a comparative study, and in fact have now information about eight further euphausiids. Unfortunately, we found no other environment as favourable as Loch Fyne or Monaco for catching easily large numbers of these animals. The numbers of specimens analysed of these species were, therefore, relatively small, but, in most instances, valid results were obtained.

We have now studied samples of *Meganyctiphanes norvegica* from several localities, and the analytical results will be compared to illustrate any geographical variations in the vitamin A and carotenoid concentrations in this species.

During our study of *M. norvegica* (Fisher *et al.*, 1953) we noted certain discrepancies in vitamin A concentrations from different groups, and these we attributed to possible impurities in the preservatives used. We discovered, however, similar differences in the vitamin A content of various groups of *Euphausia pacifica* (see p. 83) where the quality of the preservative was not in doubt and performed therefore some experiments on *Meganyctiphanes*

*norvegica* and *Thysanoessa raschii* collected from Loch Fyne to elucidate the problem. The results are given in a separate section immediately following those of the systematic studies.

#### MATERIAL COLLECTED

In all, eleven species of euphausiids were collected, some by ourselves and others by marine biologists on various research cruises or whaling expeditions.

*Meganyctiphanes norvegica* (M. Sars) was the most widely obtained species. Apart from our own collections from Loch Fyne (Fisher *et al.*, 1952, 1954) and from Monaco (Fisher *et al.*, 1953), several groups of this species were collected for us by Dr J. H. Fraser during a cruise of the Scottish Home Department's Fisheries Research ship *Scotia* in August 1951. During his researches on whale-marking at the Norwegian whaling station at Steinshamn in July 1953, Mr Robert Clarke collected samples of this species from the stomach of a 55 ft. ♂ Fin Whale.

*Thysanoessa raschii* (M. Sars) is a fjord-dwelling species (Einarsson, 1945) and we have obtained specimens only from Loch Fyne.

*T. inermis* Kröyer lives in more open waters but, apart from the groups that showed an apparent relationship between vitamin A concentration and depth, taken near the Faeroes (Fisher *et al.*, 1952) we have analysed no other free-swimming specimens. A sample of this species was, however, taken from the throat of a 72 ft. ♂ Blue Whale by Mr Clarke at Steinshamn.

Several samples of the antarctic species, *Euphausia superba* Dana, have been analysed. Specimens were collected for us during the cruise of the R.R.S. *Discovery II* in 1950–51, others were taken by tow-net from the whaling factory ship *Balaena* during her 1951–52 expedition, by Mr S. Brown and Mr R. M. Brachi, and another group of specimens was collected during the 1952–53 expedition of this ship by Mr Brachi and Mr H. W. Symons. Some of this last group were collected from the engine-room inlets of the *Balaena* and others by net from a meat-carrying ship.

During a cruise of R.R.S. *Discovery II* in the North Atlantic in August and September 1952, Mr Foxton obtained for us specimens of *Stylocheiron elongatum* G. O. Sars and *Thysanopoda acutifrons* Holt & Tattersall.

While visiting the United States in 1953, one of us (S. K. K.) spent 2 weeks at the Scripps Institution of Oceanography at La Jolla, California, and took part in a 3-day cruise (25–27 May) of the research vessel *Horizon* in waters some 150 miles west from San Diego, during which five species of Pacific euphausiids were collected with a 1 m closing net at depths from 400 m to the surface. These were *Stylocheiron maximum* Hansen, *Euphausia pacifica* Hansen, *Nematoscelis difficilis* Hansen, *Thysanoessa gregaria* G. O. Sars and *T. spinifera* Holmes.

## METHODS OF PRESERVATION

The euphausiids collected from Loch Fyne and Monaco were preserved by boiling in sea water, as previously described (Fisher *et al.*, 1952, 1953), and by keeping the specimens in deep-freeze during the journey to this laboratory. The Monaco specimens were brought, frozen, by air and so were only a few hours out of deep-freeze. Eyes and bodies were separated at Shinfield and the parts were placed in absolute alcohol and stored in the deep-freeze.

The Pacific specimens were preserved by boiling and storage in the ship's refrigerator until the return to La Jolla. With the exception of two of the three groups of *Euphausia pacifica* collected, eyes and bodies were then separated in the laboratory and preserved in absolute alcohol. The two groups of *E. pacifica* were preserved whole in absolute alcohol. All this material was kept in cold storage until despatched to Shinfield. It was at atmospheric temperature for the 6 weeks during which it was in transit.

The specimens collected by Mr Clarke at Steinshamn were preserved in absolute alcohol, and those of Dr Fraser on the *Scotia* by boiling and refrigeration. Both groups were sent to us by post. On arrival here, the alcohol specimens were immediately deep-frozen, and the boiled specimens, which appeared to be in good condition after 2 days in postal transit from Aberdeen, were dissected into eyes and bodies for separate preservation in alcohol in the deep-freeze. Specimens were sorted into size-groups.

Groups of *E. superba* collected from the *Balaena* were preserved by boiling and storage in the ship's deep-freeze. Those brought back in May 1952 were sent to us from Liverpool, where the ship docked, by post. The specimens collected in 1953 were kept in deep-freeze on arrival at Liverpool and were transferred in an insulated vehicle to similar storage in London whence they were collected and brought back to Shinfield in an insulated container. Both consignments were separated into eyes and bodies and the 1953 specimens were also size-grouped by length in the way previously described (Fisher *et al.*, 1952, 1954).

Specimens of *E. superba* collected from the *Discovery II* were separated into eyes and bodies immediately after catching and the parts were preserved in absolute alcohol. Unfortunately these specimens were inadvertently stored in a cupboard where the temperature was rather high. The euphausiids collected during the north Atlantic cruise of the *Discovery II* were preserved by boiling and storage in the refrigerator until arrival at Plymouth. They were sent to us by post and arrived in good condition. After dissection into eyes and bodies, which were preserved separately in absolute alcohol, the specimens were stored in the deep-freeze.

In this study we have found that the eyes of *E. pacifica*, when analysed separately from the bodies, had a high concentration of vitamin A, but no vitamin A at all was present in the animals preserved whole. We noted

similar discrepancies in our work on *Meganyctiphanes norvegica* from the Mediterranean (Fisher *et al.*, 1953). To investigate further this loss of vitamin A from whole preserved animals, experiments were now done on *M. norvegica* and *Thysanoessa raschii* collected from Loch Fyne on 21–22 October 1953. The specimens were separated into groups of uniform size, 23–25 mm long for *Meganyctiphanes norvegica* and 11–13 mm long for *Thysanoessa raschii*. These groups were then divided into smaller lots, each of twenty-five specimens of *Meganyctiphanes norvegica* or of twenty specimens of *Thysanoessa raschii*. Of sixteen such lots for each species, eight were boiled and eight left raw. From each group of eight lots four were separated into eyes and bodies and four left whole. All the material was then preserved in absolute alcohol. Two lots of boiled whole animals and corresponding groups of bodies and eyes and one lot of raw whole animals and corresponding bodies and eyes were kept at room temperature and all the rest of the material was stored at  $-25^{\circ}\text{C}$  in the deep-freeze. Analyses were done at intervals on successive groups.

#### ANALYTICAL METHODS

The same method of analysis for carotenoids and vitamin A was used as reported previously (Fisher *et al.*, 1952). When the samples analysed were sufficient for the purpose, as with *Nematoscelis difficilis*, *Thysanoessa inermis*, *T. raschii*, *Meganyctiphanes norvegica*, *Euphausia pacifica* and some groups of *E. superba*, chromatography on alumina columns was done before saponification in order to separate vitamin A ester from vitamin A alcohol. With species of which we had only a few specimens, only one chromatography was done, after saponification, and vitamin A was estimated all in the alcohol form. The species treated in this way were *Stylocheiron elongatum*, *S. maximum*, *Thysanoessa gregaria*, *T. spinifera*, *Thysanopoda acutifrons*, and the remaining groups of *Euphausia superba*.

#### RESULTS

##### *Meganyctiphanes norvegica* (*M. Sars*)

*Specimens from Whales.* Two samples of krill, identified as *M. norvegica*, from the stomach of a 55 ft. 5 in. ♂ Fin Whale, were analysed. The results are given in Table I. The concentrations of fat and vitamin A were considerably higher than in previous specimens of this species from whales (Fisher *et al.*, 1952), although the astaxanthin concentrations were similar to those in earlier analyses. The krill had probably been more recently swallowed by the whale than on the previous occasions and less of the vitamin A had been leached out of it.

*Specimens from Different Localities.* As previously mentioned, we have now information about specimens of *M. norvegica* from several localities. Taking into account the effects of size and season (Fisher *et al.*, 1952, 1954), it is now possible to compare some of the groups. Some of the results shown in Tables

II-IV have been previously reported (Fisher *et al.*, 1953, 1954). In Table II, groups of specimens collected in the Sandy Bank area ( $61^{\circ} 54' N.$ ,  $05^{\circ} 45' W.$ ), near the Faeroes, are compared with groups of similar weights collected in Loch Fyne in November 1950. Fat, vitamin A and astaxanthin were all in similar concentrations in specimens from both areas, apart from two Loch Fyne groups with rather higher concentrations of astaxanthin. In Table III are compared groups of animals collected during August 1951, at a station in the North Atlantic, in the port of Monaco and in Loch Fyne. Whereas the

TABLE I. OIL PER CENT, VITAMIN A PER GRAM AND PER GRAM OIL AND ASTAXANTHIN PER GRAM AND PER GRAM OIL IN EUPHAUSIIDS FROM WHALES AT STEINSHAMN, NORWAY

Species	Weight (g)	Oil (%)	Vitamin A		Astaxanthin	
			i.u./g	i.u./g oil	$\mu g/g$	mg/g oil
Specimens from stomach of 55 ft. 5 in. ♂ Fin Whale, collected 17. vii. 53						
<i>Meganyctiphanes norvegica</i>	26	2.2	7.0	310	41	1.8
<i>M. norvegica</i>	22	2.4	9.8	405	62	2.6
Specimens from throat of 71 ft. 8 in. ♂ Blue Whale, collected 16. vii. 53						
<i>Thysanoessa inermis</i>	177	0.9	9.3	1121	32	3.9

No  $\beta$ -carotene was detected in either species.

concentrations of astaxanthin were nearly the same in each set of size-groups from the different localities, the vitamin A reserves of North Atlantic specimens were lower than of those from Loch Fyne or Monaco. The fat concentration in the Monaco specimens was much lower than in those from more northerly waters. Table IV gives the analytical results for groups collected from Loch Fyne and the port of Monaco in January and February 1952. In January the concentrations of vitamin A and astaxanthin were higher and the fat concentrations lower in Mediterranean than in Loch Fyne specimens. In February the astaxanthin concentrations were again higher in the Monaco specimens but the difference between the vitamin A reserves in specimens from the two localities was reduced. The fat concentrations of these two sets of groups were similar.

*Range of Vitamin A Concentrations.* We have now analysed 258 groups of *M. norvegica*, selected by length at 2 mm intervals, in the way previously described (Fisher *et al.*, 1954). The number of specimens per group varied between 1 and 359 and the vitamin A concentrations between 3.1 and 260 i.u./g. The histogram in Fig. 1 shows the number of groups at each concentration and the bulk of these lies between 10 and 20 i.u./g, the average for the whole series, weighted for the numbers of specimens in the groups, being 15 i.u./g. The very high concentrations, namely, those over 100 i.u./g, occurred in only a few groups, all of larvae (see Fisher *et al.*, 1954).

TABLE II. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN  
AND PER GRAM IN *MEGANYCTIPHANES NORVEGICA* FROM DIFFERENT  
LOCALITIES

Locality	Date	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
					i.u./spec.	i.u./g	μg/spec.	μg/g
Sandy Bank, Faeroe	6	91	80	3·9	1·3	16	5·1	65
Sandy Bank, Faeroe	6	225	100	4·1	1·5	16	5·4	55
Loch Fyne	21	93	85	3·2	1·3	15	4·8	56
Loch Fyne	21	92	62	4·6	1·0	17	5·8	93
Loch Fyne	21	238	66	4·0	1·2	18	4·5	68
Loch Fyne	21	174	55	5·1	1·1	20	5·1	93

TABLE III. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN  
AND PER GRAM IN *MEGANYCTIPHANES NORVEGICA* FROM DIFFERENT  
LOCALITIES

Locality	Date	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
					i.u./spec.	i.u./g	μg/spec.	μg/g
63° 18' N., 18° 39' W.	29	33	66	2·9	0·5	7·4	3·1	46
Loch Fyne	16	164	55	1·2	0·6	11	2·2	40
Loch Fyne	16	210	80	2·4	0·9	11	2·8	35
63° 18' N., 18° 39' W.	29	14	222	2·9	1·7	7·8	9·3	42
Monaco	24	224	224	0·5	6·5	29	14	62
Loch Fyne	16	5	265	5·2	6·5	25	12	46
63° 18' N., 18° 39' W.	29	16	297	3·7	3·3	11	22	74
Loch Fyne	16	11	326	6·1	5·2	16	15	47
63° 18' N., 18° 39' W.	29	29	360	4·7	3·7	10	17	47
Loch Fyne	16	6	338	4·8	6·0	18	17	50

TABLE IV. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN  
AND PER GRAM IN *MEGANYCTIPHANES NORVEGICA* FROM DIFFERENT  
LOCALITIES

Locality	Date	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
					i.u./spec.	i.u./g	μg/spec.	μg/g
Loch Fyne	16. i.	69	96	2·5	0·9	10	5·2	54
Monaco	29. i.	167	83	1·6	1·5	18	5·4	65
Monaco	29. i.	113	80	1·2	1·6	20	5·6	70
Monaco	29. i.	50	95	0·9	1·6	16	5·2	55
Loch Fyne	13. ii.	8	64	2·2	1·1	17	4·2	66
Loch Fyne	13. ii.	13	97	1·5	0·9	10	4·5	48
Loch Fyne	13. ii.	12	125	0·7	3·0	24	6·1	49
Loch Fyne	13. ii.	26	129	1·2	2·0	15	6·3	49
Monaco	12. ii.	150	79	1·5	1·5	20	5·9	74
Monaco	12. ii.	109	69	1·2	1·6	24	5·6	81
Monaco	12. ii.	50	94	2·0	1·7	18	5·8	62
Monaco	19. ii.	87	94	0·8	1·2	13	5·0	54
Monaco	19. ii.	63	101	1·0	1·2	12	5·7	56

*Thysanoessa raschii (M. Sars)*

We have so far analysed 190 groups of measured specimens of *T. raschii* and the range of vitamin A concentrations is shown in Fig. 2. The concentrations were much more widely distributed than in *Meganyctiphantes norvegica* although half the groups had concentrations between 10 and 45 i.u./g., with a weighted average for the whole series of 33 i.u./g. As in *M. norvegica*, very high concentrations were found only in groups of larvae (see Fisher *et al.*, 1954).

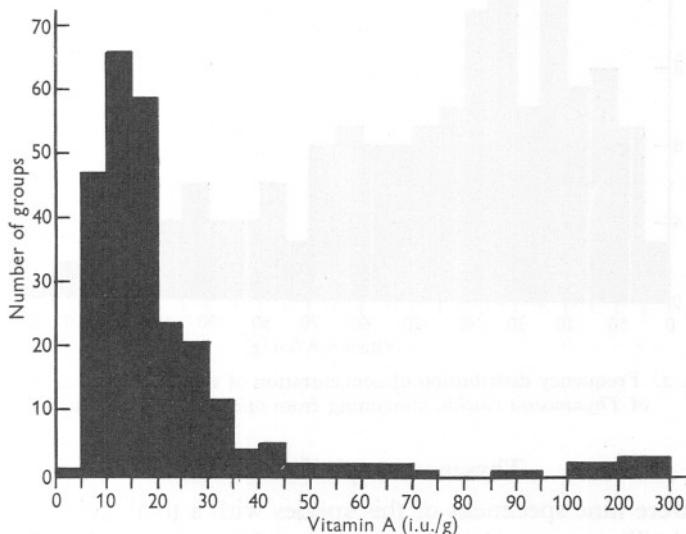


Fig. 1. Frequency distribution of concentration of vitamin A in 258 groups of *Meganyctiphantes norvegica*, containing from one to 359 specimens.

*Thysanoessa inermis Kröyer*

The results obtained from the analysis of specimens taken from the throat of a Blue Whale are given in Table I. The fat concentration was lower than in the samples of *Meganyctiphantes norvegica* collected from a Fin Whale at this station, and also lower than in free-swimming specimens of *Thysanoessa inermis* previously analysed by us (Fisher *et al.*, 1952). The concentrations of vitamin A and astaxanthin were of the same order as found in *Meganyctiphantes norvegica* from the whale and the vitamin A value was similar to that obtained from surface-swimming specimens of *Thysanoessa inermis* (Fisher *et al.*, 1952). The concentration of astaxanthin was only about half that of any of the free-swimming animals.

*Thysanoessa gregaria G. O. Sars*

A group of eight specimens, total weight 25 mg, separated into eyes and bodies, was analysed. The results appear in Table V. All the vitamin A was in the eyes, but there were only traces of carotenoid pigment present,

insufficient to be determined, though behaving chromatographically like astaxanthin.

In this small weight of material there was also too little fat for an accurate determination by our technique.

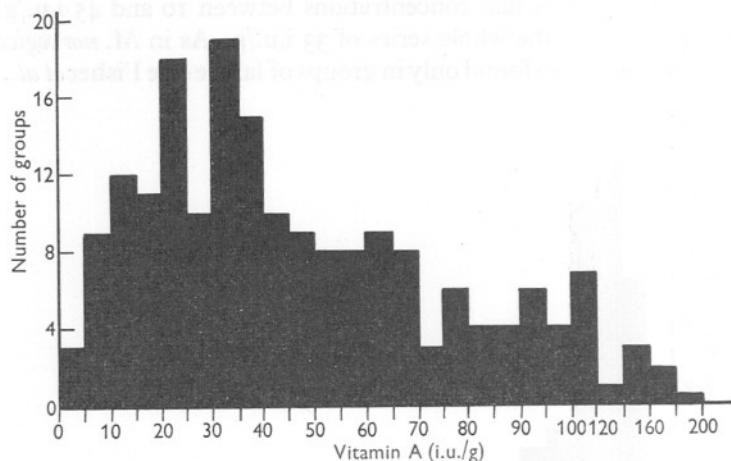


Fig. 2. Frequency distribution of concentration of vitamin A in 190 groups of *Thysanoessa raschii*, containing from one to 479 specimens.

#### *Thysanoessa spinifera Holmes*

There were nine specimens of this species with a total weight of 127 mg. Eyes and bodies were analysed separately and the results given in Table V show that vitamin A was exclusively in the eyes. The concentrations of fat and astaxanthin, which was the only carotenoid present, were of the order found in free-swimming animals of other species of *Thysanoessa*.

#### *Thysanopoda acutifrons Holt & Tattersall*

There were five specimens of this euphausiid in the group which weighed 160 mg, and bodies and eyes were analysed separately. The results in Table V show that vitamin A was not found in this species and that the concentrations of fat and astaxanthin were lower than we have found in other euphausiids.

#### *Stylocheiron elongatum G. O. Sars*

Four specimens of this species were analysed. Their total weight was 127 mg. Results of analyses appear in Table V. Vitamin A was present in a concentration similar to that in other euphausiids except *Thysanopoda acutifrons*. The concentration of fat was rather low compared with that of other species, but that of astaxanthin was much higher than usually found in euphausiids. No other carotenoids were observed.

*Stylocheiron maximum Hansen*

Only two animals of this species, with a total weight of 105 mg, were obtained. These were separated into eyes and bodies for analysis and the results are given in Table V. Vitamin A was again found only in the eyes and the concentration in the whole animals was very similar to that found in *S. elongatum*. Fat concentrations in the two species were also of the same order, but in *S. maximum* astaxanthin was found in lower concentration, resembling that in most other euphausiids.

TABLE V. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN  
AND PER GRAM IN SIX SPECIES OF EUPHAUSIID

Species	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
				i.u./spec.	i.u./g	μg/spec.	μg/g
<i>Thysanoessa gregaria</i> (P)							
Eyes (pairs)	8	—	—	0·15	—	0	0
Bodies	8	3	—	0	0	0	0
Total	8	3	—	0·15	47	0	0
<i>T. spinifera</i> (P)							
Eyes (pairs)	9	—	—	0·14	—	0·4	—
Bodies	9	14	8·7	0	0	0·5	36
Total	9	14	8·7	0·14	9·4	0·9	63
<i>Thysanopoda acutifrons</i> (A)							
Eyes (pairs)	5	0·3	—	0	0	0	0
Bodies	5	32	0·4	0	0	0·7	21
Total	5	32	0·4	0	0	0·7	21
<i>Stylocheiron elongatum</i> (A)							
Eyes (pairs)	4	2	2·3	0·4	184	2·4	1090
Bodies	4	30	0·8	0	0	1·2	41
Total	4	32	0·9	0·4	13	3·6	113
<i>S. maximum</i> (P)							
Eyes (pairs)	2	7	14	0·7	97	2·6	365
Bodies	2	46	8·4	0	0	0	0
Total	2	53	9·0	0·7	13	2·6	50
<i>Nematoscelis difficilis</i> (P)							
Eyes (pairs)	40	2	5·2	0·3	184	0·5	314
Bodies	40	35	3·3	0·1	3	0·3	10
Total	40	37	3·4	0·4	11	0·8	23

A, specimens collected in North Atlantic on 2 October 1952 from R.R.S. *Discovery II*; P, specimens collected in North Pacific on 25 May 1953 from R.S. *Horizon*.

*Nematoscelis difficilis Hansen*

A group of forty specimens of *N. difficilis*, weighing 1463 mg, was divided into eyes and bodies which were analysed separately as in previous species. The results, given in Table V, show that this species contained vitamin A in a concentration of the usual euphausiid order, but only 75% of it in the eyes, contrasting with 90–100% normally found in other species. The concentration of astaxanthin, or its esters, was lower than in most species examined.

*Euphausia superba Dana*

The results for the various groups examined are shown together in Table VI. The vitamin A concentrations were of the same order in all the groups and all lower than in other euphausiids containing it. Although the conditions of storage of the *Discovery* samples were regarded as unfavourable, the vitamin A concentrations closely resembled those in the corresponding size-group (25–26 mm) of the *Balaena* 1953 samples, which were stored at a much lower temperature. It is suspected that the *Balaena* 1952 samples may have been regurgitated by a whale and, therefore, possibly partially digested. This would account for the rather lower concentration of vitamin A in the eyes and also for the higher concentration of astaxanthin in the bodies than in the free-swimming specimens collected in 1953.

Vitamin A concentrations in the 1953 size-groups increase with size up to the group of animals 31–32 mm long, just as we have found (Fisher *et al.*, 1954) in northern euphausiids, but there is an unaccountable drop in the larger groups.

The concentrations of astaxanthin in all the groups are closely similar and about one-third of those we have found in *Meganyctiphanes norvegica*. The proportion of this carotenoid in the eyes of *Euphausia superba* is, however, as high as in *Meganyctiphanes norvegica* (Fisher *et al.*, 1954).

*Euphausia pacifica Hansen*

Three groups of this species were collected; one consisting of 100 specimens, total weight 4·99 g, separated into eyes and bodies, was taken on 25 May 1953; and two groups, of 400 and 1001 specimens, weighing 3·42 and 16·5 g respectively, were taken on 26 May 1953. These two groups were preserved whole, without dissection. Analytical results are given in Table VII. The dissected specimens contained vitamin A, all in the eyes, and the concentration in these animals was as found in most other euphausiids. The whole specimens were smaller but there was a larger bulk of material. Even so, no trace of vitamin A was detected in them. Fat concentrations were the same in the first two groups and lower in the third. Astaxanthin was the only carotenoid present in all three groups in varying concentrations, all rather lower than usually found in, for example, *Meganyctiphanes norvegica*.

*Study of Vitamin A Variations in Preserved Euphausiids*

It would appear from the results shown in Table VII that vitamin A which might have been present in the eyes of the specimens of *Euphausia pacifica* preserved without previous dissection was destroyed by some substance in the bodies released by preservation. In order to verify this supposition the experiments recorded in Tables VIII and IX were done.

TABLE VI. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN *EUPHAUSIA SUPERBA*

Part examined	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
				i.u./spec.	i.u./g	µg/spec.	µg/g
16. vii. 51. <i>Discovery II</i> station 2864							
Eyes (pairs)	197	1.4	12	0.6	437	2.3	1690
Bodies	197	195	2.3	0	0	2.1	11
Total	197	196	2.3	0.6	3.2	4.4	22
Early 1952. By net from <i>Balaena</i> . Antarctic							
Eyes (pairs)	148	0.7	5.0	0.02	34	0.2	307
Bodies*	—	—	1.4	—	1.3	—	33
Bodies + eyes	—	—	1.5	—	1.8	—	36
Early 1953. Engine room intake of <i>Balaena</i> . Antarctic							
Eyes (pairs)	148	3.3	8.4	0.9	263	2.5	753
Bodies	148	828	1.1	0	0	2.5	3.0
Total	148	831	1.2	0.9	1.4	5.0	6.0
Early 1953. By net from meat-carrying ship with <i>Balaena</i> . Antarctic							
Whole animals (< 22 mm long)	47	87	4.2	0.3	3.7	0.9	11
Eyes (pairs)	14	1.4	3.2	0.7	508	0.9	653
Bodies	14	124	4.4	0	0	1.1	9.2
Total (23–24 mm long)	14	125	4.1	0.7	5.5	2.0	16
Eyes (pairs)	22	1.4	6.0	0.7	500	2.1	1508
Bodies	22	197	2.9	0	0	0.8	4.2
Total (25–26 mm long)	22	198	2.9	0.7	3.4	2.9	15
Eyes (pairs)	II	1.7	5.8	1.1	653	2.5	1475
Bodies	II	252	3.3	0	0	2.1	8.2
Total (27–28 mm long)	II	254	3.3	1.1	4.4	4.6	18
Eyes (pairs)	22	2.2	1.3	1.3	617	2.8	1288
Bodies	22	264	4.0	0	0	1.4	5.4
Total (29–30 mm long)	22	266	4.0	1.3	5.0	4.2	16
Eyes (pairs)	17	2.9	3.9	2.5	859	4.1	1411
Bodies	17	367	4.2	0	0	2.3	6.3
Total (31–32 mm long)	17	370	4.2	2.5	6.7	6.4	17
Eyes (pairs)	II	2.0	6.8	1.2	591	3.6	1784
Bodies	II	505	2.7	0	0	1.9	3.8
Total (33–34 mm long)	II	507	2.7	1.2	2.3	5.5	11
Eyes (pairs)	23	5.2	6.0	2.0	381	3.5	677
Bodies	23	524	2.2	0	0	5.1	9.7
Total (35–36 mm long)	23	529	2.2	2.0	3.8	8.6	16
Eyes (pairs)	15	3.6	4.5	1.5	425	4.2	1293
Bodies	15	548	2.5	0.5	0.9	3.2	5.9
Total (37–38 mm long)	15	552	2.5	2.0	3.7	7.5	14
Eyes (pairs)	19	3.2	4.3	1.4	453	4.4	1379
Bodies	19	661	1.1	0	0	5.8	8.7
Total (39–40 mm long)	19	664	1.1	1.4	2.2	10	15
Eyes (pairs)	20	3.1	3.5	1.8	584	3.4	1093
Bodies	20	587	1.9	0	0	9.7	17
Total (> 40 mm long)	20	590	1.9	1.8	3.1	13	22

\* Somewhat macerated, hence uncountable.

In *Meganyctiphantes* (Table VIII), in all except two instances, the vitamin A content and concentration of the eyes and bodies separately was together greater than that of the corresponding group of whole animals. The two

TABLE VII. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN *EUPHAUSIA PACIFICA*

Specimens collected in North Pacific on 25–26 May 1953

Date	Part examined	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
					i.u./spec.	i.u./g	μg/spec.	μg/g
25. v.	Eyes (pairs)	100	1	13	0.5	515	0.7	683
	Bodies	100	49	1.9	0	0	0.4	7.7
	Total	100	50	2.2	0.5	11	1.1	22
26. v.	Whole specimens	400	9	2.2	0	0	0.3	35
26. v.	Whole specimens	1001	17	0.9	0	0	0.2	15

TABLE VIII. VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN GROUPS OF *MEGANYCTIPHANTES NORVEGICA* UNDER DIFFERENT CONDITIONS OF STORAGE

Groups of 25 specimens (length 23–25 mm) collected in Loch Fyne on 21–22 October 1953.

Date tested	Preserved	Dissected	Vitamin A		Astaxanthin	
			i.u./spec.	i.u./g	μg/spec.	μg/g
30. x.	AF	W	3.1	15	10	48
30. x.	AF	E+B	2.7	13	8.4	39
23. xi.	AR	W	0.9	4.7	8.2	45
23. xi.	AR	E+B	2.4	10	14	57
27. xi.	AF	W	1.4	6.2	11	48
27. xi.	AF	E+B	2.3	10	14	60
27. xi.	AF	W	2.6	11	13	52
27. xi.	AF	E+B	2.3	10	8.9	40
9. xi.	BR	W	1.7	6.8	10	42
9. xi.	BR	E+B	3.1	14	6.4	29
23. xi.	BR	W	2.0	9.1	6.8	30
23. xi.	BR	E+B	2.6	12	6.3	30
27. xi.	BF	W	1.6	4.5	13	54
27. xi.	BF	E+B	2.8	13	13	59
27. xi.	BF	W	1.6	6.6	14	57
27. xi.	BF	E+B	4.0	18	13	61

A, raw specimens preserved in alcohol; B, boiled specimens preserved in alcohol; R, room temperature; F, deep-freeze; W, whole specimens; E+B, eyes and bodies analysed separately and results combined.

exceptions were the alcohol-preserved group of raw specimens which had been placed at a low temperature immediately after dissection and preservation on the ship, and might, therefore, be regarded as a control group and one of the alcohol-preserved groups of raw specimens stored for nearly 5 weeks in the deep-freeze. The content and concentration of astaxanthin were not consistently altered by the various methods of preservation or by the separation of eyes from bodies.

Although differences were found between dissected and whole animals in the vitamin A content and concentration of *Thysanoessa* (Table IX), they were

not so marked as in *Meganyctiphantes*. The week-old alcohol-preserved raw specimens kept at a low temperature had a higher vitamin A content and concentration in whole than in dissected specimens. In the other groups the vitamin A concentrations were always higher in dissected than in whole animals. In all except one group the astaxanthin content and concentration were markedly higher in the dissected than in the whole specimens, in contrast to the results for *Meganyctiphantes* (Table VIII).

TABLE IX. VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN GROUPS OF *THYSANOESSA RASCHII* UNDER DIFFERENT CONDITIONS OF STORAGE

Groups of 20 specimens (length 11–13 mm) collected in Loch Fyne on 21–22 October 1953.

Date tested	Preserved	Dissected	Vitamin A		Astaxanthin	
			i.u./spec.	i.u./g	μg/spec.	μg/g
30. x.	AF	W	0.7	37	0.9	47
30. x.	AF	E+B	0.5	25	1.1	56
25. xi.	AR	W	0.5	27	0.6	33
25. xi.	AR	E+B	1.0	59	1.5	89
27. xi.	AF	W	1.4	68	0.7	33
27. xi.	AF	E+B	1.5	75	1.0	51
27. xi.	AF	W	0.6	28	1.1	51
27. xi.	AF	E+B	0.9	58	1.4	90
9. xi.	BR	W	1.1	61	1.1	64
9. xi.	BR	E+B	1.6	69	1.6	66
25. xi.	BR	W	0.9	38	0.7	33
25. xi.	BR	E+B	1.0	42	0.5	20
27. xi.	BF	W	0.6	28	0.9	40
27. xi.	BF	E+B	0.8	38	1.2	57
27. xi.	BF	W	0.4	15	1.0	38
27. xi.	BF	E+B	0.4	22	1.1	54

A, raw specimens preserved in alcohol; B, boiled specimens preserved in alcohol; R, room temperature; F, deep-freeze; W, whole specimens; E+B, eyes and bodies analysed separately and results combined.

#### DISCUSSION

Comparison of the concentrations of vitamin A, astaxanthin and oil in *M. norvegica* collected from three different localities in August 1951 (Table III) shows that the specimens taken from the open sea were poorer in vitamin A but not in astaxanthin than those from more enclosed waters. Fat concentrations were similar in the two more northerly groups but much lower in the Mediterranean animals. We have previously pointed out (Fisher *et al.*, 1953) that the low fat content might be associated with the relative poverty in plankton of the Mediterranean (Bernard, 1938), but if that were so it is surprising that the reserves of vitamin A and astaxanthin accumulated by the euphausiids were not similarly reduced. A further comparison of the Loch Fyne and Monaco specimens collected in February 1952 (Table IV) supports the earlier evidence of little difference between them in vitamin A concentration, although in January the Monaco samples were richer than the only

comparable Loch Fyne sample. Astaxanthin concentrations were again very similar in samples from both localities taken in both months. Fat concentrations in the January samples from Monaco were lower than in the Loch Fyne sample. The February samples from both places had similar fat concentrations. Samples of *M. norvegica* from Loch Fyne and The Faeroes area, two places relatively close together, differed little in their reserves of fat, vitamin A or astaxanthin (Table II).

According to Sheard (1953) some eighty-six species of the order Euphausiacea are recognized. In our studies of the vitamin A and carotenoid contents of animals of this order we have so far analysed eleven species, in nine of which vitamin A was found in high concentrations. The vitamin was absent from *Thysanopoda acutifrons*, but the amount of material analysed was small, although smaller weights of other species have been found to contain vitamin A. The eyes of *T. acutifrons* are, however, noticeably smaller than those of the other euphausiids we have examined and, since nearly all the vitamin A of euphausiids is found in the eyes, the two facts may be in some way associated, although Chun (1893) in his paper on euphausiid eyes makes no mention of any peculiarities in the genus *Thysanopoda*. Further investigation of the species with a larger amount of material is clearly indicated. The concentration of vitamin A in the samples of *Euphausia superba* we have examined was lower than in the other species of Euphausiacea containing it, but it is possible that some destruction of the vitamin A may have occurred during the passage of these specimens from the Antarctic through the tropics to this country. Even so, the concentration of vitamin A in this species is still higher than in any of the decapod Crustacea we have examined, the richest example of these being *Acanthephyra purpurea* Milne-Edwards. The average values for vitamin A concentrations of the euphausiids we have studied are compared in Table X, which shows clearly how much richer in vitamin A they all, with one exception, are than other Eucarida so far examined by us.

In view of their unique richness in vitamin A among the Crustacea it may be profitable to discuss the affinities of the Euphausiacea. In the two most recent accounts of the order (Banner, 1950; Sheard, 1953) it has been placed with the order Decapoda in the series Eucarida, division Eumalacostraca of the subclass Malacostraca. Vitamin A is present in most of the Decapoda we have studied (Kon, 1954), but in much lower concentrations (see Table X) than in the euphausiids. We have learnt from Dr I. Gordon of the British Museum that the taxonomic position of the order Euphausiacea is not yet definitely established. She informs us that her views correspond to those of the late Dr S. W. Kemp who believed, with Calman (1910), that they should be more closely associated with the Penaeidea, themselves a suborder of the Decapoda.

Comparative biochemistry has provided support for morphological evidence used in taxonomy in the work of Baldwin and his colleagues on muscle phos-

phagens in the animal kingdom (see Baldwin, 1937). It is possible that we may have in vitamin A, or the ability to accumulate it, a similar taxonomic indicator related to variations in the enzyme systems of the different groups of Crustacea. We have shown (Fisher *et al.*, 1953) that the Penaeidea have similar concentrations of vitamin A to those of other Decapoda, whereas the Euphausiacea are much richer, so that, on this evidence, the two groups are not so closely related as are the Penaeidea with the Decapoda.

TABLE X. DESCENDING ORDER OF AVERAGE VALUES FOR VITAMIN A CONCENTRATIONS IN EUPHAUSIACEA, AND IN DECAPOD RICHEST IN VITAMIN A

	Vitamin A (i.u./g)	Localities	No. of groups
<i>Thysanoessa gregaria</i>	47	P	1
<i>T. raschii</i>	33	A	190
<i>T. inermis</i>	16	A	3
<i>Meganyctiphantes norvegica</i>	15	A, M, N	258
<i>Stylocheiron elongatum</i>	13	A	1
<i>S. maximum</i>	13	P	1
<i>Euphausia pacifica</i>	11	P	1
<i>Nematoscelis difficilis</i>	11	P	1
<i>Thysanoessa spinifera</i>	9.4	P	1
<i>Euphausia superba</i>	3.1	An.	13
<i>Acanthephyra purpurea</i>	2.7	A	1

Average concentrations have been weighted for the numbers of specimens in each group. Values for free-swimming specimens only have been taken. A, Atlantic; An., Antarctic; M, Mediterranean; P, Pacific; N, Norwegian Sea. Vitamin A absent from *Thysanopoda acutifrons*.

The Euphausiacea live in similar habitats to the Mysidacea, with which they were for long classified as the Schizopoda. We have analysed four Mediterranean species of mysids (Fisher *et al.*, 1953) and *Hemimysis lamornae*, obtained from the aquarium tanks of the Plymouth laboratory, and they all lacked vitamin A. More recently we were supplied by Dr Dexter S. Haven of the Virginia Fisheries Laboratory with a large sample of *Neomysis americana* (S. I. Smith), collected from the York River, Virginia, on 9 February 1954, and found in them a vitamin A concentration of 1.9 i.u./g. Nevertheless, the evidence so far indicates that the euphausiids are biochemically very different from other Crustacea, including the Penaeidea, considered to be their nearest relatives, and the Mysidacea which are their nearest neighbours in the sea.

Einarsson (1945), Banner (1950) and Sheard (1953), who have all written at length on the Euphausiacea, are unanimous in stressing the importance of these animals in the economy of the seas. Sheard ascribes to them two general roles: 'the first being that of intercepting organic nutrient material' derived from the breakdown of phytoplankton, for example, the faecal pellets of grazing herbivores, 'and of returning it directly to the carnivore population'; 'the second (together with the pelagic larvae of bottom-living invertebrates) being that of delivering some products of the bottom cycle of organic

production to the pelagic (nutrient) cycle'. The second function would be achieved by the detrital feeders. Sheard further points out that 'this dual buffer and transference role of Euphausiacea', may explain 'the apparent excess of production of carnivores, when measured against primary nutrient values and phytoplankton production in subtropical and tropical seas'.

More detailed information about the role of euphausiids as food organisms for many whales and fishes has been provided by Einarsson (1945). He stated that the Blue Whale (*Balaenoptera sibbaldi*) lives exclusively on euphausiids, *Thysanoessa inermis* and *Meganyctiphanes norvegica* in arctic and *Euphausia superba* in antarctic seas. The Fin Whale (*Balaenoptera physalus*) also feeds largely on these species. We have already (Fisher *et al.*, 1952) drawn attention to the direct passage of the rich vitamin A supplies of these euphausiids to the stores of the whale.

Sperm Whales (*Physeter catodon*) feed on cuttlefishes and squids, in which vitamin A has been found in good concentrations in the liver (Brachi, 1953; Kon, 1954). These cephalopods live mainly on Crustacea and since ten-armed forms, important as whale food, are pelagic in habit they probably feed largely on free-swimming species, among which, by virtue of their enormous numbers and swarming habits, the euphausiids must rank as of considerable importance. Hjort & Ruud (1929) definitely stated that euphausiids form the food of the squid, *Gonatus fabricii*, which is eaten by the Bottle-nose Whales (*Hyperoodon rostratus*). It seems likely, therefore, that euphausiids are directly or indirectly the main source of vitamin A for all species of commercially valuable whales.

Copepods are the most important item in the diet of the herring (*Clupea harengus*), but they contain no more than traces of vitamin A (Fisher *et al.*, 1952). Recent work in this laboratory, so far unpublished, has shown that herring-liver oil may contain vitamin A in concentrations up to 60,000 i.u./g, and that the concentration is also high in the intestine. Einarsson (1945) in a review of the literature points out that, next to copepods, euphausiids are the food animals most favoured by the herring and, in some localities, they may form the bulk of the diet, as for example in the Skagerrak, where Poulsen (1926) reported that in May herring were feeding almost exclusively on *Meganyctiphanes norvegica*. This euphausiid is eaten by herring in the Clyde Sea area to the extent that the stomach becomes distended, the condition being described by fishermen, who reject these fish, as 'gut-poke'. Moore (1898) described *M. norvegica* as the most important food of the herring off the Atlantic coast of America.

A detailed study of the food of the cod (*Gadus callarias*) was made by Brown & Cheng (1946), who concluded that, for cod from the Bear Island and Spitzbergen Bank areas, pelagic Crustacea, especially *Thysanoessa inermis*, are the most important component from February to August, and young herring from November to December. Capelin (*Mallotus villosus*) is the principal food

of the cod off the Murman coast and sand-eels (*Ammodytes* spp.) off Andenes and Iceland. The capelin feeds on *Thysanoessa inermis*, according to Saemundsson (1937). Wiborg (1949) stated that the main food of cod of the O-II-groups from deep water along the coast of northern Norway consisted of euphausiids, especially *T. inermis* and *Meganyctiphanes norvegica*. Thus euphausiid vitamin A also seems to be the source of the rich reserves found in cod-liver oil.

Halibut-liver oil is one of the richest natural sources of vitamin A, and Lovern & Sharp (1933) studied the food of this fish in an attempt to find the source of its reserves. The torsk (*Brosme brosme*) was the commonest species eaten and torsk-liver oil proved richer in vitamin A than most cod-liver oils. Poulsen (1926) reported that torsk was one of the species to be found feeding on *Meganyctiphanes* in the Skagerrak. McIntyre (1952) more recently reported that halibut around Iceland and the Faeroes feed mainly on *Sebastes marinus*, together with other fish, especially herring, decapod Crustacea, especially *Nephrops norvegicus* and some cephalopods. Lovern, Edisbury & Morton (1933) drew attention to the wide variations between the potencies of individual samples of halibut-liver oil and these variations may be associated directly with the type of food eaten, some fish feeding consistently on food richer in vitamin A than that eaten by others. Euphausiids may, therefore, be the initial source of the vitamin A in halibut liver.

Saemundsson (1926) and Macdonald (1927) reported that the saithe (*Gadus virens*), which is the basis of important fisheries, also feeds on euphausiids. Lovern *et al.* (1933) mentioned that saithe-liver oil is of similar vitamin A potency to that of the cod and hake. The hake itself (*Merluccius vulgaris*) was reported by Hickling (1927) to feed selectively on *Meganyctiphanes*, and this euphausiid has been observed by us in the stomachs of hake caught in Loch Fyne.

An example of a number of fish listed by Lovern *et al.* (1933) as being poorer in vitamin A than the cod is the haddock (*Gadus aeglefinus*). This species has been recorded as feeding occasionally on euphausiids in Icelandic waters (Saemundsson, 1937), around Bear Island (Robertson, 1932) and in the Barents Sea (Zatsepin, 1939). In the Skagerrak, Poulsen (1926) found haddock feeding in May almost exclusively on *Meganyctiphanes norvegica*. Einarsson (1945) considers, however, that euphausiids are not of major importance as food for this fish and they were not found in any of the stomachs of haddock, from Icelandic waters or the Murman coast, examined by Brown & Cheng (1946). Einarsson (1945) names several other economically important species of fish which feed on euphausiids, and the foregoing review emphasizes the importance of this order of Crustacea not only because of the two more general roles mentioned by Sheard (1953) but also in the more specific part they play as a source of the vitamin A stores of marine vertebrates. We have previously (Fisher *et al.*, 1954) discussed, and are still investigating, possible sources of

euphausiid vitamin A, and it may be that the animals of this order possess an enzyme equipment that permits them to break down carotenoids other than those normally regarded as vitamin A precursors and in such a way that vitamin A appears as an end-product in very large amounts which are accumulated in the eyes. These reserves are passed on directly from the euphausiids to their cephalopod and vertebrate predators which store the vitamin in their livers, accumulating it throughout life (Macpherson, 1933), only a small proportion being utilized.

It certainly seems that the euphausiids form a unique reservoir in the sea of vitamin A which they accumulate from an unusual source (see Fisher *et al.*, 1954). The vitamin itself exhibits certain peculiar properties, both in its discrepant physicochemical and biological potencies (Fisher *et al.*, 1952) and in its apparent destruction under certain conditions of storage now reported. We are making an intensive biochemical study of these phenomena and also seeking material to extend our observations on the one hand to other species of Euphausiacea to determine whether high concentrations of vitamin A are a universal feature of the order, and on the other to other Crustacea to search for a species as rich in vitamin A as any of the Euphausiacea.

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#### SUMMARY

Eleven species of Euphausiacea, namely, *Meganyciphanes norvegica*, *Thysanoessa raschii*, *T. inermis*, *T. gregaria*, *T. spinifera*, *Thysanopoda acutifrons*, *Euphausia superba*, *E. pacifica*, *Nematoscelis difficilis*, *Stylocheiron maximum* and *S. elongatum* have been analysed for fat, vitamin A and carotenoids. Vitamin A was present in all species except *Thysanopoda acutifrons*, but in *Euphausia superba* the concentrations were of a lower order although still higher than found by us so far in any decapod crustacean.

Astaxanthin, or its esters, was the only carotenoid found in all the species of euphausiids examined.

Groups of *Meganyctiphanes norvegica* from enclosed waters, namely, Loch Fyne and the Mediterranean Sea, had higher reserves of vitamin A than those from Atlantic waters. The fat concentrations of the Mediterranean specimens were lower than those of animals from more northerly waters but the vitamin A or astaxanthin concentrations of the two groups were similar.

In *Euphausia pacifica*, *Meganyctiphanes norvegica* and *Thysanoessa raschii*, the vitamin A content of animals separated into eyes and bodies before preservation and analysis was higher than that of animals left whole. The possible presence in the bodies of a substance causing destruction of vitamin A is being investigated.

The role of euphausiids is discussed as a vital link in the passage of nutrient substances from detritus, phytoplankton and zooplankton to marine vertebrates, with particular reference to the possible conversion by these Crustacea of carotenoids to vitamin A which they accumulate in their eyes.

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## THE OCCURRENCE AT PLYMOUTH OF THE OPISTHOBRANCH *AKERA BULLATA*, WITH NOTES ON ITS HABITS AND RELATIONSHIPS

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(Plate I and Text-figs. 1 and 2)

### INTRODUCTION AND ECOLOGY

*Akera bullata* O. F. Müller is a tectibranch mollusc having a coiled external shell and a foot with lateral extensions used for swimming. At certain times of the year it swims actively, as was observed by Donovan, who writes that it 'sports in its watery element with all the liveliness of a butterfly, and formed a pleasing object when kept alive in a glass of sea water' (quoted by Montagu, 1803). Although sometimes abundant locally, it is not a mollusc often taken by the collector, even though it is a conspicuous object when swimming.

The first record of this species from Plymouth was the discovery of large numbers in a sea-water tank in the Naval Dockyard at Devonport, in April 1954. It has been recorded from a number of places in the British Isles, and ranges from Norway to the Mediterranean (Forbes & Hanley, 1853).

Two variants of the original species have been described: var. *nana* Jeffreys is a small form which was dredged in the Shetlands, and var. *farrani* Norman, a giant form from a locality in Ireland (Norman, 1890, p. 68). Since these so-called varieties apparently differ only in size, they would seem to be of doubtful validity. The specimens taken at Devonport were the normal form described by Müller, and listed as *A. bullata bullata* Müller by Winckworth (1932).

The specimens were found in an oval concrete tank, some 33·5 m long, 23 m broad, and 2·5 m deep. Water is continually drawn from the tank for use in the Dockyard, the level dropping perhaps 20–30 cm during the day. The tank is topped up daily with water drawn from the lower part of the Tamar estuary, opposite St John's Lake. The tank is some distance above sea level, and about a quarter of a mile from the river.

The sea-water intake is about 2 miles from the mouth of the Tamar, and at this point the surface salinity varies around 30‰ (Milne, 1938, p. 538). A single sample from the tank on 14 April 1954 had a salinity of 32·95‰

(density 1.02470 at 13.6° C), the temperature being 11.6° C. Salinity variations must be less sudden than in the estuary, since only a small proportion of the total volume is renewed daily.

The vertical sides of the tank were covered with a thick growth of green algae, mainly *Ulva*, and in the summer of 1954 there was also much filamentous green alga, both at the bottom and floating on the surface. There was a thin layer of mud on the bottom, but the water was usually quite clear, except just after pumping.

Apart from *Akera* the fauna was sparse, a few gobies, mysids, amphipods, and ascidians being taken. There were also two or three medium-sized fish, possibly pollack (*Gadus pollachius* L.). Apart from the abundance of algal food, *Akera* may have been favoured by the absence of predators. Although apparently distasteful to some fish, it has been found in the stomach of the flounder (Jeffreys, 1867, p. 432).

The outflow of water from the tank is evidently not so great as to sweep away considerable numbers of *Akera* larvae, as young specimens spawned in the tank were abundant in the summer of 1954.

The swimming habit of this animal has often been noted, and a detailed description of swimming movements is given below. On the first visit, on 14 April 1954, both adults and young were swimming in fair numbers, although there were always many more crawling on the sides and bottom of the tank. Since most individuals would swim after capture, it is fairly safe to say that at that time all, or nearly all, those in the tank were swimming intermittently. In the laboratory, adults would sometimes swim for periods of half an hour or more, and the young seemed capable of swimming for even longer periods. Swimming may be initiated by disturbing the animals, but individuals also swim spontaneously from time to time.

Swimming mainly takes place in the spring, which is the peak spawning period (cp. Guiart, 1901, p. 47). In April 1954 adults were copulating and spawning in the laboratory, and much spawn was found in the Devonport tank. *Akera* appears to have an extended breeding period, as a little spawn was found as late as 13 July, and small specimens, presumably spawned earlier in the year, were common in April. Thorson (1946) records that in Denmark egg-masses are found throughout the summer, indicating a rather later spawning season than at Plymouth.

On 29 April fewer adults and young were swimming than on the 14th. On the next visit, on 17 June, many young were crawling over the algae, but adults were very rare, and no *Akera* were seen swimming in spite of good visibility in the tank. Some of the young taken on this date did swim in a plunger jar in the laboratory, however, a few days later. On 13 July there were still none swimming, and young stages again predominated.

Observations are being continued, but the evidence so far collected indicates that the species is annual, growing to maturity and spawning after a

year, after which the majority die. Swimming, both of adults and young, is more or less confined to the spring, and may perhaps be correlated with the breeding season. *Akera* may occasionally swim in the summer.

*Akera* normally lives on mud flats, among weeds or *Zostera*, but none have been recorded from Plymouth, nor in the Yealm and Salcombe estuaries (Marine Biological Association, 1931), where conditions would appear to be suitable. It is not recorded from Torbay (Jukes-Browne, 1910) or the Exe estuary (Allen & Todd, 1902, and observations by N. A. H. during 1946-47). The nearest records are from Falmouth and Helford, 40 miles to the west (Clark, 1906, and personal communication from Mr R. Baird, who has found it in the Fal in recent years). It has been taken 70 miles to the eastward in the Fleet, Dorset, by Mr G. M. Spooner, and at Roscoff, 100 miles to the south, across the English Channel (Station Biologique de Roscoff, 1951). One or more specimens were evidently introduced to the tank via the inflow pipe, but there is no evidence as to their origin.

#### EXTERNAL FORM AND SWIMMING

A description of the external features of *Akera bullata* has already been provided by Guiart (1901), showing the division of the body into two regions—a smaller, dorso-ventrally flattened head, connected by a narrow 'neck' to a larger, ovoid visceral mass. The spirally coiled shell is yellowish brown in colour and encloses the visceral mass and the mantle. The features of the shell are well described by Jeffreys (1867) and by Forbes & Hanley (1853). In the sedentary posture, the neck and most of the visceral mass of the animal are concealed by the upgrowth of a pair of large, thin flaps, the parapodia, or 'wings' which are expansions of the ventral surface upon which *Akera* creeps. At their widest parts the wings overlap upon the dorsal surface, surrounding most of the body, so that the animal, when crawling fully extended, has a narrowly conical appearance, broadening behind, where the visceral hump and shell protrude from the parapodia. A long white filament, forming a grooved tentacle produced from the pallial margin, trails behind. In the crawling animal the right parapodium seems invariably to lie above the left, but when the parapodia come to rest between successive swimming movements, this arrangement is reversed, and it is the edge of the left parapodium which lies uppermost. *A. bullata* shows on the parapodia and ventral surface a ground colour of ashen brown or grey, closely spotted and blotched, as in an *Aplysia*, with greyish white. Inside the parapodia, and on the narrow 'neck' the colour is lighter and the head is streaked longitudinally with a series of broken lines of dark brown or black.

In crawling—which is its most frequent movement—*Akera* proceeds on the flattened ventral surface which is continuous with the parapodia. This surface is in no way marked off as a distinct sole, and is pigmented in the same way as the parapodia themselves. It is richly supplied with mucous glands and the

animal appears often to secrete about itself a tube of slime, from which it may then emerge by crawling out in front. At times the head and the visceral mass may be almost completely retracted within the cloak formed by the parapodia, and the animal sits motionless, taking on a compact flask-shape.

The swimming movements—when first seen—come as a surprise (see Text-fig. 1). They begin by the opening out of the parapodia, so as partly to display the body between them. The margins are then lifted up like a skirt and stand out freely from the body, while the anterior end of the animal rises first from the ground, so that the animal achieves an upright position, and sits making one or two tentative openings of the parapodia. The visceral mass can now be clearly seen below, like the clapper of a bell, as the parapodia are finally widely extended and the skirt is lifted high. As the edges of wings disengage the dorsal surface is also uncovered, and the skirt extends at right angles to the body, in the same plane as the creeping surface of the foot. The creeping surface is now presented uppermost, and forms a more rigid tract, terminating behind in a permanent point, while round it the flaps of the parapodia form a heart-shaped shield, with the edges slightly upcurved.

The skirt is now rapidly lowered, and its contraction against the water beneath it provides the motive power by which the animal leaves the ground and is lifted through the water. As the edges of the parapodia again come together, they enwrap the dorsal surface, the left one this time uppermost. Almost at once they are opened again and spread flat, to contract downwards in another effector stroke which carries the animal further upwards. After each swimming stroke, the animal loses way by sinking a short distance from the weight of the visceral hump, before the next movement of the wings carries it upwards in a renewed spurt. An average of forty-five strokes a minute was reckoned for one specimen at normal sea temperature. The movements have an extraordinary lightness and grace, and an impression of the swimming animal may be gained from the photographs (Pl. I) made by Dr D. P. Wilson in the Plymouth laboratory.

#### DIGESTIVE SYSTEM

Since the descriptions of the alimentary canal given by Bergh (1900) and Guiart (1901) are short and often inaccurate, an opportunity was taken to re-examine the gut, using living material and paying closer attention to functional aspects. In almost all its features the alimentary canal of *Akera* is entirely aplysioid, and the same is true of the mode of life. *Akera* is a grazer on green algae such as *Ulva*, or a deposit feeder on material already comminuted. The buccal mass does not need separate description from that of *Aplysia*, but a figure of the radula is provided here (Text-fig. 2C). Each tooth row has a series of teeth typical of the grazing and raking form of radula seen in Aplysiomorpha. The radula resembles much less that of the Bullomorpha, whether the herbivorous forms or the much more modified carnivores. Into

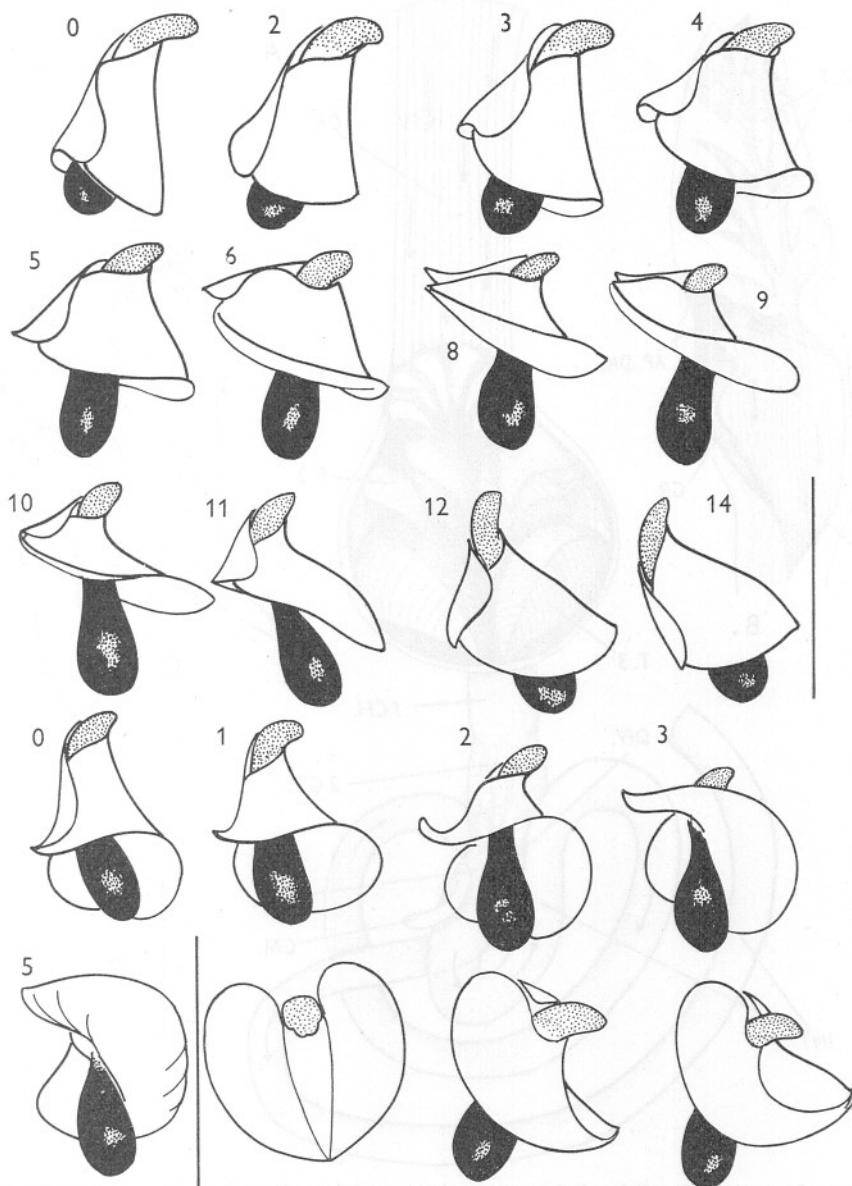


Fig. 1. Outline diagrams to show the positions of the parapodia at successive stages during the swimming movements. The drawings are constructed from a cine-film record and those of the first three rows comprise a continuous sequence, with the time intervals in sixteenths of a second shown. Those of the fourth row and the first of the fifth row form another series, and the three final drawings are selected to show further views of typical swimming postures.

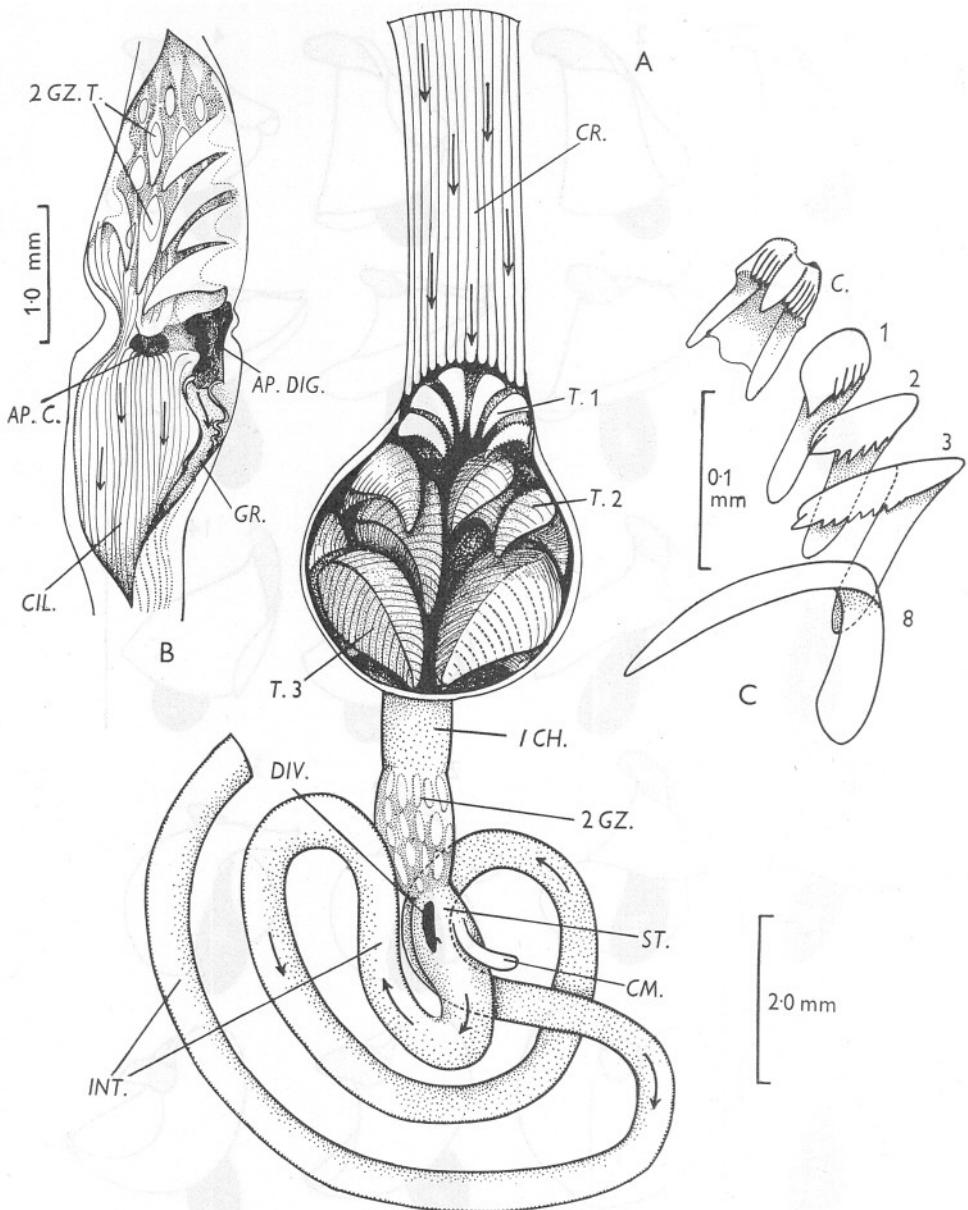


Fig. 2. A, general view of the alimentary canal removed from the animal with the buccal mass omitted, and the crop and first gizzard opened to illustrate their internal structure. B, more detailed view of the second gizzard and the stomach, dissected to show internal structure. C, central (C) and four of the marginal teeth (nos. 1, 2, 3 and 8) of the most posterior tooth row of the radula. AP. C., aperture of the gastric caecum; AP. DIG., opening of the digestive diverticulum into the stomach; CIL., ciliated folds leading from the stomach into the intestine; CM., gastric caecum; CR., crop; DIV., position of digestive diverticulum; GR., groove leading into the intestine from the digestive diverticulum; 2 GZ., second gizzard; 2 GZ. T., teeth of the second gizzard; I CH., intermediate chamber between the first and second gizzards; INT., intestine; ST., stomach; T. 1, T. 2; T. 3, teeth of the first, second and third tiers in the first gizzard.

the buccal mass open a pair of long, strap-like salivary glands, with short tubular ducts. They are orange yellow in the living animal, and their histology resembles that of *Aplysia*, as described by Howells (1942).

The next regions of the gut are contained in the narrow, neck-like part of the body, and comprise a long, very distensible crop, and a large first gizzard, both of which are oesophageal. The crop is thin-walled and is thrown into a large number of fine longitudinal ridges. It is translucent white in colour, and the contained food—fine detritus and roughly chopped-up filaments of algae—shows through its wall. The shape of the crop varies greatly, according to the position of food passing through it. It is capable of very strong peristaltic movements, so that temporary constrictions and bulges are likely to appear along its course. At times it appears like a fat sac, at others it is a narrow tube of uniform width. As well as being muscular and peristaltic, the crop is strongly ciliated, and—apart from the intestine—it is the only part of the gut where cilia appear to play an important role. All beat strongly in the same direction, along the ridges and furrows towards the first gizzard.

The first gizzard is pyriform in shape, and extremely firm-walled (see Text-fig. 2A). At its narrow anterior end the ciliated folds of the crop end abruptly, and a food string containing coarse pieces of algae and debris passes into the mill formed by the series of gizzard teeth. These teeth are arranged in three tiers, the largest ones comprising a set of four at the posterior end of the gizzard. These four are massive and broad-based, each consisting of a thick shell of cuticle applied to a strong boss of the epithelium. They are roughly pyramidal in shape, but slightly beaked so that their blunt-pointed tips, which meet at the centre of the gizzard are directly backwards towards the exit. The next tier of teeth are also four in number, slightly smaller and alternating with the posterior teeth. Their tips are sharper and much more strongly beaked, directed backwards. The teeth of the most anterior tier, immediately inside the entrance to the gizzard comprise a circlet of six to eight. They are small and slender, and form a strainer through which the food passes before being engaged and crushed between the tips of the larger teeth.

The food leaving the gizzard forms a soft, greyish green cord, much finer in consistency than when it was received from the crop. Behind the gizzard lies a smaller region of the gut, narrow and not toothed, forming an intermediate chamber by which the first gizzard communicates with the second gizzard. This intermediate chamber has a longitudinally ridged wall, and is contractile, though not strongly muscular. So far as could be made out from living material, it is not ciliated. Behind it the gut opens through a 'diaphragm' of muscle and connective tissue, by which the organs lying within the head and 'neck' are separated from those of the visceral mass. Behind the diaphragm the second gizzard opens by a slight constriction from the intermediate chamber. The second gizzard is a great deal smaller than the first, and its walls are less muscular, though still strongly contractile. The teeth are here

much smaller, the larger ones long and narrow and pointed backwards. They are hardly at all rigid, being for the most part soft and flexible to the touch. They fill most of the gizzard and form a series of papillae, serving not for the attrition of food but for filtering the stream of particles that is admitted to the stomach. These teeth are arranged in seven or eight longitudinal rows and their ovoid bases are very clear externally, through the thin wall of the gizzard. One specimen had twenty-five, most of them small and short.

Text-fig. 2B illustrates the internal structure of the second gizzard, and its relation to the stomach, which properly speaking forms that part of the gut lying immediately behind the gizzard. It receives a single digestive diverticulum, and is equipped with a short, finger-shaped caecum. It is marked off in front by a constriction from the second gizzard, and merges gradually behind into the intestine. The walls of the stomach are ciliated and thrown into fine longitudinal folds, which continue backwards from the tooth-bearing folds of the second gizzard and run on into the intestine. Some of these folds lead out from the mouth of the caecum, which is ciliated internally and in most ways resembles that of *Aplysia*. The function of the caecum appears to be to fashion segments of the faecal string which pass from its opening directly into the intestine where they merge into a continuous rope. The opening of the caecum is connected by a shallow channel with the mouth of the digestive diverticulum, which lies a little to the side of it, and immediately behind the largest (posterior) tooth of the second gizzard. Leading backwards through the stomach from the diverticulum is a broad gutter, lined with strongly beating cilia, which enters the intestine and there loses its identity. Of the sorting area, gastric shield, and style sac, represented in bullomorphs (see Fretter, 1939; Fretter & Graham, 1954) nothing remains in *Akera*.

The intestine describes a double loop, turning backwards after leaving the stomach, and running through the digestive gland to come to the surface on the right side. It then curves back again, running backwards above the stomach, around the visceral mass to the left. It finally runs forward and towards the mid-line as the rectum. The intestine is smooth-walled and finely ciliated, and carries a rope of fine, well-communited faeces. No peristaltic contractions could be observed, and the faecal string shows little sign of compression, or breaking into pellets. It issues free of the mantle cavity, breaking away from the anus at the edge of the mantle in large, irregular, coils.

#### RELATIONSHIPS

In the classification currently in use—that of Thiele (1931) which has been adopted by Hoffmann (1938)—*Akera* is placed within the suborder Cephalaspidea (Bullomorpha) of the order Pleurocoela (Tectibranchia), in the Akeratidae.<sup>1</sup> Two subfamilies are recognized by Thiele, namely the Aker-

<sup>1</sup> The family name is correctly formed as Akeratidae, as in Pelseneer (1906), not Akeridae, as cited by Thiele.

tinae, containing the single genus *Akera*, and the Cylindrobullinae. Pelseneer (1906) had previously placed the Akeratidae within the Bullomorpha, thereby reversing the decision of Guiart (1901) who had put forward a number of reasons for considering these molluscs as more properly aplysiromorphs. From a further study of the structure of *Akera bullata*, it becomes clear that Guiart was right, and that *Akera* has many more characters in common with the Anaspidae (Aplysiromorpha) than with the Cephalaspidea (Bullomorpha). Of the features regarded by Pelseneer and later writers as diagnostic of the Bullomorpha, the following are the chief:

- (i) The shell and pallial cavity are well developed and the gill is wholly or partly contained within the pallial cavity.
- (ii) The head is devoid of apparent tentacles and is modified to form a disk-shaped cephalic shield, used for digging into the sand.
- (iii) The parapodia are continuous with the ventral sole of the foot.
- (iv) The gizzard is either lacking, or single, with relatively few, sometimes calcareous plates.
- (v) The visceral commissure is usually long.

In (ii) and in (iv) *Akera* is obviously unlike the bullomorphs. Although the eyes are reduced, and obvious tentacles are lost, the head remains un-specialized and with none of the modifications associated with the habit of burrowing beneath the soil. In the structure of the gut—and this is a region that has taken on distinctive specializations in all aplysiromorphs—*Akera* has already been shown to have all aplysioid features. There is a large first gizzard, a smaller second gizzard, both with numerous teeth, and a small gastric caecum. Typical bullomorphs have none of these (see Fretter, 1939). The genitalia of *Akera* have not been investigated during this work, since they have already been dealt with in a comprehensive study by the late Dr Hilda Lloyd, which Dr Vera Fretter is preparing for publication. Dr Fretter has kindly allowed us to state that the reproductive system of *Akera* conforms in almost all respects to that of the aplysioids and has no distinctively bullomorph features. The mode of copulation with the formation of a chain of six or more individuals and the nature of the gelatinous egg-strings are also aplysioid features. The shell of the free-swimming veliger (see Thorson, 1946) has the initial sinistral twist detectable in Bullomorpha but almost suppressed in the other aplysiromorphs.

In addition, the mantle cavity in *Akera* possesses the two typically aplysioid glands, an opaline gland occupying its floor, and a purple gland located in the roof, and secreting, as in *Aplysia*, a purple-coloured fluid. Finally, as completing the list of aplysioid resemblances, Dr G. Y. Kennedy and Dr H. G. Vevers, in a forthcoming paper, will report similarities between *Aplysia* and *Akera* in the possession of the pigment Uroporphyrin I in the integument.

Pelseneer's criterion of the separation of the lateral parapodial lobes from the ventral sole would seem a much less important character. In any event,

this distinction is largely a matter of the particular height on the lateral surface at which the parapodia are given off. While creeping members of both suborders (like *Philine* and *Aplysia*) are equipped with a distinct sole, this character is surely an adaptive one, and it is likewise not surprising to find swimming members of both the Bullomorpha (*Gastropteron*) and the Aplysiomorpha (*Akera*) which have a very similar reduction of the creeping surface and enlargement of the parapodia. The parallel between *Gastropteron* and *Akera* is an instructive one, showing as it does the proneness of both these groups of tectibranchs to produce swimming members. Each group has further given rise to a section of the permanently swimming Pteropoda, the Bullomorpha producing the Thecosomata, and the Aplysiomorpha probably the Gymnosomata.

Two 'bullomorph' characters are generally relied on by those who would include *Akera* in the Cephalaspidea, namely the well-developed shell containing the mantle cavity with its pallial caecum, and the nervous system with its long visceral commissure still retaining the primitive torsion. Such features are each primitive ones: their possession implies not so much a close resemblance to the bullomorphs as a primitive position near the point where the aplysiromorphs sprang from basal tectibranchs. The pallial caecum is an elaborate structure following—independently of the visceral mass—a spiral course around the upper whorls of the shell. The earliest description was by Perrier & Fischer (1911), and Fretter & Graham (1954) have recently given a detailed account of its structure and functioning in the primitive bullomorph, *Actaeon tornatilis*. This caecum was previously regarded as a bullomorph specialization, and its presence in an aplysioid is admittedly not easy to explain. Yet many difficulties disappear if we regard it not as a peculiarly bullomorph character, but as an adaptation likely to be found generally in those tectibranchs with a large mantle cavity, where a sedimented or silty habitat raises problems of pallial sanitation.

Guiart (1901) gave a good description and figure of the nervous system of *Akera bullata*, and it is a pity that Pelseneer's later work (1906) entirely omitted mention of it. In reporting its streptoneurous condition, Guiart compares the nervous system of *Akera* with that of *Actaeon*. Undoubtedly *Akera* is much more primitive in the nervous system than any other aplysioid. It stands at the base of that group with much the same relationship to later Aplysiomorpha as *Actaeon* bears to later bullomorphs. Guiart finds in *Akera* certain characters of the nervous system in which this form already foreshadows the later aplysiromorphs. 'Il nous suffit de considérer un instant le système nerveux de l'*Acera* pour constater qu'il présente avec le système nerveux de Bulléens un certain nombre de modifications qui vont aller en s'accentuant chez les autres Aplysiens.'

The remainder of the family Akeratidae as recognized by Thiele cannot—it would seem—be placed with *Akera* in the Aplysiomorpha. Mr T. J. Evans

(personal communication) informs one of us that *Cylindrobulla* and *Volvatella* are in fact saccoglossans. The different fates of the two subfamilies of Akeratidae is a good example of how—with better knowledge—the basal parts of more advanced groups may be found to encroach into the primitive group of Bullomorpha.

We are indebted to Mr M. Little of Plymouth for bringing our attention to the presence of *Akera* in the Devonport reservoir. A film of the swimming of *Akera* was taken by Mr F. A. J. Armstrong, upon which the diagrams illustrating the swimming movements (Text-fig. 1) have been based. Dr D. P. Wilson kindly supplied the photographs reproduced in Pl. I.

#### SUMMARY

The tectibranch mollusc *Akera bullata* is recorded for the first time in the Plymouth area, where it was taken in April 1954 in a sea-water tank at Devonport. Large numbers of specimens of all sizes were present, and copulation and egg-laying were in progress. A summary is given of the records of previous occurrences in S.W. England, with ecological notes on the behaviour of the animal at Plymouth. The characteristic swimming movements of *Akera* occur mainly in the spring, and may be correlated with the breeding season. An account of the external form and structure is given and the mode of swimming is described in detail. The digestive system was investigated from Plymouth material, and the structure and mode of action of the alimentary canal described and shown to be typically aplysioid. The remaining characters of the animal, such as the structure of the reproductive organs, the pallial glands and the nervous system, also show aplysioid resemblances, and it is concluded that the family Akeratidae (in part) must be removed from the Bullomorpha and located more correctly in the Aplysiomorpha.

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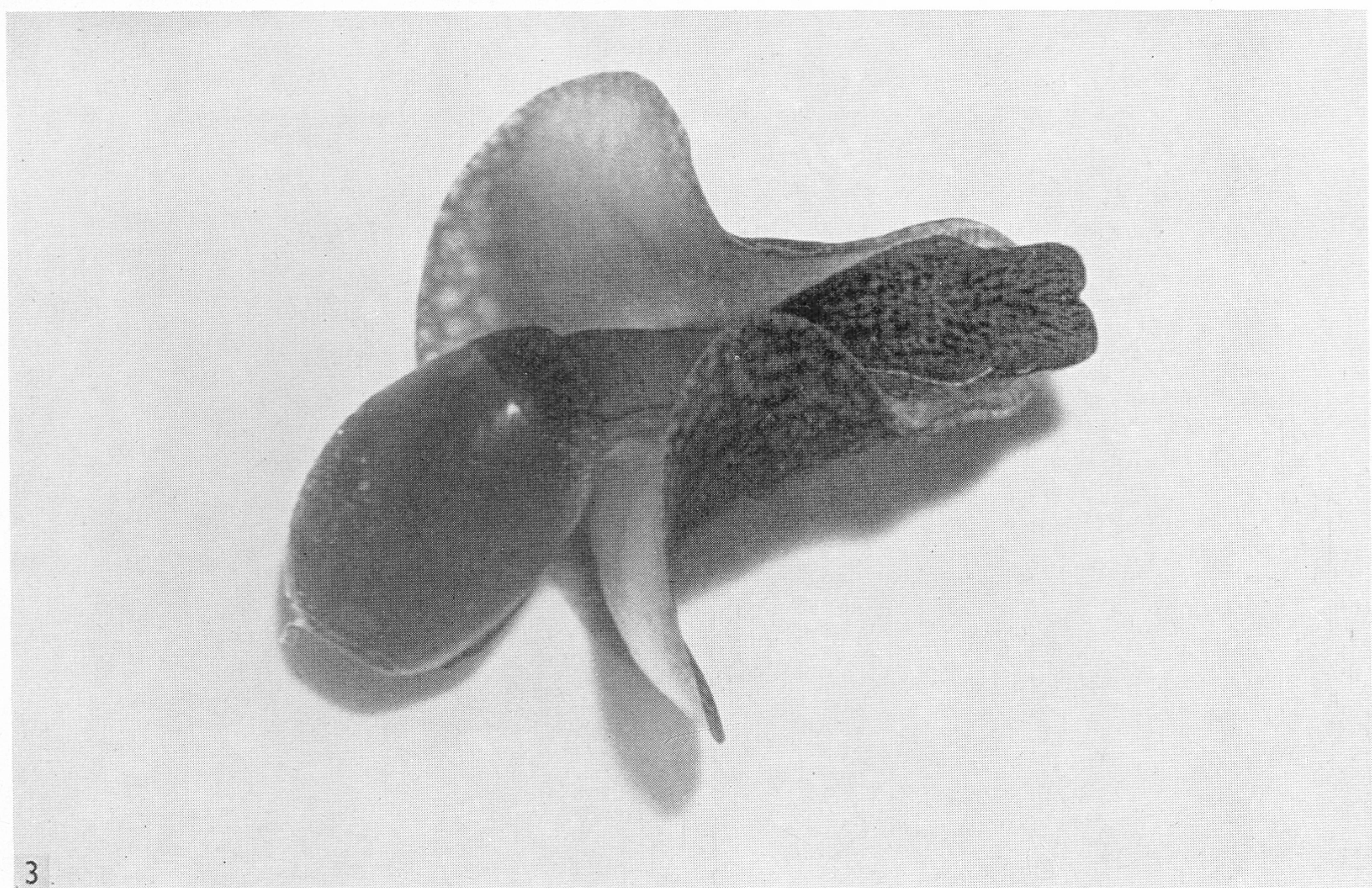
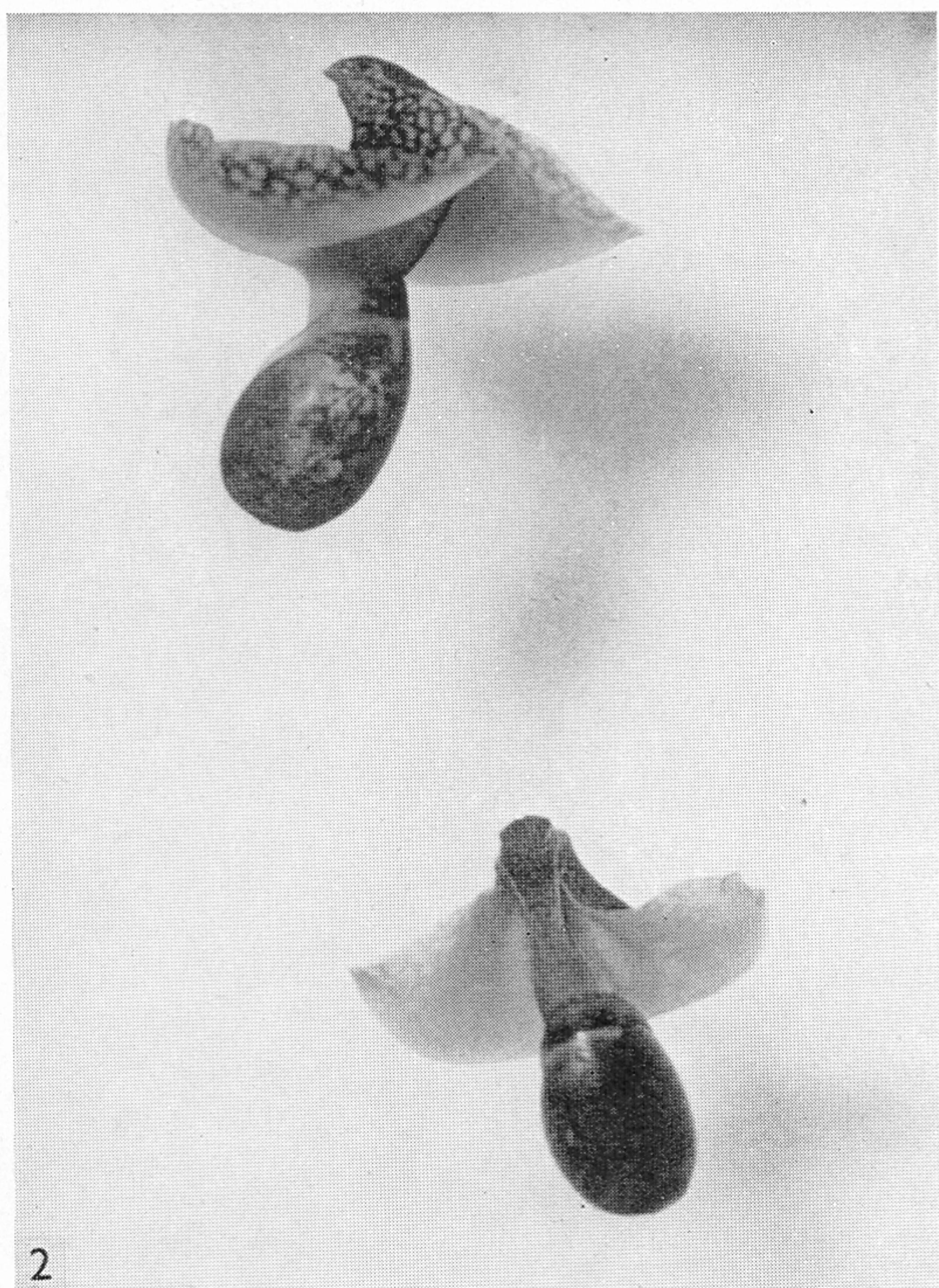
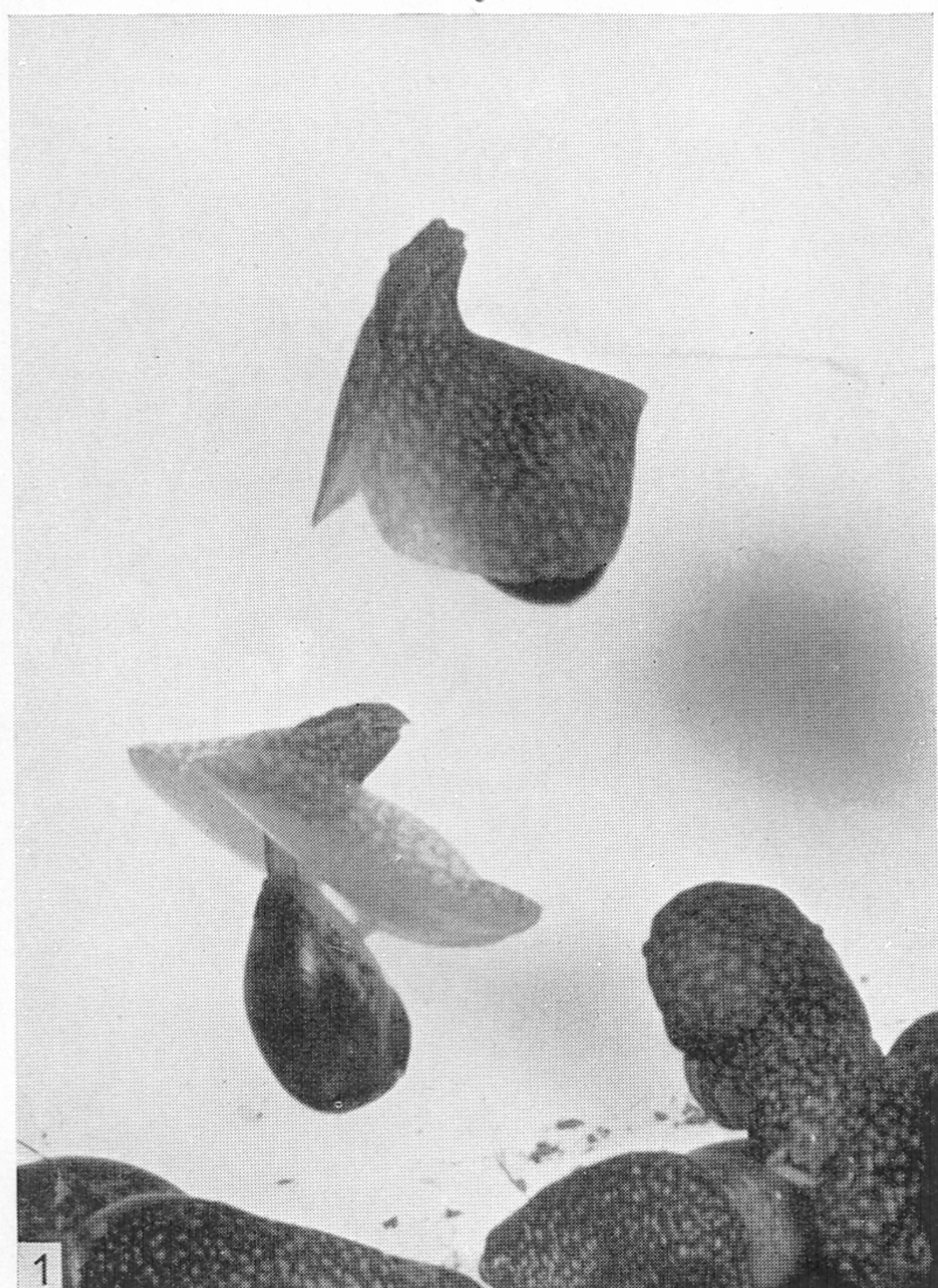
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#### EXPLANATION OF PLATE I

Swimming movements of *Akera*. 1, 2, lateral view of swimming specimens, slightly less than natural size. 3, a specimen, viewed from above, about to rise from the bottom of a dish. About twice natural size.

Photographs from Kodachrome originals by D. P. Wilson.



## THE FUNCTIONAL MORPHOLOGY OF *OTINA OTIS*, A PRIMITIVE MARINE PULMONATE

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(Text-figs. 1-12)

The family Otinidae is the smallest and probably the least known among the pulmonates. Thiele (1931) places it at the base of the Basommatophora, the more primitive order of the subclass Pulmonata, in the Stirps Actophila. It contains a single species, *Otina otis* Turton, with a geographical range confined to the coasts of the British Isles and north-west France. Its northern distribution reaches as far as the Firth of Clyde, according to Jeffreys (1869), and over the greater part of its British range it appears to follow fairly closely the distribution of the barnacle *Chthamalus stellatus*, which here reaches its northern limit. *Otina otis* is a tiny snail, and its external form is limpet-like. The shell, which is well described by Jeffreys (1869), and also by Ellis (1926), measures up to 2·5 mm in length, with a short apical visceral coil at the posterior end. It is dark chestnut brown in colour, and most resembles a minute *Haliotis*.

A description of the ecology of *Otina otis* has already appeared, as portion of a study of the crevice-dwelling animals of the upper intertidal zone at Wembury (Morton, 1954). *Otina* is rather restricted in its mode of occurrence as regards both vertical tidal range and selection of substratum. It lives a short distance within the mouths of crevices between layers of foliaceous rocks, such as Dartmouth Slate at Wembury and Whitsands, and felsite at Kingsands, and in fissures between blocks of Staddon Grit at Rum Bay and volcanic rocks at Drake's Island. Its vertical range at Wembury extends from EHWN tide to the splash zone of MHWN. A favourite site is upon the fringe of *Chthamalus stellatus*, extending just within a crevice upon a shaded north-facing side, or upon an encrusting layer of the brown alga *Ralfsia*, with the serpulid *Spirorbis borealis* somewhat deeper in the crevice. In conditions of high humidity *Otina* ventures farther out upon the exposed barnacle fringe, where its favourite habit is to hide in the empty shells of *Chthamalus*, together with specimens of *Littorina neritooides*, *L. rudis* and *Lasaea rubra*. The ecological relation between *Otina* and *Chthamalus* would appear to be very close; and the geographical distribution and the distribution of the two species upon the shore to a large extent coincide.

An essentially similar choice of habitat seems to be shown by *Otina otis* where it occurs on the coast of north-west France. The Inventory of the

Roscoff Fauna (Station biologique de Roscoff, 1951) records it under stones, in crevices between the rocks, and in empty balanoid shells. It is further stated to shelter in little tufts of algae, as it does sometimes at Plymouth in clumps of the lichen *Pygmaea pumila* (= *Lichina pygmaea*), and in the byssus of mussels in the middle to upper tidal zone.

#### METHODS

By virtue of its compact shape and small size for sectioning *Otina otis* is a relatively easy mollusc to study anatomically. During the present investigation animals were dissected alive and after fixation with aqueous Bouin's fluid and with the Duboscq modification of Bouin's. The animals survive for only a short time in the still water of a laboratory vessel and are intolerant of more than a few hours' continued submergence. Living animals were removed from the shell under the higher power of the binocular, and placed in sea water in a cavity slide, where they could be readily dissected with needles, the separate organs of the visceral mass dissociating easily and remaining intact for observation of ciliary and muscular activity in the living state. Dissected preparations were transferred to the monocular for more detailed observation with the  $\frac{1}{3}$  objective. Fixation in Bouin's for 24 h was found sufficient to dissolve the shell entirely, after which it was found advisable to remove with needles the brown-pigmented shell membrane, to assist quick penetration of the clearing and embedding media and the cutting of clean sections. Specimens were examined as cleared transparent objects, both unstained and after staining lightly with Mayer's haemalum, and were embedded for sectioning in paraffin at m.p. 52–56° C. Transverse and longitudinal sections were cut at 5 and 8  $\mu$  thickness. Staining with Heidenhain's azan was found to give ideal histological results, especially for details of the inclusions in the cytoplasm of gland cells and for general nuclear staining. For finer details of ciliary apparatus, recourse was made to iron haematoxylin without counterstaining. Mucicarmine and thionin were each used to detect mucus, and Masson's trichrome, as recommended by Pantin (1946), was found a useful alternative general stain to azan.

#### EXTERNAL FEATURES

The animal in life, as it creeps about in moist air, is not completely covered by the shell, which is raised above the substratum to expose the sides of the fleshy foot. These—like the rest of the body—are translucent white in colour; in the crawling animal they are usually kept tumid with blood. The head bears a pair of large rounded lappets, which extend forward to overlap the mouth in front, and almost reach the substratum. Behind them, the buccal mass is rounded and bulbous, appearing pinkish, with a narrow black jaw, visible through the white integument. The cephalic tentacles are short and blunt, springing from the head behind the lappets. They appear flattened and triangular from above, and the eyes are located at the centre of their bases. The sole of the foot is oval in outline. It is divided by a deep transverse groove, which cuts off the semicircular anterior third of the foot from the longer posterior portion. The animal progresses on a firm surface by advancing the front of the foot over the substratum, and fixing it in a forward position while the rest of the foot is brought up behind. The whole sole is ciliated and glandular. The most elaborate gland of the foot, however, is the

suprapedal gland (Fig. 2, *spg*) which opens on the upper or anterior surface of the front lobe. The suprapedal gland forms a compact cluster of mucus-secreting cells, embedded in the connective tissue of the anterior lobe of the foot. Their contents stain grey-green with Masson's stain, bright pink with mucicarmine. Their ducts interpenetrate between the cells of the overlying columnar epithelium, and a forward ciliary current carries a sheet of secretion to the anterior edge of the foot in the neighbourhood of the mouth. The epithelium overlying the gland is raised in an oval pad of ciliated and gland cells, which may form a cushion or a shallow trough according to the state of contraction of the foot. Farther backwards, towards the base of the foot, the subepithelial gland cells spread upwards to enclose the sides of the epithelial tract, which leads back into a recess, lined with squamous cells. Here a dense cluster of cell ducts thrusts its way to the surface, from the posterior part of the gland, where the cytoplasm differs in being less granular, the secretion staining pale green with Masson's. The whole of the secretion of the suprapedal gland appears to find its way forward to the point of contact of the mouth with the substratum. It plays the chief part in compacting particles of food into a bolus in which they are raked up by the odontophore and drawn into the mouth, with a further secretion of mucus from the buccal glands. Within the gut, the salivary glands are small, and the oesophagus almost non-glandular. The supply of mucus from the foot thus provides the chief binding substance added to the food before it is conveyed to the stomach.

The intrinsic muscles of the foot are arranged in two extensive bands inserted on the shell dorso-laterally, just inside the rim of the aperture. From either side, bundles of fibres radiate downwards into the foot, and spread out to be inserted over the whole width of the sole. They serve, on contraction, to draw the shell close to the ground, and, with the blood contained in the pedal sinus, to bring about the general movements of the foot itself. The suprapedal gland lies anteriorly in a triangular space above the convergence of the two muscle bands. Above the level of the gland, and behind it the foot is crossed by a strong sheet of transverse muscles, which form a long, shallow basin, flooring the haemocoele and containing the viscera above.

The pallial opening (Fig. 1, *p op*) is set rather far back on the right side, close to the posterior end of the foot. The anus opens immediately in front of it. The mantle forms a wide skirt, fitting closely over the head and trunk, and reflected as a thin white rim at the edge of the shell. The roof of the mantle cavity is a thin-walled, triangular area, quite transparent and with no special respiratory capillaries. It is longest on the right side, where the kidney (Fig. 2, *kd*) lies postero-dorsally to the pallial opening. The kidney is a small, pyramidal sac, with smooth, unfolded lining, its broad base applied behind to the anterior lobe of the digestive gland. The renal pore opens into the pallial cavity just inside the pallial aperture. Farther back, the pallial cavity extends as a wide, shallow recess, lying to the right of the mid-line and not

shown in Fig. 2. It is overhung by the anterior lobe of the digestive gland, which penetrates into the mantle. The pallial opening, or pneumostome, forms a short, narrow tube lined by a thick sleeve of gland cells (Fig. 4). On the lateral wall, the epithelium is shorter than on the mesial, and is continuous with the pallial skirt below its insertion on the trunk.

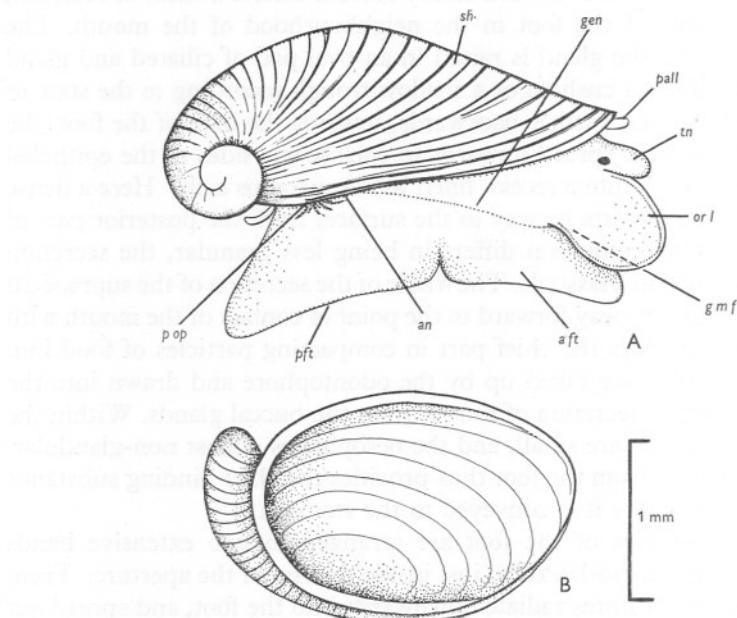


Fig. 1. *Otina otis*. A: lateral view of the entire animal. *aft*, anterior lobe of the foot; *an*, position of the anus beneath the pallial skirt; *gmf*, position of the male and female genital apertures beneath the right oral lappet at its posterior edge; *gen*, course of vagina and vas deferens beneath the integument; *or l*, oral lappet; *pall*, projecting margin of the mantle; *pft*, posterior lobe of the foot; *pop*, opening of pallial cavity; *sh*, shell; *tn*, cephalic tentacle. B: the shell, viewed from beneath.

The pallial opening in limpet-like gastropods (Graham, personal communication) appears to be always a profusely glandular region. A number of different mucoid secretions are produced, which probably have both a lubricatory and a protective role. In *Otina* there appear to be three distinct types of subepithelial gland cell (Fig. 4). The first and second types are much the greatest in length ( $150 \mu$ ) and penetrate deeply against the haemocoele on the mesial side. In the first (Fig. 4, *gl 1*) the cytoplasm forms a coarse reticulum, staining lightly brown in azan; the cell contents are often removed during fixation. The second type of gland (*gl 2*) stains bright blue in azan. The third type of gland (*gl 3*) is cylindrical or flask-shaped and is a good deal shorter. The cytoplasm stains various shades of red after azan, towards maturity the cell contents appearing deep wine red to purplish or dark blue. Intermixed

with these cells, just beneath the surface of the epithelium, are often smaller, rounded cells, uniformly packed with brown staining spherules, and probably representing younger stages of the first type of gland. Towards the pallial cavity the epithelium of the pneumostome flattens to a squamous form and

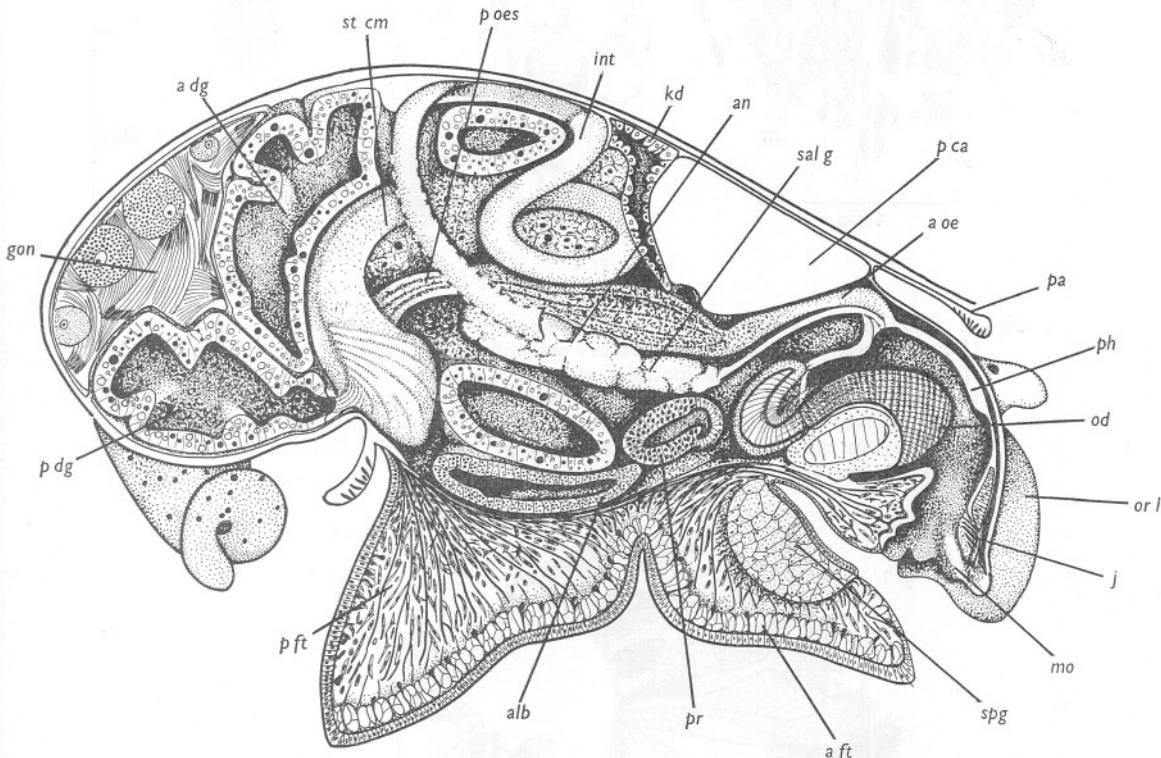


Fig. 2. *Otina otis*. Semi-diagrammatic longitudinal view of the entire left half of the animal, sectioned along the sagittal plane. The course of the rectum, from the mid-line to the anus, on the right side, has been indicated by broken lines, although actually lying outside the sagittal plane. *adg*, anterior lobe of the digestive gland; *aft*, anterior portion of the foot; *alb*, tubule of the albumen gland; *an*, position of the anus to right of sagittal plane; *aoe*, anterior region of the oesophagus; *gon*, ovotestis; *int*, intestine; *j*, jaw; *kd*, kidney; *mo*, mouth; *od*, odontophore; *or l*, oral lappet; *pa*, mantle; *p ca*, pallial cavity; *pdg*, posterior lobe of the digestive gland; *p ft*, posterior division of the foot; *ph*, buccal mass; *poes*, posterior region of the oesophagus; *pr*, prostate; *sal g*, salivary gland; *spg*, suprapedal gland; *st cm*, 'style sac'.

glands of the third type are especially numerous. At the extreme inner edge of the gland mass, there is often a nest of several large-nucleated, spherical cells (Fig. 4, *replc*), which evidently serve as replacement cells for discharged glands.

The sole of the foot (Fig. 3 B) is lined with columnar epithelium of uniform height ( $15 \mu$ ) with a central row of ovoid nuclei, and a short but dense ciliary

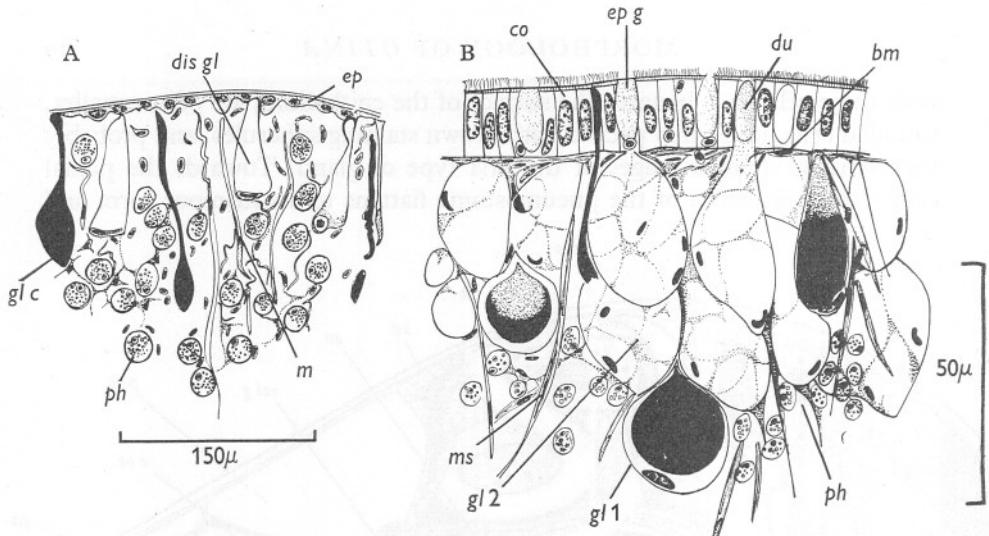


Fig. 3. *Otina otis*. A: transverse section of the integument in the lateral region of the foot (from preparations stained with Masson's and with azan). *disgl*, discharged gland cell; *ep*, squamous external epithelium; *glc*, gland cell; *m*, muscle strand; *ph*, phagocytes in blood sinus. B: transverse section through the epithelium of the sole of the foot (Bouin's; Masson's trichrome). *bm*, basement membrane; *co*, columnar epithelial cell; *du*, duct of a subepithelial gland cell; *epg*, epithelial gland; *gl 1*, *gl 2*, types of subepithelial glands; *ms*, muscle fibre; *ph*, phagocyte.

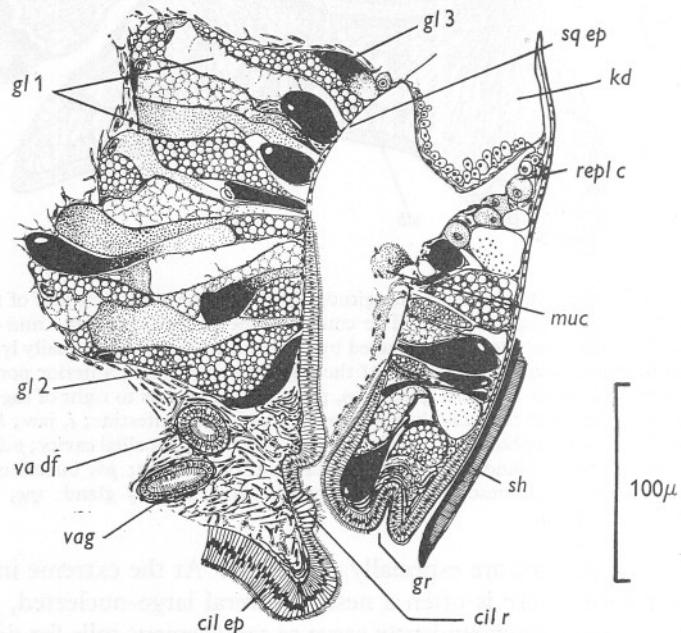


Fig. 4. *Otina otis*. Transverse section of the pallial cavity near its opening, showing the histology of the epithelium lining the pneumostome (from preparations with Masson's trichrome, and azan). *cilep*, ciliated columnar epithelium; *cilr*, ciliated ridge running along the side of the foot below the closure of the mantle cavity; *gl 1*, *gl 2*, *gl 3*, types of gland cells of the subepithelial layer as referred to in the text; *gr*, secretory groove along the pallial margin; *kd*, kidney; *muc*, mucus discharged into the pallial cavity; *replc*, replacement cells; *sh*, shell; *sqep*, squamous epithelium; *vadf*, vas deferens; *vag*, vagina.

coat. Between the ciliated cells appear the ducts of large subepithelial glands. Occasionally a whole gland cell, complete with basal nucleus, lies entirely within the columnar layer. Amoebocytes are usually numerous, wandering in from the subepithelial layers. The basement membrane of the epithelium is rather muscular, and incorporates a dense sheet of collagenous connective tissue, to which are attached narrow slips of muscle from the deeper layers of the foot. There are two types of glands, clustered thickly together in supporting connective tissue. The first (*gl 2*) are ovoid and rounded, much the more numerous and crowded in an almost continuous sheet beneath the basement membrane. They stain bluish in azan, light green after Masson's and in haematoxylin pale purple. Thionin leaves them dark brown. The nuclei are basal or parietal and the ducts are stout and cylindrical, penetrating between the overlying columnar cells. The second type of gland (*gl 1*), much less numerous, stains deeply purple or black in haematoxylin, deep red with azan. It is commonly elongate or fusiform, with a short stout duct running up through the epithelium. Sometimes one or more of these glands becomes depressed beneath the lighter staining cells, and its cell body then appears perfectly spherical with a long dark-staining duct thrusting its way into the epithelium.

The sides of the foot, which in the living animal are exposed to the moist air, are quite different in histology from the sole (Fig. 3 A). Their subepithelial connective tissue forms a large blood sinus, lying on either side laterally to the muscles of the foot, from which it is penetrated by narrow strands of muscle inserted against the epithelium. The external layer is thin and squamous, not ciliated, and with a very narrow refractile border. The basement membrane is rather crenulated by the bulging against it of cell nuclei. Glands are of a single type, staining very darkly in haematoxylin and deep red in azan. These cells form long (100–150  $\mu$ ) vesicles of secretion running deeply into the connective tissue, usually with a flattened nucleus lying horizontally at the cell base. What their function may be is not ascertained. They are probably similar in nature to the dark staining glands (*gl 1*) of the sole, and may have a protective or antiseptic function, similar to that of the multicellular glands described by Fretter (1943, p. 697) in the integument of *Onchidella*. Within the blood spaces of the sides of the foot, amoebocytes are very numerous (*ph*), rounded or slightly ovoid after fixing, with a small round, uninucleolate nucleus. They are filled with tiny granular inclusions, staining reddish brown in Masson's, as well as large amorphous clusters of particles. It seems likely that the broad lateral tracts of the foot may in *Otina* carry on accessory respiratory and excretory activity, especially in view of the reduced size of the pallial cavity, its lack of a respiratory plexus, and the reduction in size of the kidney. At the sides of the foot, blood is brought to the external surface of the body, enclosed only by the thinnest of epithelia through which respiratory interchange might easily be possible with the surrounding air.

An increase of respiratory surface by epithelial folds is probably unnecessary, in view of the small volume of the animal and its comparative lack of active movements. The amoebocytic cells probably have an excretory role, following on reduction of the kidney and the lack of a pallial water current to carry discharged renal products out of the mantle cavity. A similar occurrence of excretion through the integument by amoebocytic action has been described in several other marine gastropods, for example *Omalogyra* (Fretter, 1948) and the *Pyramidellidae* (Fretter & Graham, 1949). Here the laden amoebocytes emerge along a thin strip of epithelium into the pallial cavity. In *Osilinus lineatus*, Nisbet (personal communication) has observed the excretion of injected particles of carmine by the amoebocytes of the foot.

#### ALIMENTARY CANAL

##### *The Buccal Region*

*Otina* feeds like a limpet by the rasping action of the radula, browsing over the rock surface, and picking up wave-lodged diatoms and detritus. The places where it lives are often not rich in debris; the food tends to be more finely selected than in ellobiids (see Morton, 1955a), and the radula is kept constantly at work raking particles into the buccal cavity. The alimentary canal is not much specialized, and presents many features primitive among pulmonates and reminiscent of microphagous prosobranchs. The mouth is a long ventral slit between the oral lappets. The suprapedal mucous gland opens just below it, between the bases of the lappets, and its secretion passes forwards, helping to consolidate a food bolus as the radula works against the substratum. The buccal bulb is relatively large, and occupies the whole of the haemocoele of the convex head. When the animal is extended, the head projects forward from beneath the mantle skirt. The bulb is strongly muscular, especially in front of the odontophore and around the mouth. Its cavity leads dorsally from the mouth; when the muscles forming the oral sphincter are relaxed, the bluntly pointed odontophore is thrust down and the radular ribbon unfolded and licked across the substratum.

Fig. 5A shows a schematic view of the buccal bulb, opened from above. In front of and below the tip of the odontophore, the buccal cavity is laterally compressed; it forms a vertical cleft between two smooth, cuticle-covered side walls. The cuticle is penetrated by the ducts of mucous cells clustered beneath the epithelium, and forming on either side a lateral buccal gland, secreting binding mucus as the food is taken in. The secretion is in part released at the level of the substratum, as the radula is actually at work. The buccal glands stain pale blue with azan, red with mucicarmine and black after thionin. Just within the mouth, the anterior wall of the buccal cavity is reinforced by a small crescentic jaw, running across the expanded dorsal channel between the glandular side walls. It serves, when the mouth is opened, to grip on the substratum, providing purchase during the strokes of the radula. It consists

of a series of close-set, spinulose rods of chitin, embedded in a pad of thickened cuticle. Along the base of the jaw, runs a transverse plate of 'cartilage', consisting of long vacuolated cells, invaded by trabeculae from the surrounding connective tissue.

The odontophore (see Fig. 5 A) is bluntly pointed in front, and covered over its whole free surface by the wide radular membrane, which bears small

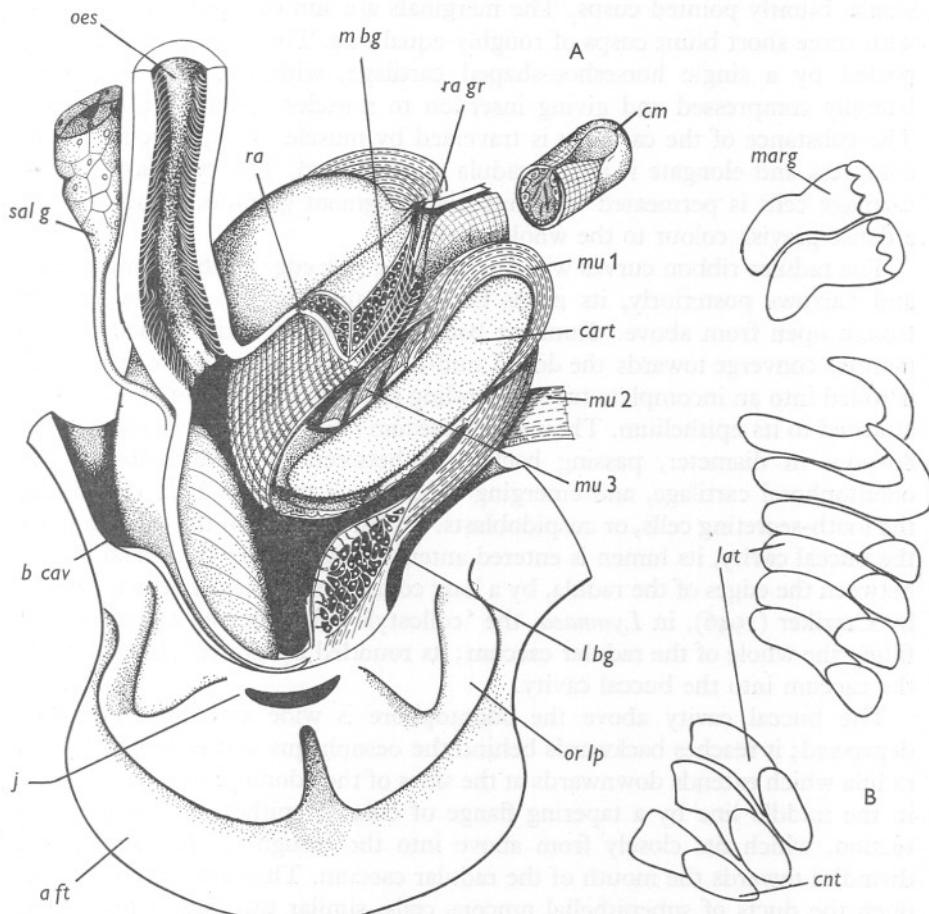


Fig. 5. *Otina otis*. A: Stereogram illustrating the structure of the buccal mass. The haemocoel of the head has been laid open from in front and the left half of the oesophagus and the roof of the buccal mass removed. The radula is represented as being cut away on the left side to show the odontophoral cartilage and the arrangement of the chief muscles of the buccal mass. *aft*, anterior division of the foot; *b cav*, haemocoel of the head; *cart*, odontophoral cartilage; *cm*, radular caecum; *j*, jaw; *lbg*, lateral buccal gland; *mbg*, median buccal gland; *mu 1*, *mu 2*, *mu 3*, muscles of the buccal bulb referred to in the text; *oes*, oesophagus; *or lp*, oral lappet; *ra*, radula; *ragr*, radular groove; *sal g*, right salivary gland. B: representative teeth of the radula. *cnt*, central tooth; *lat*, lateral teeth; *marg*, marginals.

uniform teeth. The radular structure is illustrated in Fig. 5B. Each row consists of a central tooth, flanked by a series of up to 100 laterals, with a further series of marginals towards the edges. The central tooth forms a narrow wedge, widest along its flat posterior edge, and surmounted by a single elongate cusp. The laterals are rather long and arcuate, resembling in shape the teeth of the single lateral pair of the mesogastropod radula. They have single, bluntly pointed cusps. The marginals are similar in shape, equipped with three short blunt cusps of roughly equal size. The odontophore is supported by a single horseshoe-shaped cartilage, with the two side lobes laterally compressed and giving insertion to muscles operating the radula. The substance of the cartilage is traversed by muscle fibres, which serve to compress and elongate it as the radula is protracted. The cytoplasm of the cartilage cells is permeated with fine black pigment granules, which impart a dense greyish colour to the whole cartilage.

The radular ribbon curves widely round the free edge of the odontophore, and narrows posteriorly, its edges becoming upcurved to form a shallow trough open from above. Running backwards into the radular caecum, the margins converge towards the dorsal mid-line, so that the radular membrane is rolled into an incomplete tube, extending around the lining of the caecum, attached to its epithelium. The radular caecum (Fig. 5A, *cm*) is a short tube, 80–90  $\mu$  in diameter, passing backwards between the side limbs of the odontophoral cartilage, and emerging behind as a rounded bulb containing the tooth-secreting cells, or cuspidoblasts. At the opening of the caecum from the buccal cavity, its lumen is entered anteriorly, and along the dorsal fissure between the edges of the radula, by a long column of connective tissue; called by Carriker (1946), in *Lymnaea*, the 'collostyle'. This forms a central core filling the whole of the radular caecum; its rounded tip projects bluntly from the caecum into the buccal cavity.

The buccal cavity above the odontophore is wide and dorso-ventrally depressed; it reaches backwards behind the oesophagus and is floored by the radula which extends downwards at the sides of the odontophore. It is roofed in the middle line by a tapering flange of ciliated epithelium triangular in section, which fits closely from above into the trough of the radula, and dwindles towards the mouth of the radular caecum. Through the epithelium open the ducts of subepithelial mucous cells, similar to those of the lateral buccal glands. They may be referred to as the median buccal gland (Fig. 5A, *mbg*), and their secretion probably serves chiefly to pick up particles borne backwards beyond the oesophagus.

Fig. 5A shows also the principal muscles of the buccal bulb. These comprise extrinsic retractor muscles, passing forward from the foot and the floor of the haemocoel to the base of the odontophore and the side walls of the buccal cavity. There is an especially large pair of radular retractors originating on the outer posterior aspects of the odontophoral cartilages, passing inwards

beneath the cartilages, and running forwards underneath the radular caecum, on which they are inserted ventrally. Other muscles, with insertions on the inner aspects of the cartilage pass obliquely forwards, above the cartilage, to a broad insertion beneath the radular ribbon. They contract to fold the ribbon into a trough and to draw its edges together.

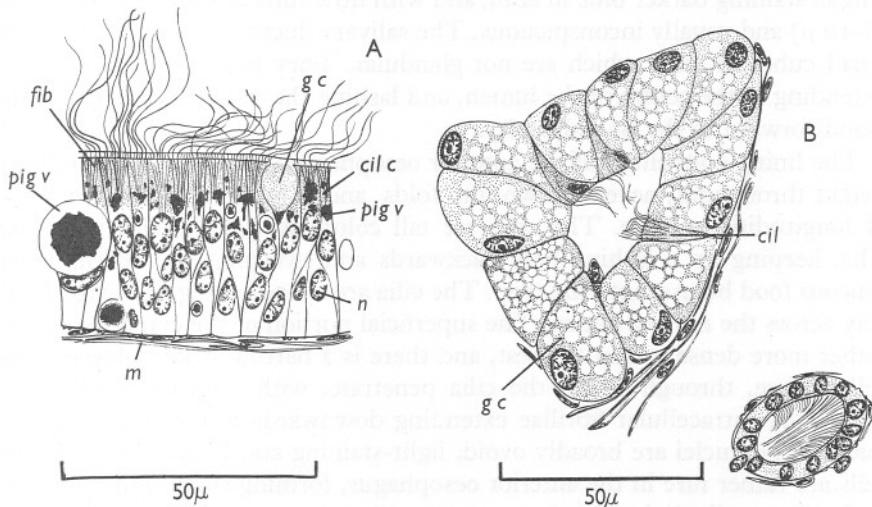


Fig. 6. *Otina otis*. A: epithelium of the anterior region of the oesophagus (Bouin's; azan) *cil c*, ciliated cell; *fib*, intracellular fibrillae; *gc*, glandular cell; *m*, muscle fibre; *n*, nucleus of ciliated cell; *pig v*, vacuole containing black pigment. B: Transverse section of a salivary gland, and of its duct (Bouin's; azan). *cil*, ciliated cell; *gc*, gland cell.

#### The Oesophagus

The oesophagus (Fig. 5 A, *oes*) arises rather far forwards from the buccal bulb in front of the odontophore, immediately above the mouth. The odontophore when retracted lies entirely behind the oesophageal opening. The oesophagus funnels upwards from the buccal bulb, and runs back in the mid-line as a narrow cylindrical or dorso-ventrally flattened tube, 100–120  $\mu$  in diameter. This region may be referred to as the anterior oesophagus. Immediately behind the buccal mass it terminates abruptly, and the gut widens into a thinner-walled fusiform crop, which may, at times, be strongly distended with a mass of food material.

Along either side of the anterior oesophagus, extending backwards beside the crop, lies a long salivary gland. This consists of a thick-walled tube, opaque white or slightly translucent, and tapers in front to a very fine duct which penetrates the pharyngeal roof. The structure of the salivary gland (Fig. 6 B) is extremely simple: it is 90–100  $\mu$  across, cylindrical or sometimes prism-shaped by compression from adjacent structures. The lumen is surrounded by up to a dozen rows of gland cells, of a single type with rather

coarsely reticulate cytoplasm, staining light blue in azan, grey-green in Masson's, jet black in thionin, and pink in mucicarmine. The cell contents are thus entirely mucoid in nature. The nuclei are rounded or ovoid, basal or pressed close to the side wall of the cell, and are uninucleolate. Two or three small ciliated cells usually appear in a transverse section, wedge-shaped and single, staining darker blue in azan, and with little tufts of lash-like cilia, short ( $8-10 \mu$ ) and usually inconspicuous. The salivary duct is  $35-40 \mu$  across, with small cuboidal cells, which are not glandular. They bear a coat of tall cilia extending to the centre of the lumen, and lashing the mucoid secretion of the gland forward to the buccal cavity.

The lining epithelium of the anterior oesophagus (Fig. 6A) is of a uniform height throughout, never thrown into folds, and is backed by a narrow layer of longitudinal muscle. The cells are tall columnar ( $40 \mu$ ), with very long cilia, keeping up a lashing beat backwards and sweeping and rolling small mucous food boluses into the crop. The cilia are  $25-30 \mu$  long and extend half way across the narrow lumen. The superficial portion of the cytoplasm stains rather more densely than the rest, and there is a narrow striated layer at the cell surface, through which the cilia penetrate, with a series of fine, perpendicular intracellular fibrillae extending downwards to the central row of nuclei. The nuclei are broadly ovoid, light-staining and binucleolate. Gland cells are rather rare in the anterior oesophagus, forming small, plump bodies or fusiform cells, light blue in azan, about a quarter or one-third the length of the ciliated cells and inserted between their distal tips. Granules of black pigment are incorporated in the cytoplasm to the superficial side of the nuclei; pigment may also be aggregated in rounded spherules, some of them quite large, within amoebocytic cells, which frequently wander into the epithelium from the underlying layers. When the crop is empty its wall is thrown into small, impermanent folds. Like the anterior oesophagus, the wall has a densely speckled appearance, from the presence of black pigment incorporated in the epithelial cells. The crop is marked off sharply from the anterior oesophagus, by its much greater diameter ( $300-400 \mu$ ) and by the reduction in size of the epithelial cells, which are short ( $12-15 \mu$ ) with cilia of about the same length. The nuclei form a single basal row, and gland cells are infrequent, as in the anterior oesophagus. Pigment is scattered in small granules in the superficial part of each cell.

#### *The Stomach and Intestine*

The stomach (Fig. 7A) is a triangular sac, lying on the left side of the animal at the base of the visceral mass. Its left aspect is visible through the body wall, tapering in front into the proximal limb of the intestine, which runs straight forward just beneath the thin external integument. The oesophagus enters towards the mid-line on the deep aspect. For the most part, the stomach is thin-walled and transparent. Its contents are clearly visible

through the wall, and it often becomes greatly distended when filled with recently ingested food. Towards the apex on the left side, the wall becomes rather more muscular, to form a short, pointed pouch (Fig. 7 A, *mp*). There are two digestive diverticula, the lobules of which make up the greater part of the

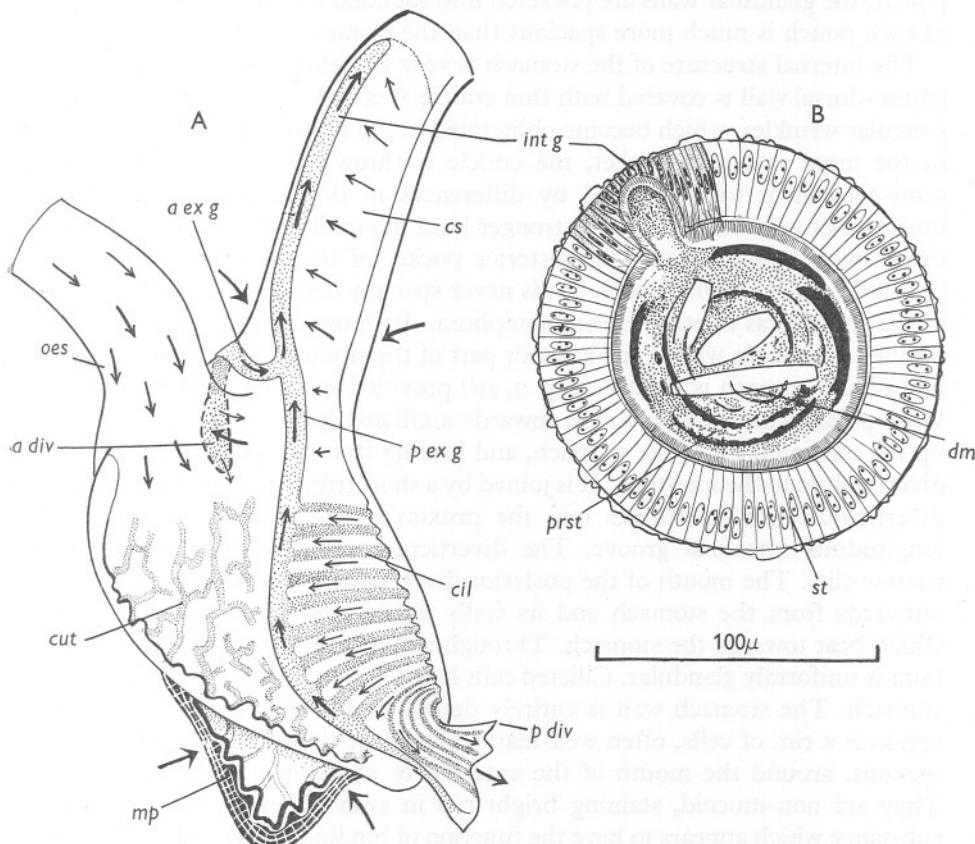


Fig. 7. *Otina otis*. A: the stomach, figured without dissection as a transparent object, showing the course of the ciliary currents. The heavy black arrows indicate the movement of contents into the diverticula, and (outside the stomach) the regions of the strongest muscular contraction. *a div*, anterior digestive diverticulum; *a ex g*, anterior excurrent groove; *cil*, ciliated area; *cut*, area of cuticle; *int g*, intestinal groove; *mp*, muscular pocket; *oes*, oesophagus; *p div*, posterior digestive diverticulum; *p ex g*, posterior excurrent groove. B: transverse section of the proximal limb of the intestine, or 'style sac'. *dm*, diatom enclosed in the mucus of the protostyle; *int g*, intestinal groove; *prst*, protostyle; *st*, epithelium of the style sac.

visceral mass. They are few in number, and each is large and rounded, or bluntly lobulate, orange or dark brown in colour, with large black spherules speckling the surface. The larger anterior digestive diverticulum opens through the antero-dorsal wall of the stomach. Its aperture is situated close to the

entry of the oesophagus and some distance behind the origin of the intestine. The somewhat smaller posterior (morphologically left) diverticulum opens at the postero-median angle of the stomach, close to the mid-line. From each diverticulum, the individual lobules run together to form a single cavernose pouch; the glandular walls are pocketed into rounded saccules, and the lumen of each pouch is much more spacious than the stomach itself.

The internal structure of the stomach is very straightforward. The left and antero-dorsal wall is covered with thin cuticle (Fig. 7A, *cut*) thrown into tiny, irregular wrinkles, which become obliterated in the full stomach. Posteriorly, in the more muscular pocket, the cuticle is thrown into several stronger, semi-permanent folds, formed by differences in the height of cells. The muscle coat is formed here by a stronger layer of circular fibres, closely intermixed with longitudinal. The posterior pocket of the stomach serves as a 'gizzard' of the simplest form, but is never strongly demarcated from the rest of the stomach as in other Basommatophora. Its muscle coat merges into the thinner contractile wall of the anterior part of the stomach. The right median half of the stomach is ciliated (Fig. 7, *cil*) provided with ten to twelve transverse plicae which sweep round towards a ciliated groove (*p ex g*) running up the median edge of the stomach, and leading from the posterior digestive diverticulum to the intestine. It is joined by a short tributary from the anterior diverticulum, and continues into the proximal part of the intestine as a longitudinal intestinal groove. The diverticula open into the stomach by narrow slits. The mouth of the posterior diverticulum is somewhat pocketed outwards from the stomach and its walls folded, and lined with fine cilia, which beat towards the stomach. Throughout each diverticulum the epithelium is uniformly glandular. Ciliated cells hardly extend outwards beyond the stomach. The stomach wall is entirely devoid of glands; the only secretory region is a rim of cells, often well-marked, at other times hardly apparent in sections, around the mouth of the anterior, or sometimes both diverticula. They are non-mucoid, staining bright red in azan. They produce a viscid substance which appears to have the function of binding the particles from the digestive gland as they are carried across the ciliated lips of the diverticula into the stomach.

The strongest cilia within the stomach are developed in the excurrent grooves from the diverticula. Here a rapid current sweeps smaller particles forward into the intestine. The grooves do not themselves clear the whole of the waste material from the digestive gland; large clusters of spherules from the digestive cells frequently appear in the general contents of the stomach. The ciliated ridges are formed by variations in the height of the cells of the columnar epithelium, which reaches  $30\ \mu$  along the folds, but less than half this length along the furrows. There is a median row of long rod-like nuclei, tending to be more compressed in the narrower cells which constitute the folds. The cilia are short ( $2-3\ \mu$ ) and very dense. Though individual particles

may be swept along the furrows, the ciliated area as a whole is evidently not adapted for sorting. The primary function of its cilia is not to deal with separate particles, but to assist the rotation of the heavy bolus of stomach contents bound with mucus. The cuticle-bearing cells are stouter than the ciliated cells, and are  $30\ \mu$  tall, with a subcentral row of oval nuclei. The cuticle layer is thin ( $1-2\ \mu$ ), staining lightly blue with azan and is attached by a delicate layer of fine tag-like processes resembling cilia.

The proximal limb of the intestine leading forward from the stomach is very distinct in histology from the stomach and from the rest of the intestine. Its lining epithelium is of brick-shaped columnar cells (Fig. 7B),  $30\ \mu$  in height, with a central row of clear-staining, ovoid nuclei, each with two nucleoli. The cytoplasm is uniformly granular and rather lightly staining. Mucous glands are never developed. The distal half of each cell is occupied by a fan of intracellular fibrillae connected with a row of short bristle-like cilia,  $5-6\ \mu$  in height. The lining of the intestinal groove is, by contrast, of extremely narrow cells, of which there are a dozen or so on either side of the groove, with compressed nuclei, darker staining cytoplasm. The cilia are longer and finer, keeping up a rapid beat aborally along the intestine. The bristle-like cilia of the rest of the epithelium beat in a transverse direction. Their action is difficult to see in the excised intestine in which they are generally inert. The direction of the effector stroke is, however, often retained in the row of cilia after fixation, and this fact, together with the concentric arrangement of layers of mucus and faeces, indicates that these cilia serve to rotate the faecal rod, which fills this part of the intestine and projects into the stomach. In short, both in its structure and function, the proximal part of the intestine in *Otina* possesses all the features of a style-sac of the most elementary kind.

It has become clear, from the work of Graham (1949) (see also Morton, 1952a) that the style-sac form of stomach is a primitive and generalized feature in the earliest members of the molluscan phylum. The most archaic members of both the gastropods (Archaeogastropoda and Cyclophoracea) and the lamellibranchs (Protobranchia) have strong cilia in the proximal part of the intestine, serving to rotate a 'protostyle', or rod of mucus and faeces projecting into the stomach. The protostyle provides the chief source of movement within the stomach, both for drawing in a food-string from the oesophagus, and for sweeping particles over the ciliary sorting area. In later evolved types in both classes, the rod may lose its faecal character and become clear and hyaline, to form the enzyme-containing crystalline style. In *Otina* waste particles from the stomach and 'faeces' from the digestive gland are compacted into a protostyle which is slowly rotated by cilia; particles are circulated within the stomach. The protostyle is much more coherent than the rest of the contents of the stomach, and while it rotates, it is gradually passed backwards into the intestine by the cilia of the longitudinal groove, and forms the first portion of the faecal string. The mucus of the protostyle is

deposited in thin layers, made up from two types of secretion, the bluish staining (azan) secretion carried into the stomach from the anterior part of the gut, and the bright red-staining secretion from the cells lining the rim of the digestive diverticula.

The mode of action of the stomach of *Otina*, a primitive pulmonate, is thus very similar to that of the lamellibranchs *Nucula* (Graham, 1949) and *Malletia* (Yonge, 1939), and of the prosobranch *Murdochia* (Morton, 1952b). As in the protobranchs, muscular action also plays an important part in the working of the stomach. Contractions of the wall, especially in the stouter posterior pocket, serve in part to triturate the contents of the food bolus, which is pressed firmly against the cuticularized wall. The cuticle forms a protection against abrasion by sharper fragments, and corresponds in location to the gastric shield. At the same time, regular contractions of the whole stomach wall serve to force semi-fluid material squeezed out of the food bolus (with the finest particles in suspension) into the digestive diverticula, which are, in *Otina*, filled chiefly by muscular action. From time to time, especially after an excretory phase of the digestive gland, the opening of the intestine shows more pronounced muscular contractions, and strong peristaltic waves nip off portions of the protostyle, which are carried backwards rapidly and added to the faecal string. Though the cilia of the intestinal groove are still active, it appears to be chiefly by these sudden muscular contractions that the faeces are driven into the intestine. In general, the food ingested by *Otina* comprises particles of a finer grade than in *Leucophytia*. Diatoms bulk large among the stomach contents, and sufficient trituration is evidently afforded by the muscular wall of the stomach, in the absence of a highly differentiated gizzard as in the Ellobiidae.

The stomach of *Otina*, in its structure and mode of action, appears to be the most primitive yet described in a pulmonate. It shows almost diagrammatically most of the landmarks regarded by Graham (1949) as typical of the stomach in the earliest molluscs: thus, for example, its division into ciliated and cuticularized halves (though there is no spur-like gastric shield), the presence of a 'style sac' with a protostyle, and the paired digestive diverticula with excurrent grooves converging to form an intestinal groove. The posterior gastric caecum (held by Graham to be an archaic feature) is not developed at all in *Otina*, unless we regard it as represented by the outpocketing of the stomach from which the posterior digestive diverticulum opens. This caecum is however present in the more primitive ellobiids (Morton, 1955b); the earliest bivalves, the protobranchs (Yonge, 1939) and the Archaeogastropoda (Graham, 1949) have it also. If a posterior caecum is to be regarded as a feature of the primitive molluscan stomach, the structural simplification going with reduction in size has probably been responsible for its loss in *Otina*.

Further, however, the stomach of *Otina* clearly foreshadows the condition in the higher Basommatophora. Already it has to a large extent lost its

reliance on cilia, and muscular action has increased in importance. At the same time, *Otina* serves to explain what has hitherto not been clear, the relation of the muscular pulmonate stomach to the style-sac type of stomach in prosobranchs, where the chief reliance is on ciliary systems and mucus secretion.

The 'style-sac' in *Otina* passes without change in diameter, into the middle region of the intestine which forms a narrow tube of uniform width ( $75\ \mu$ ). The limb running forward from the stomach passes upwards through the anterior lobe of the digestive gland, to emerge at the dorsal surface. Here it forms a short loop, commencing along the left side, then turning sharply backwards to encircle the anterior lobe of the digestive gland behind, and running forward on the right side, to open by the anus, just in front of the pallial opening. It is lined with four to five longitudinal ridges formed by differences in cell height. The cilia beat strongly out of the grooves, and towards the anus along the summits of the ridges. The epithelial cells are columnar,  $12-15\ \mu$  in height, the ciliary coat  $5\ \mu$  tall. Mucous glands are rather few, plump and fusiform in shape, and contain small clusters of secretion droplets staining bright red in azan. The subepithelial layer is only sparsely muscular, but the faecal string is moulded by intermittent muscular action, and carried backward by peristaltic waves, as well as by the continuous fast beating of the cilia. The ridges which line the intestine are temporarily flattened as the string is passed along. The rectum differs from the middle intestine in its usual lack of folds, its shorter cells ( $10\ \mu$ ) and longer cilia ( $7-8\ \mu$ ). The gland cells are of the same kind but are a good deal more numerous, filled with separate secretory spherules and constricting the narrow ciliated cells. Their secretion provides the final mucous covering of the long rope of faeces. The muscle coat is rather better developed than in the middle intestine, and the whole, or at least long portions, of the faecal string may be voided by peristaltic waves at one time. As compared with the prosobranchs, the faecal string in *Otina* is only loosely compacted. It is never compressed into separate pellets, and, by reason of the external position of the anus, there arises no problem of the fouling of the pallial cavity by voided faeces.

#### *The Digestive Gland*

The digestive diverticula in *Otina* are the site of both absorption and enzyme production; they have also an excretory role. The lining epithelium, from the stomach to the tips of the tubules, is glandular, and contains cells of two chief types, digestive cells in greater numbers, interspersed with smaller groups of excretory cells. At the absorptive phase of the digestive gland (illustrated in Fig. 8A), the cells are for the most part short and columnar, of a uniform height  $35\ \mu$ , with rounded or suboval nuclei lying slightly above the basement membrane. The largest of the excretory cells are broad-based

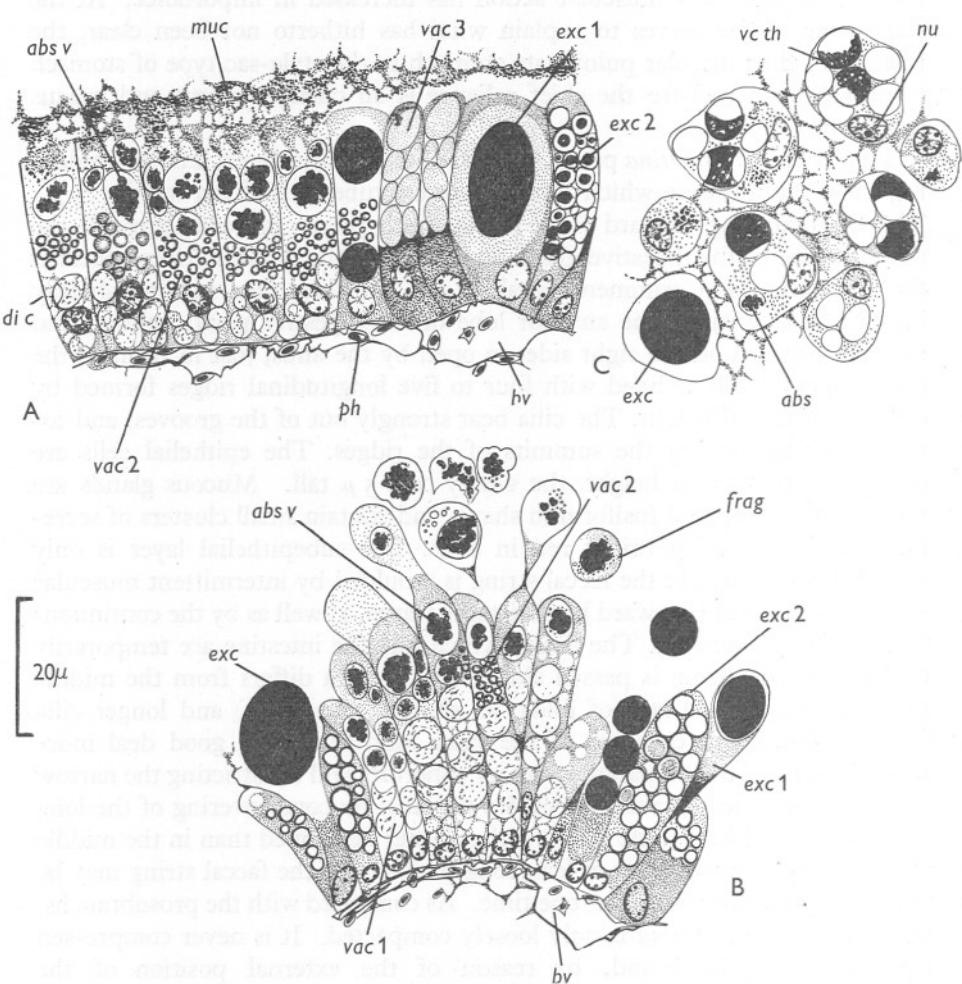


Fig. 8. *Otina otis*. A: epithelium of the digestive gland, at the phase of absorption. B: the same at the phase of fragmentation and excretion (Bouin's; azan). *abs v*, food vacuoles loaded with absorbed material from the lumen; *bv*, blood vessel; *dic*, digestive cell; *exc*, excretory spherule extruded from a cell; *exc 1*, excretory cell of the first type; *exc 2*, excretory cell of the second type; *frag*, fragmented tip of digestive cell constricted off into the lumen; *muc*, mucus from the stomach, with finely divided food material in suspension; *ph*, phagocytic blood cell; *vac 1*, basal vacuole of the digestive cell; *vac 2*, smaller excretory (?) vacuoles in the digestive cells; *vac 3*, blue-staining (azan) vacuoles in the excretory cells at the absorbing phase. C: 'fragmentation phagocytes' formed by abstraction of nucleated portions of the digestive cells, suspended in mucus within the stomach. *abs*, granules of absorbed material from stomach; *exc*, excretory spherule; *nu*, nucleus of 'phagocyte'; *vc th*, basal vacuole of digestive cell (after thionin staining) with stained surface 'membrane'.

and triangular, and as a rule may be distinguished from the digestive cells by the larger size of the nuclei ( $5-6 \mu$ ).

The digestive cell (Fig. 8, *dic*) contains several types of inclusion; its free border is flat and entire at the ingesting phase, and the cytoplasm near the surface is filled to a depth of  $4-5 \mu$  with the mucoid material containing suspended food particles, with which the lumen of the tubule is distended. This material is being freely taken up along the whole surface of the digestive epithelium, so that the cell border is often obscured, the cytoplasm taking on the same coloration (blue in azan, green in Masson's) as the contents of the lumen. Below the ingesting region, the cytoplasm is filled with large spherical vacuoles,  $6-7 \mu$  across, as a rule two or three deep in each cell, a single vacuole filling the whole cell width. Many of these vacuoles contain droplets of ingested material. They are in some places small and no more than  $1-2 \mu$  across, elsewhere clumped together in irregular clusters, or coalescing into a spherule filling the whole vacuole. The rest of the vacuoles remain colourless with most stains, including azan, haemalum, Masson's and mucicarmine. They stain strongly, however, with thionin, which could be employed to distinguish them from all other cell inclusions of the digestive gland. Their appearance after thionin is shown in Fig. 8 C; the vacuole consists of a colourless and structureless central sphere, surrounded by a thin membrane which in fixed material almost always ruptures and peels away at one or more points. With thionin the membrane becomes deep indigo blue; with other stains it presents merely an uneven refractile surface, colourless or pale golden-yellow. At the base of the digestive cell, clustered around and sometimes below the nucleus, is another zone of these thionin-staining vacuoles, of rather smaller diameter (*vac 1*), perfectly colourless, without trace of ingested contents. They sometimes extend into the middle of the cell but usually, above the nucleus, are to be found inclusion spherules of a third type, of smaller size ( $2-3 \mu$ ) pinkish brown in living macerations and retaining the same colour in azan or haemalum. These are evidently excretory in character, though it is not certain whether they are extracted from the blood or represent the final indigestible residue of material absorbed from the lumen.

Excretory cells appear, at the absorptive phase of the digestive gland (Fig. 8, *exc*) to be represented by three stages. The largest are pyriform, strongly expanded towards the tip, and each containing a large, black or dark brown, excretory sperule  $15-20 \mu$  in diameter. In each cluster of excretory cells appear one or more columnar, round-tipped cells, filled with twenty to thirty light brown or pink spherules, contained in separate, clear-staining vacuoles. These spherules may sometimes be discharged separately in the lumen, although, as a rule, the columnar cell appears to be an earlier stage of the pyriform cell, in which a single large sperule is later formed by the coalescence of the smaller ones. The remaining excretory cells are also columnar during the ingesting phase, the basal half of the cytoplasm being pinkish

in azan, or violet with thionin. The distal half is filled with rounded or ovoid vacuoles, 5–6  $\mu$  across, which do not stain with either thionin or azan.

At the excreting phase (Fig. 8 B) the epithelium of the digestive gland takes on a rather different appearance. The digestive cells become longer and round-tipped. They bulge strongly into the lumen and their free tips now begin to be constricted off, forming spherical fragments of cytoplasm (*frag*) loaded with blue-staining (azan) contents. This material would seem to represent an indigestible residue of the mucus-borne particles that were previously absorbed into the digestive vacuoles. The smaller pink spherules appear to be shed also by fragmentation or rupture of the cell wall, while the remaining basal part of the cell is occupied by the large thionin-staining vacuoles. The larger excretory cells containing the densely black spherules have now become greatly distended, some with a row of two or three spherules, others with a single large one. These are shed into the lumen by rupture or by fragmentation of the cell. The smaller columnar excretory cells are either packed entirely with purplish brown spherules; or their basal halves may be of uniformly staining cytoplasm, pink in azan, violet in thionin, while the distal end develops separate pinkish spherules, as in the excretory cells at the earlier phase (Fig. 8 A).

In *Otina* the spherical fragments of digestive cells, cast off during the excretory phase, appear for the most part to be rejected particles laden with waste material. On reaching the stomach, they become plastered on to the protostyle or pass directly into the intestine. In some individuals of *Otina*, however, sectioned after 2 days' starvation, the digestive epithelium presented a ragged, highly fragmented appearance. The stomach was filled with a watery mucus, containing, together with excretory spherules, large nucleated portions, roughly spherical, or of rather irregular shape, derived from the bases of the digestive cells. The nuclei were generally polar in position, and the cytoplasm was vacuolated and filled with clear-staining spherules, giving the thionin reaction, like those of the basal parts of the digestive epithelium (Fig. 8 C). These fragments of the digestive gland are of interest in closely resembling the 'phagocytic cells' described by Millott (1937) in the nudibranch *Jorunna*, as originating by abstraction from digestive cells. Forrest (1951) has also observed them in a number of other dorids. They differ from the fragments of the digestive gland extruded into the stomach in prosobranchs, and the constricted tips of the digestive cells in *Otina*, in retaining nuclei, and, according to Millott, presumably some power of free movement. In *Jorunna* they absorb, and in part digest, such particles as introduced blood corpuscles within the stomach, providing, as it were, a means of non-localized intracellular digestion. In *Otina*, from the small size of the stomach and the difficulty of introducing experimental foods, no evidence could be gained as to their role in digestion. Their thionin-staining vacuoles may be presumed, as in the digestive cells, to be the source of enzymes.

From the following experiments, it is clear that the stomach in *Otina* contains active enzymes.

The substrate employed was the gelatin film of a developed photographic plate. Whole digestive glands, and separate stomachs from which all traces of digestive glands had been carefully removed, were pressed out on the plate and kept moist under cover-slips. The stomachs selected were empty of food or detritus, and filled with watery mucus containing yellowish brown cell fragments from the digestive gland. After 24 h, the gelatin film beneath the crushed stomachs had been in each case completely and cleanly digested away. Digestion by the stomach fluid proceeded in fact much more rapidly than by the macerated digestive glands themselves, where the gelatin became much softened but only slightly eroded from the plate. Obviously, in *Otina*, digestion is by no means all intracellular, and material absorbed by the digestive cells has evidently undergone previous breakdown by enzymes in the stomach. Enzymes are active in the stomach after a day's starvation, and it would appear that their origin is to be sought in the so-called 'fragmentation phagocytes' derived from the digestive cells.

Wandering amoebocytic cells from the subepithelial blood cells appear to play little or no part in digestion in *Otina*, either in the stomach or in the digestive gland. In the wall of the stomach they are extremely scarce or absent. This is in contrast to their frequent presence in prosobranchs (*Struthiolaria* (Morton, 1951)); *Lunella* (Turbinidae) (Morton, unpublished) and in lamellibranchs (Yonge, 1926). They appear to be characteristic of stomachs with wide ciliary sorting surfaces, and often wander through the epithelium of the sorting area in very large numbers. Their comparative absence in *Otina* and in opisthobranchs may be correlated with the reduction in importance of the sorting area. Ciliary sorting and amoebocytic ingestion appear as a rule to go hand in hand.

#### REPRODUCTIVE SYSTEM

Like the Ellobiidae dealt with in a previous paper, *O. otis* is a protandrous hermaphrodite. The genital system is of the most primitive type found among the pulmonates; the duct is undivided and hermaphroditic throughout, except for a bifurcation into male and female channels at the extreme anterior end, in front of the glandular genital mass, and only a short distance behind the external openings. As in *Leucophytia*, the gonad shows a marked occurrence of two phases, a sperm-producing phase occupying the autumn and earlier winter, followed by an egg-producing phase culminating in oviposition. The condition of protandry is most evident from the condition of the gonad itself; the glandular genital tract reaches its female condition in advance of the completion of spermatogenesis, and at no time during sexual maturity are the female ducts absent. The prostate, however, always displays its greatest

development during the male phase, and is reduced during the female phase, when the development of the albumen gland and mucous gland is accentuated. For the sexual development of *Otina otis* over a single reproductive season, a cyclic diagram may be constructed which differs in scarcely any feature from that presented for *Leucophytia* (Morton, 1955a).

The arrangement of the accessory glands is illustrated in Fig. 9. The little hermaphrodite duct conveys sex products from the gonad, to open into the large or glandular hermaphrodite duct. The albumen gland opens into the large hermaphrodite duct at the same point. The proximal part of the large hermaphrodite duct forms the posterior mucous gland. As it runs farther forward, the duct retains its single lumen, though it is divisible on histological grounds along its whole length into an anterior mucous gland, constituting the female tract, and a prostate, forming the male channel. A non-glandular conducting tube, the distal common duct, takes its origin from the region of the anterior mucous gland and prostate, and continues forward along the floor of the haemocoele, towards the right side of the head. It divides anteriorly into a narrow vas deferens and vagina. From the vagina, just in front of this bifurcation, arises a large bursa copulatrix. A second sperm storage sac, the accessory bursa copulatrix opens by a long stalk from the distal common duct, immediately behind the origin of the vagina and vas deferens. Pelseneer's short description and figure (1901) is thus fairly accurate in outline, though the albumen gland and posterior mucous gland are indicated only diagrammatically, and the relations of the anterior part of the tract are not shown in detail. No previous account exists of the histology or functions of the genital tract of *Otina*.

The hermaphrodite gland or ovotestis forms a single pouch lying on the concave surface of the visceral spire, applied to the posterior lobe of the digestive gland. Its cavity is partly divided by thin trabeculae of connective tissue bearing the germinal cells and giving to the gonad externally the appearance of a cluster of rounded follicles. The first part of the little hermaphrodite duct is a narrow transparent tube, which courses forward ventrally, close to the mid-line, and before reaching the albumen gland becomes strongly convoluted, its coils often being distended and sacculated (*lhd*). These coils are opaque white through the greater part of the year, containing large amounts of stored sperm, and thus function as a simple vesicula seminalis. The epithelium is of simple columnar or cubical cells with round nuclei and with a long ciliary coat. There are no glands, and only a very sparse muscle coat, two or three fibres in thickness, both longitudinal and circular. Resorption of sperm was never observed, nor do the sperms become orientated.

The structure of the large hermaphrodite duct may be followed from Fig. 9. The little hermaphrodite duct opens into it at the base of the posterior mucous gland (*p muc*) which forms a rounded translucent sac, lying to the right side of the oesophagus, about half way along the trunk, beneath the thin pallial floor. The albumen gland (*albg*) is applied to the posterior mucous gland

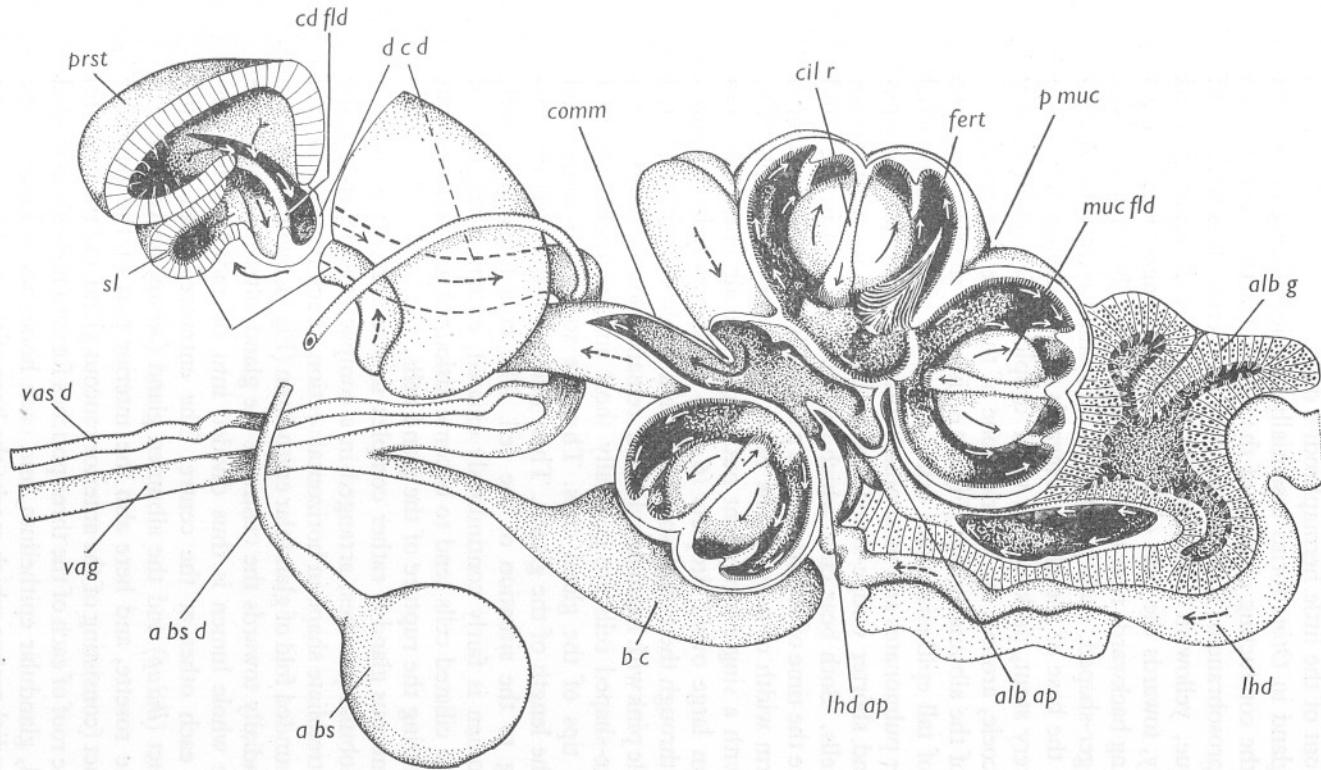


Fig. 9. *Otina otis*. Stereogram of the glandular regions of the genital tract, viewed from the median aspect and dissected to show the internal structure and the course of the ciliary currents. The anterior-most part of the albumen gland is represented in longitudinal section; the roof of the posterior mucous gland has been removed; and the prostate with the anterior mucous gland and distal common duct has been cut across transversely and the two halves separated to show the relations of the three ducts. The anterior parts of the vagina and vas deferens, with the penis, have been omitted. *abs*, accessory bursa copulatrix; *absd*, duct of the accessory bursa copulatrix; *alb ap*, opening of the albumen gland into the posterior mucous gland; *albg*, albumen gland; *comm*, communication between the anterior and posterior mucous glands; *bc*, bursa copulatrix; *cd fld*, longitudinal fold dividing the cavity of the distal common duct; *cil r*, ciliated ridge running round a fold of the posterior mucous gland; *acd*, distal common duct; *fert*, fertilization pouch; *lhd*, little hermaphrodite duct; *lhd ap*, opening of the little hermaphrodite duct into the posterior mucous gland; *muc fld*, glandular fold of the posterior mucous gland; *pmuc*, posterior mucous gland; *prst*, prostate; *sl*, longitudinal slit by which the prostate and anterior mucous gland communicate; *vag*, vagina; *vasd*, vas deferens.

behind, opening directly through its ventral wall by a short wide duct, immediately in front of the little hermaphrodite duct.

The albumen gland in *Otina* forms essentially a series of long diverticula discharging into the conducting channel of the genital tract; its cavity is no longer, as in the prosobranchs, traversed by eggs or sperm. It is built up of a series of opaque, yellowish white lobules, which are irregularly fused together anteriorly, towards the base of the gland. The longest lobule forms a thick strap passing backwards near the ventral mid-line. The smaller lobules are blunt and finger-shaped, each penetrated by a small cleft opening into a wider lumen at the base of the gland. Towards the egg-laying period, the lobules become very stout, packed with white droplets of secretion, tightly filling the haemocoele, around the anterior lobe of the digestive gland.

The histology of the albumen gland (Fig. 10A) is of the simplest type; the lining is formed of tall epithelial gland cells, and there are no subepithelial glands as in higher pulmonates and most opisthobranchs. There are two types of cell, smaller and shorter wedge-shaped cells, and much more numerous columnar gland cells. Both bear cilia, which are, however, lost in the gland cells shortly before the time of secretion. The gland cells are  $40-50\mu$  in length and have a uniform width of  $9-10\mu$ . The basal nuclei are almost spherical, darkly staining, with a single prominent nucleolus. The albumen spherules range in size from large ovoid droplets ( $6\mu$ ) to smaller rounded granules scattered densely through the cytoplasm. They stain bright red with azan, red with Masson's, pale pink with haematoxylin, and remain colourless after mucus stains. The wedge-shaped cells are generally short and triangular, inserted between the free tips of the gland cells. They are sometimes longer and attenuated, half the length of the glands. Their nuclei are spherical or compressed according to the situation of the cell. The cilia form long tufts,  $8-10\mu$  tall; the lumen is fairly continuously ciliated, except at the time of secretion, when the ciliated cells tend to become dislodged and squeezed out into the lumen, during the rupture of the gland cells.

The posterior mucous gland is rather complex in structure. It is built up of three plump globular pouches, arranged in a compact rosette, so that the whole gland is of trefoil shape in horizontal section. Each pouch is in turn subdivided by a rounded fold of glandular epithelium (Fig. 9, *mucfld*) the three folds extending radially towards the centre of the gland, alternately with the pouch walls. The whole lumen is thus divided into six radial clefts, communicating with each other at the centre. The entrances from the little hermaphrodite duct (*lhdp*) and the albumen gland (*albap*) lie ventrally, at the middle of the rosette, and here also the anterior part of the glandular hermaphrodite duct (consisting of the anterior mucous gland and the prostate) makes its exit. The roof of each of the three pouches forms a horseshoe-shaped tract of thickened, glandular epithelium, and each horseshoe is indented by a tongue-shaped radial groove which marks the base of the internal fold. The

outer wall of each pouch is of ciliated and glandular epithelium,  $15-20\mu$  in height. The dividing fold is formed by a septum of connective tissue with sparse muscle fibres. On either side of the septum arises a hemispherical group of gland cells ( $25\mu$ ) with rounded, basal nuclei. Their contents are colourless or pale blue in azan, bright red in mucicarmine and black with thionin (Fig. 10B). Interspersed with the gland cells are ciliated cells, narrow and tapered, but somewhat shorter than the glands. Their nuclei are compressed and rod-like and the cells broaden at the free surface to bear a tuft of short cilia. The mucous cells become very plump during their secretory phase, but are at other times much less developed, and the glandular fold is only slightly thicker than the rest of the lining of the pouch. Around the mid-line of the fold runs a narrow ridge (Fig. 9, *cilr*) made up wholly of ciliated cells. These form a wedge-shaped cluster in section, fanning out from the edge of the septum of connective tissue. The ciliary currents in the mucous gland are shown in Fig. 9. Over each hemisphere of gland cells, currents beat downwards towards the base of the fold, while along the bottom of each fissure runs a narrow ridge (*ri*) along which currents beat inwards towards the centre of the lumen. Along the ciliated tract *cilr*, running round each fold, the ciliary beat is also radial, towards the middle of the mucous gland.

It appears that the eggs pass through each of the three pouches of the posterior mucous gland in succession, receiving thin mucoid capsules before being carried forward into the anterior mucous gland. At the centre of the posterior mucous gland, where the little hermaphrodite duct opens, the paths followed by the eggs and sperm become separated. Sperm is directed forward into the anterior mucous gland-prostate portion of the common duct, and bypasses the mucus-secreting pouches. From the arrangement of the pouches, it would appear that the eggs first pass from the central lumen into the fertilization pocket (Fig. 9, *fert*) which is a wide dorso-ventral slit, formed simply as an outgrowth of the lateral pouch of the mucous gland. It is lined with columnar epithelium,  $30\mu$  tall, which contains no glands, and is distinguished by its very long cilia ( $100-120\mu$ ), extending in a flame-like tuft through the whole length of the slit. The fertilization pocket is directly continuous at its entrance with the wide duct of the albumen gland; its columnar cells merge into the shorter ciliated epithelium, which lines the albumen duct and isolated cells with albumen granules sometimes occur amongst the cells at the base of the ciliary flame. The cilia of the fertilization pocket could not be induced to beat in excised material; but the function is probably to expel the fertilized eggs, with their albumen coat, into the remaining portion of the mucous gland. The eggs appear to pass next through the two divisions of the posterior pouch, thence through the middle pouch, and arrive finally, surrounded by a mucoid capsule, in the anterior compartment of the lateral pouch. From the anterior wall of this pouch, a wide slit communicates with the anterior mucous gland-prostate division of the genital tract. Sperm as well

as ova are conveyed forward along this channel by a common duct which first runs towards the mid-line in a long V, then turns obliquely forwards. The sperm then pass along the prostatic side of the duct, and the eggs along the anterior mucous gland.

The anterior mucous gland (*amuc*) and the prostate (*prst*) lie together on the right side, ventro-laterally to the pharynx, and immediately beneath the thin epithelium of the pallial cavity. The prostate is the more superficial; it is broad and dorso-ventrally compressed, arched against the pallial floor, and is kidney-shaped, convex laterally and concave towards the mid-line. The prostate terminates bluntly at either end; its lumen is spacious, and leads by a wide longitudinal slit, along the concave side, into the anterior mucous gland. The two glands are in open communication for some three-quarters of their common length. The histology of the anterior mucous gland is essentially like that of the posterior. The secreting cells, however, stain deeper blue with azan, and they become distended with mucus at an earlier stage. This portion of the duct is very enlarged and tumid immediately before egg-laying; its cells produce the mucous mass with which the egg capsules are surrounded.

The lining epithelium of the prostate (Fig. 10D) is simple and unfolded, consisting of a single layer of very characteristic secreting cells. These are tall, and stoutly columnar ( $50 \times 10-20\mu$ ), and thus a good deal larger than the cells of any other region of the genital tract. Some of the cells are especially wide, and narrowly compress their neighbours. In vertical section they are rectangular or broadly ovoid; the free surfaces are either entire, or broken by the discharge of contents, often bulging convexly into the lumen. The basal nuclei are exceptionally large ( $10\mu$ ), almost spherical, though sometimes compressed against the base of the cell. They are densely granular and stain darkly in azan and iron haematoxylin, with two prominent nucleoli. The distal two-thirds of the cell are filled with rounded or irregular secretion granules, averaging  $7-8\mu$  across, closely crowded in the cytoplasm. They stain bright blue in azan, green in Masson's, remaining colourless after haematoxylin or stains for mucus. The prostatic secretion evidently serves as a nutritive component of the semen and can be recognized, persisting with foreign sperm in the bursa, long after copulation. The basal portion of the cytoplasm, in which the nucleus lies, is coarsely but uniformly granular, without separate inclusions, and stains lightly pink in azan. Ciliary cells are rather scarce in the prostate. They form little triangular wedges filled by small compressed nuclei ( $5\mu$  in length) between the tips of the glandular cells. Their cilia are extremely long, forming whip-like tufts ( $40-50\mu$ ) trailing into the lumen, and helping to lash the secretion forwards. Beneath the epithelium, the prostate has a rather strong coat of circular muscle, ten to twelve fibres deep, and reaching  $5-6\mu$  across.

The anterior mucous gland and the prostate are accompanied along their whole length, by a third, and much smaller tube, the distal common duct

(Fig. 9, *dcd*). This lies underneath the prostate and immediately lateral to the mucous gland, but is not itself glandular. It is muscular-walled and lined with long cilia, and originates anteriorly from both glands together, towards their blunt, anterior ends. Running at first backwards, the distal common duct soon becomes quite separate from both the mucous gland and the prostate. It conveys both sperms and ova to a point level with the posterior end of the prostate and then turns sharply forwards to run towards the head. The stereogram (Fig. 9) shows the relations of this duct to both the mucous gland and the prostate. Its wall is thrown into three or four impermanent longitudinal folds, and anteriorly, towards the opening from the prostate and mucous gland, its lumen becomes divided by a tall longitudinal fold of the ventral wall; and the duct dilates to about the same diameter as the tapered anterior end of the mucous gland. The prostate and the mucous gland are themselves partly divided by a large fold, which projects obliquely across their common lumen. Anteriorly, the ventral fold of the distal common duct fuses along its free edge with this dividing fold, so as to separate completely the lumen of the prostate from that of the mucous gland, and to form a separate opening from each into the distal common duct. Thus, on the right, a long fissure from the prostate opens into the right compartment of the common duct; into the left compartment opens the mucous gland by a slit-like aperture, which extends forward beneath the prostate, as far as the blind anterior end of the common duct. Farther backwards, the distal common duct narrows, and becomes quite closed off; the dividing fold along its ventral wall disappears. By the division of this duct at the openings from the glandular tract, eggs and sperm are directed separately into the common conducting channel. The subepithelial layers are strongly muscular, and access from either the prostate or the mucous gland can be closed off at need, by approximation of the edges of the slit leading to the distal common duct.

Opposite the posterior end of the prostate, the distal common duct runs down ventrally, and gives off the narrow duct of the accessory bursa copulatrix (Fig. 9, *absd*). It then plunges beneath the sheet of transverse muscle flooring the haemocoele, and runs forward, just beneath the body wall, along the right side of the foot, below the line of fusion of the mantle with the body wall. Almost at once it divides to form two narrow conducting channels, a dorsal vas deferens (*vasd*) and close beneath it, the vagina (*vag*) rather more distensible, but normally of the same diameter as the male duct (30–40  $\mu$ ). The lining of the vagina varies a good deal according to its state of contraction. As a rule, to within a short distance of the female opening, it is little folded. It is lined with tall columnar cells, with long, rather sparse cilia. There are no gland cells. The muscle coat includes both longitudinal and circular fibres. Just after the departure of the vas deferens, the vagina gives off from its ventral wall a narrow duct, of the same histological structure, with a strongly muscular wall. This duct passes back to enter the haemocoele, and, turning

towards the mid-line, expands into an ovoid, dorso-ventrally flattened sac, the bursa copulatrix. The vagina opens by a small, ventrally directed aperture, at the base of the foot, concealed beneath the right oral lappet. Close to the aperture its wall becomes much more distensible than farther back; the epithelium lower and thrown into triangular folds, mainly from differences in the size of the component cells. The long cilia beat outwards, carrying away waste secretions, and probably assisting the muscular contractions of the wall in oviposition.

The short vas deferens has a strong coat of circular muscle. It is lined with tall, ciliated epithelium rather resembling that of the vagina. The cilia are long, reaching the centre of the lumen. The vas deferens runs close to the vagina as far as the female aperture, at the side of which it turns inward to run alongside the penis. The male aperture is a small pore, separate from and just mesial to the female opening. It leads into a short muscular tube, which represents the invaginated penis. The intromittent organ in *Otina* has perhaps the simplest structure yet observed in any pulmonate. The penial tube is perforated at its tip by the vas deferens, and when everted, has a length about equal to that of the vagina as far as the bursal duct. There is no retractile papilla, and the organ forms, in the terminology of Hubendick (1945) a 'pseudopenis'. When invaginated it curves obliquely backward in the body wall, passing mesially to the penial nerve, and to the nerve arising from the cerebral ganglion and going to the oral lappet. Its apex forms a small bulb, lying in the cephalic haemocoele, in contact with the mesial surface of the right cerebral ganglion, close against the roof of the buccal mass. The retractor muscle is a slender strap, running to the left across the pharynx, and inserted on the floor of the trunk, midway along the body cavity.

The penial tube is circular in section,  $80\mu$  in diameter, near the middle of its length. Towards the male opening, its lining is sparsely ciliated, elsewhere thinly cuticularized. Its epithelium forms six triangular longitudinal folds, with underlying muscle fibres. The circular muscle layer forms a prominent external coat. The vas deferens runs to the tip of the penial tube through the connective tissue of one of the folds; it forms here a very narrow tube,  $12\mu$  across, and its dense cilia fill the whole lumen.

Of the two sacs for the storage of sperm, the vaginal bursa copulatrix is the larger (*bsc*), and receives foreign sperm after copulation. It lies closely against the floor of the haemocoele, ventrally to the mucous gland on the right side. The bursa and its duct are both muscular, serving, together with the pressure of blood in the haemocoele, for the expulsion of sperm into the vagina. The duct forms an effective sphincter controlling the release of stored sperm. After copulation, the bursa contains a mass of living sperm, never oriented or attached to the epithelial cells. There is also a large amount of prostatic secretion which may persist for a time after the sac has emptied of sperm. The lining epithelium (Fig. 10 C) is composed of simple columnar cells of uniform

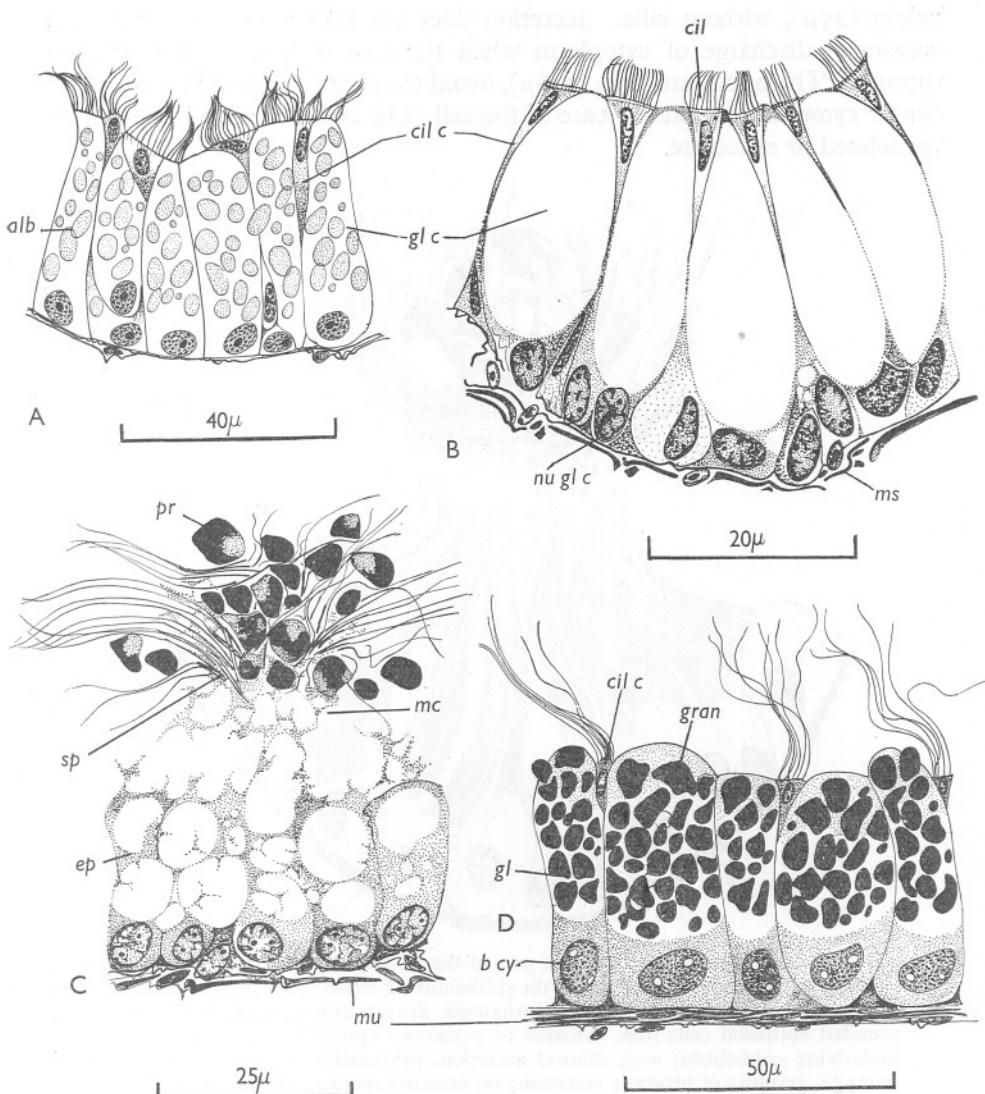


Fig. 10. *Otina otis*. A: histology of the albumen gland (Bouin's; azan). *alb*, droplets of albumen secretion; *cil c*, ciliated cell; *gl c*, gland cell. B: histology of the posterior mucus gland (Bouin's; Masson's trichrome). *cil*, cilia; *cil c*, ciliated cell; *gl c*, gland cell; *ms*, muscle fibre; *nu gl c*, nucleus of gland cell. C: histology of the bursa copulatrix (Bouin's; Masson's trichrome). *ep*, epithelial cell; *mc*, mucus material of seminal fluid; *mu*, muscle fibre of subepithelial layer; *pr*, granules of prostatic secretion; *sp*, sperms. D: histology of the prostate (Bouin's; azan). *b cy*, denser basal cytoplasm of a gland cell; *cil c*, ciliated cell; *gl*, gland cell; *gran*, granules of prostatic secretion; *mu*, coat of circular muscle.

height ( $25\mu$ ), without cilia. Secretion does not take place, except by the occasional discharge of cytoplasm when the free surfaces of the cell are ruptured. The nuclei are large ( $5-6\mu$ ), ovoid to spherical, and lie in an area of denser cytoplasm round the base of the cell. The cell contents are elsewhere vacuolated or reticulate.

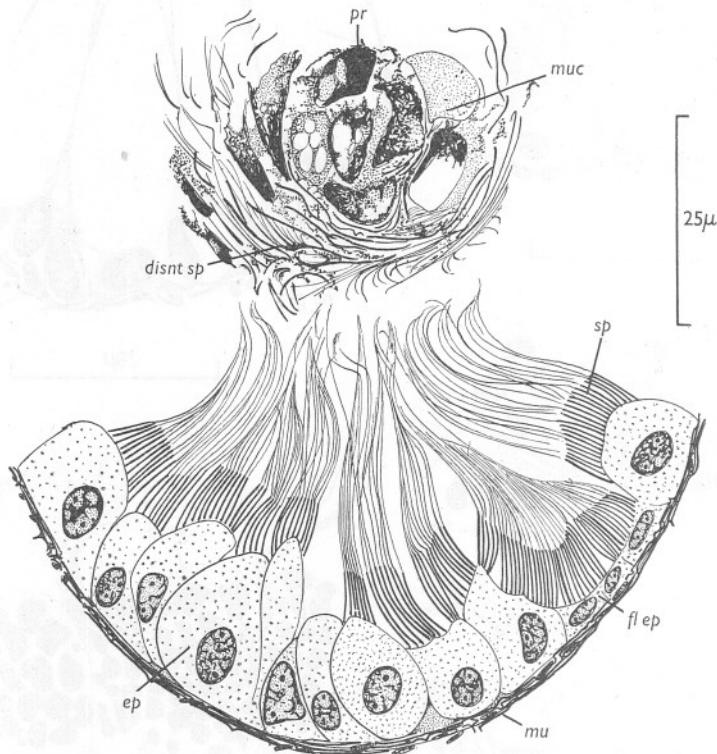


Fig. 11. *Otina otis*. Transverse section of part of the wall of the accessory bursa copulatrix, showing the attachment of sperms to the epithelium. Portion of the mass of disintegrating contents at the centre of the lumen is also figured. *disnt sp*, disintegrating sperms; *ep*, larger, rounded epithelial cell; *flep*, flattened or squamous epithelial cell; *mu*, muscle fibres underlying epithelium; *muc*, mucoid secretion, presumably derived from the genital duct; *pr*, granules of prostatic secretion; *sp*, oriented sperms. (Bouin's; azan.)

Arising from the distal common duct, the duct of the accessory bursa copulatrix sweeps across the dorsal surface of the prostate, and passes obliquely backward towards the mid-line. It expands gradually into a terminal sac,  $150-200\mu$  in diameter (Fig. 9, *abs*) which is hidden from dorsal view by an overlying lobe of the digestive gland. The lining epithelium (Fig. 11) is of a rather distinctive kind; the cells may be in places flattened or squamous, especially during distention by sperm, but more often are plump and rounded, bulging freely into the lumen, the largest cells deeply indenting

their neighbours. The nuclei are extremely large and rounded, with two nucleoli, central in position or displaced upward by compression. There are no gland cells, but, as in the vaginal bursa, cytoplasm may be released by rupture of the cell walls. From December until May, the accessory bursa is filled with sperm which are attached in clusters to the epithelial cells by their filiform heads. They also become attached to the flattened cells lining the duct, within a short distance of the terminal sac. In the middle of the lumen is usually a compact bolus of disintegrating sperm, appearing pinkish brown through the transparent wall. It consists of sperm heads, intermixed with fragments of cells, and with layers of mucoid and prostatic secretion.

Sperm do not appear to remain long in the vaginal bursa after copulation. Living sperm are evidently stored for some time within the accessory bursa; but whether sperm from this sac travel farther up the genital tract to the fertilization pouch, or whether fertilization is effected by sperm newly released from the vaginal bursa, is still uncertain. Copulation, however, seems to occur several months in advance of oviposition, and the prostate becomes smaller and ceases to secrete by the end of March. An important function of the accessory bursa is the storage and breakdown of surplus sperm. Neither of the sperm sacs in *Otina* appears to be homologous with the receptaculum seminis of the prosobranchs and of *Actaeon* (Guiaart, 1901) which lies near the site of fertilization between the albumen and capsule glands. Here sperm is stored immediately after copulation and becomes oriented on the epithelium, and in some prosobranchs it is later resorbed.

#### EGGS AND DEVELOPMENT

*Otina* breeds at Wembury during the latter half of May and the first fortnight of June. The egg capsules are typically found in crevices, on more or less clean rock surfaces, in company with groups of the ovipositing animals. Each egg mass (Fig. 12 A) forms a small irregular cluster about 4–5 mm across, loosely attached to the substratum. It consists of a single layer of twenty to thirty eggs surrounded by a tough, translucent mucoid secretion, pale yellow or straw coloured. The eggs are ovoid, 0·3 mm in length, pale yellow in colour. They are surrounded by a layer of albumen, whitish and finely granular, and enclosed in a thin tough capsule, quite smooth and transparent. The egg capsule stains pale blue in azan, as well as black in thionin, identically with the cells of the posterior mucous gland from which it is evidently derived. The investing jelly appears to be the secretion of the anterior mucous gland which is always thick and distended prior to the time of egg laying.

Embryos in eggs were abundant at the end of the first week in June. At liberation (Fig. 12B, C) they are equipped with a wide-throated, trumpet-mouthed shell, quite transparent and with only a few fine growths striae for sculpture. The visceral mass, which is yellow from the large amount of yolk, is contained in the large apical bulb, and the distinctive character of the

in Pulmonata but represents the usual condition in Prosobranchia. On either side, the parietal ganglia are closely drawn into the nerve ring, the pleuro-parietal commissure being on the right very short and on the left wholly lacking as such. There remains in *Otina* a rather long visceral loop, retaining no trace of torsion, and formed, unlike that of the ellobiids, by the parieto-visceral commissure of each side, leading back to the single visceral ganglion.

Turning to the digestive system, the simple character of the stomach may to some extent be a consequence of the small size of the animal, as well as an indication of the primitive condition of this organ. The absence of the posterior caecum, receiving the major typhlosole, seems—for instance—to be a feature in which *Otina* differs from all of the less specialized genera of the Ellobiidae. The lack of a differentiated muscular gizzard is another primitive negative character, which must be possible in *Otina* only by reason of the fine, well-communited nature of the food particles. Adding to the lack of a gastric gizzard the presence of ciliated excurrent grooves from paired digestive diverticula, and of the vestige of an intestinal 'style sac', we may recognize in the otinid stomach a most convincing transitional stage between the condition of the microphagous Prosobranchiata and that of the 'higher' Basommatophora. A 'ballooning-out' of the posterior muscular pocket, and the further strengthening of its cuticular lining, would give to this part of the stomach, in *Otina*, the gizzard-like appearance which is a feature of the ellobiids and of all the other aquatic pulmonates yet investigated.

The structure of the digestive gland agrees with what is known of other primitive pulmonates and of at least some opisthobranchs. The glandular epithelium undergoes a cycle of ingestion, excretion and fragmentation. At the last stage it would appear that 'fragmentation phagocytes' become abstricted from the digestive cells, to wander into the stomach where they carry out a non-localized absorptive and digestive function. The occurrence of such a digestive gland, with the fragmentation of its absorptive epithelium, seems to be correlated in pulmonates and opisthobranchs with the loss of the extensive gastric sorting area of the prosobranchs. Amoebocytic cells, of the type which primitively wandered into the lumen of the gut from the blood vessels underlying the sorting area, are now replaced by 'phagocytes' originating directly from the digestive epithelium.

The reproductive system, like the stomach, remains at a primitive level in *Otina*. An archaic feature, which is shared by most of the ellobiids, is the open communication between the male and female regions of the glandular genital tract. Further, there are indications that at a recent time in phylogeny the male and female genital apertures were widely separated, the female aperture near the pallial opening and the (secondary) male aperture far forward on the head, as is still found in the ellobiids and in all tectibranchs. In *Otina* these two apertures have now moved secondarily close together, both lying beneath the right oral lappet. From the original common genital aperture, giving rise to the definitive female opening, the secondary male

aperture must first have been carried forward to the head, by the usual shallow extension of the vas deferens, forming an open groove in tectibranchs and a closed tube just beneath the integument in pulmonates. In *Otina*, apparently uniquely, the female aperture has been carried anteriorly from its pallial position in the same manner, by the prolongation of the vagina as a narrow muscular tube, closely accompanying the vas deferens.

The albumen gland in *Otina*, like that of the ellobiids, but unlike the same gland in the actaeonids, has lost its primitive pallial position and has entered the haemocoele, where it no longer forms a tubular section of the genital duct traversed by the eggs, but serves as a glandular annex communicating rather narrowly with the mucous gland in the neighbourhood of the fertilization pouch. In the male portion of the glandular hermaphrodite duct, the prostate has its characteristic pulmonate position, incorporated in the main course of the genital tract, rather than—as in opisthobranchs—forming a glandular diverticulum of the male duct. The absence of a well-developed muscular penial papilla, and the functioning of the preputium as a ‘pseudopenis’ must probably be regarded as a specialization due to small size.

Most of the earliest pulmonates, as characterized by the ellobiids, retain a heterostrophic embryonic shell, held to be a common inheritance from the ancestral stock of both pulmonates and opisthobranchs (see Morton, 1955b). In *Otina* a reduced heterostrophy is still to be seen in the shell of the embryo. Such a condition resembles that of *Leucophytia* and other ellobiids mentioned by Harry (1951) and the pyramidellids (Fretter & Graham, 1949). In one ellobiid genus, *Melampus*, a well-developed velum is retained by a free-swimming larva, and in *Leucophytia* a large vestige of the velum survives in the unhatched embryo. In the embryo of *Otina* there is no trace of a velum. An operculum however persists in this, and in all ellobiid embryos studied.

A characteristic feature of pulmonates at a basal level of organization is the occurrence of a protandrous sexual succession. Such a phenomenon has already been described in *Leucophytia* and *Carychium*. A similar month-by-month survey of the condition of the gonad in *Otina* revealed an extremely close similarity to that of *Leucophytia*. It should again be pointed out that a clear-cut separation of the sexual stages with complete separation of male and female phases is never obtained. From September to December, while sperms are undergoing maturation in the gonad, small oocytes are already prominent in the germinal epithelium, and the female portions of the glandular genital duct are already fully differentiated, though of very small size. The period of female development, between December and June, is marked by the great increase in the relative size of the albumen and mucous glands, and the reduction in size of the prostatic epithelium. The earlier pulmonates thus show two sexual stages in a single season. A question not finally determined is whether *Otina* is an annual mollusc, or whether a restitution of male germinal epithelium is possible following the deposition of eggs. From the fact that animals of the whole size range were evidently equally abundant at all times

of the year, it would seem that *Otina*, like the majority of pulmonates, undergoes at least a biennial life cycle. An opposite example is that of *Skeneopsis*, a minute prosobranch, investigated by Fretter (1948) and found to have an annual life cycle, with a great falling off in the numbers of adults during winter months.

The limpet habit in the Basommatophora has been independently developed at least four times, and is associated either with resistance to wave attack on the seashore or with life in fast-flowing inland waters. With the acquisition of its 'lung', *Otina* must have evolved in a way similar to the larger limpet-like marine pulmonates, the Siphonariidae. The latter family has undergone a parallel reversion from the upper shore to a completely intertidal life (see the monograph by Hubendick, and also the recent papers of Yonge (1952) and Borland (1950). We may suppose that at the level of the origin of the first pulmonates the lung was acquired as part of the heritage of a primitive stock of supratidal gastropods. There are many examples, however, of a return to aquatic life, and among the lower Basommatophora, most of the families are characterized by an early retreat to the sea. Perhaps they never reached far beyond the shore, but acquired the lung—as, for example, in the Ellobiidae and the Amphibolidae—as an adaptation to easier aerial respiration in the turbid, oxygen-poor water of estuaries. Such pulmonates stand rather apart from the four families of the higher 'limnic Basommatophora' (the Lymnaeidae, Physidae, Planorbidae and Aculyidae). Their embryos carry opercula, and most of them bear vestiges of a velum. They retain as a mark of primitiveness a heterostrophic apex, and a relatively long visceral loop. The Siphonariidae are known to have arisen very early, according to Zittel (1923) in the Devonian. Probably the other families of primitive Basommatophora spring from an equally ancient stock; and in a reconsidered arrangement of the Pulmonata these marine families, with probably the Latiidae and Chilinidae, might be placed together in a natural assembly of 'Archaeopulmonata'.

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## UPTAKE OF RADIOACTIVE SODIUM ( $^{24}\text{Na}$ ) BY *NEREIS DIVERSICOLOR* MUELLER AND *PERINEREIS CULTRIFERA* (GRUBE)

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(Text-figs. 1-4)

### INTRODUCTION

*Nereis diversicolor* is typically euryhaline: it is in equilibrium with normal sea water (Schlieper, 1929; Beadle, 1937), and develops hypertonicity in more dilute media which it is able to maintain indefinitely. The maintenance of this steady state is preceded by a transition period. During this there is a rapid uptake of water accompanied by a loss of salts from the worm, resulting in a fall in the osmotic pressure of the body fluids, and then a subsequent water loss which, according to Ellis (1937), is not accompanied by an uptake of salts. Worms accommodated to 25% sea water weigh about 140% of their original weight (Beadle, 1931). Beadle (1937) found that when the steady state is attained the body fluid concentration in 50% sea water approaches a value which is about 5% higher than that of the external medium, whereas in 25% sea water the concentration of the body fluid is equivalent to about 44% sea water. His worms were collected from the Northumberland coast, and concentration measurements of the body fluid were determined by Baldes's modification of the Hill vapour-pressure method. The results of Schlieper (1929) on worms from Heligoland, where they may live in a salinity as low as 4‰, are based on freezing-point determinations and give a higher internal concentration in the dilute media: after 3 days in 50% sea water the concentration of the body fluid is equivalent to 68% sea water, and after 2 days in 25% sea water equivalent to 49% sea water. These varying results suggest the occurrence of local physiological races.

The method by which this hypertonicity is maintained is open to conjecture. Since the concentration of the body fluids is increased when an animal previously treated with dilute sea water is transferred to an isotonic solution, Beadle (1937) concludes that the mechanism must entail an active process which is not merely controlling the inflow of water. He cites three possibilities: the excretion of a hypotonic fluid by the nephridia; the addition of salts to the body fluids from the tissues, or, as in *Carcinus* (Webb, 1940), an active uptake of salts from the external medium. It is, of course, probable that more than one of these mechanisms is involved. Beadle favours

the first and states that the most reasonable conclusion is that the animal behaves as a normal osmotic system as regards uptake of water and change of internal concentration, but that the volume of the body fluids is continually being reduced by the removal in the nephridia of a fluid hypotonic to the body fluid. In a previous paper Beadle (1931) showed that the respiratory and the weight curves of an animal in dilute sea water are of the same form: they each have an approximately simultaneous maximum and a subsequent fall. He suggests that the extra oxygen consumption is not the result of osmotic work, but rather that of work done by the body-wall muscles in resisting swelling. That hypertonicity is maintained, at least in part, by the active uptake of salts from the environment is, however, suggested by the results of both Schlieper (1929) and Beadle (1937).

The uptake and loss of chloride by an organism is easy to demonstrate. It has been studied for a number of invertebrates as well as anamniotes. Ellis (1939) measured the chloride lost by *Nereis diversicolor* and *N. cultrifera* when placed in 20% sea water, and also its uptake when the worms are transferred back to 100% sea water. Isotopic indicators which are now in use give further scope for studying the passage of ions between an aquatic organism and its environment. By this means Jørgensen, Levi & Ussing (1947) have recorded the uptake of  $^{24}\text{Na}^+$  and  $^{38}\text{Cl}^-$  by the axolotl, and Abelson & Duryee (1949) the exchange of radioactive sodium by the frog's egg. Jørgensen & Dales (1954), by using the tracer  $^{36}\text{Cl}^-$ , have shown that at certain dilutions of the external medium there is an active uptake of chloride ions by *N. diversicolor* and *N. virens* which have been adapted to the diluted medium, and so are in a steady state with respect to their chloride content.

#### METHODS

The tracer element  $^{24}\text{Na}$  with a half-life of 14·9 h allows only short-term experiments to be carried out. It is clean to work with, however, and the slight amount adsorbed on to the surface of an organism is readily removed by washing in water. Sodium comprises the predominant cation of the environment of a marine animal, and the experiments of Ellis (1937) have shown that in *N. diversicolor* it is essential in the weight regulation associated with osmotic control. The uptake of the element by a living organism can be followed by using the apparatus designed by Arnott & Fossey (1952). It consists of 8 G.M. Tubes (20th Century, G 10 P6) set in a ring around a Perspex tube and connected in parallel. A second Perspex tube surrounds the counters and the whole is shielded by a lead castle, which can be opened at the top to the inner Perspex tube. For the present experiments a disc of sheet cork, with a hole centrally placed, was fitted horizontally into the top of the inner Perspex tube, and through the hole was slipped a boiling tube: the rim of the tube is held by the cork and the rest of it, which may contain the animal to be assayed, is exposed to the counters. A similar use of  $\gamma$ -Müller tubes as a

means of assaying different sources of radioactivity has been described by Freedberg, Ureles & van Dilla (1949) and Veall & Vetter (1952).

$^{24}\text{Na}$  obtained from A.E.R.E. Harwell as  $^{24}\text{Na}_2\text{CO}_3$  has the high specific activity of 32 mc/g. Tracer amounts never greater than 16 mg were added to each litre of sea water, which raises the sodium content by a maximum of 0.0148%. Each experimental worm was in 100 ml. of activated water in a darkened jar: the jar was shielded at the opening, and the water kept saturated with oxygen at normal atmospheric pressure. Experiments were maintained at a constant temperature, usually 14° C; others were at 5° C. Sea water used for the experiments had a salinity of 35‰. Its sodium was labelled with  $^{24}\text{Na}$ , and the activity of 1 ml., indicating the presence of 10.8 mg of inactive sodium, was recorded at the beginning of the experiment. Such a standard was used to calculate the uptake of sodium by a worm under specific experimental conditions. Distilled water was added to the sea water to give the various dilutions, and hypertonic sea water was obtained by slow evaporation of the normal sea water at a temperature not higher than 60° C.

#### UPTAKE OF SODIUM BY *NEREIS DIVERSICOLOR* AND *PERINEREIS CULTRIFERA* IN WATER OF VARYING SALINITIES

The uptake of sodium by *Nereis diversicolor* Müller was first studied on worms which were accommodated to the various salinities of sea water to be used in the experiments. These animals would be in a steady state with respect to their sodium content. Prior to the experiment the wet weight of each worm was recorded, and those weighing about 1 g were chosen. These worms were then placed in sea water of a salinity of 35, 33, 17.5, or 9‰, or in hypertonic water, which was kept saturated with oxygen at normal atmospheric pressure and was at a constant temperature of 14° C. The weight of all these individuals had reached a relatively steady value at 36 h (Beadle (1937) found that the regulation of the osmotic pressure is completed in advance of volume regulation and well within this time). Each animal was then dried on filter-paper, the weight recorded, and it was placed in the experimental vessel which contained sea water of the same salinity as that from which it had been taken, but with the tracer sodium added. After a period ranging from 1 to 2 h the worm was removed from the vessel, washed rapidly in three changes of sea water and presented to the counting apparatus. For animals in dilute sea water the uptake of sodium per g wet weight was calculated on the original weight of tissue, the osmotic uptake of water being neglected; allowance was made for the slight loss of weight due to starvation.

The results of such an experiment are shown in Fig. 1. In sea water of a salinity of 35‰ the uptake of sodium per hour ranges from 275 to 240  $\mu\text{g/g}$  wet weight; the higher figures appear to be associated with the more active worms. In a salinity of 17.5‰ the uptake per hour reaches an average of 161  $\mu\text{g/g}$  wet weight, which is rather more than might be expected if the worm

behaves as a simple osmometer; in water of about half this salinity (9%) the uptake may even exceed this figure, reaching an average of 180  $\mu\text{g}/\text{g}$  wet weight. In lesser dilutions, as in a salinity of 33.5%, the sodium influx is proportional to the degree of dilution, indicating a passive exchange of ions. For worms adapted to hypertonic sea water of a salinity of 43% the uptake is slightly less than might be expected. The hypertonicity of worms in brackish water may involve the uptake of ions in different proportions from the normal, since Cole (1940) found a differential accumulation in *Homarus* in similar conditions.

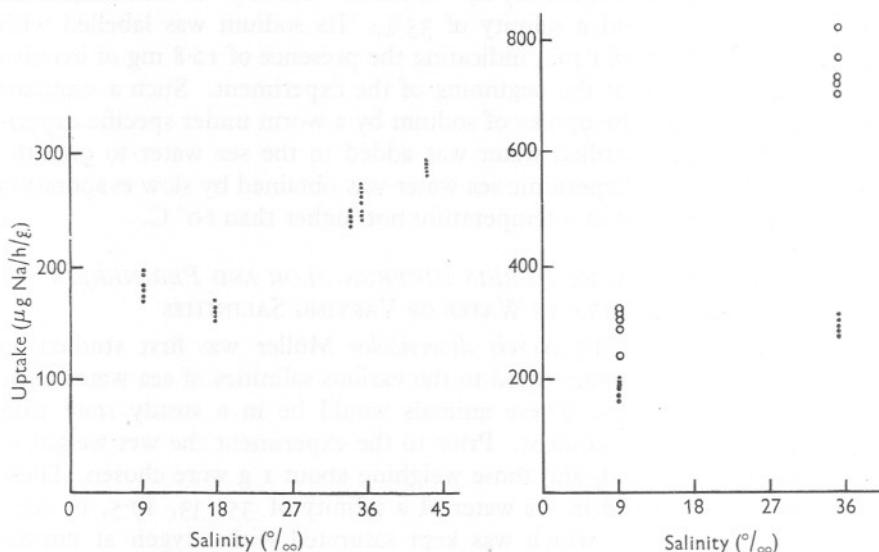


Fig. 1. *Nereis diversicolor*. Rate of uptake of sodium in sea water of different salinities. (Rate expressed as micrograms of sodium per hour per gram wet weight.)

Fig. 2. *Perinereis cultrifera* and *Nereis diversicolor*. Rate of uptake of sodium in sea water of 9% and 35% salinity. Rings indicate results for *Perinereis* and dots for *Nereis*.

Jürgens (1935) has shown that respiration takes place through the epithelium of the gut of *Nereis diversicolor* as well as through the integument. It may be that the gut wall can serve as an area of active absorption of ions for a worm in a hypotonic medium. To test this the previous experiment was repeated with a series of animals which had the body tied anteriorly and posteriorly with nylon thread to close the passage to the gut, and another series acted as a control. Both sets of worms were transferred from normal to diluted sea water, some in 50% and others in 25%, and they were weighed at intervals for a comparison of the weight regulation. When accommodation was completed the uptake of sodium was measured. The results (Table I) showed that the weight regulation of worms which had the gut obstructed, and were in

50% or 25% sea water, followed the normal course; and the uptake of sodium by these individuals showed only slight individual variations from the results of the control specimens.

These results suggest that only the integument is concerned with the uptake of salts, or that, when necessary, the integument can compensate for the loss of the gut surface as an area of transport to and from the body tissues.

TABLE I. UPTAKE:  $\mu\text{g Na/h/g WET WEIGHT}$

		Salinity	
		9%	17·5%
Gut obstructed	180	175	
	150	160	
	170	140	
Controls	183	164	
	200	150	
	170	170	

The uptake of sodium from 100% and 25% sea water was studied for *Perinereis cultrifera* in the same way as for *Nereis*. *Perinereis* is a more typically marine form, and although it can withstand a lowering of the salinity of sea water, its weight curve in brackish water increases to a higher value than that of *Nereis diversicolor*, and there is no subsequent fall. In 25% sea water the animal becomes sluggish. The results of an experiment in which both polychaetes were used for comparison are shown in Fig. 2. From these it can be seen that *Perinereis* is the more permeable to sodium ions: in normal sea water the uptake is nearly 3 times greater than in *Nereis*, and in water of about a quarter this salinity the uptake is at least 1·5 times as great. In the dilute medium even this species takes from the environment a relatively greater amount of sodium than worms in normal sea water: Wells & Ledingham (1940) state that in 25% sea water *Perinereis cultrifera* maintains a slight degree of hypertonicity. The lower permeability of the integument of *Nereis* must be of importance in diminishing the amount of work necessary to maintain osmotic independence. These results, however, are contrary to those of Ellis (1939). In measuring the chloride output of *N. diversicolor* and *Perinereis cultrifera* in 20% sea water he records a higher loss of chloride from the former than from the latter, and suggests that *Nereis diversicolor* swells less in dilute sea water because of the more rapid loss of salts relative to water intake.

The influx of ions to the tissues of worms which are transferred from dilute to normal sea water is high, and is approximately inversely proportional to the dilution of the medium from which they are taken. To estimate this the following experiment was carried out. The exchange of sodium per hour by twelve specimens of *N. diversicolor* which had been living in sea water of a salinity of 35% was calculated, and these individuals were then transferred

to a more dilute medium, four worms being placed in water of each of the following salinities, 27, 17·5 and 9‰. After 40 h they were returned to normal sea water containing  $^{24}\text{Na}$  and their uptake of sodium per hour was again measured—when returned to normal sea water the weight of the worm is reduced with the loss of water. The results are shown in Fig. 3, and the results of a similar experiment for a comparison between *N. diversicolor* and *Perinereis cultrifera* are given in Table II.

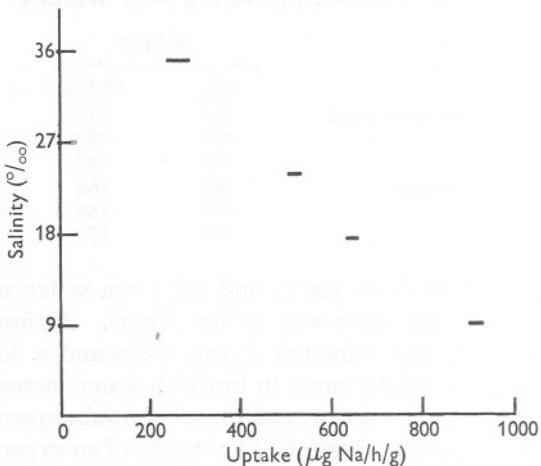


Fig. 3. *Nereis diversicolor*. The thickened bar at 35‰ salinity is the range of the rate of uptake of sodium per hour (per gram wet weight) for twelve worms. At the other three salinities is similarly shown the range of uptake by four worms when returned to sea water of 35‰ salinity from brackish water.

TABLE II.  $\mu\text{g Na/h/g}$  WET WEIGHT OF WORM

	Uptake per hour in normal sea water (salinity 35‰). Average of four worms	Uptake per hour on return to normal sea water after 26 h in dilute sea water (salinity 9‰). Average of four worms
<i>N. diversicolor</i>	260	969
<i>P. cultrifera</i>	720	2960

The increased uptake on return to normal sea water presumably compensates for the loss of salts in the lower salinities. The increase is about 3 times greater for *P. cultrifera* than for *Nereis diversicolor*.

The apparatus which has been used for these experiments makes it possible to follow the rate of uptake of radioactive sodium to the level at which equilibrium is reached, and the amount of the tracer element in the worm ceases to increase. From this level can be determined the total amount of exchangeable sodium. For *N. diversicolor* accommodated to water of a

salinity of 9% this is approximately 3·2 mg/g wet weight. For worms living in a salinity of 17·5% there may be only a slight increase over this amount, though it is doubled when the animals are in sea water of full salinity (35%) and for an average of twenty individuals was 6·3 mg/g wet weight. There would thus appear to be a correlation between the osmotic pressure of the body fluid and the amount of exchangeable sodium. In hypertonic sea water the amount increases: for the average of six worms in water of a salinity of 51% it reached a value of 7·2 mg/g wet weight. *Perinereis* has a higher permeability than *Nereis*, yet the total amount of exchangeable sodium per g wet weight in 100 and 25% sea water is slightly less than for *Nereis*.

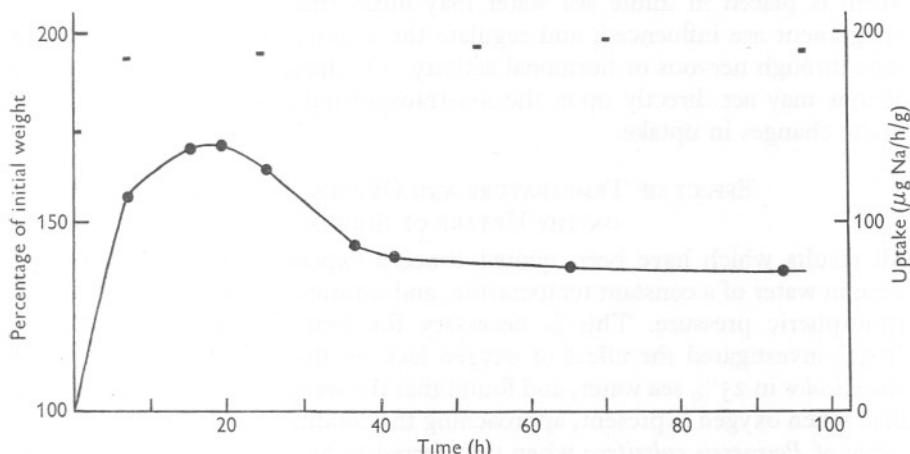


Fig. 4. *Nereis diversicolor*. Uptake of sodium per hour (per gram wet weight) during the period of weight regulation when transferred from sea water of 35% salinity to that of 9% salinity. Continuous line indicates weight regulation.

#### UPTAKE OF SODIUM BY NEREIS DIVERSICOLOR DURING THE PERIOD OF ACCOMMODATION TO DILUTE SEA WATER

No study has yet been concerned with the uptake of ions by *N. diversicolor* during the period of osmotic swelling and subsequent weight adjustment in water of low salinity. For this purpose freshly collected specimens were kept for a period of 36 h in normal sea water (35%). Twenty worms were then weighed individually and each isolated in a vessel containing water of a salinity of 9%. At a temperature of 14° C the typical weight curve followed the course shown in Fig. 4. At varying intervals of time along this curve certain worms were placed in activated sea water for an hour or two, so that an estimate of the sodium uptake at that point in the weight regulation could be made. Afterwards they were returned to the inactive water. The handling of worms in this way did not appear to have any adverse consequences. The results show that throughout the period of osmotic adjustment, which involves

varying volume changes, there is an active uptake of sodium from the brackish water at an approximately constant level. The only variation is at the beginning of the experiment when it is lower, though not sufficiently low to suggest that there is any serious time lag in the establishment of the active uptake. An experiment for worms transferred from water of a salinity of 35‰ to that of 17·5‰ gave similar results.

Thus it seems that, as in *Eriocheir* (Krogh, 1939), the passage of ions through the integument is unrelated to the passage of water, though the two events take place simultaneously. This suggests two independent transport processes. The rapidity with which the active uptake of ions is established when the worm is placed in dilute sea water may mean that chemoreceptors in the integument are influenced, and regulate the activity of the ion-transporting cells through nervous or hormonal activity. Or changes in the concentration of ions may act directly upon the ion-transporting cells and induce appropriate changes in uptake.

#### EFFECT OF TEMPERATURE AND OXYGEN DEFICIENCY ON THE UPTAKE OF SODIUM

All results which have been quoted concern experiments in which worms were in water of a constant temperature, and saturated with oxygen at normal atmospheric pressure. This is necessary for comparative results. Beadle (1931) investigated the effect of oxygen lack on the weight curve of *Nereis diversicolor* in 25% sea water, and found that the weight rises to a higher value than when oxygen is present, approaching the condition of the normal weight curve of *Perinereis cultrifera* when transferred to 25% sea water. Moreover, there is no subsequent loss of water from the tissues, which suggests that this is an active process carried out only in the presence of oxygen. Oxygen deficiency also has an effect on the uptake of sodium ions. When *Nereis* is taken from normal sea water and placed in brackish water with a low oxygen content—water through which nitrogen has been passed, or even water which is not kept saturated with oxygen—the rate of active uptake of sodium is above the normal, and the total amount of exchangeable sodium increases; these differences become greater with decreasing salinity. For a worm taken from 100% and placed in 25% sea water the influx in 20 h is twice as great as when the water is saturated with oxygen: for a worm already accommodated to 25% sea water, and therefore with a lower oxygen consumption, it is less.

A low temperature also influences the weight adjustment of worms which are transferred from normal to dilute sea water. Beadle (1937) working with *N. diversicolor* collected from the estuary of the River Blyth, Northumberland, found that 'during the winter months the increase in weight in 25% sea water was greater and more prolonged than during the summer', though his graph shows the reverse of this. He does not, however, give the winter temperatures. Results of my experiments carried out at 5°C agree with his

statement. At 5° C the weight curve for animals placed in 25% sea water rose slowly during 24 h to over 1·8 times the weight in normal sea water, which exceeds the weight increase at 14° C, and it showed no reduction for the following 24 h. At all salinities the penetration of sodium was slower at this lower temperature. Particularly noticeable is the fact that worms in 25% sea water, where more osmotic work is needed, take up less per h per g wet weight than those in 50% sea water. It is known that high temperature reduces the degree of hypertonicity which must be maintained by an animal for survival in dilute media. Pannikar (1940) suggests that it is for this reason that the most active colonization of fresh and brackish water by marine animals takes place in the tropical areas. May this not also be due to the fact that the processes concerned with osmoregulation are carried out more speedily at higher temperatures and so are less impeded?

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#### SUMMARY

In sea water of a salinity of 35‰ saturated with oxygen at normal atmospheric pressure and at a temperature of 14° C, the uptake of sodium by *Nereis diversicolor*, which is in equilibrium with its environment, ranges from 275 to 240 µg/h/g wet weight. The higher uptake appears to be associated with the most active worms. When the salinity is reduced by half the uptake, for worms accommodated to this dilution, averages 160 µg/h/g wet weight, and at 9‰ it averages 180 µg, which shows that there is an active uptake of salts against the concentration gradient. For a salinity slightly higher or lower than that of normal sea water the influx of the salt is approximately proportional to the degree of dilution or concentration. The closing off of the intestine has no effect on the rate of uptake.

The integument of *Perinereis cultrifera* is more freely permeable to ions than that of *Nereis diversicolor*, the influx of sodium being about 3 times greater in normal sea water, and at least 1·5 times as great in 25% sea water. When worms are transferred from dilute to normal sea water the uptake of sodium is high compensating for the loss of salts; it is about 3 times greater in *Perinereis* than in *Nereis*.

For *Nereis* in sea water of salinity 9‰ the total amount of exchangeable sodium approaches 3·2 mg/g wet weight: in normal sea water it is about double this amount.

During the period of accommodation to dilute sea water by *N. diversicolor* there is an active uptake of sodium, which remains at an approximately constant level throughout the period of weight fluctuation, and is of

approximately the same value as for worms already in equilibrium with their environment.

Oxygen deficiency increases the rate of uptake from dilute sea water and also the total amount of exchangeable sodium. A low temperature ( $5^{\circ}\text{C}$ ) causes a greater imbibition of water during the period of accommodation to dilute sea water, it slows down the weight adjustment and reduces the rate of uptake of sodium.

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## THE SUBLITTORAL FAUNA OF TWO SANDY BAYS ON THE ISLE OF CUMBRAE, FIRTH OF CLYDE

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(Text-figs. 1 and 2)

### INTRODUCTION

There is a considerable literature on the ecology of intertidal animals and a growing one on the sublittoral fauna. Largely because of the difficulty of taking samples in very shallow water, little attention has been paid to the continuation of the intertidal zonation below the low-water mark. Although incomplete in some respects, the results of the present survey are published, partly to help bridge the gap between studies of sublittoral and intertidal faunas, partly because it is unlikely that this survey will ever be completed and partly because the intertidal fauna of one of the bays is particularly well known. The work was begun in 1938 by one of the authors (A.M.) but was discontinued at the outbreak of the late war. Since 1949 further collections have been made and the identity of most of the species taken in the earlier sampling has been checked. The collections of animals and a full account of the results have been deposited in the Marine Laboratory at Millport.

### METHODS

None of the larger and more reliable bottom samplers can be operated from the small boat that must be used in shallow water. All samples in the quantitative survey were taken by the Robertson mud bucket, that is, a cylindrical bucket about 15 in. long and 8 in. in diameter with a sharpened edge. The bucket lies on its side and, when hauled, cuts downwards at an angle until filled. The volume of the material taken in a sample is about 8000 ml. This instrument has several advantages; it can be used from a rowing boat (with or without an out-board motor) in very shallow water, it fills rapidly even when the substratum is hard sand and, so far as one can see on a calm bright day, the bucket fills in the same way at all depths within the limits of visibility, irrespective of the angle of the hauling rope. One disadvantage is that it collects a disproportionately large number of animals living just below the

surface of the sand, though this drawback it shares with the majority of bottom samplers in current use. Another disadvantage is that densities of populations cannot be expressed in absolute terms, i.e. numbers per unit area of sea bed. However, the Robertson bucket does give reasonably accurate estimates of relative densities and that is sufficient for the purposes of this paper.

Each sample was washed through a 2 mm sieve. From the residue the larger animals were picked out by hand, while a binocular microscope was used to search for smaller animals. Formalin was added to dislodge small animals, such as *Siphonoecetes* which live in crevices of stones and shells.

A series of ten samples was taken at each of seven stations at depths of 1, 3, 5, 6½, 10½, 20 and 27 m below low water (mean ordinary tide) in each of the two bays. Kames Bay was studied in late autumn and winter (between November 1938 and January 1939) and White Bay in late spring and summer (between May and August 1939). This difference in sampling time may affect comparisons of densities for the two bays in the case of species whose young appear on the sea bottom in the early part of the year. It is impossible to say which bay is favoured in this respect: on the one hand, some young will not be large enough for retention on the sieve by May–August; on the other hand, some young will have succumbed from natural causes before November–January. In the preliminary work of 1938–39, the prime concern was to establish the composition of the fauna in the two bays and gain facility in identification. For the comparison proper, it was intended to repeat the sampling simultaneously in the two bays at the rate of 30–50 buckets per station in the winter of 1939–40. Unfortunately the Second World War frustrated this intention.

In order to collect animals living on, or just above, the surface of the substratum, samples were taken by a 4 ft. 9 in. beam trawl with a  $\frac{1}{4}$  in. mesh lined with 2 mm stramin. The trawl was hauled for a standard distance at each station in calm weather.

#### CONDITIONS IN THE TWO BAYS\*

The two bays studied were Kames Bay, facing south-east and sheltered from all other directions, and White Bay, facing north-north-east and relatively exposed to the east and north. The Isle of Cumbrae is exposed to the open sea to the south but is sheltered to some extent by neighbouring islands and the mainland in other directions. In Kames Bay the substratum consists of sand grading into fine mud at the deeper stations with only a slight admixture of stones, while White Bay is consistently coarser with a much higher proportion of stones and shells at each station. One peculiar feature of Kames Bay is the large amount of vegetable debris, consisting of fragments of algae and leafy plants, which lies on the bottom and is washed backwards and forwards with the tide. Although a certain amount of this debris is found at all stations, it is most plentiful at the three shallowest stations. Some idea of the movement

of this debris can be gathered from the volume of debris retained by a stramin covered trawl dragged over the sea bed for a standard distance at each of the three inshore stations:

Station	...	1	2	3
Low water, 15 March, 3 p.m.		27	4	2
High water, 16 March, 10 a.m.		4	2	2

(Arbitrary units of volume)

At high water the bulk of the debris is over the intertidal region of the shore. The debris carries its own fauna, particularly of crustaceans, which does not figure in the bottom samples, but must nevertheless play an important role in the economy of the infauna both in competing for and providing food.

There is also a considerable volume of debris in deeper water around station 7 and possibly extending beyond it. It is of a different character from the loose debris washed backwards and forwards by the tide in that it forms the permanent superficial layer of the substratum. Usually it is partially decomposed. The volume of debris collected by the trawl at high tide when most of the movable debris is above station 1 and in the intertidal zone is given below.

Station	...	1	2	3	4	5	6	7
Volume of debris		143	8	25	4	80	300	15,000-20,000

(Arbitrary units of volume)

#### THE FAUNA OF KAMES BAY

The collections made in Kames Bay supplement and extend those made by Elmhirst (1931), Stephen (1928, 1929, 1930) and Watkin (1942), who, between them, have described in considerable detail the zonation of animals in the intertidal part of the bay. The lowest station of Stephen (1928) corresponds approximately with the highest station in the present survey. Elmhirst (1931) also studied the zonation of the Crustacea in the upper part of the sublittoral zone. The position of the stations is illustrated in Fig. 1.

#### *Polychaeta*

The distribution of the more common polychaetes is given in Table I. On comparison with the data given by the earlier authors for the intertidal zonation, it will be seen that in no case does a species occur in large numbers both above and below the low-water mark. This discontinuity is not found in the other groups. *Nephthys hombergii* is numerous sublitorally and indeed is common in muddy deposits at all but the greatest depths throughout the Firth of Clyde. Yet although it appears in moderate numbers in the intertidal zone of other parts of the Clyde (Stephen, 1928), it does not do so in Kames Bay (Stephen, 1930; Watkin, 1942). According to these authors, *Nephthys caeca* is common in Kames Bay down to low tide level but it has not been recorded at all from the sublittoral of this bay. *Spirio filicornis* and *Phyllodoce*

*maculata* are common at station 1, very rare below that, and absent altogether above low tide level. *P. groenlandica* replaces *P. maculata* above low-water mark, but it is not nearly as common. Most species which appear to reach a

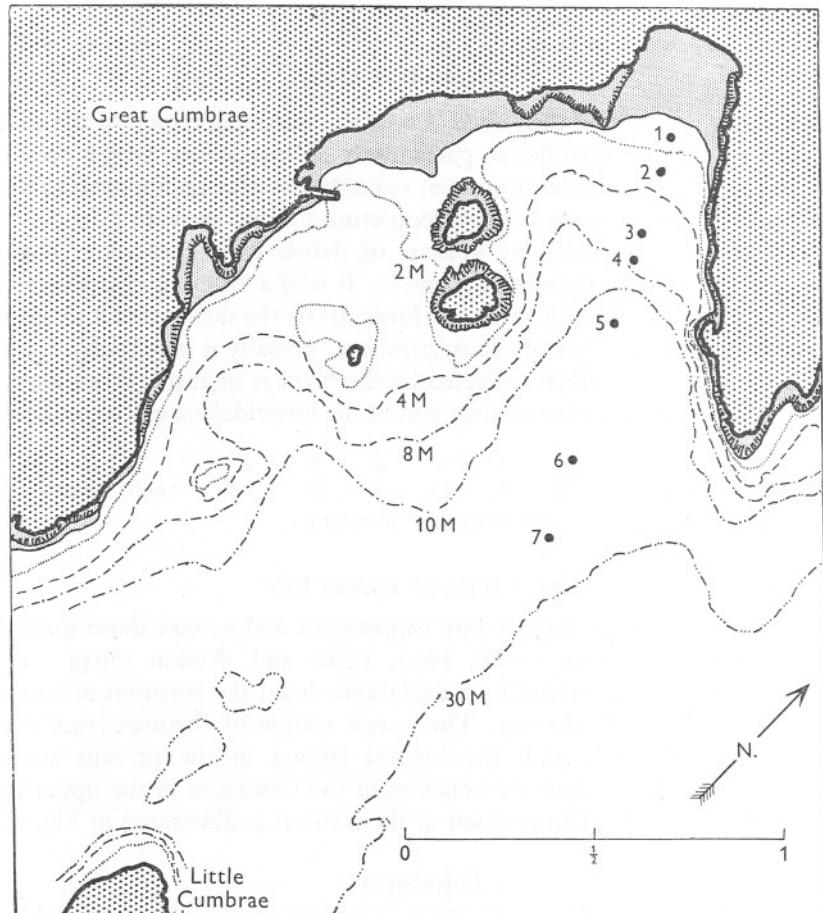


Fig. 1. Kames Bay, Isle of Cumbrae, showing the position of the stations at which samples were collected.

maximum density at station 7 are, of course, more numerous still in deeper water, e.g. *Scalibregma inflatum*, *Notomastus latericeus*, *Lumbrinereis hibernica*, *Lipobranchius jeffreysii* and *Glycera rouxii*. The last three species are well represented in muddy deposits at almost any depth throughout the Clyde Sea area. By contrast, *Melinna palmata* appears suddenly and in very large numbers at station 7 and seems to occupy a narrow belt at a depth of about 30 m in several sandy bays in the area.

*Amphipoda*

Elmhirst (1931) has studied the distribution of crustaceans in Kames Bay from the high-water mark to a depth of 6 m below low water, spring tide. The present survey therefore overlaps Elmhirst's. In the overlapping region it confirms him, and his picture of zonation can now be extended to a depth

TABLE I. DISTRIBUTION OF POLYCHAETES IN KAMES BAY

Station	...	1	2	3	4	5	6	7
<i>Nephthys hombergii</i>	92	85	42	19	43	31	45	
<i>Spiri filicornis</i>	70	—	2	—	2	—	—	
<i>Phyllodoce maculata</i>	23	—	—	1	1	—	—	
<i>Sigalion mathildae</i>	2	37	41	73	29	—	—	
<i>Owenia fusiformis</i>	—	—	9	88	89	79	117	
<i>Sthenelais limicola</i>	—	2	1	7	2	1	—	
<i>Goniada maculata</i>	—	—	—	2	—	82	71	
<i>Stylarioides plumosa</i>	—	—	—	—	—	15	5	
<i>Platynereis dumerilii</i>	—	—	—	—	1	37	4	
<i>Scalibregma inflatum</i>	—	—	—	—	—	19	17	
Maldanidae	—	—	—	13	7	245	2-400	
<i>Glycera rouxi</i>	—	—	—	—	1	12	25	
<i>Lumbrinereis hibernica</i>	—	—	—	—	—	15	20	
<i>Amphitrite cirrata</i>	—	—	—	—	—	10	14	
<i>Terebellides stroemii</i>	—	—	—	—	—	12	67	
<i>Melimna palmata</i>	—	—	—	—	—	—	787	
<i>Lipobranchius jeffreysii</i>	—	—	—	—	—	1	10	
<i>Notomastus latericeus</i>	—	—	—	—	—	14	25	

TABLE II. DISTRIBUTION OF AMPHIPODS IN KAMES BAY

Station	...	1	2	3	4	5	6	7
<i>Bathyporeia guilliamsoniana</i>	232	60	3	1	—	—	—	
<i>Iphinoë trispinosa</i>	3	40	47	30	10	—	—	
<i>Siphonoecetes dellavallei</i>	—	—	76	54	79	—	—	
<i>Ampelisca brevicornis</i>	—	—	2	13	9	3	2	
<i>A. tenuicornis</i>	—	—	—	—	—	8	24	

of about 30 m. Only five species of amphipod are numerous below low-water mark (Table II). *Bathyporeia guilliamsoniana* appears in greatest numbers about low tide level and extends for a short way into the sublittoral. In deeper water *Iphinoë trispinosa* and *Siphonoecetes dellavallei* take its place. *Ampelisca brevicornis* (*A. laevivata* of Elmhirst) occurs in small numbers from station 3 to station 7 with a small maximum at stations 3 and 4, while *A. tenuicornis* largely replaces it at stations 6 and 7 and extends into deeper water still.

*Mollusca*

*Tellina tenuis* is the dominant lamellibranch of the intertidal zone of Kames Bay (Watkin, 1942); it extends a short way into the sublittoral zone and is then replaced by *T. fabula* (Table III). In deeper water still *Abra alba* becomes dominant; *A. alba* is both common and widely distributed in the Clyde area at greater depths than those studied here. The samples of *Ensis ensis* are probably unreliable: as a rule, only the tops of shells are taken by the mud

bucket, and there is no means of telling how many individuals had retracted below the sampling depth altogether. The carnivorous gasteropod *Philine aperta* is moderately common at all stations below station 2.

TABLE III. DISTRIBUTION OF MOLLUSCS IN KAMES BAY

Station	...	1	2	3	4	5	6	7
<i>Tellina tenuis</i>		1003	45	4	—	—	—	—
<i>T. fabula</i>		15	98	147	159	124	—	—
<i>Spisula subtruncata</i>		12	69	13	4	—	—	—
<i>Donax vittatus</i>		16	29	—	—	—	—	—
<i>Ensis ensis</i>		5	20	8	13	12	—	—
<i>Venus gallina</i>		3	2	20	3	3	18	—
<i>Cultellus pellucidus</i>		—	—	2	6	25	16	4
<i>Abra alba</i>		—	—	—	5	127	2	4
<i>Dosinia lupinus</i>		—	—	1	4	—	19	83
<i>Thyasira flexuosa</i>		—	—	—	—	2	54	11
<i>Nucula nitida</i>		—	—	4	1	1	52	55
<i>Philine aperta</i>		1	2	46	44	15	56	25

TABLE IV. DISTRIBUTION OF ECHINODERMS IN KAMES BAY

Station	...	1	2	3	4	5	6	7
<i>Echinocardium cordatum</i>		7	3	12	6	3	—	—
<i>Astropecten irregularis</i>		—	1	1	1	3	1	—
<i>Ophiura affinis</i>		1	—	2	1	8	—	2
<i>O. albida</i>		—	—	—	1	7	43	50
<i>Amphiura filiformis</i>		—	—	—	—	—	32	276
<i>A. chiajei</i>		—	—	—	3	—	—	37
<i>Asterias rubens</i>		—	—	—	—	—	6	1
<i>Labidoplax thomsoni</i>		—	—	1	7	1	—	—
<i>Cucumaria elongata</i>		—	—	—	—	—	4	1

#### *Echinodermata*

The only echinoderm recorded from the intertidal zone of Kames Bay is *Echinocardium cordatum*; it extends some way into the sublittoral zone and is the only echinoid found in these comparatively shallow waters throughout the year. A marked zonation of ophiuroids exists, but only the fringe of it has been touched here. They extend into deeper water and from the trawl samples taken (*vide infra*) it is evident that the Robertson mud bucket is not ideal for collecting them. *Amphiura filiformis* is at its maximum numbers at station 7 and is replaced in deeper water by *A. chiajei*. *Ophiura affinis* is at a maximum at station 5 and is replaced by *O. albida*. In view of the unsatisfactory nature of the sampling, the figures given in Table IV must be regarded with some suspicion.

#### *The Superficial Fauna*

The superficial fauna and the bottom-feeding pelagic animals are not collected by the Robertson bucket. Some of these animals are present in large numbers and must play an important part in the economy of the bottom fauna as a whole. Some are more or less resident. Others, for example, certain pelagic crustaceans are known to migrate inshore from deeper waters at night

and at high tide (Watkin, 1941). An intensive and extended investigation would therefore be needed to give a comprehensive picture of their distribution. This has not been attempted here, but a series of trawl samples made at each station in Kames Bay revealed that, at the shallowest stations at least, some of the commonest animals living on or in the substratum had been missed completely by the Robertson bucket (Table V).

TABLE V. ANIMALS TAKEN IN TRAWL SAMPLES  
AT EACH STATION IN KAMES BAY

	St. 1		St. 2		St. 3		St. 4		St. 5	St. 6	St. 7
	H.W.	L.W.	H.W.	L.W.	H.W.	L.W.	H.W.	H.W.	H.W.	H.W.	H.W.
<i>Gammarus locusta</i>	588	4566	2	98	2	I	5	5	—	4	
<i>Nototropis swammerdammi</i>	877	2730	4	41	—	I	5	2	3	2	
<i>Pontocrates arenarius</i>	110	473	—	—	2	I	—	—	—	—	
<i>P. norvegicus</i>	31	317	I	—	—	—	—	—	—	—	
<i>Pseudocuma cercaria</i>	24	52	2	—	—	—	—	—	—	—	
<i>Idotea baltica</i>	136	182	—	—	—	—	—	I	—	—	
<i>I. viridis</i>	—	130	—	13	—	—	—	—	—	—	
<i>I. granulosa</i>	I	52	—	—	I	—	—	—	—	—	
<i>Schistomysis spiritus</i>	89	4	172	107	—	I	—	—	—	—	
<i>Crangon vulgaris</i>	21	73	12	9	14	8	I	2	—	—	
<i>Praunus flexuosus</i>	6	29	8	14	—	I	—	—	—	—	
<i>Platynereis dumerillii</i>	—	—	—	—	—	—	—	I	—	530	
<i>Asterias rubens</i>	—	—	—	—	—	—	—	—	I	59	
<i>Ophiocomina nigra</i>	—	—	—	—	—	—	—	—	—	60	

*Crangon vulgaris*, *Schistomysis spiritus*, *Idotea baltica*, *Gammarus locusta*, *Nototropis swammerdammi* and *Pseudocuma cercaria* are all plentiful at inshore stations though they are not represented at all in the mud-bucket samples. On the deeper stations the results of the trawl sampling do not alter materially the conclusions already drawn about the relative importance of the various members of the fauna, with two exceptions: (1) *Ophiocomina nigra*, which did not figure in the mud-bucket samples, is now seen to be present at station 7. (2) Contrary to the mud-bucket findings (Table I), *Platynereis dumerillii* is probably more numerous at station 7 than station 6, because of its association with matted vegetable material in the surface mud. The latter is about 50 times more plentiful at station 7. For some reason this material is not normally taken by the bucket. And it is significant that of the total thirty-seven *P. dumerillii* from station 6 (Table I), thirty-one were in the sole bucket sample (from this or any other station) containing an appreciable quantity of the said material.

#### FAUNA OF WHITE BAY AND A COMPARISON BETWEEN THE TWO BAYS

A similar series of mud-bucket samples was taken in White Bay on seven stations at the same depths as those of Kames Bay (see Fig. 2). The intertidal fauna of White Bay has never been studied. The sublittoral fauna is broadly

the same as that of Kames Bay with some interesting differences, doubtless related to the greater exposure and consequent coarser substratum together with the absence of algal debris in White Bay.

### *Polychaeta*

The only remarkable difference between the two bays is the virtual absence of *Platynereis dumerillii*. As we have already noted, this species is commonly associated with matted plant debris so that its absence is only to be expected. The other species have much the same distribution as in Kames Bay though the total numbers are somewhat smaller (see Table VI).

TABLE VI. DISTRIBUTION OF POLYCHAETES IN WHITE BAY

Station	...	1	2	3	4	5	6	7
<i>Nephtys hombergii</i>	1	28	20	24	14	33	26	
<i>Spio filicornis</i>	2	11	—	5	1	—	—	
<i>Phyllodoce maculata</i>	18	19	2	—	—	—	—	
<i>Sigalion mathildae</i>	—	—	8	3	5	—	—	
<i>Owenia fusiformis</i>	—	3	27	94	24	123	121	
<i>Glycera rouxi</i>	—	—	3	3	7	31	—	
<i>Goniada maculata</i>	—	—	2	2	—	50	65	
<i>Amphitrite cirrata</i>	—	—	—	—	—	19	12	
<i>Lumbrinereis hibernica</i>	—	—	—	1	—	36	35	
<i>Scalibregma inflatum</i>	—	—	—	—	—	37	38	
Maldanidae	—	—	6	—	18	75	75	
<i>Melitta palmata</i>	—	—	—	1	—	282	569	
<i>Notomastus latericeus</i>	—	—	—	—	—	12	38	

### *Amphipoda*

Some interesting differences appear in the crustacean fauna of White Bay (Table VII). *Bathyporeia guilliamsoniana* is much less common than in Kames Bay, but it appears in much the same part of the beach. *Iphinoë trispinosa* and *Siphonocoetes dellavallei*, both common in Kames Bay, are absent from White Bay. On the other hand, *Ampelisca typica*, which was not found in Kames Bay, is here present in moderate numbers having much the same range as *A. brevicornis*. These differences may be attributable either to the different nature of the substratum or to the lack of vegetable debris in White Bay.

### *Mollusca*

*Spisula subtruncata*, *Donax vittatus*, *Philine aperta* and *Nucula nitida* are all common in Kames Bay, but are absent or virtually absent from White Bay. *Abra alba*, *Cultellus pellucidus* and *Dosinia lupinus* are present, but in reduced numbers. *Natica alderi*, which is absent from Kames Bay, is found in White Bay where it almost wholly replaces *Philine aperta* as the important carnivorous gasteropod. Species common to the two bays occupy the same position on the beach (see Table VIII).

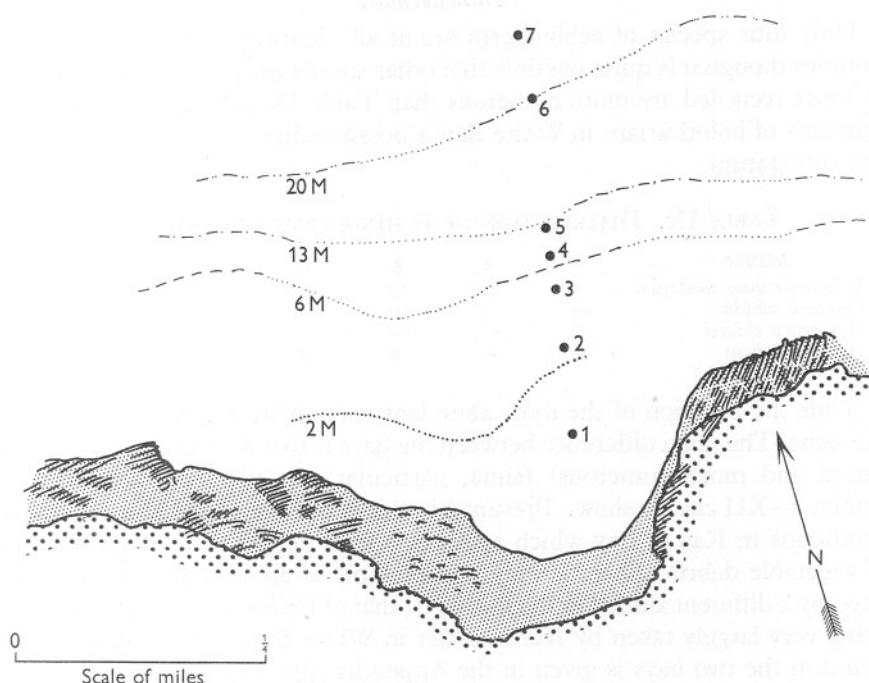


Fig. 2. White Bay, Isle of Cumbrae, showing the position of the stations at which samples were taken.

TABLE VII. DISTRIBUTION OF AMPHIPODS IN WHITE BAY

Station	...	1	2	3	4	5	6	7
<i>Bathyporeia guilliamsoniana</i>	II	35	7	—	—	—	—	—
<i>Ampelisca brevicornis</i>	—	23	27	7	21	—	—	—
<i>A. typica</i>	—	—	17	13	5	—	—	—
<i>A. tenuicornis</i>	—	—	—	3	2	6	3	—

TABLE VIII. DISTRIBUTION OF MOLLUSCS IN WHITE BAY

Station	...	1	2	3	4	5	6	7
<i>Tellina tenuis</i>	826	1169	47	2	3	—	—	—
<i>T. fabula</i>	—	83	358	216	44	—	—	—
<i>Venus gallina</i>	28	19	3	10	9	—	—	—
<i>Ensis ensis</i>	10	40	12	8	—	—	—	—
<i>Abra alba</i>	—	—	7	1	4	—	—	1
<i>Cuttlellus pellucidus</i>	—	1	8	2	2	2	—	1
<i>Dosinia lupinus</i>	2	5	11	3	1	11	6	—
<i>Natica alderi</i>	7	12	8	10	4	4	2	—
<i>Thyasira flexuosa</i>	—	—	—	5	19	4	—	—

*Echinodermata*

Only four species of echinoderm are at all plentiful in Robertson bucket samples though it is quite possible that other species are present and that some of those recorded are more numerous than Table IX indicates. The reduced numbers of holothurians in White Bay is possibly due to the coarser nature of the substratum.

TABLE IX. DISTRIBUTION OF ECHINODERMS IN WHITE BAY

Station	...	1	2	3	4	5	6	7
<i>Echinocardium cordatum</i>		5	22	11	5	—	—	—
<i>Ophiura albida</i>		—	2	10	3	4	3	1
<i>Amphipura chiajei</i>		—	—	—	—	—	—	39
<i>Asterias rubens</i>		—	—	8	5	9	3	10

Thus the zonation of the most abundant animals in the two bays is much the same. The main difference between the bays is that Kames Bay has a more varied and more numerous<sup>1</sup> fauna, particularly at the deeper stations as Tables X-XII clearly show. Presumably this is because of the more sheltered conditions in Kames Bay which result in a finer deposit with large amounts of vegetable debris. There is only one example of an ecological niche being filled by a different animal in the two bays, that of *Philine aperta* in Kames Bay being very largely taken by *Natica alderi* in White Bay. A full list of species found in the two bays is given in the Appendix (pp. 178-180).

TABLE X. NUMBERS OF SPECIES (S) AND INDIVIDUALS (I) FOUND IN TEN SAMPLES AT EACH STATION IN KAMES BAY

Station	...	1		2		3		4		5		6		7	
		S	I	S	I	S	I	S	I	S	I	S	I	S	I
Polychaeta	7	187	3	109	8	93	13	208	13	195	32	1787	31	1370	
Amphipoda	8	246	5	74	10	125	10	108	10	120	6	24	3	29	
Mollusca	8	1006	9	232	11	218	11	219	11	303	20	230	13	185	
Echinodermata	2	8	2	3	4	16	6	19	6	23	7	81	7	368	
Totals*	26	1483	19	418	35	455	45	559	42	645	70	2200	57	2026	

\* Totals include animals not in the four main groups listed above.

TABLE XI. NUMBERS OF SPECIES (S) AND INDIVIDUALS (I) FOUND IN TEN SAMPLES AT EACH STATION IN WHITE BAY

Station	...	1		2		3		4		5		6		7	
		S	I	S	I	S	I	S	I	S	I	S	I	S	I
Polychaeta	11	39	10	73	17	88	18	168	13	95	27	766	21	1043	
Amphipoda	7	33	6	63	4	53	3	23	4	29	4	12	1	3	
Mollusca	8	878	11	1340	15	488	10	257	9	87	10	37	8	19	
Echinodermata	1	5	3	26	6	47	6	19	3	15	6	17	4	51	
Totals*	32	965	31	1503	42	676	38	472	30	227	48	833	38	1146	

\* Totals include animals not in the four main groups listed above.

<sup>1</sup> But see p. 162, regarding relative numbers of individuals of some species in the two bays.

TABLE XII. COMPARISON OF THE FAUNA OF  
KAMES BAY AND WHITE BAY

	Kames Bay		White Bay	
	Species	Individuals	Species	Individuals
Polychaeta	47	3949	44	2272
Amphipoda	26	726	14	215
Mollusca	31	2393	24	2108
Echinodermata	12	518	10	180
Totals*	125	7786	102	5822

\* The total figures include a small number of animals not belonging to the four main groups.

There are practically no data on other bays with which to compare the present findings. A certain amount of collecting has been carried out in Ettrick Bay on the west side of Bute; the bay resembles Kames Bay more nearly than White Bay, but no sampling in shallow water has been possible there. So far as they go, the results from Ettrick Bay bear out the general tendencies found in Kames and White Bays.

#### DISTRIBUTION OF ANIMALS ON THE SEA BED

On examining samples from sublittoral deposits it is at once obvious that the density of some animals varies widely in successive sample units from the same station, while other animals appear to be more uniformly distributed. It is possible that the method of sampling might give a spurious impression of the distribution of some species. For instance, the mud bucket might bite deeper on one occasion than on another. But all methods of sampling the sublittoral fauna in use at present have this defect. Judging from the uniform performance of the Robertson bucket at depths where it is visible, there is strong reason for thinking that ten buckets would not vary much in character among themselves at any one station. A more important consideration is that samples of ten units are too small for certain types of statistical treatment.

In considering the nature of the distribution of organisms over an area, Fisher's 'coefficient of dispersion' may be used. It was introduced first in plant ecology, e.g. by Clapham (1936) and Blackman (1942), and later in terrestrial and marine ecology, e.g. by Salt & Hollick (1946), Holme (1950) and Barnes & Marshall (1951). The coefficient of dispersion is given by

$$\Sigma(x - \bar{x})^2 / \bar{x}(n - 1),$$

where  $\Sigma(x - \bar{x})^2$  is the sum of squares of the deviations of individual units ( $x$ ) from the mean ( $\bar{x}$ ) of all the units ( $n$ ) comprising the sample. The coefficient leads to unity when the population is randomly distributed, is less than one if the population is over-dispersed (i.e. more or less evenly distributed) and greater than one if it is underdispersed (i.e. more or less aggregated). The significance of the departure from unity is tested by

$$1 \pm 2 \times \sqrt{[2n/(n-1)^2]},$$

where, again,  $n$  is the number of units in the sample. In the present bucket samples,  $n=10$  and the limits of the coefficient for random distribution are therefore 1.9938 and 0.0062. A coefficient greater than 1.9938 may then be taken as significant evidence of aggregation. But obviously, with the lower limit at 0.0062 the coefficient can not be expected to distinguish between random and over-dispersed distribution in samples of 10 units. The sample size need only have been doubled to permit the distinction, although, of course, the larger the sample the better, as the following tabulation shows:

Sample size ( $n$ )	Limits of the coefficient of dispersion for random distribution
10	0.0062-1.9938
20	0.3342-1.6658
50	0.5918-1.4082
100	0.7142-1.2858

Departure from randomness may of course be tested by fitting a Poisson distribution from the sample mean and then applying the  $\chi^2$  test with  $n-2$  degrees of freedom. And Blackman warns that 'when the coefficient of dispersion value is not significantly different from unity the  $\chi^2$  test should still be applied since the coefficient of dispersion test, although sensitive as regards aggregation, will not detect certain types of skew distribution'. He gives a field example (a sample of 100 units) where the coefficient obtained though greater than unity is not significantly different from it, yet the evidence of the  $\chi^2$  test is that the distribution is not random. Thomson (1952) confirms Blackman. Unfortunately, with a sample size of only 10 units, the direct calculation of  $\chi^2$  for a fitted Poisson provides little or no useful information.

Some writers have pointed out that the quantity

$$\Sigma(x - \bar{x})^2/\bar{x},$$

known as the 'index of dispersion', is approximately distributed as  $\chi^2$  with  $n-1$  degrees of freedom if the data come from a Poisson population. 'For  $\bar{x} \geq 5$  the  $\chi^2$  approximation... is highly satisfactory even for  $n$ , the number of observations, as small as 5, and is fairly accurate for  $\bar{x} < 5$ , although it will tend to give too few significant results' (Bateman, 1950). With  $n=10$ , the  $P=0.05$  value of  $\chi^2$  is 16.919. And since the index of dispersion is  $(n-1)$  times the coefficient of dispersion, this  $\chi^2$  value is equivalent to a coefficient of  $16.919/9 = 1.8799$  which is practically the same as the limit 1.9938 given above. But while a coefficient  $> 1.9938$  is significant evidence of aggregation, an index  $\equiv \chi^2 > 16.919$  is merely significant evidence of non-randomness, i.e. the index apparently can not distinguish between aggregation and over-dispersion.

Three other measures of dispersion are discussed by Thomson (1952) but none are appropriate for the present data. We must therefore confine ourselves to employing the coefficient of dispersion alone in an attempt to

distinguish between aggregated and non-aggregated species, bearing in mind that animals falling into the latter group may be either randomly distributed, or distributed in a skew but non-Poisson fashion, or overdispersed.

At each station the coefficient of dispersion has been calculated for each species occurring in the ten buckets. Altogether, 459 coefficients are available from the two bays and they range from 0·2593 up to 69·99. Since only 19·4% of coefficients are above the significance limit 1·9938, it seems that non-aggregated distribution (probably chiefly random) is the general rule in the community dwelling on and in the upper layer of the sea bed. As will be seen below, this conclusion requires testing with different sizes of bucket (sample unit).

Aggregation is, of course, out of the question if there is only one individual of a species among ten buckets. But with  $n=10$ , the coefficient of dispersion is greater than 1·9938 whenever there are two or more individuals in one bucket and none at all in the remaining nine buckets. Hence significant evidence of aggregation can be obtained from densities of 0·2 per bucket and upwards. Table XIII gives the complete list of species showing evidence of aggregation in at least one sample of ten buckets. In general, the samples of these species do not show consistent evidence of aggregation until the mean per bucket is greater than about 10·0. Above a mean of 10·0, 94·1% of samples have a coefficient indicating aggregation. Below that mean level, the percentage dwindles in a smooth curve until at means of 0·2–0·9 per bucket it is only 26%. This is to be expected from the well-known empirical finding that the smaller the mean for a particular sample unit, the more nearly will a contagious or aggregated distribution conform to the Poisson Law: variance = mean. An obvious corollary is that the demonstration of aggregation will depend to some extent on the dimensions of the sample unit. Thus evidence of aggregation may disappear altogether if a smaller unit (e.g. bucket or quadrat) is used, simply because the mean per unit is reduced. Evans (1952) confirmed this by experimenting (on paper) with different unit (quadrat) sizes on the mapped data from a field in which the plants had been 'completely enumerated'. And he makes the sound point that dispersion should be investigated with more than one size of sample unit.

Partly from the nature of the data and partly from the sketchiness of biological and ecological knowledge at the present time, there is little of value to be concluded from the data on individual species showing some evidence of aggregation (Table XIII) or none at all (Table XIV).

*Polychaeta.* Ten errant and nine sedentary species of Polychaeta show some evidence of aggregation while six errant and fourteen sedentary species do not. That is 62·5% of errant species but only 39·1% of sedentary species show evidence of aggregation in the two bays. This difference of proportion is significant,  $\chi^2=4·976$ ,  $P=0·03$ . Hence errant polychaetes tend more to be aggregated than sedentary polychaetes.

TABLE XIII. SPECIES SHOWING STATISTICAL EVIDENCE OF AGGREGATION  
IN AT LEAST ONE SAMPLE\*

(Samples classified as aggregated (A, i.e. coefficient of dispersion  $> 1.9938$ ) and non-aggregated (non-A, i.e. coefficient of dispersion  $< 1.9938$ ) at different mean levels greater than 0.1 individual per bucket.)

	Mean individuals per bucket									
	0·2-0·9		1·0-1·9		2·0-3·9		4·0-9·9		> 10·0	
	Non-	A	Non-	A	Non-	A	Non-	A	Non-	A
<b>POLYCHAETA</b>										
<i>Aphrodite aculeata</i>	I	I	—	—	—	—	—	—	—	—
<i>Sigalion mathildae</i>	2	I	—	—	I	2	0	I	—	—
<i>Phyllodocia maculata</i>	I	0	0	2	I	0	—	—	—	—
<i>Eteone longa</i>	2	I	—	—	—	—	—	—	—	—
<i>Platynereis dumerilii</i>	I	I	—	—	I	0	—	—	—	—
<i>Nephthys caeca</i>	I	I	—	—	—	—	—	—	—	—
<i>N. hombergii</i>	—	—	0	2	I	5	I	3	—	—
<i>Glyceria rouxi</i>	0	3	2	0	0	2	—	—	—	—
<i>Goniada maculata</i>	0	4	—	—	—	—	3	I	—	—
<i>Lumbrinereis hibernica</i>	—	—	I	0	0	3	—	—	—	—
<i>Spio filicornis</i>	0	2	0	I	—	—	I	0	—	—
<i>Poecilochaetus serpens</i>	0	I	I	0	—	—	—	—	—	—
<i>Scalibregma inflatum</i>	—	—	0	2	I	0	I	0	—	—
<i>Notomastus latericeus</i>	—	—	I	I	I	I	—	—	—	—
<i>Owenia fusiformis</i>	0	2	—	—	I	I	3	I	2	I
<i>Ampharete grubei</i>	I	0	—	—	—	—	—	—	—	—
<i>Melitta palmata</i>	—	—	—	—	—	—	—	—	4	0
<i>Amphitrite cirrata</i>	0	I	2	I	—	—	—	—	—	—
<i>Terebellides stroemii</i>	I	I	0	I	—	—	0	I	—	—
<b>CRUSTACEA</b>										
<i>Amphelisca brevicornis</i>	0	2	0	I	I	2	—	—	—	—
<i>Bathyporeia guilliamsoniana</i>	I	I	0	I	0	I	I	0	I	0
<i>Gammarus locusta</i>	I	2	—	—	—	—	—	—	—	—
<i>Aora typica</i>	I	0	—	—	—	—	—	—	—	—
<i>Siphonoecetes dellavallei</i>	—	—	—	—	—	—	3	0	—	—
<i>Caprella acanthifera</i>	I	0	—	—	—	—	—	—	—	—
<i>Portunus puber</i>	I	I	—	—	—	—	—	—	—	—
<b>MOLLUSCA</b>										
<i>Natica alderi</i>	0	7	I	0	—	—	—	—	—	—
<i>Philine aperta</i>	I	2	0	I	0	I	0	3	—	—
<i>Elysia viridis</i>	I	0	—	—	—	—	—	—	—	—
<i>Lacuna vincta</i> ( <i>Nucula turgida</i> )	I	0	—	—	—	—	0	2	—	—
<i>Nucula nitida</i>	I	0	—	—	—	—	I	0	—	—
<i>Thyasira flexuosa</i>	0	2	I	0	—	—	0	I	—	—
<i>Dosinia lupinus</i>	0	5	I	2	—	—	0	I	—	—
<i>Venus gallina</i>	0	6	0	4	I	0	—	—	—	—
<i>Donax vittatus</i>	—	—	0	I	I	0	—	—	—	—
<i>Tellina tenuis</i>	I	2	—	—	I	0	I	0	3	0
<i>T. fabula</i>	—	—	0	I	—	—	3	0	4	0
<i>Abra alba</i>	I	4	—	—	—	—	—	—	I	0
<i>Cultellus pellucidus</i>	0	6	I	0	I	0	—	—	—	—
<i>Thracia</i> sp.	2	I	—	—	—	—	—	—	—	—
<b>ECHINODERMATA</b>										
<i>Asterias rubens</i>	I	2	—	—	—	—	—	—	—	—
<i>Amphipura chiajei</i>	0	I	—	—	2	0	—	—	—	—
<i>A. filiformis</i>	I	0	—	—	0	I	—	—	I	0
<i>Ophiura albida</i>	I	4	0	I	—	—	2	0	—	—
<i>O. affinis</i>	I	2	—	—	—	—	—	—	—	—
<i>Echinocardium cordatum</i>	0	6	I	I	0	I	—	—	—	—
<i>Cucumaria elongata</i>	I	I	—	—	—	—	—	—	—	—
<i>Labidoplax thomsoni</i>	I	0	—	—	—	—	—	—	—	—
Totals of samples	29	76	12	23	14	20	20	13	16	1
Percentage of samples aggregated	27·6	34·3	41·2	—	—	60·6	—	—	94·1	—

\* A sample is 10 buckets from any one station. The sample must not be confused with the sample unit which is 1 bucket.

TABLE XIV. SPECIES SHOWING NO SIGN OF AGGREGATION IN ANY SAMPLE WITH MEAN GREATER THAN 0·1 INDIVIDUAL PER BUCKET

	No. of samples of 10 buckets	Range of means		No. of samples of 10 buckets	Range of means
TURBELLARIA					
<i>Cryptocelis alba</i>	4	0·2-1·0	<i>Urothoë marina</i>	1	0·3
POLYCHAETA			<i>U. brevicornis</i>	1	0·2
<i>Gattyana cirrosa</i>	3	0·4-0·8	<i>Phoxocephalus holbotti</i>	1	0·6
<i>Harmothoë lunulata</i>	1	0·4	<i>Pontocerebrus arenarius</i>	2	0·7, 1·0
<i>Sthenelais limicola</i>	4	0·2-2·0	<i>Nototropis guttatus</i>	1	0·2
<i>Phyllodoces kosteriensis</i>	1	0·3	<i>Maera othonis</i>	1	0·4
<i>Eulalia sanguinea</i>	1	0·7	<i>Dexaminis spinosa</i>	1	0·4
<i>Nephtys longosetosa</i>	3	0·2-2·8	<i>Jassa oicia</i>	1	0·5
<i>Scoloplos armiger</i>	4	0·2-0·4	<i>Eupagurus bernhardus</i>	1	0·3
<i>Laonice cirrata</i>	2	0·2, 0·3	MOLLUSCA		
<i>Spiophanes bombyx</i>	1	0·3	<i>Chiton</i> sp.	2	0·3, 0·6
<i>Pygospio elegans</i>	2	0·2	<i>Aporrhais pes-pelecani</i>	3	0·3-0·5
<i>Magelona cincta</i>	2	0·4, 0·5	<i>Nassarius reticulatus</i>	1	0·2
<i>Cirratulus filiformis</i>	3	0·2-0·9	<i>Haedropleura septangularis</i>	1	0·4
<i>Stylarioides plumosa</i>	3	0·3-1·5	<i>Nucula tenuis</i>	4	0·2-0·6
<i>Lipobranchius jeffreysii</i>	1	0·2	<i>Nuculanula minuta</i>	1	0·3
<i>Ammotrypane aulogaster</i>	2	0·2	<i>Kellia suborbicularis</i>	2	0·3, 0·6
<i>Pectinaria auricoma</i>	3	0·2-0·6	<i>Montacuta ferruginosa</i>	3	0·2-0·5
<i>Lanice conchilega</i>	1	0·3	<i>Venerupis rhomboides</i>	1	0·2
<i>Pista cristata</i>	1	0·5	<i>Gari fervensis</i>	1	0·3
<i>Trichobranchius glacialis</i>	4	0·4-1·3	<i>Ensis ensis</i>	9	0·5-4·0
<i>Euchone rosea</i>	1	0·2	<i>Spisula subtruncata*</i>	5	0·2-5·5
CRUSTACEA			<i>Mya</i> sp.	1	0·7
<i>Iphinoë trispinosa</i>	5	0·3-4·4	<i>Corbula gibba</i>	2	0·2, 0·5
<i>Diastylis laevis</i>	1	0·2	ECHINODERMATA		
<i>Hippomedon denticulatus</i>	2	0·7, 0·8	<i>Astropecten irregularis</i>	1	0·3
<i>Tryphosites longipes</i>	1	0·3	<i>Acrocnida brachiatula</i>	1	0·2
<i>Ampelisca tenuicornis</i>	6	0·2-2·4	<i>Holothurian</i> sp.	2	0·4, 1·5
<i>A. typica</i>	3	0·5-1·7			
<i>Bathyporeia elegans</i>	2	0·2, 0·3			
<i>Haustorius arenarius</i>	1	0·6			

\* At the second highest mean, 1·0, *Spisula* was not aggregated (1·778) but at the highest mean, 5·5, it was very nearly to be regarded so (1·9900).

*Crustacea*. The writers see nothing to add to what the tables say.

*Mollusca*. According to Holme (1950), at lower densities *Tellina tenuis* is over-dispersed rather than randomly distributed or aggregated. This is not the case in the present work since samples with means of 0·2, 0·2 and 0·3 per bucket gave coefficients of dispersion equal to 2·0000, 0·8889 and 1·5185 respectively; and samples with means greater than 2·0 per bucket invariably had a coefficient of dispersion denoting aggregation. The disagreement with Holme is probably explained by sample size.

*Echinodermata*. It is well known that Ophiuroids tend to aggregate, and the present results, with two species of *Ophiura* and two of *Amphiura*, confirm this.

In some cases large local variations in population density may reflect the effects of local differences in the nature of the substratum on the settlement of larvae or their survival after settlement. For this to be the main cause of the marked patchiness of distribution shown by species in Table XIII it must be assumed that the sea bed is a veritable mosaic in which one square yard of the bottom may differ markedly from the next. This is hardly credible. One imagines uniform conditions existing on a sandy or muddy bottom, and certainly the bucket samples suggest uniformity of conditions within each station. The larger variance/mean ratio (coefficient of dispersion) of the species in Table XIII arises more likely from living habits which lead to aggregation of individuals.

Without knowing more about their life histories and habits, it is difficult or impossible to say what might cause aggregation in the individual species listed in Table XIII. Species lacking a dispersal phase and which are fairly sedentary in adult life would naturally tend to collect into family aggregations and one consequence of this in the speciation of polychaetes has already been suggested (Clark, 1952). However, this can not explain aggregations in two of the amphipod species which are active swimmers, leave the sand for breeding and appear in the plankton at night (Watkin, 1941). In these cases the formation of aggregations must be an active process, as it may also be in the case of the polychaete *Goniada maculata* which has a pelagic larva and which is probably an active swimmer in the adult stage. Again, none of the molluscs in Table XIII is known to have lost its dispersive pelagic larval phase; unless differential mortality takes place after the molluscan larvae have settled on the substratum, active aggregation must be assumed. No doubt the factors leading to the formation of aggregations differ not only from group to group but also from species to species. A well-known instance of active aggregation occurs among the Ophiuroidea. Allee (1927) has shown that ophiuroids disperse when living among *Zostera* and can be made to disperse in the laboratory when provided with artificial vegetation in the form of glass rods. Yet living (as they commonly do) on a fairly bare substratum, they form aggregations unless, apparently, the density is too low. Such aggregations on the sea bed have been photographed by Vevers (1951, 1952).

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#### SUMMARY

A preliminary study has been made of the composition and distribution of the macrofauna living on and in the substratum from low tide level to a depth of about 30 m in Kames Bay and White Bay on the Isle of Cumbrae in the Firth of Clyde. Samples were taken mainly by means of the Robertson mud bucket. A small amount of trawling was done as a check on results for animals dwelling at the surface of the substratum.

The main physical differences between the two bays are: (1) Kames Bay is more sheltered and has a finer deposit of sand or mud at each station with fewer stones and shells than White Bay; (2) in Kames Bay, a substantial quantity of vegetable debris is washed backwards and forwards with the tide in the upper part of the sublittoral zone and there is a large amount of decomposing organic material mixed with the superficial mud at a depth of about 30 m; both these circumstances are practically absent in White Bay.

Full lists are given of the macro-species occurring on and in the sea bed from low-water mark out to 30 m in the two bays. White Bay is somewhat poorer in variety of species and density of individuals than Kames Bay. The only other really important difference is that *Philine aperta*, abundant in Kames Bay, is replaced almost wholly in White Bay by *Natica alderi* as the carnivorous gasteropod.

The distribution of the intertidal fauna of Kames Bay, but not of White Bay, has been extensively studied in the past. The present results therefore extend the picture of animal zonation from high-water mark out to 30 m depth in Kames Bay.

When aggregation occurs, the possibility of its demonstration by means of the coefficient of dispersion depends to some extent on the size of the sample unit. With the Robertson Mud Bucket as the unit, the array of coefficients (calculated from all samples of all species occurring) suggests that non-aggregated distribution (probably chiefly random) is the general rule in the bottom community of the sublittoral. Of some 150 species comprising the invertebrate macrofauna, forty-eight showed significant evidence of aggregation in at least one sample; but the evidence is consistent over all available samples in less than half a dozen species.

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## APPENDIX

### SPECIES TAKEN IN THE ROBERTSON MUD-BUCKET SAMPLES IN KAMES BAY AND WHITE BAY.

(K, recorded from Kames Bay only. W, recorded from White Bay only.)

	TURBELLARIA
K	<i>Cryptocelis alba</i> (Lang)
	NEMERTINEA
	<i>Lineus</i> spp.
	POLYCHAETA
	<i>Aphrodite aculeata</i> L.
K	<i>Lepidonotus squamatus</i> (L.)
	<i>Gattyana cirrosa</i> (Pallas)
K	<i>Harmothoë imbricata</i> (L.)
W	<i>H. longisetis</i> (Grube)
	<i>H. lunulata</i> (Delle Chiaje)
	<i>Sigalion mathildae</i> Audouin & Milne-Edwards
	<i>Sthenelais limicola</i> (Ehlers)
	<i>Phyllodocia maculata</i> (L.)
K	<i>P. kosteriensis</i> (Malmgren)
	<i>Phyllodocia</i> sp.
K	<i>Eulalia sanguinea</i> (Oersted)
	<i>Eulalia</i> sp.
	<i>Eteone longa</i> (Fabricius)
K	<i>E. lactea</i> Claparède
K	<i>Eteone</i> sp.
K	<i>Nereis longissima</i> (Johnston)
	<i>Platynereis dumerilii</i> (Audouin & Milne-Edwards)
W	<i>Nephthys caeca</i> Fabricius
	<i>N. hombergii</i> Audouin & Milne-Edwards
W	<i>N. longosetosa</i> Oersted
	<i>Glycera rouxi</i> Audouin & Milne-Edwards
	<i>Goniada maculata</i> Oersted
	<i>Lumbrinereis hibernica</i> McIntosh
	<i>Scoloplos armiger</i> (O. F. Müller)
	<i>Laonice cirrata</i> (Sars)
	<i>Spiophanes bombyx</i> (Claparède)
	<i>Spio filicornis</i> (O. F. Müller)
	W Pygospio elegans Claparède
K	<i>Magelona papillornis</i> F. Müller
	<i>M. cincta</i> Ehlers
W	<i>Poecilochaetus serpens</i> Allen
	<i>Cirratulus filiformis</i> Keferstein
K	<i>Chaetozone setosa</i> Malmgren
	<i>Stylarioides plumosa</i> (O. F. Müller)
	<i>Scalibregma inflatum</i> Rathke
	<i>Lipobranchius jeffreysii</i> (McIntosh)
	<i>Ammotrypane aulogaster</i> Rathke
	<i>Notomastus latericeus</i> Sars
	Maldanidae
K	<i>Rhodina</i> sp.
K	<i>Axiothella</i> sp.
	<i>Owenia fusiformis</i> Delle Chiaje
W	<i>Pectinaria koreni</i> Malmgren
	<i>P. auricoma</i> (Müller)
K	<i>Ampharete grubei</i> Malmgren
	<i>Anobothrus gracilis</i> (Malmgren)
K	<i>Amphicteis gunneri</i> (Sars)
	<i>Melinna palmata</i> Grube
	<i>Amphitrite cirrata</i> (O. F. Müller)
W	<i>Lanice conchilega</i> (Pallas)
	<i>Pista cristata</i> (Müller)
	<i>Trichobranchius glacialis</i> Malmgren
	<i>Terebellides stroemii</i> Sars
W	<i>Euchone rosea</i> Langerhans
	CRUSTACEA
	Cumacea
K	<i>Iphinoë trispinosa</i> (Goodsir)
W	<i>Lamprops fasciata</i> G. O. Sars
K	<i>Dyastilis laevis</i> Norman
	Isopoda
W	<i>Cirolana</i> sp.
K	<i>Idotea granulosa</i> Rathke

- Amphipoda
- K *Acidostoma laticorne* O. Sars  
*Hippomedon denticulatus* (Bate)  
 K *Tryphosites longipes* (Bate & Westwood)  
*Ampelisca brevicornis* (A. Costa)  
*A. tenuicornis* Lilljeborg  
 W *A. typica* (Bate)  
 K *Ampelisca* sp.  
*Bathyporeia guilliamsoniana* (Bate)  
 K *B. elegans*  
 W *Bathyporeia* sp.  
 W *Haustorius arenarius* (Slabber)  
 W *Urothoë marina* (Bate)  
*U. brevicornis* Bate  
 K *U. elegans* Bate  
 W *Phoxocephalus holbølli* (Krøyer)  
 W *Leucothoë incisa* D. Robertson  
 K *Perioculoides longimanus* (Bate & Westwood)  
*Pontocrates arenarius* (Bate)  
 K *Nototropis guttatus* (A. Costa)  
 W *N. swammerdamni* (Milne-Edwards)  
 K *Eusiris longipes* Boeck  
 K *Melita obtusata* Montagu  
 W *Meara othonis* (Milne-Edwards)  
 K *Gammarus locusta* (L.)  
 K *Dexamine spinosa* (Montagu)  
 K *Orchestia* sp.  
 K *Hyale nilsonii* (Rathke)  
 K *Aora typica* Krøyer  
 K *Jassa falcata* (Montagu)  
*J. ocia* (Bates)  
 K *Siphonoecetes dellavallei* Stebbing  
 K *Caprella acanthifera* Leach  
 Decapoda  
 W *Crangon vulgaris* (L.)  
*Eupagurus bernhardus* (L.)  
 W *Porcellana longicornis* Pennant  
 K *Ebalia cranchi* Leach  
 K *Coryistes cassivelaunus* (Pennant)  
*Portunus puber* (L.)  
 K *P. holsatus* Fabricius
- MOLLUSCA
- Placophora
- W *Chiton* sp.
- Gastropoda
- W *Gibbula cineraria* (L.)  
 K *Turritella communis* Risso  
 W *Capulus ungaricus* (L.)  
*Aporrhais pes-pelicanus* da Costa  
 W *Natica alderi* Forbes  
 K *Nassarius reticulatus* (L.)
- K *Haedropleura septangularis* (Montagu)  
 K *Haedropleura* sp.  
 K *Cylchima cylindracea* (Pennant)  
*Philine aperta* Ascanius  
*Neptunea antiqua* (L.)
- Lamellibranchia
- Nucula turgida* Leckenby & Marshall  
*N. tenuis* (Montagu)  
*Nuculana minuta* (Müller)  
*Thyasira flexuosa* (Montagu)
- K *Kellia suborbicularis* (Montagu)  
*Montacuta ferruginosa* (Montagu)  
*Cardium echinatum* L.  
*Dosina lupinus* (Montagu)  
 K *Venus casina* L.  
*V. striatula* (da Costa)
- W *Venerupis (Tapes) rhomboides* (Pennant)  
 K *Donax vittatus* (da Costa)  
*Tellina tenuis* da Costa  
*T. fabula* Gmelin  
*Abra alba* (Wood)
- K *Gari fervens* (Gmelin)  
*Cultellus pellucidus* (Pennant)  
*Ensis ensis* (L.)  
*Spisula subtruncata* (da Costa)
- W *Mya* sp.  
*Corbula gibba* (Olivier)
- W *Cochlodesma praetenue* (Montagu)  
 K *Thracia* sp.  
 K *Cuspidaria cuspidata* Brown.
- ECHINODERMATA
- Asteroidea
- K *Astropecten irregularis* (Pennant)  
*Asterias rubens* L.
- Ophiuroidea
- Amphiura chiajei* Forbes  
*A. filiformis* (O. F. Müller)  
*Acrocinda brachiata* (Montagu)
- K *Ophiura texturata* Lamarck  
*O. albida* Forbes  
 K *O. affinis* Lütken
- Echinoidea
- W *Echinus esculentus* L.  
*Echinocardium cordatum* (Pennant)
- Holothuroidea
- Cucumaria elongata* Düben & Koren  
*Labidoplax thomsoni* (Herapath)
- PISCES
- K *Ammodytes tobianus* L.  
 K *Lepadogaster bimaculatus* (Donovan)

## ADDITIONAL SPECIES TAKEN BY TRAWL IN KAMES BAY

(W, species known also to occur in White Bay)

## COELENTERATA

*Actinea equina* L.

## POLYCHAETA

*Eulalia fucescens* St Joseph*Platynereis dumerilii* (Audouin & Milne-Edwards)

## CRUSTACEA

Cumacea

*Pseudocuma longicornis* (Bate)

Isopoda

*Idotea baltica* (Pallas)*I. pelagica* Leach*I. viridis* (Slabber)*I. emarginata* (Fabricius)*I. linearis* (Pennant)*I. granulosa* Rathke*Jaera marina* (Fabricius)

Amphipoda

*Orchomene humilis* (A. Costa)*Bathyporeia* sp.*Pontocrates arenarius* (Bate)*P. norvegicus* Boeck*Monoculodes* sp.W *Nototropis swammerdammi* (Milne-Edwards)*Megaluropus agilis* Hoek*Melita gladiosa* Bate(?)*Gammarus locusta* (L.)*Microdeutopus* sp.*Amphithoë rubicata* (Montagu)*Caprella* sp.

Mysidacea

*Erythrops elegans* (G. O. Sars)*Mysidopsis augusta* G. O. Sars*M. gibbosa* G. O. Sars*Schistomysis spiritus* (Norman)*S. ornata* (G. O. Sars)*Praunus flexuosus* (Müller)*Acanthomysis longicornis* (Milne-Edwards)

Decapoda

*Pandalus montagui* Leach*Pandalina brevirostris* (Rathke)

## Hippolyte varians Leach

*Spirontocaris cranchi* (Leach)*S. pusiola* (Krøyer)*Leander serratus* (Pennant)W *Crangon vulgaris* (L.)*Philocheras bispinosus* (Hailstone & Westwood)*P. trispinosus* (Hailstone)*Eupagurus prideauxii* Leach*Portunus corrugatus* (Pennant)*Macropodia rostrata* L.

## MOLLUSCA

## Gastropoda

W *Gibbula cineraria* L. (?)*Scaphander lignarius* (L.)*Aplysia punctata* Cuvier*Pleurobranchus membranaceus* (Montagu)*Acanthodoris pilosa* (Abildgaard)

## Lamellibranchia

*Chlamys varia* (L.)

## ECHINODERMATA

## Crinoidea

*Antedon bifida* (Pennant)*A. petasus* (Düben & Koren)

## Asteroidea

*Asterias rubens* L.

## Ophiuroidea

*Ophiothrix fragilis* (Abildgaard)*Ophiocomina nigra* (Abildgaard)*Ophiopholis aculeata* (L.)

## Echinoidea

W *Echinus esculentus* L.

## PISCES

*Raia clavata* L.*Nerophis lumbriciformis* (Pennant)*Syngnathus acus* L.*Centronotus gunnellus* (L.)*Callionymus maculatus* (Rafinesque)*Gobius minutus* Pallas*Pleuronectes limanda* L.*Solea lutea* (Risso).

## ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

THE ANGULAR SCATTERING OF BLUE, GREEN, AND RED LIGHT  
BY SEA WATER

By W. R. G. Atkins and H. H. Poole

*Sci. Proc. R. Dublin Soc.*, Vol. 26 (18), 1954, pp. 313-23.

Previous work on the scattering by sea water of light from a tungsten filament, and of this light filtered through a blue filter (*Proc. Roy. Soc. B*, 140, 1952, pp. 321-38), has been extended to include green and red light, a R.C.A. 1P22 photomultiplier cell being substituted for the R.C.A. 931A, which had not sufficient green or red sensitivity. To avoid fatigue the exposure was as short as possible and the current below  $0.2\ \mu\text{A}$ , usually under  $0.1\ \mu\text{A}$ . The same apparatus was used, but with a correction for the effect of light internally reflected in the experimental flask. This becomes large for angles of scatter exceeding about  $120^\circ$ , and renders doubtful the rise in scattering within a zone  $1^\circ$  wide previously deduced from measurements at greater angles. Below the minimum, which again occurred near  $120^\circ$ , the results closely resembled the previous ones, the zonal scattering rising with decreasing angle to some maximum at less than  $10^\circ$ , the lower angular limit of measurement. Blue light was more scattered than either green or red, the difference between the latter two being scarcely significant. Successive filtering through collodion filters  $1.4$ ,  $0.5$  and  $0.1\ \mu$  average pore diameter reduced the scattering by sea water to about the same value as that of the purest doubly distilled water, and increased the difference between blue light and the other colours. H.H.P.

## THE EUPHAUSIID CRUSTACEANS OF SOUTHERN AFRICAN WATERS

By Brian P. Boden

*Trans. Roy. Soc. S. Afr.*, Vol. 34 (1), 1954, pp. 181-243.

The euphausiid crustaceans from several extensive collections made in the waters around southern Africa have been examined. Forty-two species, belonging to nine genera, are described and fully illustrated. One new species, *Thysanopoda subaequalis*, is described, and sixteen new distribution records are added. Keys to the genera and species are included. A short comment is made on the hydrographic conditions in the area. The distribution of species is treated briefly, with extra emphasis on those species whose more southern representatives have been investigated by other workers. B.P.B.

QUELQUES CARACTÉRISTIQUES BIOLOGIQUES DE *LOPHIUS PISCATORIUS* L.

By L. Brull and Y. Cuypers

(avec la collaboration de L. Dubois et L. Wilsens)

*Arch. int. Physiol.*, T. 62, 1954, pp. 70-5.

The following properties of *Lophius* blood and urine have been determined:  
 (1) Systemic blood pressure is equal to 40-50 cm of water, with systolic variations of 3-5 cm. (2) There is about 2 g% haemoglobin in the blood. Variations do not depend on the size of the fish. (3)  $\Delta$  of bladder urine is lower than, or equal to, that of plasma in ten measurements out of twelve, and slightly above in the remaining two. (4) Plasma magnesium (normally 6.5 mg% or less) appears to increase from the time when the fish is caught, rising to 9 or even 16.7 mg. (5) Urine magnesium increases similarly after catching, reaching about 300 mg%. L.B.

SPICULE FORM IN *LEUCOSOLENIA COMPLICATA*

By W. C. Jones

*Quart. J. micr. Sci.*, Vol. 95, 1954, pp. 191-203.

The tri- and quadriradiates of *Leucosolenia complicata* are described in four aspects, surface view, transverse view, side view and planar view. The form and size in all four aspects vary with the distance from the oscular edge.

Each spicule ray tends to grow in a plane containing the optic axis. The oscular ray planes intersect at an angle which varies from 160° at the oscular edge to 140° at the base of the tube.

A preliminary discussion is given on some factors controlling spicule form.

W.C.J.

THE MECHANISM OF PROBOSCIS MOVEMENT IN *ARENICOLA*

By G. P. Wells

*Quart. J. micr. Sci.*, Vol. 95, 1954, pp. 251-70.

The mechanism of proboscis movement is analysed in detail in *Arenicola marina* L. and *A. ecaudata* Johnston, and discussed in relation to the properties of the hydrostatic skeleton.

Proboscis activity is based on the same three-stage cycle of movements in both species, but they differ anatomically and in their hydrostatic relationships. In *ecaudata*, the forward movement of body-fluid which extrudes and distends the proboscis is largely due to the contraction of the gular membrane

and septal pouches. In *marina*, the essential mechanism is the relaxation of the oral region which allows the general coelomic pressure to extrude the proboscis. The gular membrane of *marina* contracts as that of *ecaudata* does, but its anatomy is different and it appears to be a degenerating structure as far as proboscis extrusion is concerned.

The proboscis is used both in feeding and in burrowing; in the latter case nothing enters through the mouth; the difference is largely caused by variation in the timing of withdrawal relative to the 3-stage cycle. G.P.W.

# THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. xxvii (p. 761) and Vol. xxxi (p. 193) of this *Journal*.

The laboratory is open throughout the year and its work is carried out by a fully qualified research staff under the supervision of the Director. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology, physiology and other branches of science. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the laboratory.

## TERMS OF MEMBERSHIP

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Governors	. . . . .	500 0 0

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the *Journal* of the Association free by post; they are admitted to view the laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the library at Plymouth.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

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