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THE COMPARATIVE FORM, FUNCTION AND ECOLOGY
OF SOME NEW ZEALAND BRITTLE-STARS
(OPHIUROIDEA)

Thesis presented for the Degree of Doctor of Philosophy
in Zoology, at the University of Auckland
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by

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Section 1.
INTRODUCTION.

1.1. General considerations.

In her classic review of the echinoderms, Hyman (1955), describes the ophiuroids as "the most successful echinoderm group living today". This success has largely been attributed to both their smaller size and their remarkable agility. Many authors have commented on their great importance in the bionomics of the sea; various species participate in food chains involving demersal fish and many others serve as agents for the disposal and recycling of organic materials on the benthos. Their wide occurrence in the marine environment, coupled with their dense numbers, has led to their inclusion in the nomenclature of benthic communities and sub-communities, particularly in European waters. Some 1,900 extant species, being referred to 255 genera, have now been described (Fell, 1960).

The basis of our morphological and anatomical knowledge of echinoderms was principally laid around the turn of the century, having been extensively reviewed by Ludwig and Hamann (1901), in Bronn's Klassen und Ordnungen des Tierreichs; by Delage and Hérouard, in Traité de zoologie concrete (1903) by Cuénot, in Grassé's Traité de zoologie, (1948); and by Hyman (1955). Despite this early preoccupation with morphology, little information however is available on the functioning of even their most fundamental organ systems. Recent work has centred upon the anatomy of the podia (Smith, 1937; Buchanan, 1962; Woodley, 1967) and their method of protraction (Woodley, 1963; Buchanan and Woodley, 1963; Woodley, 1967). Particular
reference has been made to glandular secretions in both podia and spines (Fontaine, 1955, 1964; Buchanan, 1962, 1963(b)).

Other recent reviews occur in the Physiology of Echinodermata (1966), edited by Boolootian, the reviews in general covering individual aspects of the phylum as a whole - an exception being that on ophiuroid ecology by Fell (1966). Several general reviews on echinoderms have appeared recently, covering various aspects of the phylum, Nichols (1964, 1966), Clark (1962), and Millott, ed. (1967). The anatomy and physiology of the nervous system of echinoderms has also been extensively reviewed by Smith (1965). The majority of these reviews emphasise the lack of information available with respect to the ophiuroids.

A feature of ophiuroids which has often aroused comment is the lack of variety in their external appearance. With the exception of a few euryalous forms, (the basket stars), which possess branched arms, most species look remarkably alike. Their body form is stereotyped into a disc which is sharply delineated from the five (rarely more) symmetrically placed arms. The arms further possess a segmented array of variously formed spines, and small podia often referred to as papillae. It is this superficial similarity of form, plus the general lack of information on ophiuroid biology, which has prompted this somewhat varied investigation into some fundamental aspects of their structure and functioning.

Brief mention may be made of recent ophiuroid systematics. The work of Spencer (1951), Fell (1963 a and b), and Fell and Pawson (1966) argues for the derivation of both asteroids and
ophiuroids from an early somasteroid stock, and for the two classes to be combined in a new class, the Asterozoa. The correlations, as expressed by Fell (1963(a)) was questioned by Philip (1965), but Fell (1965) would appear to have given an adequate rejoinder. Barker (1967) has later cited biochemical evidence linking the ophiuroids with the echinoids, both groups also possessing plutei larvae. Nichols (1966), although supporting the close phyletic connections between the ophiuroids and asteroids, regards the somasteroids as being closer to the asteroids and thus a subclass of the Asteroidea. Therefore the exact phyletic position of the ophiuroids still remains in a fairly controvertial state and conclusive evidence has yet to be drawn.

Finally, Fell (1960) has published a synoptic key to the genera of ophiuroids, and has also revised the genera of Amphiuridae (Fell, 1962). The species of Amphipholis have further been revised by Thomas (1966). A key to the identification of New Zealand littoral ophiuroids has been published by Fell (1949).

1.2. The ophiuroids studied and their occurrence.

Ophiuroid populations are generally restricted to the offshore sublittoral where they occur in great numbers. Veevers (1951, '52), has estimated population densities near Plymouth, England, of Ophiothrix fragilis using an underwater camera. Densities of 392,000 per acre and 1.3 million per acre were recorded in two areas for this species. Fell (1961), also using photographic methods in the Ross Sea, has revealed uneven ophiuroid concentrations at depths varying from 75 to 350 meters. Population densities were estimated to be in the order of 40,000 per acre at
one station (A538/5), the population comprising mixed species.

Similarly, around the New Zealand coast large populations have been found to occur in the sublittoral zone (Hurley, 1959). The New Zealand coastline however also presents many shores where ophiuroids are to be encountered, not merely as stragglers, but forming populations of fairly stable numbers. An excellent example of such a shore is the rocky platform of Goat Island Bay, Leigh, Northland - the site of the Auckland University Leigh Marine Laboratory. This bay is situated immediately beyond Cape Rodney, a predominant headland which marks the northwestward limit of the Hauraki Gulf. The general ecology of this area has recently been described (Morton and Chapman, 1968), the rocky platform being termed the "echinoderm reef flat" owing to the conspicuous preponderance of members of this phylum.

The flat consists of a wide area of movable boulder cover which lies on the seaward side of a narrow, sandy, beach. The platform has been cut by wave action from soft, grey papa mudstone, the broken slabs of which form the loose boulder cover. Underlying the boulders, the mudstone has weathered to form a series of large slabs which break up to form broad basins, enclosing areas of standing water as the tide recedes. Such pools may in places have a depth of two feet but more frequently obtain a depth of less than one foot. The boulder cover retains smaller stones, coarse sand and shell fragments, the movement of which is further restricted by the irregularities of the platform. Clear water moves freely over the reef with the continual action of small waves approaching the shore from a northerly aspect.

It is here beneath the boulders that the principal ophiuroids of this study are to be found. By far the most abundant species
is *Ophionereis fasciata* (Hutton, 1872; Plate 1), commonly known as the mottled sand star. This greyish ophiuroid is of a moderate size with a disc diameter of up to 12 or 13 mm., the brown and white banded arms attaining a length of 60 mm. It is to be found in pools throughout the eulittoral zone and extends down into the sublittoral fringe. When moderately sized rocks resting directly on sand are raised, up to a dozen or so individuals may be revealed, either loosely buried or lying on the surface. They do not remain exposed for long but immediately seek cover. If a large boulder rests upon smaller stones it is beneath these that the animals may be found, usually with one or two arms moving freely from side to side in the water.

A smaller species may also be seen under similar stones, usually clinging to the underside of the rock surface. This is *Ophiactis resiliens* (Lyman, 1882; Plate 2), of more variable colour than *Ophionereis fasciata*, varying from red, through olive-green to brown. The disc is seldom greater than 8 mm. in diameter, the spiny arms being 35 mm. long. *Ophiactis resiliens* has a similar range to *Ophionereis fasciata* but is also found deep in crevices, which readily form in the mudstone, in algal holdfasts and between encrusting sponges.

The larger, black oar sand-star, *Ophiopteris antipodum* (E. A. Smith, 1877; Plate 3), is readily identified by its deep purplish-black colour and the flattened arm spines arranged like banks of oars on either side of the arm. Individuals may weigh up to 5 or 6 gms., with disc diameters of 20 mm. and arm lengths of 70 mm. Although common at Goat Island Bay it is not abundant and has a more restricted range being confined to the lower eulittoral and sublittoral fringe. It is also commonest under
larger, more stable boulders, clinging to the undersurface - particularly when this is encrusted by bryozoans and sponges.

These three species have formed the basis of this study, being of particular interest owing to their occupation of similar niches within the same general habitat. However, the sections on feeding and the functional morphology of the structures involved, plus the control of the podial action, have been broadened to encompass some other littoral ophiuroids found in New Zealand. A brief account of these animals now follows.

A typical member of the fauna of protected sand beaches in the Auckland area, as described by Morton and Miller (1968) is Monamphiura (≡ Amphiura) aster (Farquhar, 1901; Plate 4). This grey-brown burrowing ophiuroid occurs with a patchy distribution over the lower beach of such protected shores - particularly that of Cheltenham, on Auckland’s North Shore, which is sheltered by the offshore extinct volcano of Rangitoto. This beach contains a fairly large silt fraction while being sufficiently thixotropic to allow easy burrowing. The water table here lies on the surface preventing the drying up of infaunal animals. Monamphiura aster is found at a depth of about 10 cm. with its stiff arms maintaining burrows in contact with the surface. The disc is seldom more than 12 mm. in diameter but arms lengths may reach 140 mm.

The sheltered rocky shores of Southern New Zealand, (an example of which is described by Batham, 1956, - Aquarium Point, Otago Harbour) are often typified by the intertidal occurrence of the red-brown soft skinned sand-star, Ophiomyxa brevirima (H. L. Clark, 1915; Plate 5). This may be locally abundant under stones in the lower eulittoral zone, but occurs typically in the sublittoral area amongst the holdfasts of the bladder kelp,
Macrocystis pyrifera and the stalked ascidian Pyura pachydermatina. As its name implies this ophiuroid has a smooth, thickened epithelium which covers both the arm plates and the disc.

In contrast, the sublittoral zone of rocky shores of Northern New Zealand, such as those of Urquhart's Bay, Whangarei, commonly yield specimens of New Zealand's largest ophiuroid, the beautiful snake-tailed brittle star, Pectinura maculata (Verrill, 1869; Plate 6). This ophiuroid is very common on the benthos in certain areas around New Zealand but only encroaches into the littoral zone at the northern limit of its range.

A study of common New Zealand intertidal ophiuroids would be incomplete without mention of the ubiquitous Axiognathus (≡ Amphipholis) squamata (Delle Chiaje, 1829), which is common intertidally throughout the country.

As previously mentioned, the ophiuroids of the Leigh intertidal reef form the basis of this study, which has been broadened in sections 2 and 3 to encompass some other littoral ophiuroids of New Zealand. The remainder of the work covers the form and function of the stomach (section 4), the bursae (section 5) and the gonads (section 6). The ecological and other general data comprising section 7 is presented as much for the paucity of such information as a whole, as for its value as a coherent study.
Plate 1.
*Ophionereis fasciata*
Family
*Ophiochitonidae*

Plate 2.
*Ophiactis resiliens*
Family
*Ophiactidae*

Plate 3.
*Ophiopteris antipodum*
Family
*Ophiocomidae*
Plate 4.
*Monamphiura aster*
Family
*Amphiuridae*

Plate 5.
*Ophiomyxa brevirima*
Family
*Ophiomyxidae*

Plate 6.
*Pectinura maculata*
Family
*Ophiodermatidae*
Section 2.

FEEDING METHODS, AND THE FUNCTIONAL MORPHOLOGY
OF THE STRUCTURES INVOLVED.

2.1. Introduction.

The feeding methods of ophiuroids are far from well understood, and although several generalized accounts occur in the literature, few detailed descriptions have been given; and although detailed accounts have been given of the structure of podia and spines, for the most part such descriptions apply to species the feeding methods of which have not been fully investigated. A notable exception is that of Ophiocoma nigra, which has been extensively investigated, (Mortensen, 1927; Smith, 1937; Vevers, 1956; Nagabhushanam and Colman, 1959; Roushdy and Hansen, 1960; Fontaine, 1955, 1963, 1964, 1965).

Methods of ophiuroid feeding have recently been reviewed by Fell (1966) and Reese (1966). Detailed descriptions are mainly drawn from the studies of Magnus (1962, 1964) and Fontaine (1964, 1965) on Ophiocoma scolopendrina and Ophiocoma nigra respectively. An interesting comparative study of the feeding methods of two Amphiura species has been conducted by Buchanan (1964), A. filiformis capturing material in suspension and A. chiajei collecting deposited matter on the bottom surface.

Both microphagous and macrophagous methods have been variously described for the group as a whole, and these are briefly as follows. Microphagous feeding may be by (1) the use of a mucous net accompanied by arm waving in the water; (2) passive arm elevation; (3) water surface browsing, and (4) bottom surface sweeping. Three macrophagous methods described are (1) arm-loop capture;
(2) the collection and transport to the mouth of small particles by the podia, and (3) direct browsing of carrion and algae. It is notable that five of these seven methods have been described for one species, *Ophiocomina nigra* (Fontaine, 1965).

The structure of the podia and their glands has been investigated notably by Hamann, (1889); Cuénot, (1891); Reichensperger, (1908); Smith, (1937); and recently by Buchanan, (1962) and Fontaine, (1964). Similarly, accounts of ophiuroid spines and their glands have been given principally by Hamann, (1889); Cuénot, (1891); Reichensperger, (1908); Sokolow, (1909); Buchanan, (1963); and Fontaine, (1955, 1963, 1964). Many of these authors have commented on the various roles of the glands within these structures, both with reference to the production of mucus and the phenomenon of bioluminescence. An excellent review of the latter function is given by Millott (1966).

Unfortunately, many of the earlier investigators lacked the knowledge and access to the many histochemical methods available today. Buchanan (1962, '63), studying various British ophiuroids, has however distinguished fibrillar cells in epifaunal species and non-fibrillar ones in infaunal species. It was histochemically determined that all spine glands contain an acid mucopolysaccharide with the exception of fibrillar glands in *Ophiopholis aculeata*, which stained strongly with the periodic acid-Schiff technique. In a more detailed study of *O. nigra*, Fontaine (1955, 1963, 1964) has demonstrated two types of integumentary mucous gland cells: a massive multicellular gland secreting a highly sulphated acid mucopolysaccharide functioning in defense against predation; and secondly a unicellular gland secreting a simple
acid mucopolysaccharide functioning in suspension-feeding and sanitation mechanisms. A third type of mucous cell found in the podia was also considered to be a highly sulphated acid mucopolysaccharide.

The anatomy of the podia with particular reference to the mechanisms of podial protraction has recently been published by Woodley (1967), who also summarizes the previous literature on the subject. The observations of this paper are considered in detail in section 3, section 2 being restricted to observations on the feeding and associated behaviour of seven species plus histological and histochemical notes on the podia and spines. The structure of some gland cells of the ophiuroids found at Leigh have been further studied by electron microscopy.

2. 2. Methods.

(a) Observations. The feeding behaviour of freshly collected animals was observed in a perspex tank with the aid of a binocular microscope. Currents within the tank were created by the use of electric motors driving small propellers. A toy outboard motor, which could be clipped onto the side of the tank, proved an excellent method of providing a current parallel with the bottom of the tank, and its speed was controlled by a variable resistor. The speed of currents thus created were roughly estimated by timing water-borne particles along a centimeter scale on the side of the tank. This motor could also be rotated at right angles to the current produced, and further deflections of the current were obtained by using perspex deflectors.

The detailed behaviour of podia was best observed by using a
"dental mirror" and a water emersion objective; the latter being focused onto the image of the mirror, which was held in the desired position underneath the arms.

(b) **Histology/histochemistry.**

Before fixation, specimens were narcotized either with isotonic magnesium chloride solution or by the addition to sea water of a few drops of phenoxetol. Fixation for 8 hours in Heidenhain's 'Susa' proved adequate for most tissues, and subsequent decalcification was attained either by 2% nitric acid in 70% alcohol (renewed daily) or with a 10% E.D.T.A. (E.D.H.) solution. After dehydration, tissue was embedded in wax (58°C) after preimpregnation with celloidin as given by Páterfi's method in Pantin (1962). Serial sections were cut at 6 µm on a rotary microtome. Several general histological stains were used, the most useful being Masson's trichrome as modified by Pantin (1962); previously recommended by Nichols after his studies on echinoid and crinoid tube-feet (Nichols, 1959a and b, 1960, 1961). Other general stains of use were Mallory's Triple, Weigert van Gieson, Ehrlich's haematoxylin and eosin, and Heidenhain's azan stain.

Various histochemical stains were used, the majority being chosen to aid in the differentiation of mucous glands. An indication of basophilia and acidophilia was obtained with Ehrlich's haematoxylin and eosin (E.H. and E.). For the detection of particular reactive groups in complex carbohydrates, the best general purpose stain was the Alcian blue-periodic acid-Schiff technique (AB/PAS) as modified by Mowry (1963). With this method complex carbohydrates rich in acidic groups (especially carboxyls) are coloured turquoise blue, while neutral carbohydrates rich in
vicinal hydroxyl groups are coloured magenta. Further, carbohydrates having both acidic and oxidisable vicinal hydroxyl groups are stained in deeper blue to purple shades. The presence of vicinal hydroxyl groups and equivalent derivatives was also confirmed by using the periodic acid–Schiff (PAS) reaction after McManus (Pearse, 1961).

Alcian blue (AB) alone, was used for the detection of complex carbohydrates with free acidic groups, both by the method of Steedman (Pearse, 1961) and as given by Mowry (AB-H) (1963). Both Alcian blue and methylene blue (MB) were buffered with Walpole's acetate: HCl buffer at pH 1.5 and 3.4, using 1% solutions of the stains. Alcian blue staining at 1% conc. in 0.6N HCl (pH 0.4), and 3% in c.2N H₂SO₄ (pH 0.25) was also used.

Further differentiation of acidic carbohydrates was determined by methylation and saponification of the tissues. Fisher and Lillie (1954) found that methylation abolishes reactivity, depending on the presence of carboxyl or sulphate groups. Biochemical evidence showed that carboxyl groups were methylated but that sulphate groups were removed entirely by the procedure (in Mowry, 1963). Spicer and Lillie (1959) found that treatment of methylated tissue sections in alcoholic solutions of alkali resulted in saponification, i.e. the return of free carboxyl groups, which restored the stainability of basophilia temporarily abolished by the methylation.

Methylation was accomplished in 1% HCl in absolute methanol at 60°C. The progress of the methylation was followed by staining sections with the AB/PAS technique after 1.5, 3, 4 and 7 hours. A second batch were methylated and subsequently saponified by
immersion in 1% KOH in 70% ethanol for 30 mins. at room temperature (22°C). The progress of demethylation was also followed by staining with the AB/PAS method. Tissue staining with the Alcian blue after demethylation has usually been considered to be rich in carboxyl groups, while negative results indicate the removal of sulphate groups. However, both Kent (1963) and Mowry (1963) point out that such results do not exclude the possibility of other alterations occurring to the molecule; and it must be admitted that the lack of staining caused by sulphate removal has all the philosophical disadvantages of a negative result. Thus further confirmation of sulphate groups was sought.

Heath (1962) claimed that 0.003% solutions of basic dyes in 5% AlSO₄ produces a highly specific staining reaction for sulphated mucopolysaccharides. The best dyes were found to be nuclear fast red and methylene blue. Sections were stained in methylene blue thus prepared (MB/AlSO₄); 20 minutes usually providing adequate staining. The metachromatic dyes toluidine blue and azure A were also used for sulphate groups. Toluidine blue (Tol. B) 0.5% aqueous, buffered at pH 1.5 and 3.4 with acetate: HCl buffer, was used for periods of up to 6 hours, sections being immediately examined in water (Pearse 1961). Toluidine blue 0.1% in 30% ethanol, after Kramer and Windrum (Pearse 1961), was also used. Azure A 0.5% was used at pH 1.2, 2.1 and 3.1 in acetate: HCl buffer and in 0.6N HCl (pH 0.4) at a concentration of 0.02%. At pH values 1.2 to 3.1 staining periods were 10 to 20 minutes, but up to 40 minutes was allowed at pH 0.4. A gamma metachromasia at low pH values was taken to be indicative of the presence of sulphate groups.
Spicer (1963) claims that strong and weak sulphation in mucopolysaccharides can be differentiated by their staining with Alcian blue and azure A at different pH values. Strongly sulphated mucins are said to show strong gamma metachromasia with azure A at pH 0.5 and below, and similarly stain with Alcian blue at this pH value, lacking affinity for Alcian blue at pH 3. On the other hand, weakly sulphated mucins in general reveal a beta metachromasia with azure A, at pH 1.5 to 3.0, and also combine with Alcian blue within this pH range.

Many histochemical tests were compared with results obtained with Mayer's mucicarmine, using Southgate's method as given by Gurr (1962). Mercury-bromphenal blue (HgEPB), after Bonhag, was used for proteins, in the manner of method (2) cited by Pearse (1961, p. 792). This method was claimed by the original authors (Mazia, Brewer and Alfert, 1953) to be quantitative, in that the amount of dye bound was proportional to the amount of protein over a wide range.

(c) Electron microscopy. Isolated spines and podia were fixed at room temperature for one hour with 6% glutaraldehyde in 0.2M phosphate buffer. After washing overnight with cold buffer, and subsequent fixation with 1% buffered osmic acid, tissue was dehydrated in ethanol and embedded in Epon. Sections were doubly stained in uranyl acetate and lead citrate. Examination was with a Philips E. M. 200.
2. 3. Ophionereis fasciata.
(a) Feeding methods. When observed under water on the intertidal reef only the extended arms of this species can be seen. If placed into aquaria provided with a bottom of sand and stones (taken from the reef) their first reaction is to seek cover under stones of any size. Undisturbed animals, within a few minutes, extend two or three arms from this shelter, and where the stone rests directly on sand active "burrowing" immediately takes place. This "burrowing" takes the form of removing sand particles from beneath the stone, the particles being passed from one podium to another in a distal direction along the arm. Individual sand grains are picked up by the tip of the podium and if the particle is large the opposing podium of the same segment, plus the podia of the adjacent distal arm segment, will also aid.

While this continual removal of sand grains is taking place, the arm is moved laterally through an angle of about 25° to spread the accumulation of particles beneath the distal portion of the arm. Occasionally this accumulation falls back under the arm and the rate of removal quickens considerably, although it gradually slows down again to the original, steady pace.

Such actions may proceed for periods of 10 to 15 minutes, finally resulting in a clear passage being formed under the extended arms. However, it may begin again at any time, presumably when a "fall" has occurred somewhere beneath the stone. No other attempt was seen to maintain the passageway, such as the applying of mucus to the walls, and the coarseness of the sand would make
such a procedure difficult.

From this position three or four arms are extended from beneath the cover, although only the distal third can usually be seen. Even at night very little extension beyond this length occurs. The arms are slowly moved laterally at a rather small angle to the horizontal, usually less than 30°, although always clear of the bottom. The podia may be seen in constant action moving between the spines. Each podium primarily strokes the spines lying distally to it, the stroking action usually being restricted to the two most oral spines and most frequently concerning the oral spine alone (Fig. 1). Occasionally podia move around touching all the spines within reach, both distal and adjacent to their own arm segment.

Within a few minutes of these actions commencing, threads of mucus can be seen to cover the spines, particularly the most oral ones, and the continual actions of the podia extend these threads so that they form a loose mesh between ipsilateral spines. This spreading of the mucus is further promoted by movement of the spines, although such movements may not be inherent in the spines themselves, and is usually accomplished by the force of the extended podia exerted against them.

Frequently the podia, after stroking several spines, accumulate particles trapped in the mucus. Each podium may then be observed to undergo movements similar to those illustrated in Fig. 2 ((a) to (e)). Firstly, the podium is tightly coiled upon itself (Fig. 2(b)), resulting in the concentration of the adhering particles near the podial tip (Fig. 2(c)). This is followed by
a rapid movement by which it appears the particles are scraped off onto the tentacle scale, where they remain (Fig. 2(d) and (e)).

With a completely different action these small accumulations are added to similar ones at the bases of the neighbouring podia, usually those of both sides of two or three segments, and by the prodding of two or more podia compacted into a larger mass. The resulting brownish bolus is then manipulated by the combined action of the pair of podia in each proximal segment and hence passed to the mouth.

The movements of the arms are greatly increased by the introduction of a current and often lie almost motionless in still water. As soon as a current is introduced the arms are raised and waved laterally in the water, either one at a time or all at once. At current speeds of 5-10 cm./sec. this behaviour is maintained, but at moderate speeds of 10-15 cm./sec. the arms tend to be withdrawn slightly. No orientation to current direction was observed.

The apparently independent nature of each arm is also evident when one arm is lightly pinched with a pair of forceps, as that arm only is withdrawn, the other arms remaining extended. Stronger attack however, such as that resulting in injury, results in the withdrawal of all the arms.

If carmine particles are introduced into the tank the resulting bolus, upon being passed to the mouth, is handled by the buccal podia and then passed again distally down the arm and dropped outside the disc edge. Normally the bolus is handled by the two pairs of buccal podia which scrape it against the teeth and oral papillae (Fig. 3). It would appear that any gustatory discrimination is
a property of the buccal podia alone.

The material caught in suspension is mainly of bottom detrital origin, very little phytoplanktonic material being present. This applies not only to animals in observation tanks but also to those observed on the reef.

Other forms of feeding behaviour have been observed. Small pieces of finely chopped mussel are passed to the mouth by direct transfer from podium to podium. Larger pieces are brought closer to the mouth by the bending of the arm itself. Animals left for some time in tanks often directly ingest small shell fragments and on one occasion a length of ophiuroid arm (Ophionereis fasciata) comprising seven or eight segments, was so forcefully engulfed by direct handling with the buccal podia, that damage to the aboral stomach and disc wall resulted. Such direct ingestion may also occur on the reef and on one occasion a specimen was found in the act of ingesting a piece of alga, Liagora harveyana, a semicalcareous alga locally abundant at Leigh during the summer.

(b) Podia. The podia are the externally visible organs of the water vascular system and comprise a distinct basal area within the arm, the bulb, considered by Woodley (1967) to be occasionally analogous to the ampullae of other echinoderms; and an external distal component, the stem. The bulb is housed within the vertebral ossicle, being connected with a branch from the radial canal. A valve, situated at this junction, lies mainly within the bulb.

The stems of both arm and buccal podia of Ophionereis fasciata have an annulate appearance when relaxed or contracted (Fig. 4(a) and 5(a)). In the relaxed state the stem is about 1 mm in length in the arm podia, that of the buccal podia being only about two
thirds as long. The arm podia also differ from those of the buccal cavity by the possession of a terminal knob, which has a brownish tinge, the stem of the podium being translucent and colourless.

The main structural feature of the podium is a large cylinder of connective tissue of two distinct systems. Internally, in relaxed preparations, are what superficially appear to be a series of circular bands some 2-3\(\mu\) in diameter. As Woodley (1967) has shown for *Ammphiura* species, this inner layer is in fact double, consisting of two symmetrically opposed arrays of parallel fibres running at a steep angle to the longitudinal axis. Each fibre is itself composed of a large number of fibrils bound tightly together. In relaxed podia these two systems appear to be interwoven and are difficult to distinguish, but Woodley has shown that in fact an inner and outer spiral can be differentiated. In *Ophionereis fasciata* the angle of these fibres to the longitudinal axis is, in relaxed specimens, about 75° in the bulb, 70° at the base of the stem and increasing to near 90° along the length of the podium. It appears that such a system is capable of distortion in the manner of a set of "lazy-tongs", one spiral system sliding upon the other.

This inner envelope is surrounded by a longitudinal connective tissue layer, which is folded when relaxed, and presumably sets a limit to the protraction of the podium when it is both straightened and stretched, as discussed by Smith (1947) for asteroid tube-feet. The longitudinal connective tissue is much thicker than the inner layer, particularly in the stem where it is some 8-10\(\mu\) wide. A further layer of connective tissue lies on the
adambulacral side, external to the podial nerve, at the base of
the annulæ (Fig. 4). This thinner layer also runs longitudinally.
A similar division of the connective tissue by the podial nerve has
been noted in Ophiothrix fragilis (Smith, 1937).

Within the envelope of spiral fibres lies a longitudinal
muscular system which is attached to it. The contractile elements
of these muscle cells form a cylinder, their nuclei lying internally
and often aligned at an angle of 45° to the longitudinal axis.
The nuclei are often difficult to distinguish from those of the
coelemic epithelium, which is closely applied to the muscle cell
bodies, thus lining the inside of the podium.

There is a very striking difference between the extent of
musculature in bulb and stem. The contractile elements are 8-10 μ
thick along the stem but up to 30 μ in the bulb. The bulb is also
much wider than the lumen of the stem, internal muscle cylinder
diameters being 190 μ and 75 μ respectively.

A nerve plexus encircles the podium external, and adjacent
to, the longitudinal system of connective tissue fibres, with an
average depth of 5 μ. The epithelium is innervated by nerve tracts
along its entire length, several such tracts innervating each annu-
lus. The plexus thickens to form a podial nerve some 20 μ in
diameter on the ambulacral side of the podium where, as noted above,
connective tissue occurs external to it.

The stem is covered by an epithelium of varying depth and is
capable of considerable folding. This epithelium contains numerous
gland cells although their density is only fully apparent when a
histochemical stain such as AB/PAS is used. They are concentrated
mainly at the podial tip and along the ambulacral side of the stem.
The cells are usually as deep as the epithelium and thus extend some 60 μ at the tip, resting on the terminal nerve plexus. Many cells are swollen just below the external opening, possibly representing an accumulation of mucus prior to release. No contractile elements have been distinguished, such as those described in echinoid podia (Nichols, 1959(a)) or those of crinoids (Nichols, 1960). The accumulation of mucus near the tip suggests that their activity may be intermittent, and that only part of the cell contents are released at one time. Possibly the movement of the epithelium during the normal activities of the podium may be effective in releasing this sub-terminal accumulation, or the pressure exerted on it by rubbing against the spines and tentacle scale may suffice. The base of the cell is also swollen, but the neck of the cell canal may be as little as 2 μ in diameter. The nuclei are not easily distinguished from the many nuclei of the epithelium, but may be seen displaced to one side of the cell, slightly elongated (7 μ) and densely stained. The contents appear to be finely granulated and the histochemistry of these cells is summarized in Table 1. The histochemical results are somewhat complex. The secretion appears to be an acid mucopolysaccharide although they are positive to the PAS test. Stainability with the Alcian blue occurs after subsequent saponification of methylated sections, suggesting the presence of carboxyl groups. Sulphate groups appear to be entirely lacking and thus the acidity may principally be due to carboxyl groups alone.

The buccal podia differ mainly from the arm podia by the absence of a terminal knob (Fig. 5). They also appear wider due to an increase in width of the water vascular cavity, rather than
to any increase in the width of the tissues of the podial wall. Terminally there is also a thickening of the nervous tissue which heavily penetrates the distal epithelium. Fewer podial gland cells are present.

(c) **Spines.** Three pairs of arm spines arise from the lateral arm shields, save for the most proximal twelve or so segments where a fourth pair is present. For the convenience of this account they have been numbered from the ambulacral side as spines 1, 2 and 3, the fourth being designated as spine 2A for reasons discussed below (Fig. 6).

Spine 1 is about 1mm. long and 250/μ wide. The centre of the spine is hollow, containing a large basophilic glandular mass consisting of multicellular cells packed closely together, (Fig. 7); the mass being some 75/μ in diameter and 400/μ long. Ducts from this multicellular gland open to the exterior both at the spine tip and sporadically along the spine length. The ducts consist of squamous epithelial cells with elongated nuclei, 6 to 7/μ long and little over 2/μ wide. It is difficult to distinguish individual cells within the central area of the spine and round nuclei with a diameter of 5/μ occur scattered throughout.

The contents of these cells are intensely basophilic and fibrillar in appearance. Their histochemistry is summarized in Table 1 and implies a highly acid mucopolysaccharide. This aciddity appears to be due to carboxyl groups although a beta, tending to gamma, metachromasia with azure A at pH 0.45 indicates the presence of at least some sulphate groups. Such a conclusion is supported by the positive result obtained with MB/AlSO₄. Strong methylation at 60°C is required for at least 4 hours to reduce the
<table>
<thead>
<tr>
<th><strong>T A B L E 1.</strong></th>
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<tbody>
<tr>
<td><strong>HISTOCHEMISTRY - GLANDS OF OPHIONEREIS FASCIATA.</strong></td>
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<table>
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<tr>
<th><strong>HISTOCHEMICAL TEST</strong></th>
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<th><strong>OUTER INTEG. GLANDS AND SPINE 1</strong></th>
<th><strong>CENTRAL GLANDS SPINE 2 &amp; 2A</strong></th>
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<td>BASIPHILIC</td>
<td>ACIDOPHILIC</td>
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<td>MAGENTA</td>
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</tr>
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</tr>
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<td>pH 3.4</td>
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<td>MB pH 1.5</td>
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<td>pH 3.4</td>
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<td>+</td>
<td>-</td>
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<td>++ (RED)</td>
</tr>
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<td>+++ (RED)</td>
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<td>+ (RED)</td>
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<td>++ (RED)</td>
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<tr>
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<td>-</td>
</tr>
<tr>
<td>METHYLNR. 4 hrs. + SAPONIFICATION</td>
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<td>ALPHA</td>
<td>ALPHA/BETA</td>
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</tr>
<tr>
<td>HgBPB</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

**+** POSITIVE

**++** STRONG

**+++** INTENSE

**-** NEGATIVE

**0** NOT TESTED
affinity for Alcian blue with the AB/PAS routine and even after 7 hours the basophilia is not removed entirely, although further reduced. Saponification however, readily restores the staining reaction to its full extent.

The reason that the Alcian blue stain is not suppressed may lie in the particular configuration of the mucin molecule, requiring more than the time given for total esterification of the carboxyls (Quintarelli, 1963). The comments of Quintarelli, studying salivary mucins, may be applicable here. "Furthermore, the possibility that some chemical groups obstruct or decrease the esterification rate of the carboxyl in a given tissue seems unlikely in that the same chemical groups would also decrease the penetration rate of the dye to the available acid radicals and thus show an over-all weak staining in the untreated section", (page 355). Carboxyl groups are no doubt largely responsible for the acidity of this mucin and Alcian blue is bound intensely at all pH levels. Mucus with an identical staining reaction also covers the surface of the spine.

The spinal nerve directly innervates the cell mass at its base, branching as it does so. It has not been possible to follow any subsequent pathways within the glandular area. As there are no muscular elements within the spine the method of extruding the gland contents is not clear. It is likely that the mechanism is a physicochemical one, as suggested for Ophiocoma nigra (Fontaine, 1964), in the absence of any obvious mechanical means.

Spine 2 is both longer and wider than spine 1. Again the centre of the spine is hollow but here it is packed with large acidophil cell complexes, occupying the middle third of the spine
in longitudinal section (Fig. 8; Plate 7). At their proximal end these cell masses are separately innervated by branches of the spinal nerve, which divides into a number of discrete fibre bundles at the base of the spine.

The glandular cell masses are closely packed together and their number can only be clearly seen in transverse sections (Fig. 9). Up to twenty cell bundles may be found, of different sizes and grouped together in the centre of the spine. They range in diameter from 10 to 40 μ. Each cell mass consists of 20 or so actual cells closely packed together and bound by a membrane (Fig. 10). The number of individual cells thus bound varies with the total size of the bundle, and with the size of the cells themselves, which is not constant.

In the distal third of the spine the bundles separate and long canals from them fan out towards the surface of the tip of the spine. These gland ducts do not appear to actually pierce the spine but stop about 3 μ from the periphery, widening slightly. There appears to be only one canal per cell bundle distally, and proximally each bundle is innervated by a single nerve tract. Scattered, round, nuclei occur within the cells.

The contents of these central cells, is, in Masson's Trichrome, of a coarse granular nature of ochre colouration. When stained with mercury bromphenol blue an intense colouration is produced (Plate 8), of equal density to muscle tissue in the same section, indicating a high protein content. With the AB/PAS routine they colour magenta indicating the presence of vicinal hydroxyl groups. Negative results were obtained with tests for acid radicles and as methylene blue in aluminium sulphate (MB/AlsQ₄), and alcoholic
toluidine blue, also gave entirely negative results staining with azure A was not attempted. The histochemical results are summarized in Table 1. Methylation for 4 hours removed the staining reaction and only partial recovery was attained with saponification after the PAS routine. Thus it appears that 1, 2-glycol groups may also be affected (Pearse, 1961). The granules therefore appear to be neutral and of a complex proteinaceous nature.

Around these central cells, peripheral basiphilic cells honeycomb the outer structural part of the spine (Fig. 8), and stain with the light green of Masson's Trichrome. They stain clear blue in the AB/PAS routine and stand out clearly from the central cells (Plate 7). Their contents are fibrillar and have staining reactions of identical nature to those of spine 1. The spinal surface is also coated with this mucus and the glands occur along the entire length of the spine.

The central cells are surrounded by a ground substance, the "Kalkgrundsubstanz" of Reichensperger (1908), which does not stain with any of the histochemical tests applied. It has a heavily granulated appearance and is not removed by the process of decalcification.

As noted above, a fourth spine occurs on the first twelve or so arm segments. Where three spines occur, spine 3 resembles spine 1, only with a much reduced central basiphilic cell complex. However, where four spines occur the third spine from the ambulacrum is identical to the second, the fourth spine resembling the spine 3 of the rest of the arm. It appears therefore that a spine like spine 2 has been interpolated between spines 2 and 3 in this region and has thus been designated spine 2A in this account.
Other basophilic fibrillar cells occur scattered in the arm plates, particularly along the ventral (ambulacral) plates and also in the tentacle scales. Again, these glands appear to be essentially similar in content to those described in spine 1.

2. 4. Ophiactis resiliens.

(a) Feeding methods. Ophiactis resiliens are typically found in crevices, algal holdfasts and sponges, often in an inverted position. They have a strong tendency to climb when placed into aquaria but usually seek shelter in empty shells and beneath stones. They show a general lack of activity in still water but upon the introduction of a current the arms are quickly raised to a near vertical position with the podia stiffly extended (Figs. 11 and 12).

Each arm is orientated with the ambulacral surface facing the direction of the current flow (Fig. 12), and reversal or change in direction of the current elicits a rapid rheotactic response, turning the arm to face the new current direction. Current speeds in excess of 15cm./sec. tend to bend the arms backwards but there is no attempt to withdraw them, as seen in Ophionereis fasciata. The rigid podia are extended at an angle of about 40° to the perpendicular of the ambulacral surface in a seemingly permanent stance.

At intervals of about 2 minutes, however, the podia on both sides of the arm suddenly collapse in a wave from near the arm tip in a proximal direction. This movement is very rapid and exceedingly difficult to follow adequately particularly as the arms move slightly and go out of focus, or the field of view, during these movements. It appears that as each podium collapses it also bends to its proximal side, rubbing against its neighbour which in turn collapses and so in sequence orally. The sequence is too rapid
to be seen in great detail but the movements are inferred in Fig. 13.

The surface of each podium is covered with prominent papillae and particles in suspension accumulate primarily on these. The collapsing action of the podia has the effect of combing one podium against another and in this manner particles accumulate in greater density on the more proximal podia. The collapsing sequence occurs along the whole length of the arm and only the most proximal podia show any tendency to form a bolus. Such a bolus is formed by the podia being bent upon themselves and with a considerable amount of twisting of the podium the food particles are compacted and passed to the mouth.

In the event of larger particles being caught by the podia, these are passed by direct transference from one podium to another, although they often tend to be lost at some stage down the arm. Such a loss may be accidental or possibly some degree of gustatory discrimination is inherent in the arm podia themselves. No other form of feeding behaviour was observed, nor was it possible to elicit any response to larger pieces of chopped mussel etc. placed near them. The appearance of the particles caught by the arms is of a greenish colour and, with evidence obtained from stomach contents, appears to consist almost entirely of phytoplankton, usually spherical diatoms.

Although the addition of dilute toluidine blue to tank water stained mucus on the podia, and to some extent on the spines, mucous threads were never seen to extend either between spines or podia. Very occasionally the podia rub against the spines and thus may gather any particles which have adhered to them.
(b) Podia. The arm podia, as noted above, are both very long when protracted and also have a papillate surface. There is a small terminal knob. In the relaxed condition these podia are only a quarter or less of their protracted length. The buccal podia are short with a large terminal knob, and only the distal half of the surface is papillate.

The tissue layers of the arm podia are essentially similar to those of *Ophionereis fasciata*, although the presence of papillae present a striking difference from the annulæ, (Fig. 14; Plate 9). In the relaxed state the stem has a length of about 0.5 mm. and a diameter of 100 μm. The papillae are large and stand 50 μm high on the surface. The inner connective tissue envelope is conspicuous although even in relaxed preparations the angles of the fibre bundles cannot easily be measured owing to the small diameter of the podium. Those of the bulb vary between 70° and 80° to the longitudinal axis, depending on the degree of contraction, while those of the stem appear to be almost 90°. In view of the high degree of extension of these podia, the angles would be considerably smaller in the protracted condition. The longitudinal layer is considerably folded and only 5 μm thick.

The musculature of the bulb is again greater than that of the stem, being some 10-12 μm thick and forming a cylinder with an overall diameter of 70 μm. The stem musculature is relatively slight at a thickness of 4-5 μm, and in relaxed podia the muscle cell bodies and coelomic epithelium almost occlude the lumen, and in fact do so completely when the podium is contracted.

The podial nerve plexus lies external to the connective tissue as a very thin sheet, 2 μm, thickening to 10 μm to form the podial
nerve along the ambulacral side of the stem. Each papilla has a cushion of nerve tissue at its base which receives a fibre tract from the nerve plexus. Small pockets of connective tissue occur outside the plexus between the bases of the papillae (Fig. 14).

The epithelium, save where it is raised to form papillae, is only 8-10 μ deep. Mucous cells are confined to the papillae and terminal knob, being particularly dense in the former. The cells run the whole length of the papillae, swelling distally to 4-5 μ, with an otherwise uniform diameter of 2 μ. A slight swelling usually occurs at the distal end of the cell where mucus collects prior to its discharge. The cell contents are finely granular, the staining reactions of which are given in Table 2. Although closely resembling the reactions of Ophionereis fasciata spine 1 and integumentary glands, there appears to be an increase in sulphate groups as may be seen by the tending gamma metachromasia at a low pH with azure A, and an increased reaction with MB/AlSO₄. However, methylation does not irreversibly remove the basiphilia, suggesting that both carboxyl and sulphate groups are present.

The buccal podia have a wider central lumen and a larger terminal knob than the arm podia, with an overall relaxed stem diameter of 130 μ. The terminal knob is wider than the stem, with a large concentration of nervous tissue at its base densely innervating the epithelium. Mucous cells like those of the arm podia occur both terminally and in the distal papillae.

(c) Spines. Five pairs of arm spines occur on the lateral plates of the ten or so most proximal arm segments, and four along the rest of the arm. The most aboral spine has a vertical aspect, the remainder projecting laterally (Fig. 11). All spines have a similar
### Table 2.

**Histochemistry - Glands of Ophiactis resiliens.**

<table>
<thead>
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<th>Podia</th>
<th>Outer Integ. Glands</th>
<th>Central Glands</th>
</tr>
</thead>
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<td>BASIPHILIC</td>
<td>ACIDOPHILIC</td>
</tr>
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<td>AB/PAS</td>
<td>TURQUOISE</td>
<td>ROYAL BLUE</td>
<td>PALE PINK</td>
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<tr>
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<tr>
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</tr>
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<tr>
<td>pH 3.4</td>
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<td>-</td>
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</tr>
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</tr>
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<td>HgBPB</td>
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* ++ POSITIVE
++ STRONG
+++ INTENSE
* OCCASIONALLY PINK
histological appearance, with central granular cell bundles similar to those of *Ophionereis fasciata* spines 2 and 2A (Plate 10). Twelve bundles, varying from 40–55μ in diameter, contain large cells 12–15μ across. Distally each bundle separates, spreading out towards the tip, and at the spine surface small projections occur.

Tooth like projections on the spine surface were first recorded by Reichensperger (1908) in *Amphiura filiformis* who called them "cuticularstäbchen", since abbreviated to "stäbchen". Reichensperger considered them to be sensory and maintained that a nerve fibre ran into each projection; but Buchanan (1963), in a study of these species, noted that these projections coincided with large pyriform cells. Reichensperger had previously dismissed their possible correlation with the terminal photocyte ducts in this species, although he still considered them to be an integral part of the mechanism of light production. Buchanan has however also recorded their presence in a number of non luminescent species.

Clusters of such structures occur at the distal ducts of the acidophil cells but the nearest granules occur some 50μ proximally down the cell duct, only the compressed cell walls forming the distal part of the duct.

As in *Ophionereis fasciata* the cell bundles are proximally innervated by the spinal nerve. The granules also resemble those seen in *Ophionereis fasciata*. Of interest is the marked decrease in PAS activity, noted in several different specimens, suggesting a decrease in 'vic' hydroxyl groups. Again an intense staining reaction occurs with HgBPB, indicating a high protein content.

Basiphilic cells, 50–60μ long and 3–4μ wide, sparingly ramify
the outer calcite shell of the spine. Their fibrillar contents, by staining with AB/PAS after methylation, occasionally turn pink, presumably having reacted with the PAS component of the staining sequence. Such a reaction may be taken to indicate the presence of sulphate esters on 'vic' glycols (Spicer, 1963) although the presence of sulphate groups is not readily demonstrated with low pH metachromatic methods. Similar basiphilic cells occur in the arm plates, particularly the aboral ones.

2. 5. _Ophiopteris antipodum._

(a) Feeding methods. When placed in aquaria, _Ophiopteris antipodum_ show a strong tendency to climb, and they are often found under stones on the reef, clinging to the rock surface upside down. The podia can often be observed unattached as well as attached to the rock. To see these actions in detail, animals were allowed to cling to the underside of glass plates in a semi-darkened room. When a water current is created the majority of arms lie with their long axes at right angles to the direction of current, the podia being attached to the glass by their terminal knobs only (Fig. 15).

The spines gain a coat of mucus and particles adhere to them as the water passes through the ranks of spines. These particles are constantly removed by the podia as follows (Fig. 16(a) to (f)). At any given time a podium will release its hold on the glass plate and flex aborally between the spines, stroking each in turn (Fig. 16(a)) so that particles are thus transferred to the papillae of the podium. The podium then straightens (16(b)) and curls upon itself (16(c) and (d)) compacting the particles into a bolus (16(e)). After the bolus has been passed to the neighbouring proximal podium, the initial podium returns to clinging on to the glass (16(f)). There
is no apparent use of the tentacle scale in this process, transference of the bolus being direct from podium to podium.

There are usually three arms in a state of feeding activity — those at right angles to the current — the other two serving principally to take the weight of the animal, although particles accumulating on them are occasionally removed. Since the proximal podia of the "feeding arms" detach when the bolus is three to four podia away (Fig. 17) in order to pass it on, and other podia again are gathering particles from the spines, only 50-60% of the podia of these arms are attached to the glass at any one time. As many as three separate boluses may be in transit down an ambulacrum simultaneously. The behaviour of each podium appears to be independent of its contralateral partner, each bolus being handled by ipsilateral podia only, so that a "wave" of total arm detachment at a single level does not occur (Fig. 17).

The bolus eventually reaches the mouth, where it is delayed for a few seconds while handled by the second pair of buccal podia. The bolus may then be transferred back down the arm, or introduced into the buccal cavity. If the latter course is followed the second buccal podia, which lie on the very edge of the mouth frame, appear to scrape the bolus on to the tooth papillae (Fig. 18) from where it is finally passed to the mouth by the first pair of buccal podia. A gustatory role is no doubt played by the buccal podia, possibly more pronounced in the second pair.

Less often, *Ophiocera antipodium* may emerge from cover to extend their arms beyond the limit of the rock. This action usually occurs after animals have been in tanks for some days and may be evoked by a lack of food particles in the water. The arms are
characteristically twisted so that the ambulacrum faces the current direction, the arms being held horizontally. Again particles adhere to the spines and are removed by the podia in a similar manner to that described above.

The stomach contents often include larger food particles such as sponge spicules, isopod limbs, and other large debris the presence of which suggests some form of macrophagous feeding. A mixture of algal and organic detritus films on glass plates may be directly removed, by the combined action of tooth papillae and buccal podia. Animals are often found on sponges and bryozoans and their remnants are occasionally seen on the tooth papillae; although usually food particles in the form of a greenish bolus occupy the mouth cavity. Organic deposits on the bottom of a tank are also directly ingested by the buccal podia, larger particles being grasped by the teeth and tooth papillae.

It would appear that collection of food material in suspension, principally of a phytoplanktonic nature, by spines and podial transference forms the "basic" feeding method, but such a diet may be supplemented by direct browsing and ingestion either regularly or in the absence of the primary food material.

(b) Podia. The arm podia are stout, and as previously noted have a large terminal knob and numerous papillae. They have a stem length of 2-3 mm. when relaxed, with a diameter of up to 400 μ in large animals (15 mm. disc diameter or more). The bulb is relatively slight.

The inner connective tissue envelope, although present, is small compared to the vast bulk of the outer longitudinal fibre system. The spiral nature of the inner layer is not readily
distinguishable and it is not possible, in sections, to distinguish inner and outer spirals. In the bulb, fibres run at an angle of 75° to 80° to the longitudinal axis but in the stem all fibres merely appear circular, lying at right angles to the main stem axis. The entire envelope is some 6 or 7/µ thick.

In the stem the longitudinal connective tissue is separated from the inner envelope by the nerve plexus, which varies from 40 to 70/µ in thickness, being broader between the bases of the papillae (Fig. 19; Plate 11). Distally, a dense pad is formed at the base of the terminal knob from which bundles of fibres pass into the distal epithelial tissue (Figs. 20, 21 and 22; Plate 12). Each bundle is 5-8/µ in diameter and 40-60/µ in length, being shorter at right angles to the long axis. The terminal portion of each bundle is swollen (Fig. 20) serving to anchor it among the epithelial cells. In some preparations holes may be seen where the connective tissue had innervated this area. These bundles radiate in all directions, as can be readily seen in T/5 sections of the knob. Sections at its broadest diameter reveal an average of 50 bundles radiating in a full circle. Similarly, in L/8 sections about 25 bundles radiate the semicircle thus formed. Therefore the number of bundles over the hemisphere of the knob would be $\frac{50^2}{2\pi} = 398$. Such an approximate estimate may give some indication of the total number of bundles involved. The connective tissue in the bulb is reduced to a thickness of 10-12/µ. Numerous coelomocytes occur scattered throughout the longitudinal layer.

Within the connective tissue the muscular cylinder and coelomic epithelium are similar to those previously described. In sharp
contrast to the connective tissue, the muscle layer of the bulb (25/μ) is much thicker than in the stem (15/μ).

As previously noted the nerve plexus of the stem is uniquely applied to the inner connective tissue envelope, including the podial nerve. The base of each papilla is innervated by a discrete nerve tract which weaves its way through the bulk of the connective tissue. The plexus attains a diameter of over 30/μ to form the podial nerve, but elsewhere is little more than 7/μ deep around the podium. Distally, at the position of the penultimate papillae, the plexus turns centrafugally to run directly beneath the epithelium, external to the terminal connective tissue pad. The bundles from this pad thus pierce the nerve plexus.

The stem epithelium contains numerous long-necked gland cells, particularly where it is raised into papillae of 70/μ or more. With AB/PAS, cells staining turquoise blue are clearly seen, closely resembling those of the papillae of _Ophiactis resiliens_. With longitudinal and transverse sections of the papillae it appears that these cells are situated principally around their circumference. However, when PAS is used alone, other cells appear in the centre of the papilla, and particularly on the terminal knob where AB positive cells are few. The PAS-positive cells would be expected to stain in the AB/PAS routine, and their failure to do so is puzzling. With PAS alone the more basiphilic cells can still be distinguished as they presumably take up the Mayer's haemalum of this sequence. The histochemical reactions of these cells, based primarily on their relative positions, are summarized in Table 3. As in the podial glands of _Ophiactis resiliens_ both carboxyl and sulphate groups appear to be present. The central glands, which
<table>
<thead>
<tr>
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<th>Podia Central</th>
<th>Spines Unicellular</th>
<th>Spines Multicellular</th>
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<td>+++</td>
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<tr>
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<td>+++</td>
<td>+</td>
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<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>AB pH 1.5</td>
<td>+++</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>pH 3.4</td>
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<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
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</tr>
<tr>
<td>in 0.6N HCl</td>
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<td>-</td>
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<td>MB pH 1.5</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>pH 3.4</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
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<td>METHYLN. 4 hrs.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>METHYLN. 4 hrs.</td>
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<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+ SAPONIFICATION</td>
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<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
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</tr>
<tr>
<td>METHYLN. 7 hrs.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>+ SAPONIFICATION</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>MB/AlSO$_4$</td>
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<td>-</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Tol. B in 70%</td>
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<td>BETA/GAMMA</td>
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<td>Tol. B pH 1.5</td>
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<td>GAMMA</td>
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<td>pH 3.1</td>
<td>ALPHA</td>
<td>-</td>
<td>GAMMA</td>
<td>BETA/GAMMA</td>
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<tr>
<td>HgBPB</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ POSITIVE ++ STRONG +++ INTENSE - NEGATIVE
give a positive protein reaction, appear to be negative to all the other tests.

The buccal podia (Fig. 23) differ strikingly from those of the arm by the absence of a terminal knob. The terminal complex of connective tissue processes is thus lacking. Papillae are restricted to the distal half of the podium and these contain both types of mucous cells.

(c) Spines. The spines are long and narrow, banked six deep along the arm like rows of oars (Fig. 24). Each spine is hollow, the spinal nerve passing centrally the whole length of the spine with numerous side branches. Two types of mucous glands are evident within the integument, closely resembling, at least morphologically, those of Ophiocomina nigra, as described by Fontaine (1964), although there appear to be histochemical differences.

Unicellular glands (Fig. 25) lie in the outer calcareous layer, up to 40/μ in length, with a long and tortuous cell duct 2/μ in diameter. The cuticle is slightly indented at the pore exit. Proximally lies the ovoid nucleus, 5-6/μ in length. These cells occur on all aspects of the spine at an irregular frequency, occasionally side by side, and their contents appear finely fibrillar, staining sky blue with AB/PAS (Table 3).

Large multicellular glands (Fig. 25) are set deeper in the spine, apparently in contact with the central spinal nerve. The main body of the glands lies parallel to the longitudinal axis of the spine, turning obliquely to pierce the calcareous layer, thereby attaining a length well in excess of 100/μ. Several nuclei lie at the base of the gland and the duct is probably formed of squamous epithelial
cells, the nuclei of which lie along their length. Their strongly fibrillar contents stain turquoise blue with AB/PAS.

Table 3 summarizes the histochemical reactions of both glands. It can be seen that the unicellular glands appear to be more acidic and show a high degree of sulphation; the multicellular glands however appear to be less acidic and although sulphate groups are present, carboxyl groups are readily detected by the methylation/demethylation procedure. It is of interest to note that demethylation after 4 hours methylation results in removing the staining reaction further. This suggests that saponification had an extracting rather than esterifying effect on what were probably sulphate groups.

Other multicellular glands occur in the granules of the disc integument and in the teeth. Each granule contains a group of twenty or more glands, the group collectively receiving a discrete nerve fibre from the subintegumentary nerve plexus.
Fig. 1 Drawing of mid arm of *Ophionereis fasciata* showing relationships of spines (SP.) and podia (POD.).

Fig. 2 Particle gathering by podia of *O. fasciata*. Podium with adhering particles (a) is curled upon itself (b) to compact particles (c). The compacted mass is then scraped against the tentacle scale (d), the podium returning to rub against the spines (e).

Fig. 3 Jaw plates of *O. fasciata*.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AD. SH.</td>
<td>adoral shield</td>
</tr>
<tr>
<td>B. P. (1)</td>
<td>first buccal podium</td>
</tr>
<tr>
<td>B. P. (2)</td>
<td>second buccal podium</td>
</tr>
<tr>
<td>HALF J.</td>
<td>half jaw</td>
</tr>
<tr>
<td>OR. SH.</td>
<td>oral shield</td>
</tr>
<tr>
<td>OR. PAP.</td>
<td>oral papilla</td>
</tr>
</tbody>
</table>
Fig. 4  L/S stem of arm podium, _O. fasciata._

<table>
<thead>
<tr>
<th>Term</th>
<th>Annotation</th>
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</thead>
<tbody>
<tr>
<td>ANN.</td>
<td>annulus</td>
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<tr>
<td>COEL. EP.</td>
<td>coelomic epithelium</td>
</tr>
<tr>
<td>CONN. T. ENV.</td>
<td>connective tissue envelope</td>
</tr>
<tr>
<td>EPITH.</td>
<td>epithelium</td>
</tr>
<tr>
<td>LONG. CONN. T.</td>
<td>longitudinal connective tissue</td>
</tr>
<tr>
<td>LONG. M.</td>
<td>longitudinal muscle</td>
</tr>
<tr>
<td>M. CELL BOD.</td>
<td>muscle cell bodies</td>
</tr>
<tr>
<td>POD. NERVE</td>
<td>podial nerve</td>
</tr>
<tr>
<td>TERM. KNOB</td>
<td>terminal knob</td>
</tr>
<tr>
<td>W. V. C.</td>
<td>water vascular cavity</td>
</tr>
</tbody>
</table>

Fig. 4a Stem of arm podium, _O. fasciata._

Fig. 5  L/S stem of buccal podium, _O. fasciata._

Fig. 5a Stem of buccal podium, _O. fasciata._
Fig. 6 T/S *O. fasciata* arm (proximal) showing spine numbers as designated in the text.

Fig. 7 L/S spine 1, *O. fasciata*.

- BAS. C. COMP.  | basophilic cell complex
- PERIPH. C.    | peripheral cell
- SP. NERVE     | spinal nerve

Fig. 8 L/S spine 2, *O. fasciata*.

- ACIDO. C. B. | acidophil cell bundle
- CONN. T.      | connective tissue
- SP. NERVE     | spinal nerve

Fig. 9 T/S spine 2, *O. fasciata*.

- ACIDO. C. B.  | acidophil cell bundle
- PERIPH. C.     | peripheral cell

Fig. 10 T/S acidophil cell bundle, *O. fasciata*.

- ACIDO. C. | acidophil cell
Fig. 11 Drawing of mid arm of Ophiactis resiliens showing arrangement of spines (SP.) and extended podia (POD.).

Fig. 12 Diagram of T/S O. resiliens arm in relation to current flow.

Fig. 13 Inferred action of O. resiliens podia collapsing in series.

Fig. 14 L/S stem of arm podium, O. resiliens.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>COEL. EP.</td>
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<td>connective tissue envelope</td>
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<td>epithelium</td>
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<tr>
<td>LONG. CONN. T.</td>
<td>longitudinal connective tissue</td>
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<td>LONG. M.</td>
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<td>M. CELL BOD.</td>
<td>muscle cell bodies</td>
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<tr>
<td>PAP.</td>
<td>papilla</td>
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<tr>
<td>POD. NERVE</td>
<td>podial nerve</td>
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<tr>
<td>TERM. KNOB</td>
<td>terminal knob</td>
</tr>
<tr>
<td>W. V. C.</td>
<td>water vascular cavity</td>
</tr>
</tbody>
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Fig. 15 Drawing of podia of *Ophiopteris antipodum*, arranged between spines, and attached by their tips to a glass plate.

Fig. 16 Podia of *O. antipodum* wiping spines.
Podium strokes spine from tip to base (a) and then straightens to curl upon itself (b, c, d), compacting the gathered particles (e). Podium then returns to cling on to substrate (f) after passing the bolus to its proximal, ipsilateral neighbour.

Fig. 17 Pattern of podia of *O. antipodum* on glass plate.

- detached podium
- attached podium
- ambulacrum

Fig. 18 Jaw plates of *O. antipodum*.

- AD. SH. adoral shield
- HALF J. half jaw
- OR. ARM SH. oral arm shield
- OR. PAP. oral papilla
- TOOTH PAP. tooth papilla
Fig. 19 L/S stem of arm podium, O. antipodum.

<table>
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<td>COEL. EP.</td>
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<td>epithelium</td>
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<td>PAP.</td>
<td>papilla</td>
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<td>POD. NERVE</td>
<td>podial nerve</td>
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<td>TERM. CONN. T. BUND.</td>
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<td>TERM. KNOB</td>
<td>terminal knob</td>
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Fig. 20 Connective tissue bundles of O. antipodum embedded in terminal epithelium.

<table>
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<th>Abbreviation</th>
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<tr>
<td>TERM. NERVE PLEX.</td>
<td>terminal nerve plexus</td>
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Fig. 21 T/S O. antipodum podium at A (Fig. 19).

<table>
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<td>TERM. NERVE PLEX.</td>
<td>terminal nerve plexus</td>
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Fig. 22 T/S O. antipodum podium at B (Fig. 19).

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**Fig. 23** L/S stem of buccal podium, *O. antipodum*.

<table>
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<td>papilla</td>
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<tr>
<td>POD. NERVE</td>
<td>podial nerve</td>
</tr>
<tr>
<td>W. V. O.</td>
<td>water vascular cavity</td>
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**Fig. 24** T/S arm of *O. antipodum* showing arrangement of spines.

**Fig. 25** Glandular cells of *O. antipodum* spine.

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
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<td>SP. NERVE</td>
<td>spinal nerve</td>
</tr>
<tr>
<td>UNI. MUC. GL.</td>
<td>unicellular mucous gland</td>
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</tbody>
</table>
Plate 10.
L/S spines
O. resiliens
Mass. Tri.

Plate 11.
L/S podium
O. antipodum
Mass. Tri.

Plate 12.
Podial tip
O. antipodum
Mass. Tri.
2.6. **Electron microscopy.**

(a) **Introduction.** There are relatively few accounts of the ultrastructure of invertebrate mucous cells but several papers have recently appeared on the secretions of various asteroid podia (Chaet and Philpott, 1960, 1961, 1964; Souza Santos, 1966 a and b; Harrison and Philpott, 1966). Without exception, all podia so far studied, in five different genera, contain mucous granules within discrete "secretory packets". The packets of four genera appropriately tested were found to be PAS positive, and all genera possessed granules with internal, densely packed, rods. The podia of *Asterina stellifera* also contain secretory granules which lack an organised structure (Souza Santos, 1966 a).

This brief study attempts to indicate differences in the ultrastructure of the glandular contents of some of the cells previously described, and where possible to correlate these with their histochemical similarities. Details of the secretory cells are discussed in the account.

(b) **Podia.** Plate 13 shows a section at the surface of a podium of *Ophionereis fasciata* in which the distal portion of a mucous cell and a neighbouring epithelial cell can be seen. The epithelial cell possesses a microvillus, and a deep invagination occurs between it and the neighbouring mucous cell. Plate 14, at a deeper level, shows two mucous cells in which granules occur, apparently loose and not combined in any form of "secretory packet". Each granule is, in fact, surrounded by a loose membrane (Plate 15) which by its tripartite structure appears to be a "unit membrane". The contained granules do not all present a spherical appearance and may in fact be ellipsoidal, diameters varying from 0.41 to 0.46 /μ. The granule
consists of a central core of low opacity surrounded by a more electron dense region some 60 to 65 m/μ thick. A less dense area again is evident around the outside of the granule. Other small electron dense areas may be precipitates of lead although Harrison and Philpott (1966) have described extracellular particles around the packets of some asteroids. The central area appears to be finely granular, possibly representing rods viewed end on. One would therefore expect to see parallel bars in granules at different orientations, but none are apparent. No recognisable granules were seen on the small area of podial surface in view.

The mucous cells of *Ophiactis resiliens* (Plate 16) present a striking appearance. The cell appears to constrict at the distal extremity to form a tube occluded by a collection of microtubules, and terminally microvilli can be seen. Mucous granules, again not enclosed in packets, occur proximal to the microtubules. The microtubules (Plate 17) have an overall diameter of 280A (and an apparent internal diameter of 120A). Large empty vesicles can be seen between them. The microvilli are 0.5/μ in length and 64 m/μ in width. Dense granules along their surface could be lead precipitate.

The presence of microtubules is most interesting. In a review of these structures, Slautterback (1963) noted that in all cells containing microtubules the tubules appear to have an outer diameter of either 270A or 120 to 200A. It was proposed that, in the nematocysts of *Hydra*, microtubules are involved in the production of a change in ionic strength within the nematocyst shell; this hypothesis is supported by their presence in ion secreting cells, neurons, and the glomerular epithelium of mammalian kidney. Such a role of ion transport could assist in the firing mechanism of the
nematocyst which is known to be sensitive to the relevant concentration of divalent metal ions. Microtubules thus characterized fall within the range of 120 to 200A diameter.

Microtubules of 270A diameter appear to function as elastic bodies and have been found in cells of a wide range of function, and may be instrumental in the regulation of cytoplasmic movements within specific regions of the cytoplasm (Tilney and Porter, 1967).

It would be interesting to speculate on a possible roll of the microtubules in the ejection of mucous granules from within the cell by altering the ionic concentration as in nematocysts, but unfortunately their size of 280A diameter would appear to characterise them as elastic bodies. As such, their function remains obscure.

Microvilli appear to be a regular feature of secretory cells and are present in mucous cells in the mammalian gastric mucosa (Ito and Winchester, 1963).

At a deeper level (Plate 18) the cells are much broader, in excess of 3/μ, containing many granules and apparently empty vesicles. The granules (Plate 19) are of variable size and shape, up to 0.8/μ in length, with a central electron dense area, also of variable shape. More microtubules can be distinguished between them. The granule is closely bound by a membrane (Plate 20).

Low power sections of the papillae of an Ophiopteris antipodium podium (Plate 21) show two types of granules. A large granule about 3.5/μ in diameter, has a moderately electron dense, granular structure. Smaller granules, resembling those of Ophiactis resiliens can be seen packed together giving them a somewhat polygonal appearance. These granules (Plate 22) have an electron dense central region, and are surrounded by a fairly close membrane.
They may be ellipsoidal, up to 0.6μ in length. Large amounts of granular material occur between the granules (Plate 22) and microtubules are again present (Plate 23).

A Golgi body can be seen in Plate 24 and large vesicles are in evidence around it. The association of Golgi bodies with secretory cells is now well established (Dalton, 1961). In a study of the rat intestine, Hollmann (1963) supposed the Golgi, by virtue of its size, to play a primary role in the elaboration of mucopolysaccharide. Holland and Nimitz (1964) have discussed the probable role of the Golgi apparatus in the sulphation of mucopolysaccharide of the sea urchin gut, although actual localization of labelled compounds was only presumed. Porter (1964), however, has shown that S^{35} is taken up by Golgi vesicles.

(c) Spines of Ophionereis fasciata. The fibrillar cells of spine 1 of Ophionereis fasciata are shown in transverse section in Plate 25, where the fibrils are seen in end view. The fibrils appear to have some form of attachment to intracellular bodies lying along the cell wall (Plate 26). The fibrils themselves present an irregular pattern (Plate 27) comprising small electron dense cores, 200 to 270Å in diameter, which may represent condensed protein. An apparent irregular network connects these central cores.

The central acidophilic glands of spine 2 were similarly sectioned (Plate 28). Granules of variable size and shape occur in abundance and a well formed Golgi with budding terminal vesicles can be clearly seen. The granules (Plate 29) possess a fairly uniform, moderately electron dense interior, each granule being limited by a unit membrane. Some granules are extremely large, up to 2μ in diameter.
Plate 13. Surface section of tip of podium, Ophionereis fasciata, showing epithelial cell (E), with microvillus (V) and mucous cell (M). (x 16,500).
Plate 14. Two podial mucous cells, Ophionereis fasciata, with granules. (x 28,000).
Plate 15. Granules of *Ophionereis fasciata* showing surrounding membrane (m). Note low opacity core surrounded by a more electron dense region (D). (x 95,000).
Plate 16. Mucous cell in a papilla of the podium, Ophiactis resiliens, showing microvilli (M), microtubules (T), and granules (G). (x 32,000).
Plate 17. Higher power electronmicrograph of the microtubules (T) and microvilli (V), *Ophiactis resiliens* (x 125,000).
Plate 18. Transverse section of mucous cells, *Ophiactis resiliens*, and nuclei (N) of epithelial cells. (x 16,500).
Plate 20. Granules of mucous cells, *Ophiactis resiliens* showing closely bound membrane (m). (x 125,000).
Plate 21. Transverse section of a papilla of the podium, Ophiopteris antipodum showing large (L) and small (S) granules. (x 21,000).
Plate 22. Small granules, *Ophiopteris antipodum*, and other granular material (gm). (x 50,000).
Plate 24. Small granules, Ophiocystis antipodium, and well formed Golgi (Go). (x 33,000).
Plate 25. Transverse section of the fibrillar cells of spine 1, *Ophionereis fasciata*. (x 16,500).
Plate 24. Small granules, *Ophiopteris antipodum*, and well formed Golgi (Go). (x 33,000).
Plate 26. Fibrillar cell, spine 1, Ophionereis fasciata, showing intracellular body (1) along the cell wall. (x 33,000).
Plate 27. Higher power electronmicrograph of fibrils, Ophionereis fasciata. (x 73,500).
Plate 28. Central acidophilic cells of spine 2, Ophionereis fasciata, showing granules and well-formed Golgi (Go). (x 33,000).
Plate 29. Higher power of granules, spine 2, *Ophionereis fasciata*. (x 95,000).
2. 7. Discussion.

(a) Feeding and podial structure. All three species so far examined use the podia as an integral part of the feeding mechanism, and varying use of the spines has been observed. In Ophionereis fasciata the spines, particularly the aboral ones, function in supplying mucus for catching bottom material in suspension, the podia collecting the material thus trapped. In Ophiopteris antipodum phytoplanktonic suspension directly adheres to the spines, the podia transferring it to the mouth. Ophiactis resiliens, on the other hand, primarily uses the podia to trap material in suspension, particles being secondarily collected on the spines. These appear to be the "primary" feeding mechanisms or at least are the ones most readily exhibited when natural conditions are simulated, and may be observed in the field.

However, as has been noted in other ophiuroids, notably Ophiocoma nigra and Ophiocoma scolopendrina, more than one type of feeding mechanism has been observed. Thus in Ophionereis fasciata, (a) large particles are passed direct to the mouth by the podia, (b) the arms are bent to bring even larger particles to the mouth, and (c) some food material is directly ingested. Of the seven feeding methods mentioned in the introduction of this section only the browsing of bottom surface particles and of the water surface appear to be lacking, passive arm-waving being supplemented by the use of intraspinal mucus. Direct browsing of the bottom seems unlikely in epifaunal species, and the availability of food particles on the water surface is necessarily limited to situations where the water is relatively calm for long periods, and this cannot be said of the Leigh reef. If used at all, this mechanism
would be limited to occasions when low water occurs on calm nights in view of the photonegative behaviour of these animals. Even then the depth of water in the pools would probably be too great for such a feeding mechanism to be operated.

The feeding methods of the other two species appear to be more limited and thus may place a limit on their range of habitat. *Ophiactis resiliens* will pass larger food particles direct by podial transfer but no other form of feeding has been observed. *Ophiopteris antipodum* shows more versatility by directly browsing food material, and may possess other forms of feeding behaviour which have not been observed.

The difference between annulate and papillate podia is interesting. These may be specific to genera, and even to family level, but unfortunately the majority of descriptive papers omit to detail the form of the podia, a task of course impossible with dried material. However, a brief review of available information indicates that the form of the podia may indeed be specific to family level. *Ophiopteris antipodum* appears closely to resemble *Ophiocomina nigra* both in the external form of the podia at least, and in the morphology of the spine glands, both species belonging to the family Ophiocomidae.

The podia of *Ophionereis fasciata* show the least specialization, consisting of a simple tube which upon retraction or relaxation gives an annulate appearance. The spiral system of the envelope is well developed, the outer longitudinal connective tissue being of moderate thickness. Mucous glands for the most part are confined to the podial tip and ambulacral side of the stem. The podia of *Ophiactis resiliens* show specialization in the possession of large papillae
containing mucous glands. Such a raising of the epithelial surface serves to increase their surface area and provide sticky projections for the adherence of particles. Papillae are further employed in a combing action to transfer collected food particles. The podia themselves are capable of considerable extension and the bulb is extremely muscular, with a large volume relative to the relaxed stem.

It is in the podia of *Ophiopteris antipodum* that the greatest degree of specialization is seen. Both a large terminal knob and papillae are present, with two types of mucous glands having different distributions. Perhaps the most unusual feature is the extremely extensive longitudinal connective tissue, deeply embedded by a complex of projections into the epithelium of the terminal knob. This connective tissue is also unusual in lying external to the nerve plexus which thus penetrates it to innervate the epithelium. Such a large bulk of longitudinal fibres, placed as far from the centre as possible, gives great support to the podium, particularly in lines of stress along its longitudinal axis and to some extent to shearing forces. It is to be noted that in inverted animals only the podial tip serves as a means of attachment. The terminal array of fibres superficially suggests some form of sucker mechanism. Such a mechanism could only operate by the raising of the central surface of the knob through the translation of forces exerted by the contraction of the longitudinal musculature of the podial wall. Thus in a contracted podium one would expect to find the podial tip concave on the surface. However, this is not the case, the surface remaining flat or convex. The MacGinties (1949) reported a sucker on the podia of *Ophiactis arenosa*, but no details
were given. It is possible that this was a misinterpretation of a highly adhesive terminal knob.

Smith (1937) observed a large terminal concentration of connective tissue in *Ophiocoma nigra* but the projections into the epithelium appear to be much reduced. Also, the nerve plexus lies directly beneath the epidermis. *Ophiocoma nigra* is noted for its ability to climb (Smith, 1937; Fontaine, 1964) but there is no record of it maintaining an inverted position for any length of time. It is to be noted that both *Ophionereis fasciata* and *Ophiactis resiliens* possess the ability to climb the sides of perspex tanks by using their podia, but show a decreased inclination to do so and are easily dislodged, particularly the former species.

Of interest also is the marked difference in the form of the arm and buccal podia, a point which is seldom mentioned. A terminal knob is present in all arm podia but is absent in the buccal podia of *Ophionereis fasciata* and *Ophiopteris antipodum*. In *Ophiactis resiliens* it is not only present, but markedly larger. The precise function of this knob is not evident, except in *Ophiopteris antipodum*, but may serve as a terminal pad when the podia are used in locomotion, and to provide a large adhesive area for the handling of sand particles as in the "burrowing" of *Ophionereis fasciata*.

(b) **Gland cells and their secretions.** A wide variety of mucous secretions has been demonstrated, and although all the glands differ from one another certain similarities are apparent. In the light of the differences in behaviour shown by these species it is to be expected that mucins should differ with respect to their various uses. A difference in the origin of the basic components of mucin
molecules made available to the animal by its food material must also be taken into account.

The contents of the podial glands of *Ophionereis fasciata* are both positive to the PAS reaction and also show a high content of carboxyl groups, the latter accounting for their acidity as exhibited by an intense basiphilia with Alcian blue. Sulphate groups do not appear to be present in any quantity, as only a tendency to beta metachromasia with azure A at pH 1.2 can be seen, the dye not being bound at all at a lower pH. Some doubt however exists over the specificity of azure A for sulphate groups (Spicer, 1963; Holland and Nimitz, 1964).

Of interest is the PAS positive reaction implying that the secretion is heterogeneous, having PAS reactive residues as well as acid groups. The granules as seen by electron microscopy possess a peripheral electron dense region with a central less dense core. A similar phenomenon has been noted in the granules of *Patiria miniata* by Harrison and Philpott (1966), also using uranyl acetate and lead citrate staining. These authors considered, by further using colloidal thorium, that this electron dense area indicated the presence of an acid mucopolysaccharide. Their argument however appears confused as they also consider the staining to be "PAS-like", and the granules of all four asteroids studied by them were PAS positive. It is generally considered that when acid mucopolysaccharides are referred to as a group they are found to be PAS negative (Pearse, 1961), although weakly sulphated forms may be positive to the PAS test (Spicer, 1963). The ultrastructure of *Ophiactis resiliens* podial granules, and the smaller ones of
Ophiopteris antipodum podia, also have electron dense areas which here form the central core of the particle. These areas, although similar in Ophiactis resiliens and Ophiopteris antipodum, differ somewhat in appearance from the outer shell of Ophionereis fasciata granules. However, as the former granules are highly acidic, an electron dense area may represent the site of acid groups.

The occurrence of granules not enclosed in discrete packets, as in asteroids, suggests that they are released singly, either at a uniform or irregular rate. The granules of asteroid podia all occur in conjunction with a sucker arrangement of the podium and thus may primarily serve as adhesive packets, possibly with a catalyst as suggested by Harrison and Philpott (1966). All these packets are PAS positive and the granules contain groups of closely packed rods within them. These closely packed rods appear to be unique to asteroids although coarse transverse and fine longitudinal striations have been noted in acidophilic cells of Planaria vitta (Pederson, 1963). The majority of mammalian gut mucous cells appear to have a relatively unorganized structure (Helander, 1962; Helander and Ekholm, 1959; Ito and Winchester, 1963; Trier, 1963).

The species with the most marked ability to climb and remain in an inverted position, Ophiopteris antipodum, also possess PAS positive glands, particularly at the podial tip and in the centre of the papillae, which probably serve to supply a strongly adhesive secretion. In electronmicrographs of these papillae, large particles may be observed with a uniform, moderately electron dense interior. As the smaller granules resemble those of Ophiactis resiliens, which are acidic, the larger particles may represent
the secretion of PAS positive cells.

The basophilic glands of *Ophionereis fasciata* spines, particularly spine 1, produce a mucus which is fibrillar in fixed preparations. This may be due to its precipitation during fixation but as noted in other fibrillar ophiuroid glands by Buchanan (1963) the mucus exuded is also threadlike. Although not strongly positive to HgBPB the reactive groups are probably incorporated onto a linear protein core which may be that revealed by the electron microscope. Carboxyl groups again predominate although more sulphate groups may be present as indicated by the increased azurphilia. Carboxyl groups are also less easily split off from the mucin molecule by strong methylation.

It appears from the histochemical results that where mucus is used direct for food capture i.e. in the aboral spines of *Ophionereis fasciata*, the spines of *Ophiopteris antipodum*, the podia and to a lesser extent the spines of *Ophiactis resiliens* the mucus is acid, rich in carboxyl groups and partly sulphated. Further, the mucus in spinal glands is fibrillar while that of the podia is granular; but the similarity of reactive groups does not necessarily preclude in itself a similar overall molecular configuration.

The unicellular glands of *Ophiopteris antipodum* alone appear to be highly sulphated and their function is not clear. Fontaine (1963) describes glands of similar morphological appearance to those of *Ophiopteris antipodum* spines in *Ophiocoma nigra* but apparently the larger multicellular glands were highly sulphated, the unicellular ones being less so. Fontaine concluded that the unicellular glands provided a mucus for feeding and that the larger sulphated glands served as a defensive mechanism. Both *Ophionereis*
fasciata and Ophiopteris antipodum, and to a lesser extent Ophiactis resiliens, produce a copious flow of mucus when provoked, particularly if an arm has been severed. This may be a defensive mechanism but appears to consist of a release of all available mucus regardless of its nature.

Of particular interest are the large cell masses of acidophilic granules in the mid arm spines of Ophionereis fasciata and the spines of Ophiactis resiliens. Similar cell masses are recorded by Buchanan (1963) in Ophiactis balli and Ophiopolis aculeata although communication, or the lack of it, with the exterior is not mentioned. Harvey (1952) reports that Kato (1947) considers eosinophilic glands of Amphipura kandai to be responsible for the luminescence of this ophiuroid. The Australian Ophionereis reticulata is bioluminescent, and this species is almost identical to O. fasciata, differing only in the shape of the oral shields (Mortensen, 1924). It was of no great surprise therefore to find that Ophionereis fasciata was also luminescent.

If fresh animals are mechanically stimulated, in total darkness, a bluish flash passes along the arm in a distal direction. Quite strong stimulation is necessary and the flash is very rapid, the arms being clearly visible in profile. Isolated arms glow for 20 to 30 seconds but the intensity of illumination rapidly falls. On close examination the source of the illumination can be localized in the spines. Further localization is difficult as the intensity of illumination is low and cannot be readily detected until the observers eyes have been dark-adapted. The rapidity of the flash, plus the movement of the arm, also makes it difficult to focus
adequately on the areas concerned.

An attempt was made to clarify the matter by using ultraviolet light, as one would expect chemiluminescent molecules to be readily excited by radiant energy to fluoresce. Harvey (1952) reports that both Axiognathus (≡ Amphipholis) squamata and Ophiopsila aranea fluoresce in the ultraviolet. The arms which fluoresced showed a considerable correlation with the bioluminescent areas except that the podia of O. aranea also fluoresced.

Various areas fluoresced in Ophionereis fasciata, the podia fluorescing most of all and free mucus also fluoresces. Such fluorescence may be due to the presence of sulphate groups. The bases of the spines, where they articulate with the lateral arm plates and the integument is thin, similarly fluoresce. Even the cut ends of the arms fluoresce to some extent. The fluorescence in each case is only detectable with long wave ultraviolet light (4000 to 3000A). Mechanical stimulation of the whole animal, or the severance of an arm, fails to reveal any other areas of fluorescence.

Attempts to stimulate Ophiactis resiliens to luminesce have not been successful. There is no response to mechanical stimulation either alone or when combined with chemical irritants such as 10% solutions of sulphuric, hydrochloric and acetic acids, chloroform, NaCl, ether, absolute alcohol, ammonium and potassium hydroxides and phenpxetol. Isolated arms also fail to respond. Buchanan (1963) records that neither Ophiactis balli nor Ophiopholis aculeata luminesce.

The podia and spines of Ophiactis resiliens do not fluoresce markedly in the ultraviolet, although the proximal ventral arm
plates fluoresce a cream colour to some extent and a yellow fluorescence may be noted on the radial shields of the disc. The fluorescence here also only occurs under long wave ultraviolet light. No fluorescence has been noted in *Ophiopteris antipodum*.

The cause of fluorescence in many organic compounds is not fully understood, but this phenomenon is a regular feature of calcium carbonate containing 2-5% of mineral impurities, which depending upon the type of impurity present fluoresce in almost every colour. Thus the fluorescence in the integumentary areas of the animal may be due solely to the properties of skeletal plates. Even holes in calcite can cause fluorescence.

It is tempting to attribute the bioluminescence of *Ophionereis fasciata* to the acidophil glands of spine 2, particularly as they do not appear to open onto the spine surface and are heavily innervated by the spinal nerve. It must be admitted, however, that no direct evidence can be presented and alternative causes cannot readily be found. The existence of similar glands in *Ophiactis resiliens* further casts doubt on such surmise. The presence of a Golgi apparatus in the gland of *Ophionereis fasciata* spine 2 suggests that the granules are actively produced within the cell and thus negates any suggestion that granules represent an accumulated by-product of metabolism. It is interesting to note that granules have not been found on the spine surface itself although the fibrillar secretion of the basiphilic cells is readily detectable.

The biological significance of luminescence has been discussed and summarized by Millott (1966). As luminescence happens in response to some form of irritation, a defensive explanation appears most tenable. Such a mechanism could however be effective only against predators capable of detecting the light, principally
bottom-feeding fish. Such a method could be particularly effective in an isolated arm, combined with the release of mucus noted above.

In order to study further the role of the podia, spines and glandular cells in ophiuroids, four other littoral species were examined, occurring in different habitats around the New Zealand coast. An account of the observations on these animals now follows.
2. 8. Monamphiura aster.

(a) Burrowing and feeding. As mentioned in Section 1. 2. Monamphiura aster may be found at a depth of 5-10 cms. on protected sand beaches. These animals are extremely sluggish when placed on a smooth perspex substrate, slowly moving away from a point source of light. This slow form of locomotion is effected by folding two trailing arms which adhere to the perspex with their podia. Upon straightening the arms the disc is thus levered forward. However, as soon as the animals are transferred to a tank with fine sand taken from their locality, they rapidly burrow beneath the surface in less than two minutes. Initially the podia are thrust into the sand and then quickly "flicked" aborally; this action being particularly noticeable in the proximal part of the arm, resulting in the disc sinking below the surface. The arms are then thrown into lateral flexures and thrust distally into the sand, driving deep into the substrate. This action by two or three arms on one side of the disc pull the disc deeper, and a similar action of the remaining arms on the other side of the disc results in the complete submergence of the animal.

Ophiuroids which had thus burrowed were observed at the bottom of tanks with the aid of a mirror placed at 45° below the tank, and a system of lenses. Several animals were studied in this way and at no time were all five arms in contact with the surface. Invariably at least one arm remained below, supporting a chamber for the disc. The other arms maintain burrows with the surface (Fig. 26), characteristically bent either singly or often in pairs.

The podia of these arms constantly rub against the spines and
then stroke the burrow walls, apparently applying mucus to them. Occasionally these walls cave in, whereupon all podia combine to remove the debris. The proximal part of the arm, by its high degree of folding, prevents any large volume of sand reaching the disc chamber. To remove the intruding particles the podia co-operate with their contralateral partners in passing the sand grains distally, so that a succession of accumulated debris passes along the arm at a rate of lcm./10-15 secs. (Fig. 27). The function of the spines in maintaining the burrows is less easy to determine but may aid in maintaining its form when the arms are slowly rotated.

A considerable division of labour is evident in the arms, suggesting a high degree of "central" control. At any given time one arm is usually engaged in creating a flow of water through the burrow system. This is effected by the arm being thrown into long oral/aboral undulations (Fig. 28) of varying frequency, usually in the order of 30 to 40 per minute but occasionally almost double this rate. Woodley (1967) has remarked that the interspinal spaces in Amphiura filiformis is blocked by sand grains by the action of the podia. In Monamphiura aster sand particles also adhere between the spines but no discrete method of compacting them has been observed. The stroking action of the podia against the spines may accomplish this, although usually the podia are subsequently applied to the burrow wall. The current produced may serve to bring oxygenated water in contact with the disc.

On a level with the disc one arm is often thrust horizontally into the substrate. Particles of fine sand are passed proximally by the podia until they reach the disc. Here the second buccal podia, which have an oral aspect as in Ophiopteris antipodum, handle
the particles as they pass over the mouth rim. It has not been possible to observe whether some grains are ingested but the stomachs are often packed with detritus and sand particles. Organic particles may possibly be thus engulfed. After passing over the mouth the particles are further carried distally down another arm by the podia and deposited on the surface.

Animals kept for several weeks were never observed to raise their arms above the surface of the sand, and normally no more than the distal two or three cms., if that, protrude at all. The surface of the sand around the burrows also shows no evidence of being swept by the arms. Thus the behaviour noted above may represent the feeding method of this species, selectively sorting detrital material from sand particles beneath the surface. Interstitial fauna may also be digested off the sand particles although as the gut is blind and of limited volume, some sorting of prospective food particles must take place outside.

The burrows (Fig. 29) formed by Monamphiura aster appear to be temporary only, the whole animal frequently changing its position below the surface, usually following the arm which is thrust into the substrate. Similarly, the employment of the arms is also varied and they are rapidly switched from one task to another eg. from creating a current to removing sand particles.

(b) Podia. The podia (Plate 30) are annulate and have a stem length of some 800 μ when relaxed, with an overall diameter of 130-140 μ. The general form is much the same as that described for Amphiura filiformis by Woodley (1967), with inner connective tissue spiral angles of 75° and 85° to the longitudinal podial axis
in bulb and stem respectively. The longitudinal connective tissue layer is rather slight compared to the species described previously, being only 3-4 μ thick. The muscular tissue of the bulb is again at least twice as thick as that of the stem. Mucous cells are present, particularly on the ambulacral side, in the epithelial layer, and their histochemistry is summarized in Table 4. The secretion is acid and sulphate groups appear to be either few or absent.

The podia are rather pointed in both arm and buccal podia and there appears to be little morphological difference between them.

(c) Spines. Six pairs of spines occur on the most proximal twenty or so arm segments but only five pairs occur along the rest of the arm. All of the spines are similar in shape and of about the same length (0.5 mm.), fanning out around the arm owing to the shape of the lateral arm plates. Histologically they present a complicated picture and with Masson's trichrome a variety of cells appear (Fig. 30).

One of the most striking tissues is the ground substance ("Kalkgrundsubstanz") which forms an inner cylinder around the central cells and is very granular. External to this cylinder the calcareous shell of the spine possesses numerous channels in which lie a number of secretory cells. The most noticeable of these are basiphilic fibrillar cells, (Fig. 31) staining strongly with the light green of Masson's trichrome, and turquoise with AB/PAS. The cells have a polynuclear origin and the neck of the gland consists of squamous epithelial cells. The whole syncytium lies outside the ground substance cylinder and may reach 70-80 μ
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<tr>
<td>pH 3.4</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>METHYLX. 4 hrs.</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>METHYLX. 4 hrs.</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>+ SAPONIFICATION</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>METHYLX. 7 hrs.</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>METHYLX. 7 hrs.</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>+ SAPONIFICATION</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>MB/Al₂SO₄</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tol. B in 70%</td>
<td>BETA</td>
<td>GAMMA</td>
<td>BETA</td>
<td>-</td>
</tr>
<tr>
<td>Tol. B pH 1.5</td>
<td>BETA</td>
<td>GAMMA</td>
<td>BETA</td>
<td>-</td>
</tr>
<tr>
<td>pH 3.4</td>
<td>ALPHA</td>
<td>BETA/GAMMA</td>
<td>ALPHA</td>
<td>-</td>
</tr>
<tr>
<td>AZURE A pH 0.45</td>
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<td>GAMMA</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>BETA</td>
<td>BETA</td>
<td>ALPHA</td>
<td>-</td>
</tr>
<tr>
<td>pH 2.1</td>
<td>BETA</td>
<td>BETA</td>
<td>ALPHA</td>
<td>-</td>
</tr>
<tr>
<td>pH 3.1</td>
<td>ALPHA</td>
<td>ALPHA</td>
<td>ALPHA</td>
<td>-</td>
</tr>
<tr>
<td>HgBPB</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ POSITIVE ++ STRONG +++ INTENSE - NEGATIVE
in length and 10-12 μ in width. Distally the cell duct narrows and pierces the cuticle through a raised projection on the spine surface (not to be confused with "stabchen", see below). The histochemistry of these fibrillar cells (Table 4) indicates the presence of a highly sulphated mucus, methylation irreversibly removing the basophilia. Mucus on sand particles from the burrow wall similarly give a gamma metachromasia with the metachromatic dyes.

Large granular acidophil cells often occur (Fig. 32) and tangential sections of the outer spine layer indicate that these cells are often semi-wrapped around the fibrillar cells (Fig. 33). These cells are blind and do not pierce the spine surface.

The centre of the spine contains large turgid cells, up to 10 μ in diameter at their base, with neck canals running the length of the spine. Also within the ground substance cylinder a fourth cell type occurs. These cells are basophilic and finely granulated, with a long cell body running the length of the spine, (Fig. 34). They occur in clusters of nine or ten, and possess a terminal swelling below the spine surface. Morphologically they closely resemble the photocytes of *Amphiura filiformis* as described by Reichensperger (1908) and Buchanan (1963). However, as with *Ophiactis resiliens* all attempts to stimulate *Monamphiura aster* to luminesce have been negative. As recorded by Buchanan in *Amphiura filiformis* the cells contain an acid mucopolysaccharide as shown by their staining with Alcian blue. The histochemical results have failed to demonstrate sulphate groups and strong methylation has no effect on these cells. In fact after methylation they are most clearly seen due to the suppression of staining of
the other secretory cells.

"Stabchen" in Monamphiura aster are confined to the distal third of the spine. If an opening occurs through them it is certainly less than 1/μ in diameter. Both the central cells and the finely granulated cells appear to be closely associated with these areas and it has been impossible to resolve exactly which, if either, of the cells open into them. The correlation differs from section to section but the finely granulated cells usually show a closer correlation than the central cells.

Thus only the function of the fibrillar cells appears to be readily discernible. Possibly the finely granular cells may produce a secretion for adhering sand particles between the spines. The central cells, which stain weakly basophilic with Masson's trichrome, giving a brownish mauve colour, are also weakly positive to the PAS reaction. They do not appear to open to the spine surface and neither to the large granular acidophil cells, which resemble the oxyphil cells described in Amphiura filiformis, A. chiajei and Acronida brachiata by Buchanan. Their close association with the fibrillar cells may be significant.

2.9. Ophiomyxa brevirima.

(a) Feeding. This species appears to be solely carnivorous. An examination of the stomachs of several animals revealed a variety of animal remains, the most abundant being the small ophiuroid Axiognathus squamata. The arms of this species within the stomach had the intervertebral musculature completely digested away. Some animals contained as many as four such digested skeletons. A variety of amphipods were also found, of which some
were identified as *Aura typica* and *Lembos kergualani*. Pieces of *Ulva reticulata* were also commonly found.

If large food particles such as pieces of chopped mussel are introduced into tanks, they are readily detected from distances of 12" or more downstream. The chopped mussel is picked up by the podia, two or three combining for a large piece, but no attempt is made to pass the morsel from podium to podium, the movements of which are restricted (Section 2. 9. (b)). Instead the arms are bent, often coiling laterally in a helical coil (Fig. 35). All of the arms may gather around the food and combine to push it into the mouth, which is capable of considerable extension. If all the arms are employed in the action the whole animal may roll over onto its aboral surface and the entire body is arched over the food. The material is then grasped by the teeth, which are serrated, as are the oral papillae, and then passed into the mouth by the buccal podia.

(b) Podia. The whole body of *Ophiomyxa brevirima* is covered by a thickened epidermis which overlies the skeletal plates. Similarly, the usual tentacle scale is also covered and further modified into a barrel completely surrounding the podial stem, being supported by two calcareous spicules (Fig. 36), probably representing the tentacle scales. The barrel is a characteristic feature of this species. It is the same length as the neighbouring, covered, spines, and can only be distinguished by being hollow at the tip. Through this barrel only the terminal knob protrudes (Plate 31), and at maximum protraction the podia can barely touch each other or the spines.
The podia, however, are used in locomotion, the terminal knob serving as a means of attachment to the substrate. Due to the flexibility of the barrel surrounding the podia, the whole structure can be moved to some extent. When the podia are used in locomotion up to six of them together move in phase. *Ophiomyxa brevirima* readily climb the sides of perspex tanks and are very active in general.

There is very little differentiation between bulb and stem and the podium in general is slight. The inner connective tissue spiral is just detectable and the longitudinal connective tissue is only 4-5/μ in width. It was not possible to determine the angles of the spiral system. The muscle tissue is also slight, with a tissue thickness of some 6-8/μ. The podium is unusual due to its almost negligible covering of epithelial tissue except at the terminal knob where it is greatly enlarged to a depth of 40-60/μ, with an apparent external cuticle. The nervous tissue is only readily detectable in the terminal knob, being much thinner along the stem. Nerve tracts radiate in all directions in the knob from a central nerve pad.

Two types of mucous cells are detectable in the terminal knob with AB/PAS. Thus PAS positive cells stain red and are similar histochemically to those of *Ophiocopteria antipodium* podia. The histochemistry of mucous glands staining blue with AB/PAS is summarized in Table 5. The results indicate that acidity is principally due to sulphate groups.

The buccal podia are annulate, resembling those of *Ophionereis fasciata* and lack the surrounding barrel of the arm podia. Young specimens removed from the bursae also lacked the barrel structure
**TABLE 5.**

**HISTOCHEMISTRY - GLANDS OF OPHIOMYXA BREVIIRIMA.**

<table>
<thead>
<tr>
<th>HISTOCHEMICAL TEST</th>
<th>PODIA BASIPHILIC</th>
<th>SPINES WIDE FIBRILLAR</th>
<th>SPINES NARROW FIBRILLAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.H. &amp; E.</td>
<td>BASIPHILIC</td>
<td>BASIPHILIC</td>
<td>BASIPHILIC</td>
</tr>
<tr>
<td>AB/PAS</td>
<td>BLUE</td>
<td>TURQUOISE</td>
<td>PURPLE</td>
</tr>
<tr>
<td>PAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AB</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>AB-H</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>AB pH 1.5</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>pH 3.4</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>AB in 2N H$_2$SO$_4$</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>in 0.6N HCl</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>MB pH 1.5</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>pH 3.4</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>METHYLN. 4 hrs.</td>
<td>-</td>
<td>+</td>
<td>RED</td>
</tr>
<tr>
<td>METHYLN. 4 hrs.</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>+ SAPONIFICATION</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>METHYLN. 7 hrs.</td>
<td>-</td>
<td>+</td>
<td>RED</td>
</tr>
<tr>
<td>METHYLN. 7 hrs.</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>+ SAPONIFICATION</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MB/ALSO$_4$</td>
<td>+</td>
<td>++</td>
<td>+</td>
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</tbody>
</table>

<table>
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<tr>
<th>Tol. B in 70% alc.</th>
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<th>GAMMA</th>
<th>BETA</th>
</tr>
</thead>
<tbody>
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<td>ALPHA</td>
<td>BETA/GAMMA</td>
<td>BETA</td>
</tr>
<tr>
<td>pH 3.4</td>
<td>ALPHA</td>
<td>ALPHA/BETA</td>
<td>ALPHA</td>
</tr>
<tr>
<td>AZURE A pH 0.45</td>
<td>-</td>
<td>GAMMA</td>
<td>BETA/GAMMA</td>
</tr>
<tr>
<td>pH 1.2</td>
<td>ALPHA</td>
<td>ALPHA/BETA</td>
<td>BETA/GAMMA</td>
</tr>
<tr>
<td>pH 2.1</td>
<td>0</td>
<td>ALPHA</td>
<td>ALPHA</td>
</tr>
<tr>
<td>pH 3.1</td>
<td>0</td>
<td>ALPHA</td>
<td>ALPHA</td>
</tr>
<tr>
<td>HgBPB</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ POSITIVE
++ STRONG
+++ INTENSE
- NEGATIVE
0 NOT TESTED
around the arm podia, although the podia were operational and used for locomotion.

(c) Spines. The spines are short and stubby, being irregularly spaced along the arm. Each spine is just over 600/μ in length and over 400/μ in diameter, with a large central calcareous plate over a 100/μ in diameter (Fig. 37). The thickened epidermal covering is 50-60/μ deep and contains a dense layer of connective tissue. Large granules also occur scattered throughout.

Three types of secretory cells are present (Fig. 38), the most noticeable being wide fibrillar cells which appear to be highly sulphated (Table 5). These cells are 35-40/μ in length and up to 10/μ in diameter, opening onto the spine surface at the bottom of a slight depression. Two large nuclci are situated at their base. A second type of fibrillar cell stains purple with AB/PAS and upon methylation stain with the PAS part of the AB/PAS sequence, indicating the presence of sulphate esters on 'vic' glycols (Spicer, 1963). These cells have a single nucleus near their base, are of the same length as the sulphated mucous cells, but only 2-3/μ in diameter. They are less frequent and occur every 15-20/μ over the integument. Coarse granular cells also occur scattered throughout the integument. These cells are acidophil and are PAS positive. All of these cell types occur in the integument of the barrel around the podium.

_Ophiomyxa brevirima_ has a general slimy texture and mucus on the surface of the animal consists principally of the sulphated form.

2. 10. _Pectinura maculata_.

(a) Feeding. The first published observation of the feeding of _Pectinura maculata_ was the strange report by Fell (1952) that
specimens collected from deep water of the south-western New
Zealand fiordland were found to have the stomach completely filled
with the anthers of the southern beech (*Nothofagus* sp.). Fell
concluded that the ophiuroids had been selectively feeding on this
terrestrial plant material, but later (1966) retracted this state-
ment and now considers them to merely collect detrital material,
though no mechanism of feeding was suggested.

The stomach contents of several *Pectinura maculata* from the
Leigh and Whangarei areas have been examined and in most cases they
have revealed little of large, identifiable, material. The
majority of them have been collected by skin divers and the animals
have probably thrown out their stomach contents when handled.
Specimens have however lived for two or three months in aquaria on
chopped mussel. Apparently *Pectinura maculata* congregate near
patches of fish offal at depths of 4-10 meters off Mount Maunganui
near Tauranga.

Pieces of mussel are readily detected and grasped by the podia
of the distal part of the arm. The arm itself is then coiled up
in an oral/aboral direction (Fig. 39) and thus at right angles to
the manner of *Ophiomyxa brevirima*. The disc is raised by the
proximal part of the remaining arms and the tip of the food carrying
arm is introduced into the mouth cavity where the buccal podia
remove the food and pass it into the mouth.

The arm podia are extremely small and it is the spines which
play a major role in locomotion. The spines are broad and flat,
being normally closely adpressed against the lateral arm plates.
As the arms are thrown forward the spines are raised where they
come into contact with the substrate giving added grip and
supplementing the leverage of the arms. If animals are suspended by their disc the spines normally lie flat. When they are stroked on their outer surface they are rapidly erected and this response extends to the adjacent five or six arm plates both proximally and distally. The response is ipsilateral only. Each spine is supported from lateral bending by being proximally supported by notches in the lateral arm plates (Fig. 40).

(b) Podia and Spines. The arm podia are extremely small, extending some 500 μ in length when relaxed. The buccal podia however attain a length of 2 to 2.5 mm. and are over 600 μ in diameter. Both podia are annulate and are of a similar histological pattern. The inner spiral envelope is well developed, running at an angle of 70° to 75° to the longitudinal axis in both bulb and stem. All of the other histological layers are present with little differentiation, the nerve plexus lying external to all connective tissue components. Mucous cells present in the epithelium closely parallel those of Ophionereis fasciata, being both PAS positive as well as possessing carboxyl groups, and a detailed analysis need not be figured.

The spines posses a large, central, plate and mucous cells appear to be lacking here although they occur elsewhere scattered in the integument of both arm and disc, particularly around the jaw plates and teeth. These cells contain a sulphated mucin, histochemically resembling those of Ophiopteris antipodum multicellular glands.

2. 11. Axiognathus squamata.

(a) Feeding. Axiognathus squamata is usually found either in
coralline algae or amongst coarse sand particles and shell fragments over the entire reef flat. They show a marked response to currents introduced into experimental tanks although no rheotactic response has been noted. The arms are stiffly raised and quickly become coated with mucus. Particles adhering to the spines are passed direct from podium to podium to the mouth. No attempt to form an intraspinal mucous network was observed, the spines merely acting as sticky rods. Due to the small size of the animal in general, the spines are arranged along the arms at intervals of only 70–90 μ and thus serve as a filtering device themselves. The podia also extend beyond the spines and are arranged to interdigitate with them. The tips of the podia are often characteristically bent and are frequently rubbed against the spines.

Martin (1968) has also observed Axiognathus squamata to directly engulf particles with the buccal podia, and considers this method to be used more than any other. Feeding by the use of mucus is almost always observed in animals amongst the coralline algae and thus the method observed by Martin may be primarily used in those animals that are amongst the sand particles. Martin also examined the stomach contents of a number of specimens, which contained a variety of organisms including unicellular algae, small gastropods, foraminifera and isolated amphipod limbs.

(b) Podia and Spines. The arm podia are small being little over 100/μ in length and only 25/μ in diameter. They are annulate and have a large terminal knob. The connective tissue envelope is prominent but appears circular in relaxed podia; the longitudinal system is so slight that it appears superficially to be lacking.
The muscle fibres consist of isolated cells 2-3 μ in width which are terminally embedded in a narrow channel penetrating the centre of the terminal knob (Fig. 41). The insertion of these muscles possibly accounts for the apparent isolated control of the terminal knob, as ipsilateral contractions would tend to pull the knob to one side or the other. The external epithelium comprises a single cell sheet of 6 μ, deepening to 25 μ to form the terminal knob.

The terminal knob alone contains mucous cells which extent proximally to rest upon a thickened nerve plexus. Their histochemistry closely parallels that of Ophionereis fasciata podia, although only a pale positive reaction with the PAS test can be detected.

The buccal podia are broader than those of the arm (45 μ) and have a large terminal knob forming the distal third of the entire podium (Plate 32).

The spines (Fig. 42) have recently been described by Buchanan (1963), and were previously described by Reichensperger (1908) and Sokolow (1909). All three authors have described fibrillar cells occupying the centre of the spine and Sokolow regarded them as photocytes. Buchanan found these cells to be metachromatic with aqueous toluidine blue and positive to Alcian blue. The present investigation has shown only a beta metachromasia with azure A at pH 1.2 and methylation/demethylation indicates a preponderance of carboxyl groups. Indeed the staining reactions show all the indications of a similar secretion to that of Ophionereis fasciata spine 1. Areas of "stabchen" are closely associated with the terminal ducts of these cells.

Both Reichensperger (1908) and Buchanan (1963) have reported
basophilic granular cells around the raised base of the spine, and concluded that these cells are the true photocytes as they correlate with the areas of observed light production recorded by Mangold (1908) and Reichensperger (1908). The light produced is yellowish in colour.

Other granular cells occur scattered in both spines and arm plates. These are granular, acidophil, and are positive to the PAS test.
Fig. 26 Surface view of Monamphiura aster burrow, showing protruding arm tip.

Fig. 27 Arm of M. aster in burrow, removing sand particles (SA. PA.).

Fig. 28 Arm of M. aster in burrow, creating irrigatory current by oral/aboral flexures.

Fig. 29 Oral view of M. aster within burrow. Two arms are retained within the disc chamber (DISC CH.).
Fig. 30 Composite drawing of *M. aster* spine.

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AREA OF &quot;STAB&quot;</td>
<td>area of &quot;cuticularstabchen&quot;</td>
</tr>
<tr>
<td>CENT. C.</td>
<td>central cell</td>
</tr>
<tr>
<td>CONN. T.</td>
<td>connective tissue</td>
</tr>
<tr>
<td>DECAL. SP. SHELL</td>
<td>decalcified spine shell</td>
</tr>
<tr>
<td>FIB. C.</td>
<td>fibrillar cell</td>
</tr>
<tr>
<td>FINE. GRAN. C.</td>
<td>finely granulated cell</td>
</tr>
<tr>
<td>GR. SUB.</td>
<td>ground substance</td>
</tr>
<tr>
<td>LARGE GRAN. C.</td>
<td>large granular cell</td>
</tr>
<tr>
<td>SP. NERVE</td>
<td>spinal nerve</td>
</tr>
</tbody>
</table>

Fig. 31 Fibrillar cells, spine of *M. aster*.

<table>
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<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIB. C.</td>
<td>fibrillar cell</td>
</tr>
<tr>
<td>GR. SUB.</td>
<td>ground substance</td>
</tr>
<tr>
<td>N.</td>
<td>nucleus</td>
</tr>
</tbody>
</table>

Fig. 32 Large granular cell, spine of *M. aster*.

<table>
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<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LARGE GRAN. C.</td>
<td>large granular cell</td>
</tr>
</tbody>
</table>

Fig. 33 Tangential view of spine surface, *M. aster*.

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIB. C.</td>
<td>fibrillar cell</td>
</tr>
<tr>
<td>LARGE GRAN. C.</td>
<td>large granular cell</td>
</tr>
</tbody>
</table>

Fig. 34 Distal portion of finely granulated cells, *M. aster* spine.

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FINE. GRAN. C.</td>
<td>finely granulated cell</td>
</tr>
<tr>
<td>&quot;STAB&quot;</td>
<td>&quot;cuticularstabchen&quot;</td>
</tr>
</tbody>
</table>
Fig. 35 Drawing of *Ophiomyxa brevirima* arm, coiled laterally.

Fig. 36 L/S stem of arm podium, *O. brevirima*.

- **COEL. EP.** coelomic epithelium
- **CONN. T.** connective tissue (both inner envelope and longitudinal components)
- **EPITH.** epithelium
- **LONG. M.** longitudinal muscle
- **NERVE PLEX.** nerve plexus
- **W. V. C.** water vascular cavity

Fig. 37 L/S spine, *O. brevirima*.

- **CONN. T.** connective tissue
- **NARROW FIB. C.** narrow fibrillar cell
- **SP. NERVE** spinal nerve
- **TH. EPITH.** thickened epithelium
- **WIDE FIB. C.** wide fibrillar cell

Fig. 38 Gland cells of spine, *O. brevirima*.

- **GRAN. C.** granular cell
- **NARROW FIB. C.** narrow fibrillar cell
- **WIDE FIB. C.** wide fibrillar cell
Fig. 39 Arm of *Pectinura maculata* showing oral/aboral coiling.

Fig. 40 Arm spines of *P. maculata*, both adpressed to side plate, and raised (---).

POD.  podia
SP.  spines

Fig. 41 L/S podium, *Axiognathus squamata*.

COEL. EP.  coelomic epithelium
CONN. T.  (both inner envelope and longitudinal components)
EPITH.  epithelium
LONG. M.  longitudinal muscle
M. CELL BOD.  muscle cell bodies
N.  nucleus
NERVE PLEX.  nerve plexus
W. V. C.  water vascular cavity

Fig. 42 L/S spine, *A. squamata*.

FIB. C.  fibrillar cell
GRAN. C.  granular cell
SP. NERVE  spinal nerve
Plate 30.
L/S podium
M. aster
Mass. Tri.

Plate 31.
L/S podium
C. brevirima
Mass. Tri.

Plate 32.
L/S buccal podium
A. squamata
Mass. Tri.
2.12. **Discussion.**

In the animals studied in Part 2 an even greater variety of mucus cells occur, although once again certain similarities are apparent. The podial glands of *Monamphiura aster*, *Pectinura maculata* and *Axiognathus squamata* all resemble those of *Ophionereis fasciata* in having a heterogeneous PAS positive and acidic mucus. This mucus is viscous enough to give adequate adherence to both sand and food particles. The podial glands of *Ophiomyxa brevirima* contain solely PAS positive cells in addition to the carboxyl/sulphate cells as has been observed in *Ophiopteris antipodium*, the PAS positive component probably being important in providing an added adhesive secretion.

The spine glands of *Axiognathus squamata*, as in *Ophionereis fasciata*, contain fibrillar cells with a carboxyl/sulphate mucus utilized in a feeding mechanism, even though this feeding mechanism differs somewhat in detail. The particular configuration of the mucus may be correlated with its physical property to form a sheet which can be spread over the body surface, and/or its subsequent breakdown in the stomach. Unfortunately, little is known of the physical and chemical properties of mucus and recent summaries of glycoproteins by Brimmacombe and Webber (1964) and Gottschalk (1966) refer principally to mammalian tissues.

It is known, for example, that bovine submaxillary gland mucus, which contains sulphate esters in only small amounts, has a high proportion of sialic acid. These sialoproteins are highly branched, globular structures in which polypeptides are chemically linked to oligosaccharides (Kent, 1964). It is not possible to histochemically detect many of the monosaccharide components of the mucus but these
may be as important when the mucus is ingested and has to be broken down as the side groups determining its physical properties on the surface of the animal.

Algae are known to contain a high proportion of acidic carbohydrates with a considerable number of sulphate esters (Kent, 1964). Sulphated mucins, however, are not confined to phytoplankton feeders, occurring in a fibrillar form in Monamphiura aster, Ophiomyxa brevirima and Pectinura maculata. Their function in Monamphiura aster appears to be principally in coating the burrow wall, but both Ophiomyxa brevirima and Pectinura maculata are epifaunal. It is possible that these sulphated mucins serve to coat the animal with an acidic slimy film in order to discourage settling larvae and parasites on their exposed surfaces which the diminutive podia cannot clean. An animal such as Pectinura maculata presents a large surface area and Ophiomyxa brevirima is not small.

Recent work by Pequinat (1966) has suggested that "skin digestion" occurs in echinoderms, including ophiuroids, by enzymes carried in spherule-coelomocytes. Pequinat's methods have been repeated on the ophiuroids of this study but no conclusive occurrence of enzymatic activity has been observed.

The variety of mucus types cannot be correlated with the infaunal or epifaunal mode of life of the animal, as suggested by Buchanan (1963), and it is also unsatisfactory to attribute one mucus type to one function; similar mucins playing different roles in different species. Histochemical methods only aid in bringing attention to the similar reactive properties of side groups, and in view of the present knowledge of a great variety in the ultrastructure of mucous granules in asteroids, as well as the ophiuroids studied here, the
protein configuration and its physical properties would also appear to be important. The problems are only beginning to be unravelled.
Section 3.
THE RADIAL CANAL AND OPERATION OF THE PODIA.

3.1. Introduction.

The mechanisms of protracting the podia in ophiuroids have recently been given renewed investigation. Buchanan and Woodley (1963) and Woodley (1963) contended that the proximal bulb of the podium had a muscular system which was generally antagonistic to that of the stem, and thus served as the functional equivalent of the ampulla of other classes. A valve was described at the head of the bulb which could be opened against the pressure within the podium. Also, a dorsal vesicle in the radial canal of Amphiura filiformis was described, which could maintain by its inherent elasticity, the volume of the podial fluid when the podium was retracted. Sphincter muscles in each arm segment prevent the dissipation of pressure from one segment to another and thus each podial pair could operate independently.

These views were subsequently modified by Woodley (1967), as the result of a study of Amphiura filiformis and A. chiajei; Woodley considers that in these species at least, the extension of the podia is brought about by the collapse of the radial canal rather than by an ampulla-like action of the bulb. However, Woodley concedes that reciprocal activity of the bulb and stem muscles in combination with elastic distortion of the podial envelope, may serve to protract the podia of other species. The following section attempts to relate the relative significance of the radial canal, bulb, and valve systems, in the activities of Ophionereis fasciata, Ophiactis resiliens, Ophopteris antipodium, Monamphiura aster and Ophiomyxa brevirima, as described in section 2.
Some notes on the possible extent of nervous control of the podia are also presented.

3. 2. The podial valve.

The podial valve has been described in a generalized manner by Woodley (1967). The valve lies within the bulb of the podium, consisting of two nearly equal flaps which are extensions of the podial wall. The flaps lie proximally and distally in relation to the longitudinal axis of the arm, and are invested with muscle fibres running in two distinct groups. Woodley considers the two groups to contract synergically and thus open the valve.

In *Ophionereis fasciata* all components of the valve are present (Figs. 43 and 44). Only two diagonal muscle fibres run across the podial side of the valve flap but seven or eight fibres in a sheet run parallel to it. On the radial canal side of the valve the podial branch canal narrows to a diameter of 25/μ from its diameter of 40/μ along most of its length. A series of muscle fibres 2/μ in width run around this area and may assist in the restricting of the canal to aid the closing of the valve. Other muscle fibres run around the canal at irregular intervals.

*Ophiactis resiliens* has a similar arrangement with a large sheet of parallel fibres, running parallel to each other (Fig. 45). However, the podial branch canal does not narrow near the junction with the bulb, and although scattered muscle fibres occur in its wall no distal concentration near the valve is discernible.

Similarly, *Ophiopteris antipodum* lacks an actual concentration of fibres but other fibres surround the branch canal along its length. In the valve of *Ophiopteris antipodum* the diagonal fibres are more in evidence than the parallel ones, and in *Ophiomyxa brevirima* the
latter appear to be lacking entirely. The valve is very slight in this species.

Finally, the valve of Monamphiura aster is essentially similar to that of Ophionereis fasciata. Both diagonal and parallel fibres are present although the two systems tend to merge one into the other.

3. 3. The radial canal and its musculature.

The walls of the radial canal are usually referred to as being elastic (Cuénot, 1948) although this property has been effectively demonstrated only by Woodley (1967) for Amphiura filiformis. The form of the canal is basically similar in all species. It is lined by an inner very sparsely ciliated epithelium, except along the floor. Outside the epithelial tissue lies a hyaline layer no more than 2-3μ thick, staining with the light green of Masson's trichrome. The roof of the canal is variously thickened with an apparently structureless layer staining with the xylidene de Ponceau of this staining sequence. In Ophionereis fasciata two such thickenings run parallel along the dorsal surface of the canal. Similar thickenings occur in Ophiopteris antipodum and in Ophiactis resiliens. It is in Monamphiura aster that these thickenings are well developed and may be up to 10μ thick, particularly on the shoulder of the dorsal accessory vesicles of the radial canal which occur in this species.

Woodley (1967) has shown that this tissue in Amphiura filiformis is acidic with protein and polysaccharide components. It resembles elastin in being relatively impermeable, non-birefringent, insoluble in weak acids and alkalies and is probably co-valently linked with the disulphide bonds of cystine. Apparently formaldehyde fixed
sections were negative to dyes which demonstrate vertebrate elastic tissue. *Monamphiura* aster tissue, however, stained in Verhoeff's elastic stain (Pearse, 1961), after being fixed in Heidenhain's "suza", stain heavily; but Weigert's elastic stain (Pearse, 1961) had little or no effect, even with only brief immersion in aqueous picric acid. Unfortunately the mechanism by which both of these stains work is not clearly understood (Pearse, 1961) and elastin, as such, has not been identified in invertebrate tissue, although elastic properties of tissues are frequent.

The radial canal furthermore possesses its own musculature. This for the most part comprises a collection of narrow (2/μ) muscle bands running around the canal, but not across the floor; they are situated internal to the epithelial lining. Larger muscle fibres form sphincters in each segment, occurring distal to the branch canals to the podia (Fig. 46). In *Monamphiura aster* these sphincters are particularly well developed, lying 100/μ distal to the origin of the branch canals. Each sphincter consists of fibres some 2-3/μ in width, closely packed together along 80-85/μ of the canal wall. Transverse sections show that when contracted the canal is restricted by a "pincer-like" action (Fig. 47).

The floor of the canal contains a series of longitudinal fibres. There are five or six in *Ophiactis resiliens*, eight or nine in *Ophionereis fasciata*, twelve in *Monamphiura aster*, up to twenty in *Ophioptera antenna* and more than thirty in *Ophiomyxa brevirima*. These fibres lie along the centre of the floor of the canal (Fig. 48) and in *Monamphiura aster* they are not attached to the floor of the canal between sphincter muscles. They may be attached in the region of the sphincter muscles but the sphincter itself obscures
the longitudinal fibres. The fibres are little over 1 μ in diameter, the contractile elements lying within the radial canal and their muscle cell bodies among the epithelium. In Monamphiura aster, Ophionereis fasciata and Ophiopteris antipodium the fibres bulge into the canal, but this is less evident in Ophiactis resiliens and Ophiomyxa brevirima. The fibres thus have characteristic profiles in transverse sections.

The radial canal of Monamphiura aster differs from all other species in the possession of a dorsal accessory vesicle, as in Amphiura filiformis (Figs. 46 and 51). This vesicle lies at the junction of the branch canals to the podia and is accommodated within a cavity passing through the middle of the vertebral ossicle. The floor of the canal is also slightly depressed in this region, although the diameter does vary along its length being narrowest midway between vesicles. The entire vesicle and the associated parts of the canal are heavily supported with the elastic-type material, which runs in a circular manner around the vesicle itself. The canal widens to a diameter of 70 μ near the vesicle which, in relaxed preparations, has an internal diameter of 20 μ and is 70 μ in height.

As mentioned above, narrow muscle fibres also occur around the epithelium of the branch canals which run to each podium.

3. 4. Extension and retraction of the podia.

The previous theories and speculations about the mechanisms for protracting the podia of ophiuroids have been adequately reviewed by Woodley (1967). A summary may be useful here of the functions of the various components involved.

Firstly, the radial canal as Woodley has shown, appears to act
as an elastic radial chamber which stores the fluid, and its potential energy, created by the retraction of the podia, either singly or both together. The sphincter muscles prevent the dissipation of fluid pressure from one arm segment to another and also allows each podial pair to function independently. The pressure thus developed appears to originate principally from the expansion of the canal as the ciliation is extremely slight and the muscle fibres are sparse and of narrow diameter. It is difficult to imagine that the canal would collapse much more than its relaxed volume, when the muscles of the podia themselves are relaxed. The longitudinal fibres may, however, aid in restricting the canal volume but the interpretation of their function depends on the appreciation of a moving component. As the fibres do not appear to be attached along the canal floor, except possibly in the sphincter muscle area, then their contraction would apply tension to the floor of the canal and tend to raise it slightly, particularly as it is depressed at the junction of the branch canals. The canal is surrounded by an apparently fluid filled space and would therefore not be anchored securely enough to allow the floor of the canal to be depressed by the change in length of the fibres during their contraction. In either event the width of the fibres is not great and they appear to function under considerable mechanical disadvantage.

Assuming that the elasticity of the vesicle is constant over the whole range of its inflation then the rate of extension for one podium would be the same as for both. The actual rate of protraction of the podia may not depend so much on the pressure developed within the inflated canal as by the very high frictional
coefficient of the branch canal which, at a diameter of about 50/µ, must approach unity. The fluid from the canal provides the initial inflatory mechanism of the podia.

The valve at the head of the podium prevents the return of the fluid from the podium to the canal. Its closing may be assisted by the contraction of fibres around the distal portion of the branch canals, although the pressure within the podium would effect the return of the flaps when their muscles relaxed. The branch canal, at least in *Ophiactis resiliens*, is separately innervated by a branch from the hyponeural region of the radial nerve. The valves must also be opened against the pressure within the podium and thus the muscles are arranged within the bulb to exert maximum force against this pressure. The diagonal fibres would force the lips of the valve apart by shortening them, presumably allowing the parallel system to draw them even further apart.

The inner spiral envelope of the podium ensures a rapid translation of pressure to the distal end of the podium. This could be attained if the inner layer consisted of entirely circular fibres. However, circular fibres impose a limit on the extent of distortion of the cylinder when inflated. Harris and Crofton (1957) have demonstrated a spiral system in the wall of the nematode *Ascaris*. These authors have discussed the relevance of the angle of the spiral fibres to the longitudinal axis; the volume of the cylinder decreasing upon contraction of the longitudinal muscles within it if the angle is greater than 55°. The angles measured in Section 2 apply only to relaxed podia and would thus be smaller when the podium is extended, but are unlikely to be less than the critical 55°. As Woodley (1967) has inferred, such a system can
be used simply to shorten and re-extend the podium as well as allowing it to be bent from side to side. Also, a localized contraction can cause extension elsewhere by raising the internal pressure, providing the system remains closed.

By fixing one arm in a relaxed state, and a neighbouring arm with the podia in a contracted state, it has been attempted to compare the tissue widths of the longitudinal connective tissue and muscle layers by taking transverse sections of mid arm podia. Thus in *Ophiactis resiliens*, where the podia exhibit a considerable change in length, the connective tissue width alters from 4 /μ in the relaxed state to 6 /μ when contracted, an increase of 150%. The longitudinal muscle alters from 5 /μ to 9 /μ, an increase of 180%. Similarly in *Ophiopteris antipodium*, where little overall change in length occurs, the connective tissue only alters from 80 /μ between the bases of the papillae to 100 /μ, (125% increase) and the muscle from 15 /μ to 25 /μ (167% increase). The overall diameter of the enclosed water vascular cavities increase by 180% and 160% respectively in the two species.

The function of the bulb in the protraction of the podia raises the most difficult problems. Woodley (1967) has found that in *Amphiura filiformis* the extended podium, in narcotized specimens, has an extended bulb musculature. If the bulb plays no part in the extension of the podia then their relaxed length should closely parallel the length of extended podia in an active state. This is in fact the conclusion Woodley has drawn after measuring both active and narcotized podia. One would imagine that in relaxed preparations the musculature of the radial canal would also be relaxed and thus not in a completely deflated state,
although nearly so. The situation may also be somewhat specialized in this species owing to its ability to accommodate extra fluid in the accessory vesicle of the radial canal. Podia would be longer, and thinner, when protracted if both the radial canal and the bulb were deflated.

The musculature of the bulb is often considerably larger than that of the stem. In both Ophiactis resiliens and Ophionereis fasciata the bulb musculature is up to three times that of the stem; in Ophiopteris antipodum and Monamphiura aster half to twice as thick; and in Ophiomyxa brevirima there is hardly any difference. As the podia of Ophiactis resiliens may be four times as long in the extended state as they are when relaxed, contraction of the bulb may play a considerable role in their extension. The podia of Ophionereis fasciata may extend to twice their relaxed length. The podia of Ophiopteris antipodum are certainly longer when extended to a maximum but are normally fairly stout, particularly when attached to the substrate upside down, implying that the musculature may be in a state of tonus when the tip is attached. There is little observable difference in length in Monamphiura aster and Ophiomyxa brevirima.

In connection with the possible deflation of the bulb it is interesting to note the presence of a group of muscle fibres which extend from the top of the bulb on its exterior surface and run to the lateral arm plate. They are found in Ophionereis fasciata and Ophiactis resiliens, (Figs. 49 and 50). In Ophiactis resiliens this sheet of fibres is a little over 60μ wide and 150μ long. The fibres run over the surface of the bulb at an angle of 30° to the bulb musculature. They are restricted to the
proximal side of the bulb in relation to the longitudinal axis of the arm. Their contraction could decrease the bulb longitudinally by compressing it against the ventral arm plate. A similar, less extensive, muscle occurs in *Ophionereis fasciata*, but appears to be absent from the other species examined.

Woodley also comments upon the extent by which podia can be withdrawn. The podia of *Monamphiura aster*, as in *Amphiura filiformis*, can only be contracted to about half their extended length. The podia of *Ophiopteris antipodum* can be retracted only to about two thirds of their extended length and are then laid between the spines for protection. The podia of both *Ophionereis fasciata* and *Ophiactis resiliens* however can be fully retracted behind tentacle scales on the ventral arm plate. It is therefore of interest to compare the relative volumes of stem and bulb, as the volume that could be held by the bulb is severely limited by its enclosure in the vertebral ossicle. The relative volumes of stem and bulb are presented in Table 6 and have been calculated by considering each part as a cylinder. The figures represent the average of five separate calculations.

It can be seen in *Ophiactis resiliens* that when the total volume of the stem is absorbed by the bulb, it only increases its volume by a factor of 1.185 i.e. volume of stem plus volume of bulb/volume of bulb. Similarly in *Ophionereis fasciata* the volume of the bulb is increased by a factor of 1.285. In the other three species however the volume of the stem would more than double the volume of the bulb. This is perhaps why the podia of *Ophiopteris antipodum* are only slightly retracted, because a limit is set primarily by the volume of excess fluid that can be taken
by the radial canal. The podia of *Ophiomyxa brevirima* require only slight contraction to withdraw them into the protective barrel, and the podia of *Monamphiura aster* can be considerably retracted owing to the accommodation afforded by the accessory vesicle.

**TABLE 6.**

**VOLUME RATIOS OF PODIA.**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>VOL. OF BULB mm$^3$</th>
<th>VOL. OF STEM mm$^3$</th>
<th>RATIO OF BULB:STEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. resiliens</td>
<td>$11.563 \times 10^{-4}$</td>
<td>$2.144 \times 10^{-4}$</td>
<td>1:5.4</td>
</tr>
<tr>
<td>O. fasciata</td>
<td>$93.547 \times 10^{-4}$</td>
<td>$26.680 \times 10^{-4}$</td>
<td>1:3.5</td>
</tr>
<tr>
<td>M. aster</td>
<td>$14.190 \times 10^{-4}$</td>
<td>$14.472 \times 10^{-4}$</td>
<td>1:0.9</td>
</tr>
<tr>
<td>O. brevirima</td>
<td>$7.560 \times 10^{-4}$</td>
<td>$9.004 \times 10^{-4}$</td>
<td>1:0.8</td>
</tr>
<tr>
<td>C. antipodum</td>
<td>$52.906 \times 10^{-4}$</td>
<td>$87.634 \times 10^{-4}$</td>
<td>1:0.6</td>
</tr>
</tbody>
</table>

The activities of *Ophiactis resiliens* podia, particularly the action of collapsing the podia in series, suggest that a rapid change in length is important. This could be attained by the antagonistic contraction of bulb and stem musculature, with the valve closed, as the amount of fluid to be exchanged is relatively small. The podia of *Ophionereis fasciata* similarly change length to some extent when active and this could also be accomplished by the exchange of fluid with the bulb.

The podia of both *Ophiopteris antipodum* and *Monamphiura aster*
undergo little change in length, particularly in the latter species where the stiff podia are thrust into the substrate, and in *Ophiomyxa brevirima* only the podial tip is protracted. In these species almost total extension could be attained by the release of fluid from the canal when the podial muscles relax in both stem and bulb. Contraction of the bulb musculature, which protrudes distally beyond the arm plates in *Monamphiura aster* and *Ophiopteris antipodium* could control postural activities of the stem as a whole and also cause a slight increase in its length. Other activities of the extended podia can be attained by the ipsi- and contralateral contractions of the longitudinal musculature of the stem distorting a podium containing a constant volume of fluid when the podial valve is closed.

The method of podial protraction in *Ophiactis resiliens* and *Ophionereis fasciata* may therefore depend upon the use of the bulb to provide full extension and to effect a rapid change in length. In the other species however the radial canal may provide the means of podial protraction with only minor action by the bulb which may be primarily postural, causing the podium as a whole to be moved in any direction.

Brief mention may be made of the extent of nervous control of the podia. It is often stated that the neurones of the nerve ring dominate the activities of the arms of ophiuroids (Smith, 1965) and that there is a greater dependence of behaviour on the integrity of the nervous system. This may be true of complex activities of the arm during locomotory activities but the movements of the podia exhibit a remarkable degree of behavioural autonomy.
The arms of six specimens of *Ophionereis fasciata*, *Ophiactis resiliens* and *Ophiopoteris antipodum* were severed from the disc and placed in well-aerated tanks of water. The immediate reaction in all species was one of violent movement for several minutes. Within 5 minutes the arms of *Ophiopoteris antipodum* lay motionless although the podia remained active. After 21 hours the podia remained active where some form of peripheral contact was afforded but after 30 hours only slight movement was observed. Response to touch was readily elicited after 42 hours but after 75 hours (3.1 days) little response occurred.

The isolated arms of *Ophionereis fasciata* continue to flex after 75 hours and the podia perform all the actions of those of an intact animal such as rubbing the spines, collecting particles and forming a bolus. Even after 167 hours (6.9 days) the podia continue to show spontaneous, although sluggish, movements. After 300 hours (12.5 days) the podia still responded to touch.

The arms of *Ophiactis resiliens* exhibit the most remarkable degree of isolated behaviour. The severed arms are raised in response to a current, being supported by the proximal third of the arm which coils around to form a base. The distal third orientates into currents although this may be only a physical effect of the pressure of the water on the extended podia. After 167 hours (6.9 days) 30% of the arms still exhibited the feeding posture, the podia carrying out all the actions of an intact arm. The other 70% responded to touch and most of them reared up into a "feeding" position. By the end of 300 hours (12.5 days) the majority of the arms had completely disintegrated. Examination of them showed that the intervertebral musculature had broken down.
Presumably the podia and general activities of the arm had drawn upon the muscles as a respiratory substrate, resulting in their eventual dissolution.

The experiment was repeated with the modification of leaving half the arms with a fifth of the disc, incorporating the junction of the radial nerve and the central nerve ring. The difference between the two sets of arms occurred in the first half hour, the arms containing the disc nerve junction displaying a tendency to locomote. In Ophiopteris antipodum and Ophionereis fasciata the arms attempted to climb the side of the tanks, with the proximal end foremost, but in Ophiactis resiliens, in 90% of the individual arms, it was the tip of the arm that was thrust forward. After this initial half hour the behaviour of all arms closely paralleled those of the first experiment.

Thus a considerable degree of local control is exhibited by the podia, particularly following local tactile stimulation. Attempts to interfere with nervous connections, either ipsilaterally or contralaterally, have not proved successful as the radial water canal is often damaged as well. Moreover, the functioning of the various layers of the nerve cord are not sufficiently known to infer the experimental results. It would appear however that the actions of the podia of Ophionereis fasciata and Ophiactis resiliens are not dependent on central control although the various actions such as moving sand grains, locomotory and other activities are presumably initiated by a connection with the nerve ring. The podia of Ophiopteris antipodum display a marked decrease in spontaneous activity in isolated arms although readily respond to tactile stimulation. The more complicated actions of these podia
Fig. 43 Valve of *Ophionereis fasciata* podium as seen in a vertical section of the arm.

- **BR. CAN.** branch canal
- **CIRC. M. FIBRES** circular muscle fibres
- **COEL. EP.** coelomic epithelium
- **DIAG. FIBRES** diagonal fibres of valve
- **PARA. FIBRES** parallel fibres of valve

Arrow indicates direction of water vascular fluid when valve is open to inflate podium.

Fig. 44 Valve of *Ophionereis fasciata* podium as seen in a horizontal section of the arm.

- **DIAG. FIBRES** diagonal fibres of valve
- **PARA. FIBRES** parallel fibres of valve
- **V. FLAP** valve flap

Arrow indicates direction of water vascular fluid when valve is open to inflate podium.

Fig. 45 Valve of *Ophiactis resiliens* podium as seen in a vertical section of the arm.

- **PARA. FIBRES** parallel fibres
- **V. FLAP** valve flap

Arrow indicates direction of water vascular fluid when valve is open to inflate podium.

Fig. 46 Longitudinal section of the radial water-vascular canal of *Monamphiura aster*.

- **ACC. VES.** accessory vesicle
- **HAEM. STR.** haemal strand
- **RAD. CAN.** radial canal
- **RAD. NERVE** radial nerve
- **SPH. M.** sphincter muscle
- **VENT. ARM PLATE** ventral arm plate
Fig. 47 Transverse section of the radial canal of *Ophionereis fasciata* through the sphincter muscle.

- COEL. EP.
- N.
- RAD. FIBRES
- SPH. M.

coelomic epithelium
nucleus
radial muscle fibres
sphincter muscle

Fig. 48 Floor of the radial canal of *Ophionereis fasciata*.

- COEL. EP.
- N.
- RAD. FIBRES

coelomic epithelium
nucleus
radial muscle fibres

Fig. 49 Horizontal section of the bulb of a podium, *Ophionereis fasciata*.

- BULB M.
- INTERV. M.
- POD. NERVE
- PROX. EXT. M. FIBRES
- RAD. NERVE
- SP. NERVE

bulb musculature
intervertebral muscle (L/S)
podial nerve
proximal external muscle fibres
radial nerve
spinal nerve

Fig. 50 Transverse section of an arm of *Ophiactis resiliens*, on the proximal side of the bulb of the podium.

- BULB M.
- CONN. T.
- INTERV. M.
- PROX. EXT. M. FIBRES
- SP. NERVE
- VENT. ARM PLATE

bulb musculature
connective tissue of bulb
intervertebral muscle (T/S)
proximal external muscle fibres
spinal nerve
ventral arm plate

Fig. 51 Transverse section through the dorsal accessory vesicle of the radial canal of *Monamphiura aster*.

- ACC. VES.
- BR. CAN. TO POD.
- COEL. EP.
- ELAST. T.
- RAD. CAN.

accessory vesicle
branch canal to podium
coelomic epithelium
elastic tissue
radial canal
Section 4.
MORPHOLOGY OF THE GUT AND A QUALITATIVE REVIEW OF DIGESTIVE ENZYMES.

4.1. Introduction.

The scant amount of available information on the structure of the ophiuroid gut has been summarized by Hyman (1955), being drawn mainly from the work of Hamann (1889). A more recent review is that of Anderson (1966) who draws attention to the general lack of information on the subject. The classic description of the stomach wall shows a ciliated epithelium with a brush border, and an underlying nervour layer. Beneath this runs both connective tissue and muscle fibres and an internal, ciliated, coelomic epithelium. Remarkably, neither mucous nor gland cells have been described. Anderson in his review, however, mentions personal observations indicating the presence of tall cells filled with secretory granules in the epithelium of Ophiura brevispina.

A recent paper on the morphology of the gut of Ophiothrix quinquemaculata by Roubaud (1965), appears to be the only detailed study in the literature. Both mucous and secretory cells are described, as well as coelomocytes, all lying within the epithelial layer. The presence of an alkaline phosphatase was also demonstrated. The only other information on digestive enzymes stems from a thesis by Wintzell (1918), quoted in Hyman (1955), which reports the presence of a strong proteinase, acting in both acid and alkaline media, in Ophiura texturata; an amylase and probably a lipase were also detected. Further information appears to be wanting.
4.2. Material and methods.

The discs of *Ophioneresis fasciata*, *Ophiactis resiliens* and *Ophiopterus antipodum* were fixed in 'Susa', the disc being punctured between the radial shields to ensure adequate internal fixation. Subsequent decalcification was carried out as given in Section 2. As well as the general histological stains of Section 2, Mallory's phosphotungstic acid haematoxylin was also used, being particularly useful for the staining of secretory cells. Deposits of glycogen were determined by using the PAS method with diastase control, and the sudan black B method (for paraffin sections) was used to demonstrate lipid deposits (Pearse, 1961).

Some enzyme sites were demonstrated histochemically. Tissues were both dissected out, and fixed in situ, with decalcification of the disc in Lorch's fluid (Pearse, 1961), a method designed to minimise enzyme loss. For esterases, gut tissue was fixed in cold (4°C) absolute acetone and embedded in 1% celloidin. Sections were incubated for 15 minutes in an alpha naphthyl acetate medium (pH 7.4) and stained with Fast B salt (Pearse, 1961). Cold acetone fixation was also used for acid phosphatase demonstration, sections being incubated and stained by the modified lead nitrate method of Takeuchi and Tanoue (Pearse, 1961). For alkaline phosphatase the modified coupling azo dye method of Pearse (1961) was used, with good results.

Other digestive enzymes were determined by using gut extracts. The complete gut wall of several animals of each species, which had been kept in filtered sea water for 24 hours, was removed, washed thoroughly in several changes of sea water and ground up with a few drops of glycerol. The suspension was diluted by adding an equal
volume of filtered sea water and centrifuged for 15 minutes. The supernatant liquid was drawn off and an equal volume of filtered sea water was added and mixed with the residue. After further centrifuging for 15 minutes the supernatant liquid was again drawn off and added to the first.

For the determination of a proteinase (gelatinase, an endopolypeptidase) 0.1 ml. of the gut extract was placed in the centre of a Petri dish containing 1% agar gell with 0.5% gelatin (Reid, 1966), which had been soaked in the required buffer solution for 30 minutes. After 6 hours incubation the substrate was soaked in a saturated solution of mercuric chloride in 1N HCl which precipitates the undigested gelatin.

A wide range of enzymes hydrolysing glycosidic linkages were also determined by using gut extract. For amylase, 0.1 ml. of extract was incubated for up to 24 hours at room temperature on a 0.5% soluble starch/1% agar gell substrate which had been soaked in the required buffer solution for 30 minutes. The action of the enzyme was followed by staining with 1% iodine solution. All other "carbohydrases" were determined by incubating 1 ml. of extract, 2 ml. of substrate solution and 2 ml. of phosphate buffer at 22°C for 16 hours, subsequently testing 1 ml. of the solution with Fehling's solution. Negative results were repeated at 37°C for 1½ and 2½ hours. Although only qualitative, some comparative measure of activity was noted by using 0.5 gm. of stomach tissue, ground for 5 minutes, in each case. Naturally the estimate is of comparative value only and does not take into consideration any varying states of activity even though specimens were starved prior to fixation.
4. 3. Morphology of the gut.

(a) *Ophionereis fasciata*. The ophiuroid gut consists of a single sac with little differentiation save for its division into ten pouches; five deep and well folded interradial pouches lying between the bases of the arms, and five shallow radial pouches lying over the arms. These pouches are confined to the disc except in the curious species *Ophiocanops fugiens* (now considered by Fell (1963) to be a surviving member of the Oegophiurida) in which the stomach sends out a diverticulum into each arm. The mouth is a circular opening in the centre of a peristomial membrane and leads into what is termed a very short oesophagus. There is no intestine and no anus.

The stomach is slung from the top of the disc to which it is attached by two types of supporting strands. The main support is afforded by thick strands (30 μ diameter) which consist of a central core of connective tissue fibres running from the connective tissue of the casing layer of the stomach to that of the integument. This core is surrounded by a muscular cylinder, with an outer covering of cuboidal epithelial cells (Fig. 52). Apart from these main supports, other more slender strands, (7-8 μ diameter), of connective tissue only are interspersed between the larger ones. The stomach is further supported, laterally, by what have been termed "bridles" running from the internal bases of the arms to the stomach wall, and orally by the peristomial membrane.

Apart from the coelomic epithelium, the other tissues which comprise the stomach wall differ little in thickness, usually being in the order of 15 μ thick although occasionally as little as
3 or 4 μ. The main supporting tissue of the stomach consists of a number of different tissue layers which show a considerable degree of interweaving. Nearest the integument is a layer of cuboidal epithelium upon which lies mixed layers of connective, muscle and nervous tissue. The connective tissue lies beneath the digestive epithelial cells and contains varying amounts of nerve fibres which in places come to lie directly beneath the basement membrane of the epithelial cells. These nervous elements are particularly prominent around the mouth. Nerve fibres also innervate this layer from the general sub-epithelial layer of the body wall, particularly on the aboral surface. The connective tissue also interweaves between the bundles of muscle tissue lying directly below it.

The muscle fibres, lying below these layers, are in general, orientated in a radial direction, running from the aboral centre of the stomach around to the mouth, enclosing the other tissue. Two other muscle systems are distinguishable, both being situated around the mouth. When looking at the stomach lining from the aboral side five trans-ambulacral tracts can be seen (Fig. 53). Vertical sections through this area reveal a large muscle orientated in this direction, having a cup-shaped profile, bordered internally by the peristomial coelom within which the cell bodies of these fibres lie (Fig. 54, Plate 33). The muscle is effectively in two parts, possessing a cleft in which is inserted a separate muscle system, only 15 μ in width, having an origin behind the nerve ring. The radial muscle fibres run outside these muscle systems. The larger muscle fibres by their contraction would tend to close the
mouth, drawing its five ambulacral edges together in the manner of a "purse-string". The narrower fibres could function to open the mouth by pulling it back, and downwards, aided perhaps by the contraction of the radial fibres. Hamann (1889) suggests the possible use of the peristomial coelom in the opening and closing of the mouth but it is difficult to see in what manner, if any, it could be used, especially as its form varies considerably between species as described below.

The epithelium consists of cells forming an integral part of this tissue layer plus migratory coelomocytes. Its height varies considerably. On the aboral side it is less folded, ranging from 70-80 μ, with occasional, simple, invaginations. Elsewhere, in both radial and interradial pouches, the height of the epithelium varies from 90-160 μ with some cells of only 30 μ at the bottom of some of the folds.

With Ehrlich's haematoxylin and eosin the epithelium displays a general uniformity, as described in the earlier descriptions, although some cells appear more granulated than others. With either Masson's or Mallory's trichrome stains a variety of large, heavily granulated, cells stand out clearly. The typical cells of the epithelium are tall and columnar presenting a generally hexagonal appearance on the surface. Each cell has a distal brush border of microvilli (striated plateau) some 6-8 μ in height. The nuclei are ovoid, 5.5 μ long and 3 μ in width, occupying the proximal part of the cells, a slight basal area clear of nuclei being just discernable.

Interspersed between these cells are heavily granulated cells, their contained secretions being readily observed distally (Fig. 55).
The cells open to the stomach lumen at the base of the microvilli of the neighbouring epithelial cells, and are usually swollen either subterminally or irregularly along their entire length. Occasionally the secretion can be traced at least 100 μm in from the microvilli region of the epithelium. These cells are far more in evidence on the oral side of the gut but there appears to be little difference in distribution between ambulacral and interambulacral pouches, where they occur at an average frequency of one cell per 14 μm linearly in any one section. Although these cells stain readily in trichrome stains, and the granulations stand out clearly in phosphotungstic acid haematoxylin, there is a negative result with AB/PAS. As they do not therefore appear to contain mucus of any description it is possible that they are zymogen cells. Somewhat similar cells have been described by Anderson (1960) in the stomach of asteroids, particularly the pyloric portion.

Staining with AB/PAS however does stain larger droplets in a few scattered cells, which colour blue with the Alcian blue of this sequence. The layer of microvilli over the stomach surface also stain heavily with the Alcian blue. These mucus cells exhibit only an alpha, tending to beta, metachromasia at low pH values, whereas the mucus on the microvilli exhibit a more intense beta reaction and is probably the mucus taken in with the food particles.

The PAS procedure with diastase control has revealed only scant deposits of glycogen in the layers beneath the epithelial cells. Staining with sudan black B reveals two distinct bands of lipid deposits. Immediately below the microvilli lipids stain up in a black band across the epithelium, some 3-4 μm deep, extending deeper in some cells and entirely absent in others. The deposit
is in the form of exceedingly fine droplets. Another layer, more diffuse than the first, occurs at the base of the cells in certain areas, as if accumulated, the drops being somewhat larger (Fig. 56).

Coelomocytes, averaging 8 µ in diameter, occur scattered throughout the epithelium. These cells display a gradual degeneration from the basal area of the epithelium to the layer of microvilli. The cells near the basal area are usually acidophilic although slight basiphilia is exhibited by some cells. Nearer the proximal layer the cytoplasm becomes clear and the nucleus deforms, becoming pycnotic and adpressed against the cell wall. The coelomocyte is finally released into the stomach lumen by passing between the epithelial cells (Fig. 57). A similar method of coelomocyte discharge has been noted by Roubaud (1965), in *Ophiothrix quinquemaculata*, who also reports another method whereby the cell breaks up into droplets before passing out into the gut lumen. This latter method has not been observed in the present study.

Where stomachs have been fixed with food particles in the stomach, and when they have been dissected in this state, it can be seen that the particles are isolated in discrete folds of the epithelium which is wrapped around them. This ensures that the food is kept in close contact with the epithelial lining, an important consideration in such a single undifferentiated digestive organ of the magnitude of the ophiuroid gut.

(b) *Ophiactis resiliens*. In general form the stomach of *Ophiactis resiliens* differs only slightly from that of *Ophionereis fasciata*
in that the interambulacral pouches show a considerable degree of oral/aboral folding. The gut is also attached to the integument of the disc in a similar manner, although the larger strands are less in evidence.

The casing layer of the gut is rather slight at a thickness of 15/\mu, nervous tissue being barely discernable except around the mouth. The connective tissue is 6 or 7/\mu thick, the coelomic epithelium and muscle layer accounting for the rest.

Around the mouth the peristomial coelom is subdivided into inner and outer cavities. The large trans-ambulacral muscle fibres seen in *Ophionereis fasciata* are again present, the partition of the peristomial coelom subdividing this muscle block (Fig. 58). The more oral muscle fibres running at right angles to the muscle block are 125/\mu in length and only 8/\mu wide, again extending behind the central nerve ring.

The epithelium varies in height, being shorter on the aboral surface of the stomach where it averages 60/\mu. On the oral surface it varies from 140/\mu down to 30/\mu, generally being in the region of 90/\mu and is thrown into large folds. Clefts between the folds are both more frequent and deeper than in *Ophionereis fasciata*.

The details of the epithelial cells differ only slightly from those of *Ophionereis fasciata*. The nuclei are usually more centrally placed displaying a larger clear basal area in the epithelial cells. A layer of microvilli is again present, staining intensely with Alcian blue. Secretory cells also occur, the granules being less dense but the cells occurring at a greater frequency at an average of one cell per 5/\mu linearly in any one section. Cells containing
larger mucous droplets are considerably scattered and not readily stained. Coelomocytes also abound in the epithelium.

The PAS procedure with diastase control does not reveal deposits of glycogen. Lipid deposits are confined to the distal areas of the epithelial cells, forming a distinct band across the epithelium. Basal lipid deposits appear to be lacking.

(c) *Ophiopetria antipodum*. The ambulacral pouches of the gut of *Ophiopetria antipodum* are only slightly folded, while the inter-ambulacral ones show a high degree of folding, both laterally and in an oral/aboral direction. This may be an allowance for the lack of depth in the rather flat disc of this species. Both large and small strands attach the stomach to the integument of the disc.

The peristomial coelom is subdivided, as in *Ophiactis resiliens*, being further complicated by a second coelomic cavity situated aboral to the first, which is also subdivided. The trans-ambulacral muscles are also present but the oral/aboral fibres are extremely slight, only a few strands occur and they are not arranged in a discrete muscle system. Other fibres also running between the ambulacral areas occur within the second coelomic cavity.

A fairly constant variation in height is exhibited by the stomach epithelium. Along the aboral wall it is generally rather shallow at 35-40/μ but increases markedly to 90/μ opposite the mouth, and to 65-75/μ over the ambulacral pouches. The oral epithelium of the ambulacral pouches is somewhat shallower, at 40-50/μ, than the interambulacral pouches where it attains a height of over 120/μ. The epithelial cells differ somewhat from those of the other two species in the arrangement of the microvilli.
These structures fan out distally from the cells into the stomach lumen rather like the bristles of a shaving brush, thus leaving a gap at the bottom (Fig. 59).

Despite the variation in height of the epithelium, the distribution of gland cells is still the same in both ambulacral and interambulacral areas, although the frequency of their spacing is much more variable. Of twelve such cells measured in one section the distances between cells varied from 6-40μ, with an average of 20μ. The secretory cells open to the stomach lumen between the tufts of microvilli. There is some variation in the size of the granular inclusions within these secretory cells but all give similar staining reactions with the trichrome stains. Scattered mucous cells are present, however, with large droplets staining in a similar manner to those of Ophionereis fasciata. Coelomocytes are again present.

Histochemical tests have not revealed glycogen in the gut wall but very dense deposits of lipid abound in the distal half of the epithelial cells (Plate 34).

4.4. Peristomial organ.

In the mouth cavity, between the first buccal podium and the mouth, is a highly glandular area which appears to have escaped the attention of earlier workers. This is understandable in that it is only in Ophiopteris antipodum that it is differentiated into a discrete, readily observable area (Figs. 60 and 61; Plate 35). In this species there are five such areas situated in an ambulacral position on the mouth side of the podia, appearing as a fleshy pad, 500 by 400μ, the long axis running around the mouth. It also
lies just aboral to the nerve ring (Fig. 61).

This area has been termed the peristomial organ because it is composed of several cell types. The epithelial cells are elongate (Fig. 62) some 60-70 μ in length and 4-5 μ in diameter. The nuclei are 7 μ in length, situated fairly regularly at about 20 μ from their distal ends, forming a nuclear band across the area. From the surface of these cells short cilia-like projections, a little over 10 μ in length, can occasionally be seen. Interspersed between these cells are large mucous cells with more rounded nuclei at their basal ends. The mucous droplets are large and often coagulated. These cells stain intensely basophilic and turquoise with AB/PAS. With the metachromatic dyes many cells exhibit an intense gamma metachromasia indicating a high degree of sulphation. Some few cells however are only beta metachromatic. A third type of cell present are numerous bipolar cells, which are seen quite clearly in Heidenhain's azan stain.

All of these cells are situated on a large neuropile, 20 μ deep, which is connected directly with the nerve ring. The fibres of this nerve pad are principally orientated at right angles to the epithelial cells and the bipolar cells appear to interconnect with them.

Although well compacted in form in *Ophiopteris antipodum* this area is less readily identifiable in *Ophiactis resiliens* and cannot be adequately distinguished from the general epithelium in *Ophionereis fasciata*. It must also be noted that other epithelial areas of a somewhat similar nature occur around the mouth, the mouth papillae being particularly prominent, but these thickenings lack both mucous cells and a concentration of bipolar cells.
The function of such a specialized area of epithelium can only be surmised. Ophiuroids are noted for their lack of any specialized sense organ and yet the preponderance of bipolar cells implies a sensory function for this area. Indeed, the presence of both sensory and mucous cells implies some form of chemoreceptor, located strategically near the mouth. Such a tentative proposition however, can only be based upon its morphological appearance. Its well developed condition in *Ophiopteris antipodum* is also puzzling, although this species is far more mobile than the other two, which tend to inhabit more seclusive niches.

4. 5. Qualitative review of enzymes.

(a) Esterases. Ambulacral and interambulacral pouches of each species were dissected out and fixed separately. After 15 minutes incubation in an alpha naphthyl acetate medium both gut pouches of *Ophionereis fasciata* exhibited intense esterase activity in the microvilli of the epithelial cells in the form of a heavy black precipitate. In *Ophiactis resiliens* a light precipitate was seen in the microvilli layer, plus a further diffuse layer in many epithelial cells in the distal region above the nuclei. In *Ophiopteris antipodum* a precipitate occurs in the microvilli only and appears to be much greater in the ambulacral pouches. With all three species activity is far greater on the oral side of the stomach lumen.

(b) Acid and alkaline phosphatases. Tests for acid phosphatases yielded only very poor results in all three species and it was not possible to confirm positively any site of activity. Alkaline phosphatase activity was, however, readily detectable, occurring
in Ophionereis fasciata as a dense precipitate in the microvilli after the coupling azo-dye method, and a less dense area is also apparent in the basal region of the epithelial cells. In Ophiactis resiliens a diffuse region of activity is detectable in the tissue layers lying beneath the epithelium as well as in the microvilli. The precipitate in Ophiopteris antipodum is confined to the microvilli. In all cases the enzymatic activity appeared to be identical in both ambulacral and interambulacral pouches.

(c) Proteinase (gelatinase). Gut extracts of all three species were tested for gelatinase at pH 5.3, 5.9, 6.6, 7.4 and 8.0. After 16 hours incubation at 20°C enzyme activity had occurred at all pH levels with Ophionereis fasciata extract, but was only just detectable at pH 8.0. The reactions with Ophiactis resiliens and Ophiopteris antipodum indicated a marked increase in activity at pH 6.6 and 7.4 in both species.

(d) Enzymes hydrolysing glycosidic linkages. The results of these tests are presented in Table 7. It can be seen that the activity of laminarinase is only detectable after 2.5 hours incubation at 37°C in Ophionereis fasciata and Ophiopteris antipodum; with Ophiactis resiliens 1.5 hours incubation at 37°C is required to reveal the presence of a pectinase, alginase and beta glucosidase.

As a negative result was obtained with the use of sodium carboxy-methyl-cellulose to detect any cellulase activity the experiment was repeated using powdered cellulose, washed cotton wool and filter paper but no positive result was obtained.

A test for an amylase was conducted separately at pH 5.3, 5.9, 6.6, 7.4 and 8.0. Amylase activity was apparent after 2.5 hours
at all pH levels with *Ophionereis fasciata* gut extract but after 5 hours activity was most marked at pH 6.6, 7.4 and 8.0. A similar range of activity was recorded with *Ophiactis resiliens* but with *Ophiomeris antipodum* activity at pH 8.0 was barely detectable.

**Table 7.**

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>SUBSTRATE</th>
<th><em>O. fasciata</em></th>
<th><em>O. resiliens</em></th>
<th><em>O. antipodum</em></th>
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<tbody>
<tr>
<td>INVERTASE</td>
<td>SUCROSE 5%</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>α GLUCOSIDASE</td>
<td>MALTOSE 5%</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>β GLUCOSIDASE</td>
<td>CELLOBIOS 2%</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>SALICIN 1%</td>
<td>-</td>
<td>**</td>
<td>+</td>
</tr>
<tr>
<td>α GALACTOSIDASE</td>
<td>MELLIBIOSE 1%</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>β GALACTOSIDASE</td>
<td>LACTOSE 2%</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>PECTINASE</td>
<td>PECTIN 1%</td>
<td>+</td>
<td>**</td>
<td>+</td>
</tr>
<tr>
<td>CHITINASE</td>
<td>CHITIN 1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LAMINARINASE</td>
<td>LAMINARIN 1%</td>
<td>+**</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>β MANNOSIDASE</td>
<td>MANNAN 1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ALGINASE</td>
<td>Na. ALGINATE 1%</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>XYLANASE</td>
<td>XYLANCE 1%</td>
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<td>-</td>
</tr>
<tr>
<td>CELLULASE</td>
<td>Na. C. M. C. 1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>TREHALASE</td>
<td>TREHALOSE 1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Faint Positive Reaction  **AFTER 2.5 hours at 37°C**
++ Strong Reaction    **AFTER 5 hours at 37°C**
+++ Intense Reaction

4.6. Discussion.

In general form the gut of all three species investigated is very similar, differing only in the extent of folding of the oral floor of the interambulacral pouches. The arrangement of the muscles around the mouth however differ from one species to another although in all three muscles which could close the mouth, running across the ambulacral edge, have been noted. It is the oral/aboral
fibres which show considerable variation in size.

Of interest is the presence of numerous gland cells containing refringent droplets, which may be zymogen cells. Mucous cells are few and the mucous coat over the epithelium is probably that ingested with the food particles. In his study of *Ophiosthrix quinquemaculata* Roubaud (1965) also noted the presence of granular inclusions within some epithelial cells but these appeared to be situated in the basal half of the cells. This author also records the presence of mucous cells although he further describes mucous droplets within other epithelial cells.

Coelomocytes appear to be a regular feature of echinoderm gut epithelia (Anderson, 1966; Endean, 1966). There has been much speculation on their function, particularly as to their possible role in transferring absorbed food substances, foreign particles and excretory matter. Only in some holothurians is there direct evidence of the participation of coelomocytes in the carrying of the products of digestion (Endean, 1966). In asteroids, Ferguson (1964 (a) and (b)) has shown by using C\(^{14}\)-labelled nutrients that the coelomocytes of *Asterias forbesi* only ingest sufficient material to satisfy their own metabolic requirements and show no indication of being special vehicles of nutrient transport. Coelomocytes have been shown by several authors to take up particulate matter artificially introduced into the stomach, but as Anderson (1966) has indicated, the fate of inert particulate matter may not be related in any way to the normal pathway of uptake and distribution of food. There is some evidence that coelomocytes may serve to transport enzymes (Endean, 1966; Pequinat, 1966).

The coelomocytes of the ophiuroids studied here appear to
undergo some degree of degenerescence in the epithelium. Roubaud (1965) also noted coelomocytes which actually broke up into droplets before being released into the gut lumen in *Ophiothrix quinquemaculata* and considered both types to serve in the elimination of waste products. Certainly any role played in the form of either enzyme secretion or uptake of nutrients seems superfluous in view of the abundant secretory cells and the presence of microvilli in the epithelium.

Only small deposits of glycogen were demonstrated in *Ophionereis fasciata* but large deposits of lipid appear in all three species. Both lipid and glycogen occur in echinoids and asteroids (Anderson, 1966). Greenfield et al (1958) found that lipid constituted 32 to 34% of the dry weight of the pyloric caeca in *Pisaster ochraceus* and 48 to 49% in *P. giganteus*, while glycogen represented only 1 to 2% of the dry weight. Rodegker and Nevenzel (1964) also found a high percentage of lipid in *P. ochraceus*. Recently, Fish (1967) has demonstrated lipid in the gut wall of the holothurian *Cucumaria elongata* but failed to detect glycogen.

Unfortunately, the distribution of digestive enzymes in echinoderms is still but poorly understood. Esterase activity, effective on short chain (C₂-C₄) fatty acids, has been noted in *Cucumaria elongata* (Fish, 1967), and proteases have been noted in echinoids (Lewis, 1964), asteroids (various authors, see Anderson, 1966) and holothurians (Oomen, 1926; Fish, 1967). Roubaud (1965) recorded strong alkaline phosphatase activity in *Ophiothrix quinquemaculata* and Fuji (1961) in *Strongylocentrotus intermedius*, an echinoid.

A wide range of enzymes hydrolysing glycosidic linkages
"carbohydrases") has been demonstrated in the three ophiuroids studied. Amylase, invertase and maltase have been recorded in both echinoids and holothurians (Lewis, 1964; Fish, 1967) but only amylase has so far been reported in asteroids (Anderson, 1966). It can be seen from Table 7 that all three species possess both alpha- and beta-glucosidases and alpha- and beta-galactosidases, although salicin was not hydrolysed by Ophionereis fasciata gut extract, and only in Ophiactis resiliens after 1.5 hours incubation at 37°C. The presence of a beta-galactosidase (lactase) is interesting in that it has not been found in either echinoids or holothurians although tests to demonstrate its presence have been employed (Lewis, 1964; Fish, 1967). Of the other enzymes pectinase (polygalacturonase) is present in all species, and laminarinase also, although requiring 2.5 hours incubation at 37°C to indicate its presence. Such a procedure of incubation at temperatures above normal is open to question, but may be taken to be a means of accelerating the action of an enzyme which is present but possibly retarded by artificial experimental media at normal temperatures. Ophiactis resiliens also possesses an alginate.

It is apparent that a greater variety of "carbohydrases" are present in Ophiopteria antipodum and Ophiactis resiliens, both of which appear to be predominately algal feeders. The absence of a cellulase is, however, interesting. In a study of the occurrence of this enzyme, Yokoe and Yasumasa (1964) failed to demonstrate its presence in the ophiuroid Ophioplatus (species not given) and failed to detect it in a number of asteroids also. Three of six echinoids tested were found to display cellulase activity. It is possible
that the breakdown of cellulose may be attained within ophiuroids by bacteria or other organisms. The gut of all three species studied contain numerous ciliates moving freely between the folds of the epithelium.

There are many records of the association between ciliates and echinoderms, particularly with the digestive tract (Hyman, 1955).
Fig. 52 Supporting strand of the stomach wall of *Ophionereis fasciata*.

- **CONN. T.** — connective tissue
- **CONN. T. OF INTEG.** — connective tissue of integument
- **CUB. EPITH.** — cuboidal epithelium
- **M. FIBRES** — muscle fibres

Fig. 53 Aboral view of the oral region of the stomach of *Ophionereis fasciata* showing the trans-ambulacral tracts.

- **AMB. POUCH** — ambulacral pouch
- **INTER-AMB. POUCH** — interambulacral pouch
- **PAP.** — papilla of mouth
- **TRANS-AMB. TRACT** — trans-ambulacral tract

Fig. 54 Vertical section through mouth of *Ophionereis fasciata*.

- **AB. RAD. M.** — aboral radial muscle
- **GUT EPITH.** — gut epithelium
- **NERVE T.** — nerve tissue
- **ORAL/ABORAL M. FIBRES** — oral/aboral muscle fibres
- **PAP.** — papilla
- **PERIST. COEL.** — peristomial coelom
- **RAD. M. FIBRES** — radial muscle fibres
- **TRANS-AMB. M. FIBRES** — trans-ambulacral muscle fibres
Fig. 55  Distal portion of gut epithelium of *Ophionereis fasciata*.

MICROV.  microvilli
MUC. CELL  mucous cell
SECR. CELL  secretory cell

Fig. 56  Gut epithelium of *Ophionereis fasciata* stained with Sudan black B.

N.  nuclei

Fig. 57  Distal portion of gut epithelium of *Ophionereis fasciata*.

MICROV.  microvilli

Fig. 58  Vertical section through mouth of *Ophiactis resiliens*.

AB. RAD. M.  aboral radial muscle
GUT EPITH.  gut epithelium
NERVE T.  nerve tissue
ORAL/ABORAL M. FIBRES  oral/aboral muscle fibres
PAP.  papilla of mouth
PERIST. COEL.  peristomial coelom
RAD. M. FIBRES  radial muscle fibres
TRANS-AMB. M. FIBRES  trans-ambulacral muscle fibres
Fig. 59  Microvilli of the gut epithelium, *Ophiopteris antipodum*.

Fig. 60 Diagram showing location of Fig. 61.

Fig. 61 Vertical section through mouth of *Ophiopteris antipodum*.

GUT EPITH.  
NERVE T.  
ORAL/ABORAL M. FIBRES  
PAP.  
PERIHAEM. RING  
PERIST. COEL.  
PERIST. ORGAN  
TRANS-AMB. M. FIBRES  
gut epithelium  
nerve tissue  
oral/aboral muscle fibres  
papilla of mouth  
perihaemal ring  
peristomial coelom  
peristomial organ  
trans-ambulacral muscle fibres

Fig. 62 Detail of the specialized epithelium of the peristomial organ, *Ophiopteris antipodum*.

EPITH. CELL  
NERVE PLEX.  
epithelial cell  
nerve plexus
Plate 33.
V/S mouth
C. fasciata
Mass. Tri.

Plate 34.
Gut epithelium
C. antipodum
Sudan black B

Plate 35.
Peristomial organ
C. antipodum
Mass. Tri.
Section 5.

RESPIRATORY SURFACES AND RESPIRATION.

5.1. Introduction.

Ophiuroids are unique among echinoderms in the possession of ten saciform invaginations of the oral wall of the disc at the base of the arms. These invaginations have variously been termed burase; respiratory bursae, although no respiratory function has been demonstrated; and genital bursae, because of their close association with the gonads. These bursae project into the interior of the disc, occupying the spaces not occupied by the stomach pouches or the gonads. The MacGinties (1949) proposed that the bursae provide a respiratory surface for the disc and that the podia serve as a respiratory surface for the arms. Support for the bursae playing a respiratory role is the occurrence, at least in Ophiocoma and Ophiothrix genera, of diverticula from these pouches into the external interradial muscle, the movements of which would aid in the circulation of water through them (Cuénot, 1891; Smith, 1940).

Information on the respiration rates of ophiuroids is scant. Hyman (1955) reports, from a thesis by Wintzell (1918), respiration rates of five species, higher figures being obtained for the most active ones. Montuori (1913) (quoted by Farmanfarmaian, 1966) gives respiration rates for Ophioderma longicauda and Ophioglypha lacertosa. Recently, Buchanan (1964) has given respiration rates for Amphipura filiformis and A. chiajei; the former, more active species having a respiration rate five times greater than the latter.

The present investigation has attempted to examine various
aspects of respiration in Ophionereis fasciata, Ophiactis resiliens and Ophiapteris antipodum, including an evaluation of the relative respiratory functions of the bursae.

5. 2. Possible respiratory surfaces.

(a) **The body wall.** By using the diffusion coefficient for connective tissue, as determined by Krogh (1959), Farmanfarmaian (1966) estimated from data obtained on the respiration of the echinoid Strongylocentrotus purpuratus, that under natural conditions the distance at which the respiratory rate of this species could be sustained by diffusion is approximately 1.5 mm. Farmanfarmaian considered that such an approximation applies to the entire phylum.

The body wall of most echinoderms, however, possesses a large number of calcareous plates, and in the ophiuroids these plates almost completely cover the arms, and a large part of the disc. As all cells are permeable to oxygen, it is probable that the cells of the epidermis at least obtain their oxygen directly from the sea water by diffusion, but such diffusion is unlikely to provide sufficient oxygen for the other tissues of the animal.

(b) **The epithelium of the gut.** Several authors, even fairly recently (Cuénot, 1948) have suggested that the digestive tract may serve as a respiratory surface. This hypothesis has also been discussed for the echinoderms by Farmanfarmaian in his review of respiration in the phylum (1966); he dismisses the possibility on three grounds. These are, (1) digestive and respiratory surfaces differ in histological appearance; (2) many species of holothurians eviscerate their intestines and have survived to regenerate these
organs, and some asteroids regenerate the rest of the body from one arm only; and (3) experimental evidence has demonstrated in holothurians and asteroids that closure of the digestive tracts alone results in only a slight change in respiration.

(c) The bursae. As stated above the bursae have often been presumed to act as respiratory chambers, and it is pertinent to review their structure in the three species studied.

In *Ophionereis fasciata* each bursa opens at the base of the arm through a bursal slit, supported on either side by an elongated calcareous plate. Within the disc the pouch extends as far as the large external interradial muscle (Fig. 63) and aborally to the aboral limit of this same muscle (Fig. 64). Each bursa widens to form a rectangular area some 1.25 x 0.75 mm. in adult specimens (8 mm. disc diameter) in the horizontal plane, although fairly shallow (100-200 /u) in the vertical plane (Fig. 64). On the radial side a further extension occurs over the most proximal three or four arm "vertebrae", and interradially a similar, much folded, extension occurs beneath the stomach tissue (Fig. 65). Yet another extension occurs over the respective interradial third of each external interradial muscle (Fig. 65).

Histologically, the bursal wall consists of an outer epithelial layer overlying a thin, 8-10 /u, connective tissue layer. A slight muscle sheet, 5-6 /u, lies underneath the connective tissue, the fibres of which are principally orientated in an oral/aboral direction. Some fibres do, however, run in other directions. Beneath the muscle fibres lies a cuboidal coelomic epithelium. The outer epithelium, which is relatively smooth towards the
exterior, becomes increasingly folded within the disc, being thrown into folds 50 or 60 µ in height where the bursae ramify beneath the stomach tissue. This epithelium is only sparingly ciliated near the exterior.

The bursae of *Ophiactis resiliens* differ somewhat from those of *Ophionereis fasciata*. Each bursa forms a pouch with a maximum width of 400 µ, length of 800 µ (Fig. 66) and a height of 100 µ over the arms and beneath the stomach. An extension occurs on the aboral side of the external interradial muscle, and there is further involvement with this muscle by means of an extension which ramifies in its interior (Fig. 67). This diverticulum extends some 250-300 µ into the muscle in a radial plane sending two branches in a vertical plane, each branch being 150 µ long and 100 µ wide, so that the muscle itself becomes subdivided when viewed in horizontal sections (Fig. 68). There are also five pairs of muscles running from the aboral disc wall to the third arm ossicles, the contraction of which would compress both the disc and the bursae.

The most striking histological feature of the bursal wall, in comparison with that of *Ophionereis fasciata*, is the marked decrease in an intrinsic muscle layer. Muscle fibres are lacking in the vertical section of the bursae and where horizontal diverticula extend into the disc scattered muscle fibres occur, usually one fibre layer thick. The epithelium is again extensively folded, particularly beneath the stomach tissue.

The bursae of *Ophiopeteris antipodum* resemble in general form those of *Ophiactis resiliens*, in that a diverticulum extends into the external interradial muscle. This diverticulum (Fig. 69) is
far more extensive, extending tangentially to a width of 500/μ, the overall width of the muscle block being 1.4 mm. (Fig. 70). In the vertical plane one branch of the diverticulum penetrates the muscle in an oral direction while two extensions occur in an aboral plane to a depth of 500/μ (Fig. 70). The rest of the bursa also extends over both the proximal vertebrae of the arms and beneath the gut. The interradial extensions are folded in a vertical plane (Fig. 71) becoming intimately involved with the oral side of the stomach. The extensions are up to 0.75 mm. in length.

These bursae also lack an extensive muscle layer, a sheet of fibres occurring around the lateral extensions. The external epithelium varies from a thickness of 18/μ near the exterior to 5/μ underneath the stomach, where the entire wall may be only 12/μ thick. With AB/PAS staining scattered mucous cells appear in the epithelium, staining royal blue, each cell being 15-30/μ in length. They give a beta, tending to gamma, metachromasia with azure A at low pH levels. No mucous cells were observed in the other two species.

(d) Podia. The podia of echinoderms have often been cited as a possible respiratory surface and Nichols (1966) has suggested that their probable origin was one of increasing the efficiency of gaseous exchange across the walls of the ambulacral canal of the ancestral echinoderm. Farmanfarmaian (1966) has obtained direct evidence of oxygen transport across the ampullae of tube feet in the echinoid Strongylocentrotus purpuratus by using luminous bacteria. When the podia were covered, oxygen consumption dropped
to an average 60.9% of the original. Similarly, Meyer (1935) indicated that oxygen uptake in *Asterias rubens* drops to between 40 and 50% of the normal rate when all five ambulacral grooves are closed. The remainder presumably being that resulting in gaseous exchange across the papulae of the aboral body surface, and integumentary metabolism.

The structure of the podia of the ophiuroids in this study have been described in Sections 2 and 3. An attempt was made to estimate the possible surface area afforded by the podia in each species. Such a task is necessarily difficult in that the podia vary in size along the arm and are at different stages of extension at any one time. It may be tenable however, to estimate the surface area of a relaxed podium of a mid-arm segment, treating such a podium as a simple cylinder, and multiplying this figure by double the total number of arm segments of the arms. In this manner the following figures were obtained. They are possibly overestimates of the actual area, in view of the diminution in size of the podia towards the distal portion of the arm.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MEAN NO. OF SEGMENTS PER ARM</th>
<th>PODIAL SURFACE AREA PER WET WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. fasciata</em></td>
<td>94</td>
<td>0.7265 cm²/gm.</td>
</tr>
<tr>
<td><em>O. antipodum</em></td>
<td>82</td>
<td>0.4294 cm²/gm.</td>
</tr>
<tr>
<td><em>O. resiliens</em></td>
<td>75</td>
<td>0.1473 cm²/gm.</td>
</tr>
</tbody>
</table>

The possession of papillae on the surface of the podia in *Ophiopoteris antipodum* and *Ophiactis resiliens* would serve to
increase considerably the surface area in these two species.

5.3. Respiration: material and methods.

Adult animals as uniform in size as possible were used in comparative experiments, except where a size range was required. In view of the different sizes of the three species, different sized vessels were employed in order to maintain a similar oxygen tension in each experiment. Thus 150 ml. stoppered flasks were used for Ophiopteris antipodum, 100 ml. flasks for Ophionereis fasciata and 50 ml. flasks for Ophiactis resiliens. At the end of the experiments subsamples were drawn off into stoppered titration flasks of 50, 25 and 10 ml.s respectively, into which were pipetted the reagents of the Winkler method for oxygen determination, manganous sulphate and alkaline iodide. For the three sizes of subsamples 2, 1 and 0.5 ml. of each reagent were pipetted into the 50, 25 and 10 ml. flasks.

The stoppered flasks containing well-oxygenated, filtered sea water were placed in a constant temperature water bath and experiments were run for either two or three hours, depending on the experiment. The volumes of these flasks were estimated by weighing, applying a correction factor for the specific gravity of sea water. After the determinations of the oxygen content of control flasks of the same size as those containing the animals, and that of the experimental flasks, the oxygen consumed by the animal was estimated. Respiration rates were calculated by either using the blotted wet weight, or the blotted wet, decalcified, weight, as given in the results.
5.4. Respiration: experimental results.

It is to be expected that the total oxygen consumption of an animal increases with its weight. When the rate of consumption is expressed in terms of a power function of weight it becomes approximately constant for animals of different sizes. This relation has been given by Prosser and Brown (1961) as \( M = K W^b \), where \( M \) is the total oxygen consumption per unit time for the animal, \( W \) is the wet body weight, \( b \) is the slope of the log-log plot of \( M \) against \( W \), and \( K \) is the point at which this extrapolated line intercepts the ordinate.

Thus where \( M = K W^b \)

\[
\log M = b \log W + \log K
\]

The value for \( b \) is in fact the same as that obtained in the regression formula when regressing the total oxygen consumption against wet weight where

\[
Y = aX^b
\]

becomes \( \log Y = a + b \log X \)

The calculated regression lines for oxygen consumption per hour against wet body weight for the three species studied are given in Fig. 72. The values obtained for \( b \) are as follows:

- *Ophionereis fasciata* 0.503
- *Ophiactis resiliens* 0.706 at 20°C.
- *Ophiopteris antipodum* 0.417

It is also to be expected that the rate of oxygen consumption per unit weight would be higher the smaller the animal, and the fitted regression lines for the respiration rate per wet weight are given in Fig. 73.

It is customary to plot such information as respiration rate
per unit wet weight, and this convention has been adhered to in
the graphs given. Such a method however does not take into
consideration any varying amounts of non-respiring skeletal material
from one species to another. The percentage of body weight
represented by skeletal material removable by the process of
decalcification of the species involved is given below (Table 8).
The figures represent the mean values followed by the standard
error.

**TABLE 8.**

**PERCENTAGE OF NON-RESPERING**

**SKELETAL MATERIAL.**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>NO. OF ANIMALS</th>
<th>% CALCIFIED WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0. fasciata</td>
<td>25</td>
<td>61.53 ± 0.79</td>
</tr>
<tr>
<td>0. resiliens</td>
<td>25</td>
<td>66.91 ± 1.34</td>
</tr>
<tr>
<td>0. antipodum</td>
<td>25</td>
<td>66.94 ± 0.87</td>
</tr>
</tbody>
</table>

By a calculation of the standard error of the difference
between the mean values, it appears that the difference between
Ophiactis resiliens and Ophiopteris antipodum is not significant,
but that the difference between both of these species and Ophionereis
fasciata has a high probability (20:1) of being significantly
different, owing to the structure of the animals. The regression
lines of Ophionereis fasciata in Figs. 72 and 73 would thus be
slightly lower in comparison to the other two species than the
position given.

For the calculations of respiration rates of isolated arms and discs, differences in percentage calcification must be taken into account not only between species but also between the discs and arms of the same species. The percentage calcification of the discs and arms of the three species are given in Table 9 plus the decalcified weights of both as a percentage of the total decalcified weights of the whole animals.

<table>
<thead>
<tr>
<th>DISC</th>
<th>O. fasciata</th>
<th>O. resiliens</th>
<th>O. antipodum</th>
</tr>
</thead>
<tbody>
<tr>
<td>% calcification</td>
<td>53.01</td>
<td>54.31</td>
<td>51.66</td>
</tr>
<tr>
<td>% of body weight (decalcified)</td>
<td>30.57</td>
<td>25.98</td>
<td>24.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ARMS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>% calcification</td>
<td>64.13</td>
<td>68.22</td>
<td>67.67</td>
</tr>
<tr>
<td>% of body weight (decalcified)</td>
<td>69.43</td>
<td>74.12</td>
<td>75.64</td>
</tr>
</tbody>
</table>

It can be seen that in all three species the arms are far more heavily calcified than the discs, and that they also represent a much higher percentage of potential respiratory tissue. In order to measure the relative respiration rates of isolated arms and discs the tissue was therefore subsequently decalcified and the respiration rates given for these experiments are considerably
higher than those given on a wet weight basis for the intact animals in Fig. 73. For each experiment the isolated disc was placed in one flask and the five isolated arms collectively placed in another. The figures obtained for these respiration rates are given in Table 10.

The considerable variation in the results is perhaps only to be expected in view of the extraordinary conditions of the isolated body components. As the experimental vessels were maintained in a closed constant temperature bath it was not possible to observe the behaviour of the arms and discs during the 2 hours of the experiment. At the end of the experiments however, the isolated arms behaved as described in Section 3 and the discs often attempt to climb the sides of the vessel, occasionally falling onto their aboral surfaces, and to move around the bottom.

It is to be noted that the respiration rates of the isolated parts as a percentage of the whole animal as given in Table 10 do not reflect the relative total amount of oxygen used by each part. Thus although the discs have a high respiration rate they use up only a small amount of oxygen relative to the arms. As shown in Table 9 however the decalcified disc weights represent only about a quarter of the whole animal.
<table>
<thead>
<tr>
<th></th>
<th><strong>O. fasciata</strong></th>
<th></th>
<th><strong>O. resiliens</strong></th>
<th></th>
<th><strong>O. antipodum</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( O_2/ ) decalcified wt./hr.</td>
<td></td>
<td>( O_2/ ) decalcified wt./hr.</td>
<td></td>
<td>( O_2/ ) decalcified wt./hr.</td>
</tr>
<tr>
<td><strong>DISC</strong></td>
<td>0.3789</td>
<td><strong>DISC</strong></td>
<td>0.9010</td>
<td><strong>DISC</strong></td>
<td>0.0799</td>
</tr>
<tr>
<td></td>
<td>0.0424</td>
<td><strong>ARMS</strong></td>
<td>0.0532</td>
<td><strong>ARMS</strong></td>
<td>0.1592</td>
</tr>
<tr>
<td></td>
<td>0.0759</td>
<td></td>
<td>0.5837</td>
<td></td>
<td>0.1904</td>
</tr>
<tr>
<td></td>
<td>0.2192</td>
<td></td>
<td>0.2090</td>
<td></td>
<td>0.0639</td>
</tr>
<tr>
<td></td>
<td>0.2097</td>
<td></td>
<td>0.8427</td>
<td></td>
<td>0.0456</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td>0.1852</td>
<td><strong>MEAN</strong></td>
<td>0.6609</td>
<td><strong>MEAN</strong></td>
<td>0.0918</td>
</tr>
<tr>
<td>( \pm 0.0599 )</td>
<td>0.1019</td>
<td></td>
<td>0.2063</td>
<td></td>
<td>0.0476</td>
</tr>
<tr>
<td></td>
<td>( \pm 0.0263 )</td>
<td></td>
<td>( \pm 0.1248 )</td>
<td></td>
<td>( \pm 0.0254 )</td>
</tr>
</tbody>
</table>

**MEAN RESPIRATION RATE, WHOLE ANIMAL**

- **O. fasciata**: 0.2495
- **O. resiliens**: 0.1711
- **O. antipodum**: 0.0813

<table>
<thead>
<tr>
<th></th>
<th><strong>DISC</strong></th>
<th><strong>DISC</strong></th>
<th><strong>DISC</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of WHOLE</td>
<td>% of WHOLE</td>
<td>% of WHOLE</td>
</tr>
<tr>
<td><strong>O. fasciata</strong></td>
<td>108.3%</td>
<td>56.6%</td>
<td>112.8%</td>
</tr>
<tr>
<td><strong>O. resiliens</strong></td>
<td>264.9%</td>
<td>82.7%</td>
<td>58.5%</td>
</tr>
<tr>
<td><strong>O. antipodum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As an example of the relative total amount of oxygen used by the isolated parts the following figures are given for *Ophiopteris antipodum* at 20°C.

<table>
<thead>
<tr>
<th>ml. O₂ UPTAKE/HR.</th>
<th>ml. O₂ UPTAKE/HR.</th>
<th>ml. O₂ UPTAKE/HR.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHOLE ANIMAL</td>
<td>DISC</td>
<td>ARMS</td>
</tr>
<tr>
<td>0.1235</td>
<td>0.0377</td>
<td>0.0333</td>
</tr>
<tr>
<td>0.0405</td>
<td>0.0442</td>
<td>0.0321</td>
</tr>
<tr>
<td>0.1300</td>
<td>0.0237</td>
<td>0.0627</td>
</tr>
<tr>
<td>0.1179</td>
<td>0.0143</td>
<td>0.0394</td>
</tr>
<tr>
<td>0.0621</td>
<td>0.0199</td>
<td>0.0420</td>
</tr>
<tr>
<td>MEAN: 0.0948</td>
<td>MEAN: 0.0279</td>
<td>MEAN: 0.0419</td>
</tr>
</tbody>
</table>

It can be seen that the isolated disc uses only 29.4% of the oxygen of the whole animal; but as the disc represents only 24.36% of the decalcified body weight its respiration rate is slightly higher than that given for the whole animal, i.e. 112.8%. Similarly, the arms use 44.2% of the oxygen of the whole animal but as they represent 75.6% of the decalcified body weight their respiration rate is only 58.8% of that for the whole animal.

In order to estimate the relative amount of oxygen uptake through the bursae a series of experiments were carried out with *Ophionereis fasciata* and *Ophiopteris antipodum*. The total oxygen uptake, over a two hour period, was first determined for each animal. The specimens were then removed and the bursal slits covered with Cital aquapaint (obtainable from Colebrand, Ltd.). This paint can be applied underwater, or onto a wet surface, and
dries hard in about one hour. After a few minutes however it becomes "tacky" and sufficiently adhered to the integument. Care was taken to avoid applying any paint over the mouth as possible toxic effects of the paint are not known. However, all the animals remained active, although to a lesser extent than before, for at least four hours after the termination of the experiment. The decreased activity may have been due to the paint or as a result of interference with the functioning of the bursae. Trials were made with both Vaseline and nail varnish but these failed to adhere to the wet surface of the animal. After application of the paint each specimen was maintained for a further two hours and its oxygen consumption determined. The results are given in Table 11 ((a) and (b)). The respiration rate per wet weight for each animal as recorded in the first part of each experiment before the bursae were closed is also given.

**TABLE 11.**

**EFFECT OF BLOCKING OFF BURSAE (20°C).**

(a) *Ophionereis fasciata.*

<table>
<thead>
<tr>
<th>WET WEIGHT (GMS.)</th>
<th>TOTAL 0.2 hrs O₂</th>
<th>BURSAE BLOCKED</th>
<th>DROP IN O₂ CONSUMPTION AS % OF NORMAL</th>
<th>RESPIRATION RATE 0₂/WET wt./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>0.13340</td>
<td>0.12703</td>
<td>73.25%</td>
<td>0.0555</td>
</tr>
<tr>
<td>1.0</td>
<td>0.17107</td>
<td>0.14377</td>
<td>84.04%</td>
<td>0.0777</td>
</tr>
<tr>
<td>1.3</td>
<td>0.09396</td>
<td>0.07879</td>
<td>83.87%</td>
<td>0.0408</td>
</tr>
<tr>
<td>2.1</td>
<td>0.17769</td>
<td>0.14574</td>
<td>82.02%</td>
<td>0.0415</td>
</tr>
<tr>
<td>2.6</td>
<td>0.16345</td>
<td>0.12666</td>
<td>77.49%</td>
<td>0.0312</td>
</tr>
</tbody>
</table>

**MEAN**

80.13 ± 2.09
(b) *Ophiocystis antipodum.*

<table>
<thead>
<tr>
<th>WEIGHT (GMS)</th>
<th>TOTAL O₂/2 hrs.</th>
<th>BURSAE BLOCKED</th>
<th>DROP IN O₂ CONSUMPTION AS % OF NORMAL</th>
<th>RESPIRATION RATE O₂/WET wt./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7</td>
<td>0.17309</td>
<td>0.12186</td>
<td>70.40%</td>
<td>0.0504</td>
</tr>
<tr>
<td>3.6</td>
<td>0.23239</td>
<td>0.17150</td>
<td>56.77%</td>
<td>0.0320</td>
</tr>
<tr>
<td>2.5</td>
<td>0.17032</td>
<td>0.12406</td>
<td>72.84%</td>
<td>0.0552</td>
</tr>
<tr>
<td>1.5</td>
<td>0.15057</td>
<td>0.10216</td>
<td>67.85%</td>
<td>0.0497</td>
</tr>
<tr>
<td>1.7</td>
<td>0.14291</td>
<td>0.12321</td>
<td>68.97%</td>
<td>0.0423</td>
</tr>
</tbody>
</table>

MEAN

67.36±2.78

Intertidal animals are exposed to a considerable variation in temperature. The temperature of the pools on the Leigh reef flat are considered in Section 7, and vary from 26° in February to 15° in August. Figs. 74, 75 and 76, give the increases in respiration rates of the three species at 15 and 25°C, over a size range for each species. Of interest is the low respiration rates of *Ophiocystis antipodum* at 25°C. Fortunately the animals were not killed immediately and the experiment was repeated at 20°C. A second series of animals were maintained at 25°C and gave comparable results to the first series. Q10 values were therefore calculated in this species from the 15° and 20°C results. The Q10 values for all three species are given in Table 12.

As might be expected, the larger *Ophiocystis antipodum* has the lowest Q10 figure, and *Ophiactis resiliens* the highest.
TABLE 12.

Q10 VALUES.

<table>
<thead>
<tr>
<th>O. fasciata</th>
<th>O. resiliens</th>
<th>O. antipodum</th>
</tr>
</thead>
<tbody>
<tr>
<td>WET WEIGHT</td>
<td>Q10</td>
<td>WET WEIGHT</td>
</tr>
<tr>
<td>GMS.</td>
<td></td>
<td>GMS.</td>
</tr>
<tr>
<td>0.31</td>
<td>2.59</td>
<td>0.15</td>
</tr>
<tr>
<td>0.40</td>
<td>2.16</td>
<td>0.23</td>
</tr>
<tr>
<td>0.51</td>
<td>1.86</td>
<td>0.29</td>
</tr>
<tr>
<td>0.58</td>
<td>2.36</td>
<td>0.33</td>
</tr>
<tr>
<td>0.87</td>
<td>2.54</td>
<td>0.39</td>
</tr>
<tr>
<td>1.51</td>
<td>3.00</td>
<td>0.46</td>
</tr>
<tr>
<td>1.60</td>
<td>2.80</td>
<td></td>
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<tr>
<td>MEAN</td>
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<td>MEAN</td>
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<tr>
<td>2.47</td>
<td></td>
<td>2.62</td>
</tr>
<tr>
<td>±0.146</td>
<td></td>
<td>±0.121</td>
</tr>
</tbody>
</table>

5.5. Discussion.

The values given for $b$ in the relation of $M = kW^b$ fall within the range of other echinoderms. Choe (1962), quoted in Farmanfarmaian (1966), has shown that the value for $b$ in Stichopus japonicus is 0.73 at 13.5°C, and that the value decreases with rise in temperature. In the same review, Farmanfarmaian has replotted the data of Koller and Meyer (1933) for Asterias rubens and obtained the relatively low value of 0.31 for $b$ at 15°C, which is considerably lower than the values reported for various poikilotherms, although similar low values appear to exist for other echinoderms. The precise relationship between size and respiration however, has not been calculated (Farmanfarmaian, 1966).

The mean respiration rates for the three species also lie
close to figures given for other ophiuroids and asteroids. The mean values, at 20°C are 0.053 ml. O₂/gm. wet wt./hr. for *Ophionereis fasciata* with a range of 0.031 to 0.078; for *Ophiactis resiliens*, 0.06 ml. O₂/gm. wet wt./hr. (range 0.061 to 0.129); and for *Ophiopteris antipodum* 0.039 ml. O₂/gm. wet wt./hr. (range 0.017 to 0.080). Montuori (1913) gives values of 0.03 ml. O₂/gm./hr. for *Ophioderma longicauda* and 0.05 ml. O₂/gm./hr. for *Ophioglypha lacertosa*, both at 25°C. Buchanan (1964) records a respiration rate of 0.058 ml. O₂/gm./hr. for *Amphiura filiformis* at 6°C with a range of 0.032 to 0.079, very close to the figures given here for *Ophionereis fasciata*. A mean of 0.011 ml. O₂/gm./hr. with a range of 0.004 to 0.016 at 6°C was given for *Amphiura chiajei*, an infaunal species. Such differences in the respiration rates of epi- and infaunal species are interesting. Recently, Lewis (1968) records that the oxygen consumption of three species of epifaunal regular echinoids were significantly higher than the rates of two infaunal, irregular species.

Interpretations of the results of respiration rates of isolated arms and discs are not easy to make. It is obvious that the rates need not necessarily parallel those which occur in the intact animal, and if they did the amount of oxygen uptake per hour for the separate organs would collectively approximate that for the intact animal. Such results were recorded by Wells (1952) for the polychaete worm *Sabella pavonina* where the oxygen consumption of the crown and body of the worm were separately determined. In *Myxicola infundibulum* Wells noted that the oxygen consumption of isolated crowns and bodies did not approximately total that for the intact animal and thus concluded that the crown was
responsible for supplying a considerable amount of oxygen for the whole animal. If parallel conclusions are to be drawn here i.e. that the arms, or more specifically the podia, procure oxygen for the intact animal, it must be assumed that the isolated arms and discs expend energy at the same rate as in the intact animal. However, the respiration rates calculated for the isolated parts indicate that the normal respiration rate of the arms is probably depressed as a result of severence from the disc, and that the rate for the disc on its own is higher than normal, considerably so in *Ophiactis resiliens*, probably because the discs attempt to move around on their own without the aid of the arms, occasionally falling over and attempting to right themselves, thus expending more energy than usual.

When the bursae are closed there is a greater drop in oxygen consumption per hour in *Ophiopteris antipodum* than in *Ophionereis fasciata*. Attempts to close the bursae of *Ophiactis resiliens* were not successful as for some reason the paint used did not adhere well to the integument of this species, and, owing to the extremely small size of the disc, application of paint over the bursal slits without covering the mouth was also not easy.

It is of interest to compare the relative structure of the bursae in the three species. The thin wall of these structures, even thinner than the walls of the podia, could easily serve as areas of oxygen diffusion. Their involvement with the oral side of the stomach tissue would provide a relatively thin diffusion path for gaseous exchange with this organ, and the ramifications of the bursal wall within the disc generally produces a large surface area for gaseous exchange with the coelom. The results
of experimentally closing the bursae also lend support for a certain amount of oxygen intake through them. The two aspects of morphology which are most striking are (a) the involvement of the bursae with the large external interradial muscle in *Ophiopteris antipodum* and *Ophiactis resiliens*; and (b) the relative paucity of intrinsic muscle fibres in the walls of the bursae in these two species and their extensive development in an oral/aboral direction in *Ophionereis fasciata*. The two factors may possibly be related in that the natural movements of the external interradial muscle in *Ophiopteris antipodum* and *Ophiactis resiliens* would serve to expand and contract the extensions of the bursae within the muscle and thus flush water through the bursal pouches. The water within the bursae of *Ophionereis fasciata* on the other hand could be replenished by regular contractions of the oral/aboral muscle fibres which would alter the volume of the bursae. Such contractions need not necessarily be evident externally, and no regular raising and lowering of the disc is noticeable, nor is it evident in the other two species although irregular movements of the disc in *Ophiactis resiliens* can be seen. Any such pumping mechanism in *Ophiopteris antipodum* and *Ophiactis resiliens* might also take place when the bursal slits are closed, forcing water further into the system.

It might also be argued that the extensions into the external interradial muscles may serve as a means of providing adequate gaseous exchange for these relatively large muscles. This may certainly apply in the large *Ophiopteris antipodum*, but *Ophionereis fasciata* is considerably larger than *Ophiactis resiliens* and lacks these extensions.
The drop in oxygen consumption after closure of the bursae in *Ophiopetris antipodum* is considerably greater than in *Ophionereis fasciata*. This may be due to a greater dependence on a bursal supply of oxygen in this species, possibly due to greater efficiency as a result of the extensions into the external interradial muscle. As noted in sub-section 5.2(c) however, the surface area of the podia in *Ophionereis fasciata* is possibly greater per gram of tissue than in *Ophiopetris antipodum*, although such approximations are very loose and it is extremely difficult to estimate the increase in surface area of the podia due to the papillae on its surface. In both species the remainder of the oxygen uptake is presumably that absorbed and utilized by tissue on the surface and that taken up by the podia. One would imagine that the presumably high oxygen requirements of the podia as a result of their constant activity would be met by direct diffusion into their tissues.

Koller and Meyer (1933) assumed that oxygen which had diffused into the lumina of the podia of *Asterias rubens* was further carried by the water vascular fluid to the internal organs of the animal. As Farmanfarmaian (1966) has previously noted however, this is unlikely in that the activities of the podia of this asteroid, as shown by Smith (1947) occur when the valve isolating the podial fluid from that of the radial water canal is closed. Oxygen diffusion therefore probably occurs across the wall of the ampulla. Farmanfarmaian (1966) has further presented direct evidence for such an occurrence in the echinoid *Strongylocentrotus purpuratus*. In view of the activities of the podia in ophiuroids as discussed in Section 3 however, it is possible that a certain degree of oxygen which has diffused into their lumina may be relayed into
the radial canal as a result of regular exchange of fluid between the two.

The Q10 values given in Table 12 again fall within the range given for other echinoderms. Crozier (1916) gives a value of 2.4 for the Q10 of several holothurians in the temperature range of 12.5 to 22.5°C, and Meyer (1935) gives values of between 2 and 3 for various individuals of Asterias rubens in the range of 10 to 25°C. Farmanfarmaian and Giese (1963) give values of 1, 2.78 and 3.99 for the Q10 of the echinoid Strongylocentrotus purpuratus at temperature ranges of 5 to 10, 10 to 15, and 15 to 20°C respectively. Recently, Boolootian and Cantor (1965) give the Q10 of another echinoid, Arbacia punctulata, as 2.24 between 10°C and 20°C and between 20°C and 30°C. The Q10 values for the ophiuroids studied here are higher, as might be expected, for the smaller species, as were the respiration rates noted previously.

Finally, the strange drop in respiration rate of Ophiocotis antipodum at 25°C is of particular interest. As will be discussed in Section 7 this species is notably absent from pools on the Leigh reef at high levels where the temperatures in summer are much higher. Also, this species has been noted as a southern form in New Zealand waters and before the present study had not been recorded further North than Rangitoto Island outside the Auckland harbour. It is also a predominantly sublittoral species. Meyer (1935) has reported that oxygen uptake in Asterias rubens increases to a maximum at 25°C beyond which it decreases steeply. Crozier (1916) also records that the time taken for 10 cloacal pulsations in the holothurian Stichopus moebii is least at 26°C but thereafter increases markedly.
Fig. 63  Horizontal section of the interambulacral area of the disc of Ophionereis fasciata at the level of C in Figs. 64 and 65.

EXT. INTER. M.  external interradial muscle
INTERAMB. POUCH  interambulacral pouch

Fig. 64  Vertical section of part of the disc of Ophionereis fasciata in the plane of A in Fig. 63.

EXT. INTER. M.  external interradial muscle

Fig. 65  Vertical section of part of the disc of Ophionereis fasciata in the plane of B in Fig. 63.

INTERAMB. POUCH  interambulacral pouch
Fig. 66 Horizontal section of the interambulacral area of the disc of *Ophiactis resiliens* at the level of B in Fig. 67.

EXT. INTER. M. external interradial muscle
INTERAMB. POUCH interambulacral pouch

Fig. 67 Vertical section of part of the disc of *Ophiactis resiliens* in the plane of A in Fig. 66, showing extension of bursa into the interradial muscle.

EXT. INTER. M. external interradial muscle

Fig. 68 Horizontal section of the disc of *Ophiactis resiliens* at the level of C in Fig. 67.

INTERAMB. POUCH interambulacral pouch of stomach
Fig. 69 Vertical section of the disc of Ophiopteris antipodum in the plane of B in Fig. 70, showing the complex bursal extension into the external interradial muscle.

EXT. INTER. M.  external interradial muscle
INTERV. M.  intervertebral muscle

Fig. 70 Horizontal section of the disc of Ophiopteris antipodum at the level of A in Fig. 69.

INTERAMB. POUCH  interambulacral pouch of the stomach

Fig. 71 Vertical section of the disc of Ophiopteris antipodum in the plane of C in Fig. 70, showing the bursal extensions under the stomach and over the proximal portion of the arm.

PROX. ARM SEG.  proximal arm segment
Fig. 72 Graph of calculated regression lines of oxygen consumption per hour against wet body weight.

O. f. *Ophionereis fasciata*
\[ \log Y = -1.80584 + 0.50229 \log X \]

O. r. *Ophiactis resiliens*
\[ \log Y = -1.326 + 0.70629 \log X \]

O. a. *Ophiopteris antipodum*
\[ \log Y = -1.91124 + 0.41721 \log X \]

Fig. 73 Graph of calculated regression lines of respiration rate against wet body weight.

O. f. *Ophionereis fasciata*
\[ \log Y = 2.6569 - 0.90482 \log X \]

O. r. *Ophiactis resiliens*
\[ \log Y = 1.44245 - 0.3526 \log X \]

O. a. *Ophiopteris antipodum*
\[ \log Y = 1.94607 - 0.60385 \log X \]
Fig. 74 Graph of respiration rate of *Ophionereis fasciata* against wet body weight, over a size range of animals, at 15 and 25°C.

Fig. 75 Graph of respiration rate of *Ophiactis resiliens* against wet body weight, over a size range of animals, at 15 and 25°C.

Fig. 76 Graph of respiration rate of *Ophiopterus antipodum* against wet body weight, over a size range of animals, at 15, 20 and 25°C.
Section 6.
GONADS AND REPRODUCTION.

6.1. Introduction.

Echinoderms have been used as a source of food, when the gonads are ripe, for a long time; and a common knowledge of the breeding periods of many species has thus developed. The use of echinoderm eggs in embryological studies has also resulted in a reasonable documentation of the breeding periods for many animals. Such information has been supplemented by data obtained from specimens collected during the course of various expeditions around the world. Detailed studies on reproductive cycles however are few and limited to the asteroids, holothurians and echinoids. The present knowledge of these cycles has been summarized recently by Boolootian (1966), who also notes the lack of such a detailed study for the ophiuroids. Boolootian does however figure a table (page 591) summarizing the reproductive periods for many species. The majority appear to have a breeding season, when mature gametes are present, of one to three months, comparatively short in contrast to other echinoderms.

Many authors have provided histological and morphological information on the structure of the gonads themselves and their contained gametes, and these have been adequately summarized by Hyman (1955). The most recent detailed study is that of Smith (1940) on the British species Ophiothrix fragilis. Smith concluded that this species has a breeding season which extends from March to October, spawning occurring at monthly intervals for females although mature spermatozoa are present in the males
all the year round.

The following account describes the breeding cycles of *Ophionereis fasciata*, *Ophiactis resiliens* and *Ophiopteris antipodum* over a fifteen month period based on animals collected regularly from the Leigh reef.

6.2. **Structure of the gonads, development of the gametes, and the breeding cycle.**

(a) *Ophionereis fasciata*. The gonads of this species consist of a number of discrete lobes which are attached along the edge of the bursal slit. The wall of each lobe is composed of a sheet of connective tissue fibres external to which lies a layer of coelomic epithelial cells. Muscle fibres also occur outside the connective tissue layer in mature animals, consisting of a series of parallel fibres running radially around each lobe. The lumen of the gonad is packed with either developing male or female gametes, the species being dioecious. The course of development of these will now be discussed.

The primordial germ cells originate from the genital rachis. As shown for *Ophiothrix fragilis* by Smith (1940), this structure consists of a cylindrical strand of germinal tissue, approximately circular in cross section, which originates from the right axial organ. Cells lying in the rachis are similar in size to the smallest observable gametocytes within the actual gonad tissue, at a diameter of 6 or 7μ. In view of the lack of a germinal epithelium within the gonad itself it is highly likely that a migration of cells occurs from the rachis into the lumen of the gonad, where further development occurs.
Spermatogenesis. The spermatogonia have relatively large nuclei about 5\(\mu\) in diameter, which contain diffusely staining material. These cells develop directly into spermatocytes which possess a smaller, more basophilic nucleus some 3\(\mu\) in diameter. Early spermatids appear as even smaller cells with nuclei only 2\(\mu\) in diameter, again densely stained. The spermatids form columns of cells running at right angles to the wall of the gonad. Late spermatids are void of cytoplasm and a flagellum develops opposite the clear eccentric acrosome which is now observable. Mature spermatozoa occupy the central lumen as they develop. The respective layers of cells in the process of development form distinct bands around the testis lobe and by measuring the width of each layer the relative abundance of each can be ascertained.

When spermatogonia alone are present, the gonad lobes are small and inconspicuous. During the winter months of July and August spermatogonia are abundant although in some individuals there are many spermatocytes and even some spermatids in August. These early spermatids develop during September and October and the lobes become increasingly swollen. By November the layer of spermatocytes is some 45\(\mu\) deep while the spermatids lie 160-180\(\mu\) deep, with a few mature spermatozoa occupying the central lumen. The number of spermatozoa steadily increases through the summer months until by March almost the entire lumina of the lobes are filled with fully mature spermatozoa, although late spermatids are still evident. Samples taken at the end of May still show residual amounts of spermatozoa but their gonads are almost empty. It is therefore presumed that by this time all viable spermatozoa have been shed. By July the lobes are once again very small when
spermatogonia increase in number and the cycle begins again.

The cycle of sperm development is accompanied by the occurrence of granular, eosinophilic clumps of cells. In July these cells occupy, and occlude, the central lumen of the gonad. They do not superficially appear to be attached to any of the germinal cells and persist until November when numbers of mature spermatozoa begin to occupy the centre of the gonad. From this period onwards they are entirely absent. As noted by Smith (1940) in Ophiothrix fragilis, each lobe possesses a ciliated gonoduct which pierces the wall of the bursal slit.

Oogenesis. Oocytes develop from oogonia which initially resemble spermatogonia and indeed the sex of some animals is difficult to determine when only small gametocytes are present. Throughout most of the year gametocytes of various sizes are present. The frequency of the different sizes of oocytes was estimated by counting and measuring the diameters of 50 oocytes per animal. Only oocytes which had been sectioned through the nucleolus were measured as this ensures that each oocyte is not measured twice, although it does not necessarily represent the greatest diameter of the cell. A linear micrometer was used to measure the oocytes and the ones measured were those that intercepted the micrometer as it transversed each lobe. Wherever possible sections which incorporated the junction of the genital rachis and the gonad were used in order to take into account the relative number of oogonia in that region.

The gonad cycles of asteroids and echinoids are often followed by means of a gonad index, based upon measurements of the volume of the gonad relative to the body weight. No attempt was made
to arrive at a gonad index for the ophiuroids studied here for several reasons. Firstly, the volume of the gonads is almost too small to measure accurately by the usual displacement methods, especially when a large number of discrete lobes comprise each gonad. Secondly, the ripe gonads usually rupture upon handling; and even if their volume could be ascertained there is nothing to which it can be related, except perhaps the disc. The whole weight of the body varies considerably with the loss of whole or part of the arms and the subsequent varying degrees of their regeneration. The frequency polygons of oocyte diameters for Ophionereis fasciata are given in Fig. 77.

Samples taken in December indicate that two populations of oocytes are present. The majority of the cells are greater than 50 μ in diameter but a few oogonia are present. These small cells are present throughout the summer months but only show a marked increase in number in April. During the months of May, June and July their size gradually increases until in August many oocytes have a diameter in excess of 35 μ and some are twice this size. At a diameter of 40 μ the cytoplasm becomes less basiphilic and increasingly eosinophilic. The cells also now stain with the PAS procedure indicating an increase in carbohydrate content. Lipid is also evident at this stage and appears to be deposited initially in the outer part of the oocyte. The deposits become increasingly intense and are quite dense from a diameter of 65 μ and above.

As can be seen from Fig. 77 the size of the oocytes during the months of September through to December become increasingly larger although a wide range of diameters is apparent. The maximum size of oocytes does not increase markedly after this month.
but more oocytes attain larger diameters although not necessarily to the maximum.

The centre of the gonad lobes, as seen in the testes, contain a collection of eosinophilic cells when the oocytes are small, and these cells persist until December. During March and April however the ovaries contain spherical droplets which have similar staining reactions to the oocytes themselves. These droplets may therefore represent broken down oocytes. Pearse (1965) has recorded that in the antarctic asteroid *Odontaster validus* a considerable number of mature oocytes break up and serve to provide material for further developing oocytes. It is difficult to tell whether the droplets represent oocytes which have broken down naturally or have been ruptured as a result of the procedures of histological treatment.

From the frequency polygons it may be correct to assume that full development of the oocytes takes one year. Thus the small oogonia present in the December samples become mature in the following December although they may not be released until April or May. If all the oocytes mature at the same rate then those present during the first three months of the year reach a maximum size during the same three months of the following summer. On the other hand the small gametes present during the summer may represent only a small percentage of the total number, which lie dormant until all the primary oogonia have accumulated within the gonad, by April or May, when further development of all oocytes begins, the larger oocytes having been shed.

It is therefore possible to estimate when each population of gametes will be shed, and the dates on Fig. 77 indicate the probable
year of spawning. Thus the small oocytes of the December 1966 samples will be released in 1968, the larger ones during the late summer of 1967. The frequency polygons only illustrate the percentage of each size group throughout the year and thus after spawning small oocytes obviously make up the entire population, but their actual number need not necessarily be increased. Samples taken in May and June however do indicate that the numbers of oogonia are increased as the region where the genital rachis expands into the gonad becomes increasingly packed with these cells.

As in the testes, each lobe of the ovary possesses a ciliated gonoduct which opens into the side of the bursal slit. These gonoducts are rather short at a length of 100 μ and have an internal diameter of 10 μ, although they obviously expand greatly to allow the release of the gametes into the water. The gonoducts penetrate the body wall, pass through the cavity of the genital sinus which surrounds each gonad, eventually opening into the lumen of the gonad lobe. The cells lining the gonoduct superficially resemble those of the external epithelium but possess long cilia and pear shaped nuclei, 6 μ in length, the epithelium having more rounded nuclei. The internal portion of the gonoduct is complicated as its point of entry into the gonad coincides with the genital rachis, the developing oogonia of which may obscure the gonoduct.

(b) **Ophiactis resiliens.** There are ten gonads in this species, one to each bursa. The gonad when swollen lies over the bursa and becomes subdivided into lobes by the invasion of connective tissue which form septa across the lumen. The development of both male and female gametes is essentially similar to that described
in *Ophionereis fasciata* but the actual cycle of events is slightly different.

The lobes of the testes become packed with spermatogonia during the winter months of June, July and August but the gonad is very small. During September spermatozoa develop and even a few early spermatids are present. The number of spermatids increases markedly in October and November. In December spermatocytes lie to a depth of 14 μ, the spermatids being 120-130 μ deep and the centre of the lobes occluded by mature spermatozoa. By February more spermatozoa are present and in March occupy most of the testis. Spermatogonia persist in small numbers throughout the year but spermatocytes disappear in April. By May the testes are empty and small again, having presumably spawned.

Only a few weakly staining eosinophilic cells appear in the testis lobes during September when few spermatids are present, but they are absent during the remainder of the year. Similar cells occur in the ovaries.

The period of oocyte development is greatest during May and June (Fig. 78) when the small gonads are packed with oogonia less than 10 μ in diameter. During February, March and April only a few oogonia are present where the genital rachis swells into the gonad. The number present is in fact so small that it seems unlikely that these cells alone could further develop into the full complement of mature oocytes the following year.

In July and August there is an upward shift in the frequency polygons which is accelerated in September when some oocytes are 90 μ in diameter. Through the summer months the lobes are packed with oocytes in excess of 70 μ in diameter until the May samples
indicate that the gametes have been shed. The entire cycle may again therefore be of only one years duration, from May to the following April, when the mature cells are shed. As noted in Ophionereis fasciata droplets with the same staining property as the oocytes are present in the ovaries during February, March and April.

A single, ciliated gonoduct is present for each gonad, penetrating the wall of the bursa near the bursal slit. The duct is longer than that of Ophionereis fasciata, usually more than 200μ, and slightly wider at a diameter of 15μ. The gonoducts are present all the year round in adult specimens.

(c) Ophiopteris antipodum. The gonads of Ophiopteris antipodum consist of a cluster of discrete lobes. Male animals possess a few spermatogonia throughout the year, but there is an increase during the period of April to July. During August and September spermatocytes increase and some early spermatids are present. By November the spermatogonia and spermatocytes occupy a layer less than 20μ in depth, the spermatids extending into the lumina to a depth of 150μ while the centre contains mature spermatozoa. From December to March spermatozoa occupy the entire centre of the gonads but by April the entire contents have been shed.

Oogonia are present from April onwards but only in July is there any marked increase in their diameter. A gradual growth period extends until December when a few oocytes exceed 100μ in diameter although a few are still extremely small (Fig. 79). By March however oocytes less than 50μ in diameter were not seen and all cells appear to have been shed before the April samples were taken. The oocytes differ from those of the other two
species by the possession of two nucleoli, one basophilic and the other acidophilic. They also stain more intensely with both the PAS and lipid tests from a diameter of 40 μ onwards than the other two species.

Gonoducts are present which are short (100 μ) and open onto the bursal wall near the bursal slit. As in the previous two species eosinophilic cells are present in the lobes of the testis prior to the development of spermatozoa, and broken down oocytes can be seen in the ovaries prior to spawning.

The actual act of spawning has only been seen in Ophionereis fasciata. This occurred with a specimen collected at Kaikoura, in the South Island, on 8th January, 1968. The animal, a male, had just been collected from the shore and spawned at 8.20 p.m. The disc was raised to a height of one inch by the elevation of the proximal third of each arm. Sperm were shed from each bursa and quickly dispersed in the tank, the period of release lasting only twenty seconds. Three other animals in the same tank produced no apparent response and did not spawn.

In the limited volume of the disc the annual increase in gonad size obviously has an effect on the other organs, principally the stomach. Figures 80 and 81 indicate the restriction of the inter-ambulacral pouch of the gut of Ophionereis fasciata when the gonads are ripe in April. It can be seen that although the stomach lumen is compressed in the horizontal plane the effect in a vertical plane is less severe. Food particles are present within the pouches of the stomach and animals exhibited feeding postures throughout the breeding season.

The constriction of the stomach is much more marked in
Ophiactis resiliens as can be seen in Figs. 82 and 83 of the inter-ambulacral pouches of a specimen collected in December. The compartments of each gonad greatly restrict the gut lumen in both horizontal and vertical planes. Again however food particles are present in the gut.

The effect of gonad enlargement is least noticeable in Ophiopteris antipodum. There is some restriction of the gut in a vertical plane (Fig. 84) but in a horizontal plane the restriction is only slight. This species also continues to feed throughout the entire summer.

6. 3. Discussion.

In a review of the reproductive physiology of echinoderms, Boolootian (1966) defines the reproductive period, or breeding season, as that period when mature fertilizable gametes are present. The reproductive cycle on the other hand was defined as the total course of events from the resting gonad through activation of gametogenesis, spawning and back to the resting gonad regardless of the time period over which these events occur. Thus in some animals the cycle may take a matter of days, weeks, months or years.

By these definitions therefore it would appear that the reproductive cycle for the three ophiuroids studied takes a complete year. It is difficult to estimate a "resting gonad" and although in each species there is a period when little increase in diameter of the oocytes occurs, more oogonia are being added to the gonad lumen. The period of oocyte growth is certainly less than a year but the actual reproductive period is less easy to define. Again in all three species mature gametes (as far as can be histologically
ascertained) are present over a period of several months. This could indicate that the gametes are shed at intervals during this period, as inferred for Ophiothrix fragilis by Smith (1940). The size range of female gametes however is not uniformly aligned within the gonad, and it is difficult to imagine how the largest oocytes could be released, by the dilation of the gonoduct, without the loss of the smaller immature cells. Also one would expect to find individuals amongst each sample which lacked the largest oocytes, but this is not the case.

It is therefore possible that, although of a maximum size, the oocytes are withheld for some time prior to their eventual release, and the same may be said for the mature spermatozoa. This may be up to four months in Ophionereis fasciata and Ophiactis resiliens, and three months for Ophiopteris antipodum. During this period smaller oocytes attain a maximum diameter. A similar "resting period" prior to spawning has been recorded in a thesis by Chia (1964) (quoted by Boolootian, 1966) for the asteroid Leptasterias hexactis, where the period lasted five months. Although mature as regards size, it has not been ascertained whether the gametes are in fact fertilizable during this period.

Pearse (1964) in a study of the antarctic asteroid Odontaster validus, also noted that oocytes attained a maximum size some months before spawning. This author made the additional observation that after one years growth three quarters of the oocytes disintegrate and apparently act as storage cells which hold nutrients for other growing oocytes.

The oocytes however require a two year growth period to attain maturity, as Chia (1964) also noted for Leptasterias hexactis.
The observed breakdown of oocytes noted in the ophiuroids of this study do not therefore readily compare as the period of development is only one year and as previously mentioned it is highly probable that the broken cells are a result of the methods of histological preparation.

The accumulation of eosinophilic cells during the early stages of gamete development indicates that these cells may possibly play a nutritive role. Cells have been described in the ovaries of the antarctic echinoid Sterechinus neumayeri by Pearse and Giese (1966) as nutritive phagocytes. These cells were considered to store nutrients in order to permit the growth of oocytes during the winter when no phytoplankton occurs, and thus parallel in function the pyloric caeca of the asteroid Odontaster validus (Pearse, 1965). Similar cells have been described by Liebman (1950) in the echinoid Arbacia. Greenfield et al. (1958) demonstrated that the amount of lipid within the gonad correlated with the reproductive cycle. Similarly in echinoids Giese (1961) and Giese et al. (1964) have demonstrated that a large amount of lipid is accumulated in the gonads. This observed accumulation of food material indicates the ability of the gonads to act as storage organs but it is not clear whether the compounds are stored in phagocytes or in gametes which are later broken down as observed by Pearse (1965).

Roubaud (1965) considered that amoebocytes observed in the ophiuroid Ophiothrix quinquemaculata played the opposite role i.e. of removing excretory products from the developing gonads. It cannot be assumed however that the cells described by various authors are necessarily of a similar nature or function and a
detailed study is required of these cells occurring within echinoderm gonads.

The accumulation of carbohydrate and lipid from a diameter of 45 μ in all three species is of interest. Both Cognetti and Delavaault (1962) and Pearse (1965) have recently noted the change in the staining properties of asteroid oocytes at a diameter of 75 μ (approximately half the maximum diameter), when they become increasingly eosinophilic. Cognetti and Delavaault described this as a transition from a uniformly stained cytoplasm to a meshed form. The latter state in the ophiuroids studied indicates that nutrients are being absorbed.

The beginning of the spawning period of all three species is far from clear but it is evident that it has occurred prior to April in Ophiopteris antipodum, before May in Ophiactis resiliens and by the end of May in Ophionereis fasciata for the year 1967. The observation of the spawning specimen of Ophionereis fasciata in the South Island during the month of January cannot necessarily be related to the spawning time of animals from the more temperate latitude of the Leigh population. The larval forms of the species studied have not, unfortunately, been described. Jillett (1966) records unidentified plutei larvae in the Waitemata Harbour, near Leigh, from October to May, and in the Jellicoe Channel off the Leigh shore from November to July. The latter samples are probably more relevant.

Echinoderms are noted for their lack of synchronization of spawning with changes of sea temperature. The monthly mean surface temperatures of Leigh have been figured in Figs. 77, 78 and 79 and have been kindly supplied by Dr. W. J. Ballantine. A far more
detailed study is required however to elucidate, if any, correlation.

Finally, the presence of gonoducts in all three ophiuroids is of particular interest. It is commonly stated that ophiuroids shed their gametes through a temporary opening in, or a rupture of, the bursal wall. This statement was attributed by Smith (1940) to Chiajei (1841) and has since been quoted in various works. Smith however described gonoducts in *Ophiorthrix fragilis*. The occurrence of muscle fibres around the gonad implies that the gametes are forcibly ejected and it is likely that all the gametes are shed at one time.
Fig. 77 Gonad cycles of *Ophionereis fasciata* collected from the Leigh reef showing the relative abundance of male and female gametes from 16th October, 1966 to the 3rd December, 1967.

The top series indicates the relative abundance of male gametes.

- **FEW**
- **COMMON**
- **ABUNDANT**

The lower series indicates, by frequency polygons, the varying percentage of different sized oocytes. The inner scale is the diameter of oocytes in μm, for the polygons, while the outer scale is of temperature in °C for the superimposed graph of the mean monthly sea surface temperature at Leigh. The dates on the polygons indicate the probable year of spawning for these oocytes.
Fig. 78 Gonad cycles of *Ophiactis resiliens* collected from the Leigh reef showing the relative abundance of male and female gametes from 16th October, 1966 to the 3rd December, 1967.

The top series indicates the relative abundance of male gametes.

- **FEW** S'OZOA spermatozoa
- **COMMON** S'ATIDS spermatids
- **ABUNDANT** S'OOCYTES spermatocytes
  
  S'OCONIA spermatogonia

The lower series indicates by frequency polygons the varying percentage of different sized oocytes. The inner scale is the diameter of oocytes in μ, for the polygons, while the outer scale is of temperature in °C for the superimposed graph of the mean monthly sea surface temperature at Leigh. The dates on the polygons indicate the probable year of spawning for these oocytes.
Fig. 79 Gonad cycles of *Ophiopteris antipodum* collected from the Leigh reef showing the relative abundance of male and female gametes from 16th October, 1966 to the 3rd December, 1967.

The top series indicates the relative abundance of male gametes.

- **FEW** S'OZOA  spermatozoa
- **COMMON** S'ATIDS  spermatids
- **ABUNDANT** S'OCTYES  spermatocytes
  S'OGONIA  spermatogonia

The lower series indicates by frequency polygons the varying percentage of different sized oocytes. The inner scale is the diameter of oocytes in $\mu$m, for the polygons, while the outer scale is of temperature in °C for the superimposed graph of the mean monthly sea surface temperature at Leigh. The dates on the polygons indicate the probable year of spawning for these oocytes.
Fig. 80 Horizontal section of the disc, *Ophionereis fasciata*, of a specimen taken in April, showing diagrammatically the restriction of the gut lumen by the gonads.

Fig. 81 Vertical section of the disc, *Ophionereis fasciata*, of a specimen taken in April, showing diagrammatically the restriction of the gut lumen by the gonads.

Fig. 82 Horizontal section of the disc, *Ophiactis resiliens*, of a specimen taken in December, showing diagrammatically the restriction of the gut lumen by the gonads.

Fig. 83 Vertical section of the disc, *Ophiactis resiliens*, of a specimen taken in December, showing diagrammatically the restriction of the gut lumen by the gonads.

Fig. 84 Vertical section of the disc, *Ophiapteris antipodium*, of a specimen taken in February, showing diagrammatically the restriction of the gut lumen by the gonads.
Section 7.
SOME ECOLOGICAL FACTORS.

7.1. Introduction.

During the course of this study a number of observations of relevance to the general ecology of the species studied have been made. It is apparent from the recent review of Fell (1966) that many factors of the biology of ophiuroids are of importance to gain a full understanding of their ecology. Some of these, namely feeding, metabolism, and reproduction have been discussed in the preceding sections. This section presents some additional information on distribution, intertidal abundance and the loss of arms and their subsequent regeneration, subjects upon which the literature is particularly lacking.

7.2. Distribution.

The three species which form the basis of this study are not restricted to the intertidal zone and have a wide distribution around the New Zealand coast. Mr. D. G. McKnight of the Oceanographic Institute has kindly supplied the following information (1966). Ophionereis fasciata occurs throughout New Zealand but has not been reported from the west coast of the North Island, possibly due to the lack of collecting. It is also reported from the Kermadec Islands, 500 miles north-east of the North Island. Nearly half of the 48 records obtained by the Institute were in depths of less than 20 metres. The maximum depth recorded was 209 metres and specimens were found to occur on all sediments except mud.

Ophiactis resiliens ranges from the Three Kings Islands, 29
miles north of the North Island, to the Snares Islands, 70 miles south-west of Stewart Island, and has also been reported from Lord Howe Island and off south-eastern and southern Australia. There are 23 records from New Zealand waters and the species appears to be most common between the Three Kings Islands and the East Cape of the North Island. It ranges from the intertidal zone to 210 metres with 26% of the records from less than 20 metres. It occurs on all sediments except mud and is commonly found in algal holdfasts.

There are fewer records of *Ophiopteris antipodum* although this species too appears to have a considerable range, occurring from the Auckland region to the Snares Islands. The 9 records obtained by the Institute have all been taken from less than 84 metres depth, specimens being found in algal holdfasts, on rocky bottoms and on muddy shell-sand.

Information is also available on *Pectinura maculata*. There are many records of this species, all from south of Cook Strait. It is particularly abundant in Foveaux Strait and ranges to a depth of 168 metres although the majority of the records are from less than 50 metres. A wide range of substrates is inhabited, being most frequently found on sand or gravel. It has not been found on sandy mud or mud. As noted in Section 1 this species may be found intertidally at Whangarei and is commonly reported by divers around the Auckland area. The lack of *P. maculata* in offshore dredging hauls taken by the Oceanographic Institute may be due to a change in habitat in the North. Off the South Island coast, specimens are readily identified in underwater photographs, where they occur exposed on the bottom. Around the Auckland area
however they are usually found in crevices, beneath overhangs and other sheltered places, and would thus not be taken by any form of boat-operated collecting device.

At Leigh the several species are by no means restricted to the intertidal reef flat. *Ophionereis fasciata* extends to a depth of at least 10 metres where suitable areas exist with adequate boulder cover. *Ophioactis resiliens* is typically found in areas where the current is strong, on rocky outgrowths provided with suitable algal holdfasts and also deep inside crevices in weathered rock. Mr. R. V. Grace of this department has reported an area on the seaward side of Matheson Bay just south of Leigh where the whole of the boulder strewn slope, from 10 to 15 metres depth contained numerous small crevices each occupied by an *Ophioactis resiliens*.

*Ophiopteris antipodum* also occurs subtidally beneath boulders but is always less common than the other two species.

Large reef flats similar to that of Leigh occur around the Kaikoura peninsula on the east coast of the South Island. *Ophionereis fasciata* is also found here in suitable areas and the specimens are noticeably larger. Of four animals collected from beneath a single rock the disc diameters were 2.3, 2.0, 1.8 and 1.7 cm. The mean arm lengths were 12.9, 11.7, 11.6 and 9.9 cm. Average Leigh specimens have a disc diameter of 10 cm. and an arm length of 6 cm. *Pectinura gracilis* also occurs in the holdfasts of *Macrocystis pyrifera* and *Ophiomyxa brevirima* is common sub-littorally under stones. It is at Portobello, near Dunedin, however that *Ophiomyxa brevirima* is most commonly found and specimens of *Pectinura gracilis* were also collected intertidally.
7.3. **Abundance at Leigh.**

In order to obtain information on the relative abundance of ophiuroids on the reef flat at Leigh throughout the year, four pools at three different levels on the reef flat were marked and the number of each species in the pools were counted regularly throughout the year. The temperature of each pool was also noted and a sample of water drawn for a salinity test.

It is difficult to apply zoning terms to the reef flat as the whole area is broadly speaking the midlittoral zone and due to its very flat topography there is little difference in the total time of emmersion. The first four pools however were chosen at the upper limit of the reef flat, the next four across the middle of the flat while the last four bordered the sublittoral fringe.

Each pool was approximately one metre square and at least six inches deep. As might be expected with such a large volume of water there is little variation in salinity. The salinity of one of the top four pools was, after heavy rain, as low as 31.5°/oo but the usual range was from 35.2°/oo to 35.5°/oo. The middle pools varied from 35.2°/oo to 35.8°/oo while the lower ones differed only slightly from the sea with a variation of 35.0 to 35.4°/oo.

Temperature ranges however showed considerable variation. The mean temperatures for each level are given in Table 13.

The total numbers of each species in the pools at each of the three levels are given in Figs. 85, 86 and 87. There is an obvious increase in all three species further down the shore and at all three levels *Ophionereis fasciata* is far more abundant.
Ophiopteris antipodum is absent from the higher pools and was not found on occasions in the middle pools. Ophiactis resiliens, although not always present in the upper pools, is usually found at the lower levels.

**TABLE 13.**

**MEAN POOL TEMPERATURES, LEIGH.**

(Approx. vol. of water 150 ltrs.)

<table>
<thead>
<tr>
<th>Date</th>
<th>TOP</th>
<th>MID</th>
<th>LOWER</th>
</tr>
</thead>
<tbody>
<tr>
<td>26th Feb.</td>
<td>27.1°C</td>
<td>25.5°C</td>
<td>25.2°C</td>
</tr>
<tr>
<td>27th March</td>
<td>24.5</td>
<td>24.1</td>
<td></td>
</tr>
<tr>
<td>29th April</td>
<td>17.1</td>
<td>17.0</td>
<td>16.9</td>
</tr>
<tr>
<td>27th May</td>
<td>17.7</td>
<td>16.8</td>
<td>17.0</td>
</tr>
<tr>
<td>24th June</td>
<td>15.8</td>
<td>15.9</td>
<td>16.0</td>
</tr>
<tr>
<td>22nd July</td>
<td>17.5</td>
<td>16.9</td>
<td>15.3</td>
</tr>
<tr>
<td>19th Aug.</td>
<td>16.1</td>
<td>15.7</td>
<td>14.8</td>
</tr>
<tr>
<td>17th Sept.</td>
<td>21.2</td>
<td>19.7</td>
<td>17.1</td>
</tr>
<tr>
<td>7th Oct.</td>
<td>19.3</td>
<td>19.1</td>
<td>18.3</td>
</tr>
<tr>
<td>4th Nov.</td>
<td>22.7</td>
<td>22.8</td>
<td>20.7</td>
</tr>
<tr>
<td>3rd Dec.</td>
<td>27.2</td>
<td>26.1</td>
<td>23.9</td>
</tr>
</tbody>
</table>

The variation in the number of each species throughout the year is also more marked at the higher levels, which are more subject to variations in temperature. The absence of Ophiopteris antipodum from these pools, in view of the apparent depression of their respiration rate at 25°C, is worthy of note. The highest temperature recorded in one of these pools was 28.1°C on the 26th February, 1967. Both upper and middle level areas are also subject to wave action during heavy weather and a considerable amount of boulder movement occurs.
7. 4. Arm loss and regeneration.

Advantage was taken of the salt water circulation tanks of the Leigh laboratory to maintain animals over an extended period in order to gain an estimate of the regeneration rates of each species. Of 50 animals of each species gathered from the reef the percentage of arms to be observed in the process of regeneration is given in Table 14.

**TABLE 14.**

**PERCENTAGE OF REGENERATING ARMS.**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. fasciata</td>
<td>54.5%</td>
<td>20.5%</td>
<td>13.6%</td>
<td>6.8%</td>
<td>4.5%</td>
<td>0.0%</td>
</tr>
<tr>
<td>O. resiliens</td>
<td>60.0%</td>
<td>16.7%</td>
<td>6.7%</td>
<td>3.3%</td>
<td>10.0%</td>
<td>3.3%</td>
</tr>
<tr>
<td>O. antipodum</td>
<td>21.3%</td>
<td>25.5%</td>
<td>38.3%</td>
<td>8.5%</td>
<td>4.3%</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

In order to estimate the rate of arm regeneration sixteen animals of each species were maintained in the circulation tanks. At the start of the experiment the diameter of the discs were measured and one, two, three and four arms were severed near the disc of each of four animals. At monthly intervals the animals were removed and the length of regenerated arms and the diameters of the discs were measured.

Figure 88 shows the average regenerated arm length of Ophionereis fasciata for each arm removed. The data has been
presented in the form of calculated regression lines for simplicity and the individual points have been omitted as all 96 of them would obviously obscure the graph. It can be seen from Fig. 88 that the average regeneration rate is fairly similar regardless of the number of arms involved. The total length of regenerated arm is, however, a greater indication of the amount of tissue involved. Figure 89 gives the total length of regenerated arm and it is obvious that a greater total length of arm is produced when more arms are involved. Also given in this figure is the total disc diameter of each group as a percentage of the total disc diameter at the beginning of the experiment. When only one or two arms have been lost the diameters soon revert to their initial size, but where four arms have been lost the disc diameter continues to fall. Thus presumably the arms are either regenerated at the expense of tissue within the disc or, the gains of the reduced feeding capabilities are directly utilized to provide new arm growth while general metabolism is carried out at the expense of disc tissue.

The calculated regression lines of the total lengths of new arm growth of *Ophiactis resiliens* and *Ophiactis antipodum*, plus their changes in disc diameter, are given in Figs. 90 and 91. With all three species total length is not in itself an indication of whether more tissue is laid down in any one instance, that is, whether some arms are long but thin and thus do not actually contain more tissue by weight. At the end of the experiments the regenerated arms were cut off and weighed, and the figures obtained are given in Table 15. As each weight is only relevant to each species it is also given as a percentage of the mean total
body weight of the animals.

**TABLE 15.**

**MEAN WEIGHTS OF REGENERATED ARMS.**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>NO. OF ARMS REGENERATING</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. f. A. ARM WT. (GMS.)</td>
<td></td>
<td>0.015gm.</td>
<td>0.0267gm.</td>
<td>0.045gm.</td>
<td>0.0510gm.</td>
</tr>
<tr>
<td>B. ARM WT. AS % of body wt.</td>
<td></td>
<td>1.78%</td>
<td>3.98%</td>
<td>7.32%</td>
<td>7.52%</td>
</tr>
<tr>
<td>O. r. A. ARM WT. (GMS.)</td>
<td></td>
<td>0.0037gm.</td>
<td>0.0112gm.</td>
<td>0.0094gm.</td>
<td>0.0172gm.</td>
</tr>
<tr>
<td>B. ARM WT. AS % of body wt.</td>
<td></td>
<td>1.35%</td>
<td>3.14%</td>
<td>4.49%</td>
<td>7.89%</td>
</tr>
<tr>
<td>O. a. A. ARM WT. (GMS.)</td>
<td></td>
<td>0.0516gm.</td>
<td>0.026gm.</td>
<td>0.071gm.</td>
<td>0.037gm.</td>
</tr>
<tr>
<td>B. ARM WT AS % of body wt.</td>
<td></td>
<td>4.09%</td>
<td>1.34%</td>
<td>3.47%</td>
<td>5.87%</td>
</tr>
</tbody>
</table>

From Figs. 89, 90 and 91 it is apparent that the regeneration rate is greatest in *Ophionereis fasciata*. At the end of the seven month experiment the arms had reached approximately half their previous length. The arms of *Ophiactis resiliens* had also reached about half their previous length but it is obtained at a greater expense of the disc. *Ophiopteris antipodum*, although possessing a slower regeneration rate, the arms having reached only about a third of their original length, appears to achieve this rate with a minimal effect on its disc diameter.

Previous information on the rate of ophiuroid arm regeneration is rather scant. Zeleny (1903, 1905) using *Ophiura texturata* found that after 33 and 46 days the rate of arm regeneration was greater the larger the number of arms regenerating, and that the rate was also higher in medium sized animals. Morgulis (1909)
stated that the rate of regeneration was faster the greater the length of arm removed in the Bermudan species *Ophiocoma pumila*. Milligan (1915) recorded the rather low rate of 17mm. per year for the British species *Ophiothrix fragilis*. The rates given for the Leigh species are probably lower than those of animals in their natural habitat.
Fig. 85  Total numbers of ophiuroids in four pools along the upper midlittoral zone of the reef flat at Leigh throughout the year, 1967.

O. f.  *Ophionereis fasciata*
O. r.  *Ophiactis resiliens*

Fig. 86  Total numbers of ophiuroids in four pools along the mid midlittoral zone of the reef flat at Leigh throughout the year, 1967.

O. f.  *Ophionereis fasciata*
O. r.  *Ophiactis resiliens*
O. a.  *Ophiocryptis antipodum*

Fig. 87  Total numbers of ophiuroids in four pools along the lower midlittoral zone of the reef flat at Leigh throughout the year, 1967.

O. f.  *Ophionereis fasciata*
O. r.  *Ophiactis resiliens*
O. a.  *Ophiocryptis antipodum*
Fig. 88 Calculated regression lines of the average length of new arm growth per arm (in mm.) against time (in weeks) of *Ophionereis fasciata* after 1, 2, 3 and 4 arms have been severed near the disc.

1 \( Y = 0.76 + 0.8789X \)
2 \( Y = 1.67 + 0.9038X \)
3 \( Y = -1.09 + 1.4114X \)
4 \( Y = -1.24 + 1.2047X \)

Fig. 89 Calculated regression lines of the total length of new arm growth (in mm.) against time (in weeks) of *Ophionereis fasciata* after 1, 2, 3 and 4 arms have been severed near the disc. The total diameters of the discs of these animals, as a percentage of the total disc diameter at the beginning of the experiment, are given below these lines.

1 \( Y = 2.87 + 0.8789X \)
2 \( Y = 6.69 + 1.8080X \)
3 \( Y = -7.02 + 4.5280X \)
4 \( Y = -4.95 + 4.8191X \)
Fig. 90 Calculated regression lines of the total length of new arm growth (in mm.) against time (in weeks) of Ophiactis resiliens after 1, 2, 3 and 4 arms have been severed near the disc. The total diameters of the discs of these animals, as a percentage of the total disc diameter at the beginning of the experiment, are given below these lines.

1  \[ Y = 2.46 + 0.8077X \]
2  \[ Y = 3.06 + 1.9328X \]
3  \[ Y = 7.81 + 1.9638X \]
4  \[ Y = 5.07 + 2.8541X \]

Fig. 91 Calculated regression lines of the total length of new arm growth (in mm.) against time (in weeks) of Ophiopteris antipodum after 1, 2, 3 and 4 arms have been severed near the disc. The total diameters of the discs of these animals, as a percentage of the total disc diameter at the beginning of the experiment, are given below these lines.

1  \[ Y = 3.01 + 0.6435X \]
2  \[ Y = 7.92 + 1.1189X \]
3  \[ Y = 7.67 + 2.5691X \]
4  \[ Y = 14.88 + 1.8920X \]
Section 8.

SUMMARY.

Of the five living classes of echinoderms the ophiuroids have been the most successful both in number of species and individuals and in geographical distribution. Surprisingly little however is known of their general biology. Advantage has thus been taken of the abundance of intertidal species of the New Zealand shores and three species have been studied in order to gain a greater understanding of some principal aspects of their biology. Additional information on four other species has been given.

The feeding methods of all seven species have been examined in detail, and the methods correlated as closely as possible with the functional morphology of the organs concerned, namely the podia and spines. The histochemistry of the various mucins produced by these organs has also been studied. Four species, Ophionereis fasciata, Ophiactis resiliens, Ophiopteris antipodum and Axiognathus squamata have been found to utilize mucus directly in the capture of food particles in suspension. This mucus varies slightly from species to species but is generally acidic with a high proportion of carboxyl groups. A similar mucin occurs in the podia of Ophiomyxa brevirima which also contain a less acid mucin, as does Ophiopteris antipodum. It is probable that the latter type is correlated with the ability of these two large species to adhere strongly to the substrate. More sulphated, acidic, mucins have been found in abundance in Monamphiura aster, Ophiomyxa brevirima and Pectinura maculata. Other non-acidic granular cells have been noted but their precise function has not
been elucidated. The secretions of some of these cells have been investigated further with the electronmicroscope.

The comparative histology and morphology of the podia have also been interpreted in connection with their functions and method of operation. The valves operating the system have been described as well as the radial canal and its role in the extension of the podia.

The structure and functioning of the stomachs of *Ophionereis fasciata*, *Ophiactis resiliens* and *Ophiopteris antipodum* has also been studied. Both secretory and mucous cells have been described. Evidence was found for the presence of a wide variety of enzymes, particularly enzymes hydrolysing glycosidic linkages.

The bursae have also been studied from a functional point of view and their probable role as a respiratory surface demonstrated. The respiration rates of both whole animals and isolated discs and arms have been examined and an estimate of the metabolic rates of the species obtained.

The other main organs within the disc, the gonads, have been studied over a fifteen month period and the relative cycle of gonad growth followed. Finally, some additional notes on ecological factors have been given. It is hoped that these studies will contribute to a greater understanding of ophiuroid biology, which is of significant importance to the study of benthic ecology as a whole.
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