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THE FILTER-FEEDING OF *ARTEMIA*

III. FAECAL PELLETS AND THEIR ASSOCIATED MEMBRANES

By M. R. REEVE
The Department of Zoology, University of Southampton

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Food ingestion in *Artemia* having been studied in some detail, attention was next directed towards certain aspects of the movement of food through and eventually out of the gut, in order to discover whether this process had any effect upon the method of feeding.

Faecal-pellet production was used by Harvey, Cooper, Lebour & Russel (1935) as a measure of the grazing activity of the zooplankton in the sea. Raymont & Gross (1942) found that the shape of the pellets produced by *Calanus* varied with the nature of the food and was different for males as compared with females. Such differences have also been reported by Marshall & Orr (1955) who could even tell two animals apart by their characteristic pellets. They also noted that with increasing food concentration pellet production rose rapidly at first and then levelled off, becoming rather variable.

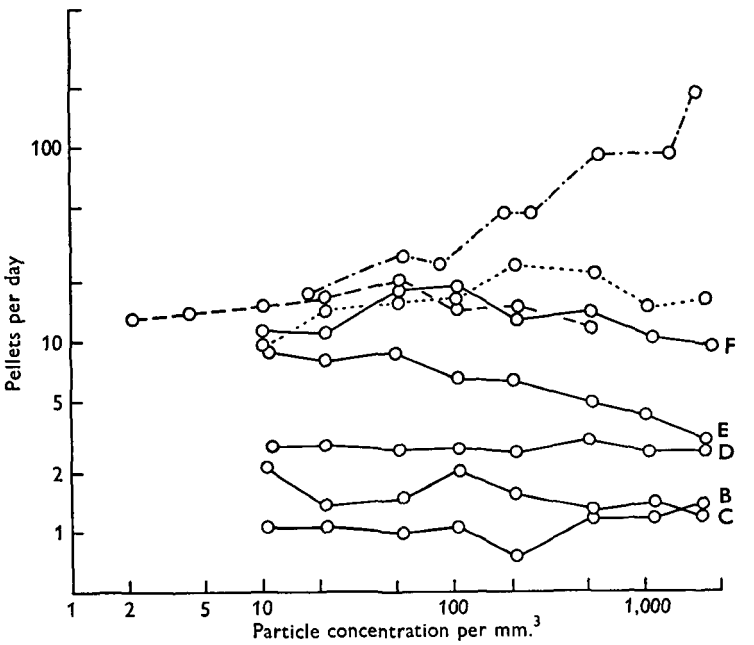
Chatton (1920) investigated the gut membranes of *Daphnia* and concluded that the fore- and hindguts produced a membrane but that the midgut did not. The presence of a membrane around the food in the midgut was due to the backward movement of the membrane produced in the foregut. Forster (1953) described in several Caridea a membrane which was produced by the anterior midgut and was chitinous, while Gauld (1957) found in *Calanus* that the posterior part of the midgut was responsible for membrane formation.

METHODS AND RESULTS

In the experiments on the effects of food concentration on filtration rates in relation to age (Reeve, 1963*a*) counts were made of the number of faecal pellets produced at the end of each experiment and the figures were standardized to number of pellets produced per animal per day. No results were obtained for series A of that work as the pellets were too small to be identified with certainty. Similar counts were made subsequent to the experiments with *Dunaliella* and *Chlorella* reported in the same paper, and also subsequent to the experiments with sand grains (Reeve, 1963*b*). As stated in the relevant paper, animals in series B–F were of progressively increasing size and were fed on *Phaeodactylum*, and the animals of series F were of the same population and size as those feeding on *Dunaliella* and *Chlorella*. Those ingesting sand were slightly smaller. In addition an experiment was also run with animals of average length 12 mm. feeding on *Phaeodactylum* to provide additional data.

Text-fig. 1 presents the daily faecal pellet production plotted against particle concentration for all these experiments, except those on the 12 mm. *Artemia* which have been omitted to avoid excessive overcrowding of the figure. The overall picture sug-

gested by curves B–F for the animals of increasing size (1–10 mm.) is that as the animal grows it produces more faecal pellets. No general trend for increase of pellet production with increasing particle concentration is obvious in these curves. Of the experiments with *Phaeodactylum* series F, *Dunaliella*, *Chlorella* and sand, in which animals of nearly the same size were used, all but the last show a rate of pellet production of the same order, i.e. between 10 and 20 per animal per day over the whole range of concentrations. Thus pellet production appears independent of both concentration and nature of organic food. When the animal is presented with low concentrations of sand particles it produces pellets at much the same rate as when presented with plant cells; but with the highest concentrations of sand particles the rate of



Text-fig. 1. Daily faecal pellet production plotted against particle concentration (both axes logarithmic) for animals of various ages (increasing in size from B–F) feeding on *Phaeodactylum*, and for adult animals feeding on various particles. ·—·—, Sand; ....., *Chlorella*; ----, *Dunaliella*; —, *Phaeodactylum*.

pellet production is increased by a factor of 10. The relationship between rate of pellet production and concentration of sand particles is shown in Fig. 1 plotted on logarithimic axes (to allow all the data to appear in the same figure) and could be represented by a straight line.

If the plots shown in Fig. 1 may be represented by straight lines, and if these lines are extrapolated production of pellets is implied even when no particles are available for ingestion. In fact when animals were deprived of food for some time pale membranous cylinders of similar shape to the usual faecal pellets were found to be scattered over the bottom of the vessel in which they had been living. The cylinders were generally not completely empty but mottled with what appeared to be colonies of bacteria. In order to investigate this further, three male and three female animals (average length 9·0 mm.) were put in each of two vessels, one containing *Phaeo-*

actylum at 10 cells/mm.^{3} and one containing filtered water. The cell concentration in the former was checked and maintained daily. Pellets were counted at the end of the 1st, 2nd, 3rd, 6th, 9th, 12th, 15th and 20th days. At each count the medium in both vessels was completely changed. Results in Table 1 giving pellet totals for each period show quite fair agreement in most cases between the fed and starved animals, and after 20 days the grand total produced was 1525 and 1630 pellets respectively. Taking into account that the experiments were performed on different groups of animals, these figures indicate that animals feeding on a cell concentration of 10 cells/mm.^{3} do not produce a materially different number of pellets as compared with those ingesting no food at all.

Table 1. *Totals of faecal pellets produced by six fed and six unfed animals within each period, and the grand totals*

Day	Fed	Unfed
1	94	127
2	54	36
3	77	88
6	280	199
9	185	360
12	240	200
15	225	270
20	370	350
Total	1525	1630

Table 2. *Mean length and diameter with* ± 95 % *confidence limits of pellets produced by* Artemia *feeding on three different food organisms*

	Phaeodactylum		*Dunaliella*		Sand	
	Length	Diameter	Length	Diameter	Length	Diameter
Mean (mm.)	1·250	0·3900	1·530	0·4250	1·370	0·3650
S.D.	0·102	0·0472	0·351	0·0201	0·258	0·0472
± 95 % limits	0·070	0·0300	0·240	0·0150	0·190	0·0340

To test whether the nature of the ingested particle had any effect on the shape and size of the pellet, three females of length 10 mm. were placed one in each of three vessels containing an adequate amount of *Phaeodactylum*, *Dunaliella* and sand respectively (at least 200 particles/mm.^{3} in 1000 ml.). On the following day the length and diameter of 10 faecal pellets of each were measured. Reference to Table 2 will show that at the 95 % confidence level the mean lengths of pellets are not significantly different. This is also true of mean diameters with the exception that a barely significant difference has been found between *Dunaliella* and sand.

There were no differences in the shape of the pellets, which was in all cases a regular cylinder.

Though there are certainly membranes surrounding pellets containing organic material, their presence could not be demonstrated in association with pellets of sand. These pellets were more frequently found fragmented, although they did not easily disintegrate and on being teased apart it was clear that individual particles were held together by material of a mucoid nature. Sometimes animals which had been left for long periods in water which had deteriorated in condition were found to have a faecal

string emerging from the anus. This string showed up to six regular constrictions along its length, and appeared to be made up of incompletely separated faecal pellets.

Artemia kept without food do not quickly empty their guts, which may appear coloured with food even after 2 weeks' starvation; but microscopical examination revealed the contents to be almost entirely liquid by this time.

Following on from these observations on pellet production, a histological and histochemical examination of the membranes was undertaken to discover more about their nature and origin.

Isolated membranes were first subjected to the chitosan-iodine colour test for chitin. This test has been reviewed by Richards (1951) who states it to be the most reliable test known for chitin. The method used here was largely that of Campbell (1929). Membranes were obtained by keeping twenty large animals in 500 ml. of filtered sea water, from which a copious supply of almost empty membranes could be filtered off. On applying the test the membranes developed the characteristic reddish-violet colour by which chitin is recognized.

Several sections were prepared of the whole animal after fixing in alcoholic Bouin's reagent, dehydrating in alchohol, clearing in xylol and embedding in wax. These sections could not be tested for chitin by virtue of their inevitable destruction in the hot alkali which the method entails. However, selective histochemical stains for polysaccharides in general will stain chitin, so that knowing its existence outside the animal, its position inside the gut can be checked. Such a staining test is the periodic acid/Schiff reaction first used to identify mucin by McManus (1946). Details of the method together with a critical review of past literature are to be found in Casselman (1959).

In animals well fed and with a full gut it was not possible to demonstrate convincingly the presence of a membrane, as the latter was always closely apposed to the gut wall. Similar difficulties were reported by Forster (1953) in *Palaemon*. This problem was overcome by using animals previously starved for 2 weeks. Under such conditions this staining method yielded positive results and the membranes described below were coloured magenta. In addition the pale pink staining reaction of the other material in the gut suggested the presence of mucoid substances.

Sections for a general histological examination were stained with Ehrlich's haematoxylin and counterstained with eosin. These sections revealed that there is little histological differentiation of the gut wall, which along almost its whole length consists primarily of regular columnar granulated cells with large granulated nuclei, and constitutes the midgut (Pl. 1, figs. A and C). The foregut represented by the short narrow oesophagus, and the hindgut occupying the posterior abdominal region, were recognizable by their flatter and more irregularly shaped cells, which were surrounded by a layer of muscle (Pl. 1, figs. B and D). All the regions appear to possess the ability to produce membranes from the inner surface of the epithelium, though the two ends are characterized by membranes which are relatively thicker and follow the cell border more closely, and do not duplicate themselves even in starved animals (Pl. 1, fig. B). The midgut cell walls in animals that have been starved appear to have delaminated several membranes of a more delicate nature which have progressively moved inwards to surround what remains of any food material in the gut lumen (Pl. 1, fig. A). Up to ten layers may be seen. These membranes are present in the guts of

animals which are feeding actively as can be seen from Pl. 1, fig. C, where a membrane is in the process of delaminating from the gut wall. The mechanism of the anal sphincter can be clearly made out from Pl. 1, fig. D. The epithelium is surrounded by a layer of circular muscle to constrict the anal orifice, and radial muscle bands run from the gut to the body wall to open the anus so that a faecal pellet may pass.

DISCUSSION

The membrane described above, being chitinous and produced by the midgut, is equivalent to the peritrophic membrane of Insecta (cf. Forster, 1953). In his recent review on insect digestion Waterhouse (1957) concluded that in view of the fact that many primitive insects and representatives of the Onycophora, Crustacea and Myriopoda possess midgut epithelia with the ability to secrete chitinous membranes, it is probably only secondarily that the capacity has been lost or limited to a small zone at the anterior end of the mid gut. Such an interpretation is supported by the situation in *Artemia*, where the property of secretion of chitinous membranes is possessed by the entire midgut and which belongs to the most primitive group of living Crustacea. Probably the heavier membranes of the ectodermal fore- and midgut are simply the usual chitinous ectodermal secretion continued inside these regions of the gut, and are replaced only as often as the animal moults.

The function of these midgut membranes in insects is commonly stated to be the protection of the gut epithelium from the abrasive action of the food, to replace the mucous secretions which are lacking. However, some liquid-feeding insects also have them, including adult Lepidoptera, Nematocera and Orthorrhapha (Waterhouse, 1957). If such a function is necessary in *Artemia* then the membranes may presumably be useful in this way, but the gut of the animal certainly contains mucoid substances though whether these were derived from the animal itself or its food could not be definitely established. Gauld (1957) suggested that copepod faecal pellets compacted in the membranes quickly fell out of the feeding zone of the animals so that re-ingestion of the food remains is avoided. The pellets of *Artemia* sank very rapidly and if digestion values as high as 90% occurred, as found in *Calanus* by Marshall & Orr (1955), the avoidance of waste material of low food value would be advantageous.

On the basis of the evidence presented here a further function may be suggested for these membranes, namely that of assisting in preventing the food from passing through the gut too quickly. It is here suggested that the back pressure of a maximum rate of egestion is responsible for limiting the rate of ingestion to a certain maximum level. The implication of the observations that all pellets of organic material are invested by a membrane which is produced within the alimentary canal is that the speed at which food passes out of the gut may only equal the speed at which the membrane can be produced to invest it. Nevertheless, the continued pressure of food from the forward regions would force it to be egested faster were it not for the muscular anal region acting as a sphincter in keeping the exit closed. Possibly it relaxes rhythmically with a period corresponding to the time needed for the formation of a length of membrane equal to that of a pellet. On relaxation of the sphincter, the pressure of food from in front together with the contractions of the gut wall, which in this terminal region is provided with a layer of circular muscle, allow a pellet to be extruded. The

sphincter, on contracting again, stops the flow and severs the pellet and its membrane. Failure to do this adequately results in a string of pellets trailing behind the animal.

The only exceptions to this course of events are the phenomena associated with sand ingestion. For some reason, perhaps an irritant action or an effect of the greater weight of compacted sand, the anal sphincter cannot resist pressure upon it and relaxes as frequently as necessary to prevent this pressure building up. Consequently there is no barrier to the much higher ingestion rates recorded (Reeve, 1963*b*) and corresponding rates of pellet production are over ten times the rate to be expected on the basis of size of sand particles. Emerging at this greatly increased rate most sand pellets are without an investing membrane. The ultimate limiting factor (for eventually a maximum ingestion rate is reached) may be the rate of operation of the mouth parts.

The importance of the pressure of the food, caused by the action of the oral appendages tightly ramming particles into the mouth, in moving the food mass through the gut was demonstrated by keeping well fed animals in filtered water. Their guts do not empty as in *Calanus* (Gauld, 1951); instead the food remains in all but the muscular posterior region, and although its contents are almost entirely liquid by the end of 2 weeks the gut still retains a green colour.

When the food is being ingested at anything less than its maximum rate each faecal pellet must contain relatively less material, until ingestion ceases altogether and transparent empty membranes or 'ghosts' are avoided. This fact means that the use of pellet production rate as a measure of food consumed (Harvey *et al.* 1935) is a matter for extreme caution unless the species of animal has been carefully studied with respect to these two factors.

SUMMARY

1. The effects of animal size and of type and concentration of food particles upon the rate of faecal pellet production in *Artemia* have been investigated.

2. The rate of pellet production in starved animals, and the relation between the nature of ingested particles and the size and shape of the pellet, have been determined.

3. Chitinous membranes enclose the pellets. The origin of these membranes from the gut wall has been studied.

4. The possible function of these membranes in relation to the passage of food through the gut is discussed.

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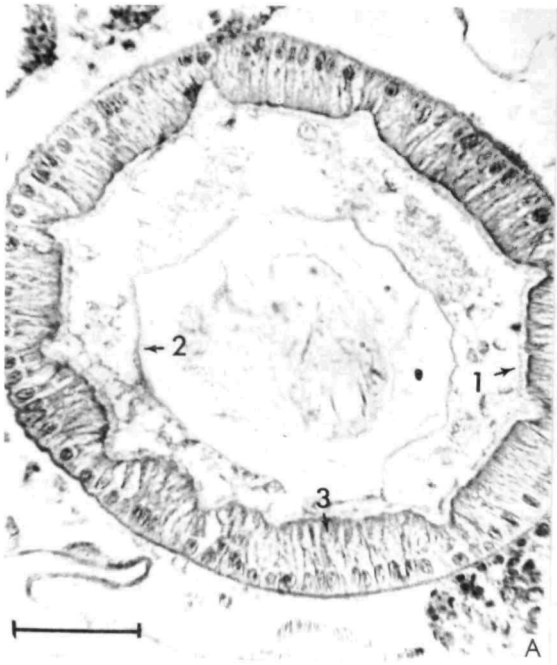
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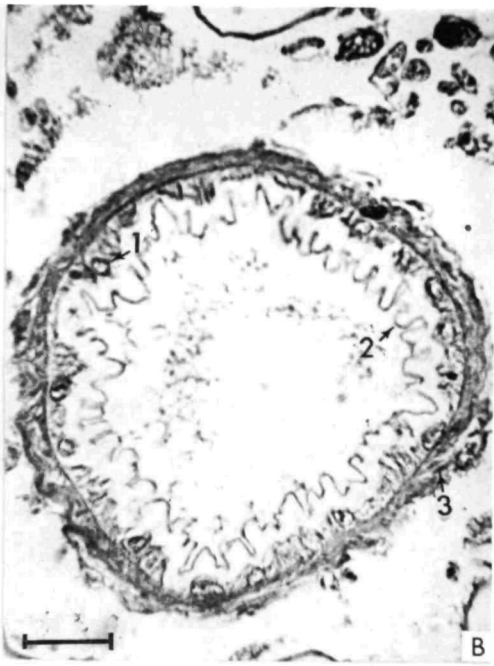
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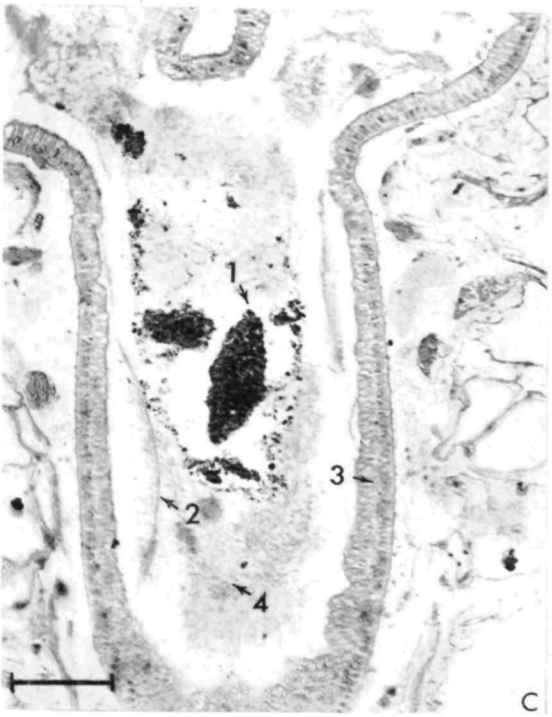
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EXPLANATION OF PLATE

Photomicrographs of transverse sections through the gut of *Artemia*.

A. Midgut, thoracic region showing membrane (1) in process of delaminating from gut wall (3). Several inner membranes (2) are visible. Scale, 30 μ.

B. Hindgut, posterior abdominal region; with flatter irregular cell wall (1), an outer muscle layer (3), and a single thick membrane within (2). Scale, 30 μ.

C. Midgut, head region anterior to mouth, containing food (1), and a membrane (2), delaminating from the cell wall (3). Mucoid substances are also visible (4). Scale, 200 μ.

D. Hindgut through anal opening (4), showing ectodermal membrane (1), muscle band, (3) surrounding gut, and radial muscles (2) to body wall. Scale, 30 μ.