The Digestive Mechanism of the European Squids *Loligo vulgaris, Loligo forbesii, Alloteuthis media,* and *Alloteuthis subulata*

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With two Plates

CONTENTS

SUMMARY

1. The digestive mechanism has been investigated in *Loligo forbesii, L. vulgaris, Alloteuthis media,* and *A. subulata.* Histology and morphology were examined on living and fixed material and the feeding of *L. vulgaris* was observed in the living animal.

2. The food is bitten by the jaws and rapidly swallowed; the radula has no rasping action.

3. Preliminary digestion takes place in the stomach, which is cuticle-lined.

4. The caecum is a complex organ, containing the opening of the mid-gut gland. This opening is connected with the closing mechanism of the caeco-intestinal opening in such a way that the hepato-pancreatic secretion can be directed either to the caecum or to the stomach. The anterior part of the caecum contains a ciliary collecting mechanism whose main groove leads along the intestine towards the anus.

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5. The posterior part of the caecum is a simple ciliated sac where digestion is completed, food absorbed, and pancreatic secretion stored between meals. Solid food other than particulate is never found in the caecum.

6. The intestine is a short, straight tube, lined with a ciliated and mucous epithelium. Absorption also takes place here, and continues after the caecum has ceased absorbing.

7. The junction of intestine and rectum is considered to be defined by the replacement of the ciliated epithelium by one with a hyaline border.

8. The rectum is short; in the region of the rectal sphincter it is lined with cells bearing curious retractile processes, whose possible function is discussed.

9. The mid-gut gland secretes all digestive enzymes; it is divided into two unequal and markedly different glands: the so-called 'liver' and 'pancreas'. These are connected in series, so that hepatic secretion flows through the main lumen of the pancreas to reach the caecum.

10. Hepatic secretion is passed into the caecum only during digestion. The liver has a single type of cell in which food reserves also accumulate. The hepatic duct can be closed by a sphincter.

11. Pancreatic secretion accumulates in the caecum between meals and apparently passes to the stomach during digestion. The structure and rhythm of the cells of the pancreas and the nature of its blood-supply suggest some second activity, possibly directed towards the blood-stream, alternating with that of enzyme-secretion.

12. Food is not absorbed in the mid-gut gland.

13. The structural and functional peculiarities of the digestive system of these squids may be related to an exceptionally speedy and efficient digestive mechanism, well fitted to the life of a perpetually swimming, active predator.

Introduction

THE anatomy and physiology of the Cephalopoda present a fascinating problem to the student of functional morphology.

It is classic to correlate their active predatory life and highly developed organs of locomotion with eye and brain, heart and blood-system, prehensile tentacles and powerful beak; and it has long been known that prey of astonishing size and activity can be captured and killed by these means. The problem of how and where the food so obtained is digested and absorbed, of the extent to which the cephalopod digestive system resembles and differs from that of other Mollusca is clearly one of considerable interest.

The preliminary study described in these pages was made on the two European species of the genus *Loligo*, *L. forbesii* Steenstrup 1856 and *L. vulgaris* Lam. 1799, with supplementary observations on *Alloteuthis subulata* (L. 1798) Naef 1921 and *A. media* (L. 1758) Naef 1921: no significant difference was detected between the European species of these two genera. These forms were originally selected by the present writer because a study of the larval function of the gut during the period of yolk-absorption (Portmann and Bidder, 1928) drew attention to the whole question of the digestive mechanism in the adult. The material proved to be ideal in that the great transparency of the tissues makes it possible to observe much of the digestive mechanism in the living, feeding animal as it swam in a tank. Against this rare advantage must be set the great disadvantage that these animals are so delicate and highly strung that they seldom survive capture unless very special precautions are

observed, and then will often die in captivity before they have recovered sufficiently to feed. As a result, much of the histological material was not derived from animals at a known stage of digestion, beyond the very crude measure given by an examination of the stomach contents, while the observations on the ciliary currents of the gut were largely made on moribund material.

Nevertheless it has proved possible to reconstruct a digestive mechanism which proved to have so many interesting features as to justify the publication of its description, despite the many gaps which, it is hoped, may one day be filled.

The actual observations on living material on which this account is based were largely made in the years 1929–31, with a few additional observations in the summer of 1939 and spring of 1947.

Literature

Very little published work exists on the gut of *Loligo* and none on that of *Alloteuthis*. Short accounts of the morphology are given in Cuvier's monograph on the Mollusca (1817), in the *Leçons d'anatomie comparée* (1805, 1837–40), and in Bourquelot's second paper on the digestive enzymes of the Cephalopoda (1885), and a full account of the digestive system is contained in Williams's monograph (1909) on *Loligo pealeii* Les., and can for the most part be applied to the European species. This monograph did not reach my notice until most of my work on *Loligo* was completed. Through the kindness of Miss Grace Pickford I was able to examine some specimens of *L. pealeii* and confirm that the major differences between my account and that of Williams correspond, in fact, to specific differences. The action of the jaws is discussed by Jordan (1913, p. 353) and the jaws and radula are figured by Naef (1923), for *L. vulgaris* and for *L. forbesii*. (Heinrich's (1904) account of the jaws of '*Loligo todarus*' refers in fact to *Ommatostrephes sagittatus* (Lam.) d'Orbigny.) The histology of the alimentary canal is described shortly by Williams (1909), of the liver briefly by Cuénot (1907), and of the pancreas by Vigelius (1881, 1883). The histology of the alimentary canal is described in detail by Gariaeff (1915) in a long comparative paper on the histology of the Cephalopod gut. This paper is entirely in Russian; through the kindness of Dr. B. P. Uvarov I was able to obtain a translation of the greater part. The digestive enzymes are discussed very briefly by Bourquelot (1882, 1885) and mentioned by Williams (1909); both these authors and Gariaeff (1915) discuss the function of the various parts of the gut; Sellier (1910) gives an account of the proteolytic enzymes. Romijn (1935) investigated the carbohydrate-splitting enzymes. The part played by the larval liver and pancreas in the absorption of the yolk in both *Loligo* and *Alloteuthis* is discussed by Portmann and Bidder (1928). Most of the best known studies of Cephalopod digestive mechanisms either do not mention *Loligo*, or do it so passingly that it is impossible to refer any statement from them to this genus.

Material

Loligo forbesii (Steenstrup 1856) and *Alloteuthis subulata* (L. 1758) Naef 1921 were obtained at Plymouth, *L. vulgaris* Lamark 1799 and *A. media* (L. 1758) Naef 1921 at Naples. No difference in structure or histology has been detected between any of these species, which are therefore treated together. The Plymouth species were chiefly used for morphology and the ciliary currents, which were worked out almost entirely on *Loligo*; some histological material was also obtained from both species, and compared with that obtained at Naples. The living Naples material was all *L. vulgaris*. This species appears to be slightly less sensitive than *L. forbesii*, and it was possible to keep half-grown specimens (less delicate than sexually mature animals) in captivity, and carry out the feeding experiments described on p. 5 and the observations described on pp. 9 ff. In addition, a certain number of young forms (mantle-length 15–50 mm.) were obtained, many of them alive, in which the movements of the digestive organs could be watched under the binocular dissecting microscope. Some of these were probably specimens of *Alloteuthis*, as the two genera are difficult to distinguish when young; but the observations were consistent, so that this possibility only shows the close similarity between the digestive mechanisms of all four species.

This agreement is all the more remarkable as the common American species of *Loligo*, *L. pealeii*, differs from all four European species in a significant point of gut structure (see p. 20).

Technique

The *morphology* was studied in fresh and preserved material of two distinct types: that which was received alive and decapitated for examination, and that which had died in the trawl, or after capture, apparently from 'shock', without visible injury. In material of the first type the tissues remain alive and transparent for a considerable time after death, and the gut shows active and even violent peristaltic contractions, while dissection or manipulation of any kind is liable to start local tonic contractions, producing conditions which are never found in freshly killed material, and are therefore to be regarded as post-mortem effects. In material of the second type the body becomes quickly rigid and opaque, and it was rare to find any but the faintest traces of peristaltic activity in the gut, but post-mortem contraction is of frequent occurrence.

For general morphology material was fixed and preserved in 5 per cent. formalin (2 per cent. formaldehyde), neutralized, and usually made up in sea-water; this was found to preserve the organs with very little shrinkage or loss of colour and the slight maceration did not interfere with the study of general structure, which was confirmed on fresh material. For preservation the mantle was always opened ventrally, so that only the thin body-wall covered the digestive organs.

Serial sections of complete small digestive systems, and plasticine models on an enlarged scale were used in interpreting the details of structure.

The *feeding experiments* were carried out on *Loligo* by the use of traceable foods, originally in the hope of tracing absorption. *Loligo* is difficult to maintain in captivity but living *Loligo* were obtained at Naples, where fishing by man-hauled nets from small boats made it possible to transfer the animals directly from the mouth of the net to a bucket of sea-water. Small shoals of immature animals about 70 mm. in mantle-length, taken in this way, will live for some weeks in the deep, cool tanks of the aquarium and will feed fairly readily. The tanks used for the feeding experiments, however, were, for practical reasons, smaller, shallower, and lighter than the aquarium tanks and the squid were not really healthy or at ease in them; they died more quickly than in the aquarium tanks, often refused to feed at all, and were clearly overstimulated. The capture of one member of a shoal threw the others into a state of agitation which apparently reduced the likelihood of their feeding at a later time.

Fragments of small fish were used as food, and treated with a mixture of iron saccharate and finely ground carmine, and with nile blue (sulphate). These substances were embedded in agar-agar jelly or gelatine jelly, and strips of jelly were inserted in the fish.

It was not easy to persuade the squid to feed, as the mere approach of an observer to the tank, and the dropping of food into the water, frightened them so much that the food had often fallen to the bottom of the tank before they had recovered. Once food had fallen on the bottom, it was rarely taken again. Pieces of food suspended on threads in mid-water were always ignored. The most successful method was to balance food on a loop of wire, fixed into the end of a long piece of glass tubing, so that neither operator nor tool was visible to the squid, and to let the food fall in as gently as possible. Chiefly by this means, between fifteen and twenty animals were fed, during a total period of 3 months.

Animals so fed with iron saccharate were decapitated immediately after capture and opened ventrally. Small fragments of the mid-gut gland were fixed in various fixatives and the whole animal then immediately fixed in ammonium sulphide with Bouin-Dubosq. The solution used was: ammonium sulphide 1 part, 90 per cent. alcohol 11 parts, absolute alcohol 3 parts, an equal volume of alcoholic Bouin being added just before use. This avoided the precipitate obtained with aqueous Bouin (Yonge, 1926, p. 710) or with 90 per cent. alcohol, without the addition of absolute alcohol. After 24 hours the fixative was changed, and the whole digestive system cut transversely into blocks, which were embedded after washing well in 95 per cent. alcohol. Sections were stained with carmalum and treated with 10 per cent. potassium ferrocyanide followed by 1 per cent. hydrochloric acid.

Considerably longer times were required than those given by Hirsch (1925, p. 4) for hydrochloric acid or Yonge (loc. cit.) for potassium ferrocyanide and hydrochloric acid. About 1 hour in potassium ferrocyanide and 10–20 minutes in the acid were necessary to bring out the blue reaction for the iron. It is not certain how much of the colour obtained was derived from the iron saccharate, as some colour was found in animals which had received no iron. This colour

probably represents iron normally present and revealed by the technique. Three types of colouring were obtained: a brilliant greenish-blue in the pen and iridescent sheath of the ink-sac, present at all stages; a diffuse, very pale blue, rapidly fading, and appearing in the blood-system 2–4 hours after feeding and in the muscle after a longer interval; finally, some granules in the liver cell showed the characteristic Prussian-blue reaction, both with and without administered iron. This was the only reaction of interest to the present discussion (see p. 33). Material for histology was obtained from *L. vulgaris*, as already stated, by taking small fragments from each of the animals used in the feeding experiments and therefore killed at known intervals after the taking of food. Material from *L. forbesii* and *A. subulata* was obtained from animals decapitated as they came out of the trawl. The number of minutes which had elapsed since death was noted for each individual fixation.

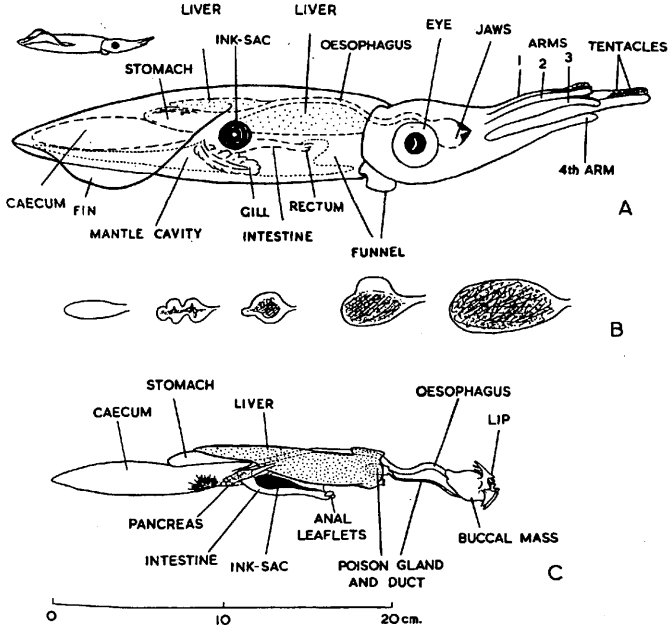
The material used was fixed in Zenker's fluid, Helly's Zenker-formol, Bouin-Dubosq with ammonium sulphide, 5 per cent. formalin, Flemming-without-acetic, and Champy. Sections were cut usually about 6μ thick, and stained with haemalum and eosin or orange G., Mallory, or (most generally useful) with iron haematoxylin (Geigy or Grübler), counter-stained with eosin and light green after Hollande's variation of Prenant's triple stain (Portmann and Bidder, 1928, p. 320) (the method is there attributed to Dubosq; the late Professor Dubosq later informed me that the method was due to Hollande). I have recently received from Professor Portmann the following modification: stain with iron haematoxylin; wash in running tap-water; distilled water; 0·25 per cent. aqueous eosin 2–6 hours (watch); rinse well with distilled water; 1 per cent. phosphomolybdic acid on the slide 10–15 minutes; rinse with tap-water; distilled water; alcohol series to 95 per cent. very quickly (differentiation of eosin); 0·5 per cent. light green in 95 per cent. (watch carefully); absolute alcohol on the slide; xylene; balsam. I have found that Mayer's haemalum can often profitably replace iron haematoxylin.

After Flemming or Champy, sections were sometimes stained with safranin and light green. Ethyl alcohol was used throughout the histology. The pictures obtained with the different fixatives agreed closely on many points, and could usually be equated to what had been seen in the living cell. The best results in general were obtained after fixation with Zenker or Helly. Bouin tended to produce shrinkage of the cytoplasm and a vesicular appearance in the nuclei.

Vital staining was principally confined to the liver and pancreas, but the mucous cells of the ciliated organ of *L. forbesii* were examined at Plymouth with methylene blue. A preliminary examination of the cells of the liver and pancreas was made at Naples with *L. vulgaris*. The living (and dying) cells were examined for general structure unstained, and with neutral red (Gurr), nile blue (sulphate), brilliant cresyl blue, and Janus green B (Höchst). Fresh tissues were tested for fatty substances with nile blue, Sudan III, and osmium tetroxide; *L. forbesii* and *A. subulata* were used at Plymouth, *L. vulgaris* at Naples and Banyuls-sur-Mer.

Observations on the Living Animal

Loligo and *Alloteuthis* are pelagic animals and *L. vulgaris* spends its life, when in captivity, swimming ceaselessly to and fro. The slender, torpedo-shaped body and wide, undulating fins, are shown in Text-fig. 1A. The



Text-fig. 1. A. Young living *Loligo vulgaris* as seen swimming in tank. Optical section of mantle-cavity in dotted outline. Pancreas and details of gills not visible in life. Oesophagus, stomach, and caecum only visible when containing food.

B. Stomach empty, half-full and actively churning, and fully distended.

C. Digestive system from animal's right side.

A, B, and C to same scale.

mantle-musculature is powerful and the animal, besides swimming gently to and fro with the fins, can swim very rapidly either backwards or forwards by ejecting water through the funnel. In swimming forwards the stream from the funnel is directed backwards, as described by Williams (1909, p. 43) for *L. pealeii* and by Russell and Steven (1930) for *Sepia*.

The main parts of the digestive system can be seen through the transparent body-wall and mantle: the jaws, the slender oesophagus and intestine, which

together form a U-shaped alimentary canal, the sac-like stomach and caecum which open side by side into the bend of the U, and the liver, which occupies the anterior half of the body, as the caecum occupies the posterior half. The orientation used in this paper is that of the swimming animal: the pen is 'dorsal', the funnel and mantle-cavity 'ventral', the head and arms 'anterior' the pointed end of the body 'posterior'.

The only parts of the digestive system not visible in the living animal are the buccal glands and poison glands and the 'pancreas' (Text-fig. 2A, B), which is a spongy elaboration of the duct by which the liver opens into the caecum.

Loligo's natural food is fish, crustacea, and smaller squid, all of which have been found in the stomachs of captured animals. The cannibal habit of *L. pealeii* was observed in life by Verrill (1882, p. 354) and recorded from stomach-contents by Williams (1909, p. 2). Examination of the stomach-content of *Alloteuthis* showed a diet and method of feeding exactly as found for *Loligo*; i.e. the stomach contained bitten pieces of fish and crustacea. In the stomach of one female, of mantle-length 80 mm., the entire pen of another squid was found, measuring 19 mm. in length! The viscera had not been rejected as they would have been with a fish (see p. 9), and the stomach and ciliated organ of the prey were found nearly complete, together with the buccal mass, optic ganglia, &c. The body of a squid is soft compared with that of a fish or crustacean of the same size, yet it remains a mystery how such large fragments as those just described could pass through the brain and skull, by a relatively narrow passage, without inconveniencing the eater almost as seriously as the eaten.

In the work here described, only small dead fish and pieces of fish were used. A healthy captive squid will eat once or twice a day; when at liberty, the squid may feed more often, as shown by the stomach-contents of captured animals; but a large number of animals brought in have the stomach empty or containing only indigestible residue, so that (as would be expected with predators) the animals are not continuous feeders even in freedom.

Williams (1909, p. 43) states that *L. pealeii*, when capturing living fish, may lie in wait for them on the bottom of the tank, to which they anchor themselves by their tentacles, and Drew (1911, pp. 337–8) speaks of a squid lying on the bottom 'in the attitude habitually assumed by resting squid'. I have not seen *L. vulgaris* in pursuit of living fish, but, in my experience, it never lies on the bottom unless it is sick and within a few hours of death. There is a clearly marked difference in behaviour between the two species, and I was once informed by the late Dr. E. Sereni, who had worked both at Naples and Woods Hole, that *L. pealeii* is far hardier and more resistant to handling and operation than *L. vulgaris*. The differences may be correlated with their differences in distribution: *L. vulgaris* is usually to be found in 40–50 m. or in deeper waters, while *L. pealeii*, according to Verrill (1882) and Bigelow (1925), habitually occurs in shallow littoral waters. Thus to *L. pealeii* the bottom might be a familiar object, and contact with solid objects a familiar experience, while neither would ever enter into the life of *L. vulgaris*. This would account

for the difficulty shown by *L. vulgaris* in recognizing a piece of food when it has fallen on to the bottom, since, in this squid's normal life, an object which has fallen has gone for ever.

Once a piece of food has been taken by *L. vulgaris*, the procedure is always the same. The fish is seized behind the head and held obliquely with the tail uppermost and is so carried until the head is bitten off and dropped. This takes from 2 to 5 minutes. The trunk of the fish is then held horizontally in the arms, in line with the body of the squid, with the tail sticking out between the arms, and is carried round in this position until the end of the meal. For the first 5 minutes or so the *Loligo* swims backwards only, round and round the tank, often appearing nervous and excitable, with the chromatophores rapidly expanding and contracting in uneven patches. Afterwards it becomes quieter and, for the rest of the meal, swims gently to and fro with contracted chromatophores.

The movements of the jaws (Text-fig. 1A) during the meal are clearly visible. Usually they keep their normal vertical position in the head and work about a horizontal axis; sometimes, however, the buccal mass rotates about the oesophagus, until the jaws are almost horizontal, and working about a vertical axis.

The squid bites through the fish from head to tail by a series of transverse bites. The alimentary canal of the fish is not eaten, but is left hanging down until the anus is reached, when the rectum is bitten through and the whole digestive tract of the fish violently rejected. After this, the meal proceeds steadily until the tail is almost reached. The morsel is then turned obliquely and the flesh bitten off the bones, first on one side and then on the other. (It is then that the twisting movements of the jaws are often seen.) The terminal fused vertebrae and the tail fin are finally rejected.

The duration of the meal is usually 15–20 minutes, but it may take longer if difficulties are encountered in getting rid of the head, or if the prey be unusually large.

Even while the head is being bitten off pieces of flesh can be seen passing down the oesophagus, in rapid procession, to the stomach. The latter becomes visible as soon as the first greyish food particles reach it, and immediately on their entry begins to contract violently, changing its shape rapidly and continually, becoming now oval, now spherical, or ballooning out, now on one side, now on another (Text-fig. 1B). As the meal proceeds and the stomach grows larger, the changes in shape become less marked, but the contractions continue to be plainly visible. By the end of the meal, when the tail vertebrae are rejected, the stomach is about a third of the length of the mantle and half its breadth; contractions cannot then be detected. Almost as soon as the meal is ended the stomach begins to diminish in size, and may become almost invisible (though not necessarily empty) after 2 hours.

At about the same time that the stomach becomes invisible the caecum, usually invisible, becomes a bright tan-brown; this colour remains in the caecum for about 2 hours. During that time the caecal sac appears to be fully

distended, and no contractions of its walls can be observed, though it is possible that slight contractions might be masked by the larger movements of the mantle. About 4 hours after the beginning of the meal the animal becomes, in general, indistinguishable from one which has not been fed. Shortly before the caecum becomes visible the liver often, though not invariably, appears darker and more swollen than usual; and when the caecum is coloured the liver matches it exactly, so that the two organs appear to be continuous. The liver returns to its normal appearance before the caecum; the changes in it are not so rigidly associated with the meal as those of the caecum, and may appear in a hungering animal.

The times given are only a rough indication of what appears to be the normal rate of digestion in captivity, a rate which can vary considerably. Of two animals fed at the same time with meals of the same size, the smaller animal digested its food more than half as fast again as the larger. In another brought into captivity with the stomach full of undigested food, digestion was suspended for at least 4 hours, and then proceeded at something less than the normal rate. This inhibition, which must have been the result of capture, illustrates the highly strung nature of these animals.

Investigations on newly decapitated animals showed that, in early stages of the meal, the stomach is a limp bag, filled with fluid containing lumps of food. Immediately after the lumps are swallowed these are squarish and clear-cut, measuring 2–3 mm. each way, and showing no sign of any rasping radular action or of previous digestion. These lumps are rapidly broken down and the liquid in the stomach becomes filled with flocculent grey masses of digested food and, if a fish has been taken, with a glittering suspension of silvery particles from its scales. As digestion proceeds this suspension disappears, the flocculent matter diminishes in amount, and the contents become more and more compact, until only a mass remains of perfectly clean and transparent skeletal parts of crustacea or fish. These are not necessarily ejected before the next meal, and may be found present with newly swallowed food.

In decapitated animals there may take place a sort of reversible peristalsis, up and down the sac, as well as the violent churnings observed during the meal.

The whole organ can also contract violently, emptying its contents; in hunger it goes into a state of tonic contraction. Death may overtake the stomach when in active contraction and, as a result, stomachs of varying shapes may be found in material which has been dead some time. There can be no doubt that these forms represent what are, in life, but transitory phases of activity. The shape of the stomach in freshly killed material is always a smooth oval.

The caecum of a freshly killed hungering squid is always partly or fully distended and full of colourless liquid, sometimes slightly milky, usually perfectly clear. Within 35 minutes of the beginning of the meal a yellowish or pinkish tinge appears in the liquid, which may also contain a few small, often glittering particles. As the meal proceeds the colour of the caecal liquid deepens in intensity, until it is visible in the living animal (see above); the suspension of

particles also thickens and then disappears; there is some evidence that at least one later influx of particles takes place and is again removed. During the latest stages of the meal, when absorption is proceeding, the caecal contents are milky and yellowish and free from particles. When the meal is ended the caecal contents return to the colourless, limpid condition associated with the hungering condition (Text-fig. 1). No large food masses have ever been observed in the caecum: on this all authors are agreed (Bourqelot, 1885; Williams, 1909; Sellier, 1907, 1910).

It may be assumed that the colour which appears in the caecum during the meal represents hepatic secretion, since the brown of the caecum matches that of the liver exactly (p. 10); a yellowish or brownish colour has never been seen in the stomach.

The suspension of particles comes, as its appearance strongly suggests, from the stomach, and its passage has been observed in decapitated specimens; this was confirmed by a specimen which was killed 35 minutes after feeding, and showed, when sectioned, particles of carmine from the food free within the caecum. (Ample evidence for the penetration of food into the caecum was also obtained from the morphology, the injection experiments (pp. 18 ff.), and histology (pp. 25, 26), which revealed cells of the caecum crammed with absorbed fat.)

The intestine is sometimes found to contain half-digested food, which was certainly prematurely ejected from the stomach at capture or death; it is also often found distended with liquid resembling the caecal contents.

These observations on the living and newly killed animal confirmed those of Williams on *L. pealeii* (1909, p. 44) in so far as the action of the jaws is concerned, and showed that breakdown of the food, to particulate form at least, takes place in the stomach and that liquid and particulate food is then passed into the caecum. It remained to determine where digestion is completed and where the digested food is absorbed: whether in the intestine as maintained by Bourquelot (1885, p. 69), in the caecum, as supposed by Williams (1909, p. 39), or in the liver and caecum, as claimed by Cuénot (1907, p. 237) and Sellier (1910), and where and in what manner the digestive enzymes are produced. Evidence on these questions and details of the mode of action of the various organs were obtained by examining the gross structure and histology of the digestive system, both during the meal and during hunger and starvation.

The Structure and Action of the Digestive System

The Buccal Mass

The general arrangement of the digestive organs is shown in Text-figs. 1 and 2. The buccal mass with its inner and outer circular lips is freely movable and even protrusible, and is surrounded by a well-developed, sucker-bearing buccal membrane (cf. Naef, 1923, p. 179, Text-figs. 77, 78). The jaws are well developed and powerful. The radula is present, and it and the jaws are

figured by Naef (loc. cit., Text-fig. 14, Pls. 14, 17). It will be seen from the description of the meal (p. 9) that the food is swallowed too quickly for any rasping action of the radula to take place: this agrees with the texture of the radular teeth, which are delicate, flexible structures, pale gold in colour, and presenting the strongest possible contrast to the hard, dark jaws. The radula of *Loligo* is a tongue, used in swallowing like the rough tongue of the cat; the rough surface may in some cases be used, as Williams said (1909, p. 44), 'upon resistant objects'. Such an interpretation was already put forward by Owen (1836, p. 532) and by Cuvier (1805, p. 345), who described the movements of the radula, which raises and lowers the teeth 'et passe insensiblement les aliments dans l'oesophage'.

The Glands of the Fore-gut

No detailed study of these organs has been made by the present writer.

The 'anterior salivary', 'buccal', or 'mandibular' gland is small, and lies partly applied to and partly within the buccal mass (Wülker, 1910); the 'posterior salivary' or 'poison' gland, which arises as a paired structure but, in *Loligo*, is single in the adult, lies partly embedded in the anterior end of the liver (Text-fig. 1c) and is connected with the buccal mass by a long duct, running parallel with the oesophagus. The function of the small buccal gland is unknown. Williams (1909, p. 34) found no lipase nor amylase and suggested a proteolytic action. It seems probable that the chief function of this organ is to provide the lubricant necessary for swallowing.

The poison gland has been shown by Bottazzi (1916) and by Bottazzi and Valentini (1924) to secrete tyramine, which poisons the prey, and by Sereni (1928) to have an endocrine effect on the tone of the squid's own muscle. Although most of the results were obtained on *Octopus* and *Eledone*, sufficient comparative data exist to justify extending these results, in this case, to *Loligo*.

Accounts of structure of the buccal gland have been given by Wülker (1910, p. 44, Figs. 43, 44, 45) and of both the buccal and the poison gland by Joubin (1887).

The Musculature of the Digestive Tract

The whole of the alimentary canal is lined with a continuous epithelium, whose cells and their nuclei (generally in the middle of the cell) vary greatly in shape with the degree of contraction of the organs. The lining epithelium of the oesophagus and stomach is cuticle-secreting, that of the caecum and intestine ciliated. The basement membrane is always in intimate connexion with a surrounding network of connective tissue, by which it is separated from the muscular coats with which the alimentary canal is everywhere supplied. These muscle layers are in turn surrounded by a second layer of connective tissue covered by a pavement epithelium which, in the stomach and caecum, is part of the coelomic lining. In the stomach and caecum, layers of connective tissue are interspersed with the layers of muscle-fibres. The connective tissue forms a delicate mesh-work with big, oval nuclei; nerves and

blood-vessels run through it, and strongly developed connective-tissue fibres with long, very slender nuclei; these fibres are seen especially well developed immediately under the lining epithelium. (Gariaeff (1915) states that these are too fine to be easily seen, but after staining with Prenant-Hollande their brilliant green colour makes them easily visible.) The muscle-fibres show the characteristic structure described by Marceau (1904, 1906), i.e. a central core of lightly staining cytoplasm, surrounded by a more darkly staining fibrillar sheath. This sheath consists of very fine longitudinal fibres, arranged parallel to the long axis of the cell or slightly twisted round it and thus showing none of the transverse striation due to close twisting described by Marceau (1905) for the mantle cells of *Loligo vulgaris* and *Sepia officinalis*, and for the mantle and heart cells of *Octopus vulgaris* (but not found by him in the arm muscle-fibres), and plainly visible in the heart cells of *Loligo*. Only in one region of the muscular part of the hepatic duct (pp. 32, 36) some fibres are to be found, lying between the lining epithelium and non-striated fibres, which have the striated appearance. All the muscle-cells are very long, so that nuclei but rarely appear in the sections. Gariaeff describes spiral striation in the muscles of the alimentary canal, but the present writer has not been able to confirm this.

The Oesophagus and Stomach

The *oesophagus* (Text-fig. 1) lies in a groove between the liver and pen, and passes through the liver in an obliquely ventral direction to open into the stomach. It is a muscular organ by whose active peristalsis the fragments of food are passed rapidly from mouth to stomach. There is no crop.

The musculature consists chiefly of a well-developed layer of circular muscles; the inner longitudinal muscle-fibres are for the most part confined to a series of longitudinal strands, which are reinforced by connective-tissue fibres, show as faint ridges in the dilated organ, and, in the contracted state, form deep folds obliterating the lumen of the oesophagus. Nerves and blood-vessels run in both of the connective-tissue layers. The great depth of connective tissue figured by Gariaeff (1915, Pl. IV, fig. 48) between the lining epithelium and muscle layers has not been found by the present writer.

The *stomach* (Text-fig. 2) is a very muscular sac, lying on the animal's right side, with the oesophagus and intestine opening side by side into the anterior end. The muscles (whose action is described on pp. 9, 10) are arranged in wide, interwoven bundles, many of which run obliquely; the mesh-work of connective tissue in which the blood-vessels run penetrates the muscle layers and surrounds and separates the bundle of muscle-fibres.

Both oesophagus and stomach are lined with a soft, smooth, colourless, distendable cuticle. Miss C. H. Brown kindly examined the fresh cuticle of *Loligo* and reports as follows:

'Millon's, the xanthoproteic and ninhydrin tests for proteins all gave positive results, indicating the presence of proteins in the membrane. The lead acetate test for sulphur was also very slightly positive.

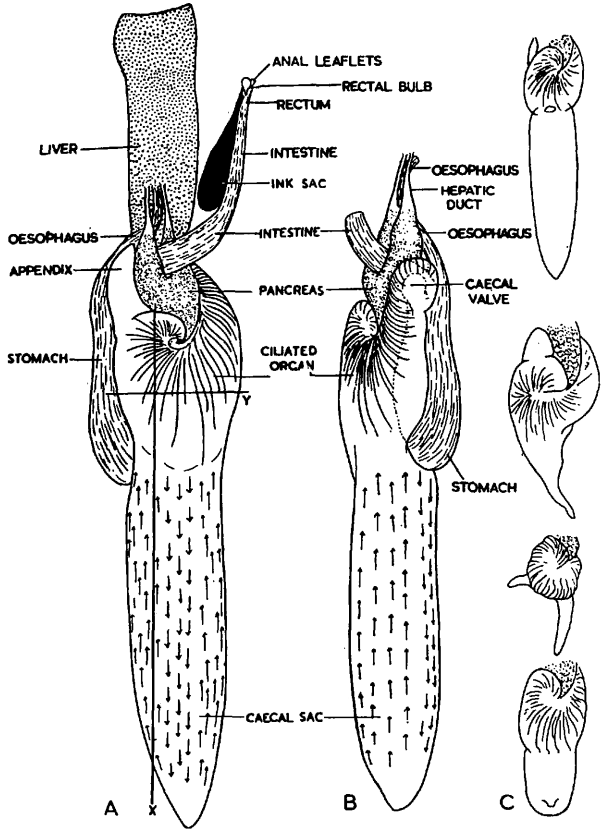
'The material would not dissolve in boiling water but thickened and contracted. Dilute acid had no apparent effect, but concentrated hydrochloric acid dissolved the membrane completely. On boiling in dilute or concentrated sodium hydroxide solution there was always an insoluble residue and this residue gave a positive chitin test with iodine followed by dilute sulphuric acid. Swelling agents such as lithium iodide and urea caused the membrane to swell slightly but not dissolve. The argentaffin and diazo tests for polyphenols in the membrane were negative.

'It is concluded, therefore, that the stomach lining of *Loligo* is composed of a chitin protein complex.'

The cuticle varies greatly in thickness with the state of contraction of the organ; this variation is much greater in the stomach than in the oesophagus. In the distended stomach the cuticle is about 10μ thick; in the contracted stomach the cuticle is both greatly thickened (reaching, in the adult organ, a thickness of 350–400μ) and thrown into rounded folds, which become sharp and hard after most fixatives. Similar ridges in other cephalopods have been described as 'grinding ridges': in *Loligo* the observations on the living and freshly dead animal show that the action of the stomach is here a churning, not a grinding action, and, indeed, the ridges are only fully developed when the stomach is empty, or contains only indigestible residue. This residue (p. 10) consists of sharp, hard fragments of bone and chitin, and the thickened, contracted cuticle will afford the gastric epithelium the same protection as can be given to the intestine during defaecation by copious mucus-secretion (p. 27).

The lining epithelium of both the oesophagus and stomach shows no sign of activity other than that of cuticle-secretion. The histology of the two epithelia is closely similar. The cells are cubical to columnar, according to the state of contraction, and the cytoplasm finely fibrillar. The fibrillae are continuous with the basement membrane, and through it are in such intimate contact with the underlying connective tissue that sections have been found in which the cells, torn away by the knife, left their fibrillae adhering, brush-like, to the underlying layers. In the stomach these fibrillae extend through the whole depth of the cell and appear to be continuous with a fibrillar formative layer which is present everywhere between the epithelium and the finished cuticle. At times, indeed, the cell-border disappears altogether, and the intra- and extracellular fibrillae can only be distinguished by the acidophil staining of the formative layer (Pl. I, fig. 5). The finished cuticle appears to be formed of two substances: one, the acidophil fibrillar sheet, the other represented by irregular, basiphil masses (staining red with Mallory), sometimes visible within the cell, sometimes just outside the cell-border, sometimes amongst the strands of the fibrillar layer (Pl. I, figs. 3, 4). This double method of secretion may be compared with the mixed nature of the cuticle found by Miss C. H. Brown and indicated by a rather muddy acidophil staining often shown by the cuticle. (The formative layer is described and figured by Gariaeff for other Cephalopod genera (1915, p. 73, Pl. VIII).)

In the oesophagus the formative layer is not always present, and, when present, is very narrow. The double staining found in the gastric formative



TEXT-FIG. 2. A, B. Mid-gut and mid-gut glands of *Loligo*; A, ventral view, B, seen from the animal's left side. The arrows mark alternative systems of ciliary currents in the caecal sac (see p. 22). (Lines X and Y mark planes of section of Text-figs. 3 A and 4 A respectively.)

C. Four sketches of the caecum showing post-mortem variations in size and shape—all drawn to the same scale.

layer has occasionally been seen in that of the oesophagus, and basiphil granular fragments occasionally detected within the cells. When the formative layer is absent, the cells have a very narrow but brightly acidophil cell-border. The fibrillar layer cannot be traced to the cell-border (this is confirmed by Gariaeff, 1915, p. 49) and there is no visible connexion between it and the cuticle. This may be a qualitative difference; the other differences are probably purely

quantitative, representing a lower rate of secretion. This difference may be related to a very much lower rate of wear and tear in the oesophagus than in the stomach: food passes rapidly along the oesophagus, and at a stage at which the sharp parts are still largely protected by flesh. Since, however, the oesophagus is ectodermal, the stomach mesendodermal in origin (Ranzi, 1928, p. 93), some qualitative difference between the two epithelia is not wholly improbable.

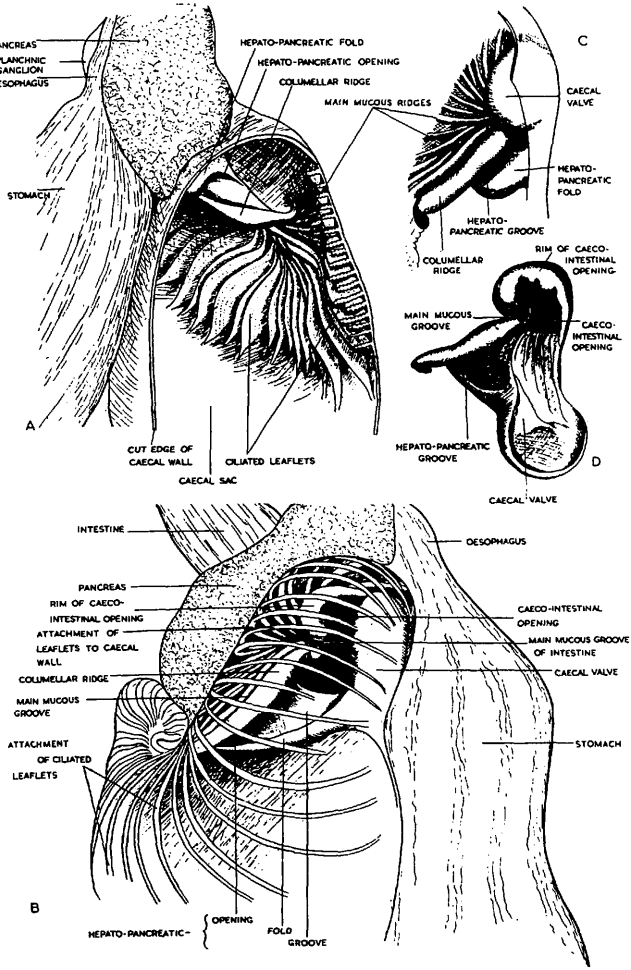
The oesophagus is thus purely a passage: the stomach is a place of preliminary digestion where, under the action of enzymes formed elsewhere (p. 36), the food is broken down to particulate form and separated very completely from larger indigestible residue. The only gastric secretion is that of the protective lining cuticle.

The Caecum

The caecum has three activities: first, as has long been recognized, it is the ante-chamber between the mid-gut gland, which is the source of digestive enzymes (p. 31), and the stomach, where the preliminary digestive breakdown takes place (p. 10); secondly, it is, as will be shown, the place of final digestion and of absorption; thirdly, associated with its absorptive function, it contains an elaborate ciliate and mucous collecting mechanism whose function would seem to be to clear the nutrient fluid contents of all solid particles. These three independent functions modify and complicate the internal anatomy of the organ, which is illustrated in Text-figs. 3 and 4.

The caecum of *Loligo* consists of two parts: an anterior, spiral portion, of about one and a half turns, and a long sac, blown out, as it were, from the posterior side of the spiral (Text-fig. 2). The spiral portion has its axis obliquely dorso-ventral, and the last half-turn is separated from the rest by the wedge-shaped pancreas (p. 32) whose thin end runs in the columella of the spiral and opens at its apex; the caeco-intestinal opening is at the other end of the spiral, at the mouth, as it were, of the snail-shell. These two openings are connected by a groove, the 'hepato-pancreatic groove', which runs round the columella of the spiral, and is bounded on one side by a well-marked ridge, the 'columellar ridge', and on the other by a fold, the 'hepato-pancreatic fold'. This fold can shut down on the columellar ridge, and convert the hepato-pancreatic groove into a tube, leading direct from the hepato-pancreatic duct into the intestine.

The ventral surface of this part of the caecum is set with a number of leaf-like ciliated folds, projecting across the spiral, and alternating in length, so as to define a system of converging 'interleaflet grooves', all ultimately leading into a 'main mucous collecting groove' (Text-figs. 3, 4), which runs round the columella, parallel to the hepato-pancreatic groove, and separated from it by the columellar ridge. The leaflets are themselves ridged and grooved with 'primary' and 'secondary' leaflet grooves (Text-fig. 5; Pl. II), so that each interleaflet groove is itself a collecting groove for the two leaflet faces which bound it, and the whole forms a ciliate and mucous collecting mechanism, whose



TEXT-FIG. 3. A. Anterior end of caecum (plane of section shown by Text-fig. 2A, *X*) showing hepato-pancreatic opening.

B. The same, showing the caecal valve; the ciliated leaflets indicated by their bases only.

C. The caecal valve shut. D. Open.

A, B, drawn from living post-larvae.

action is described on pp. 22 ff. Williams (1909, p. 36) described the arrangement of the leaflets in *L. pealeii*, but regarded them as radiating from, not converging towards, the columellar ridge and caeco-intestinal opening. Gariaeff (1915, p. 51) regarded them as glandular. Where the form of the spiral is unaffected by the sac, the leaflets run across the spiral, so that their lines of attachment, seen from the outside, resemble the lines of growth on a snail-shell.

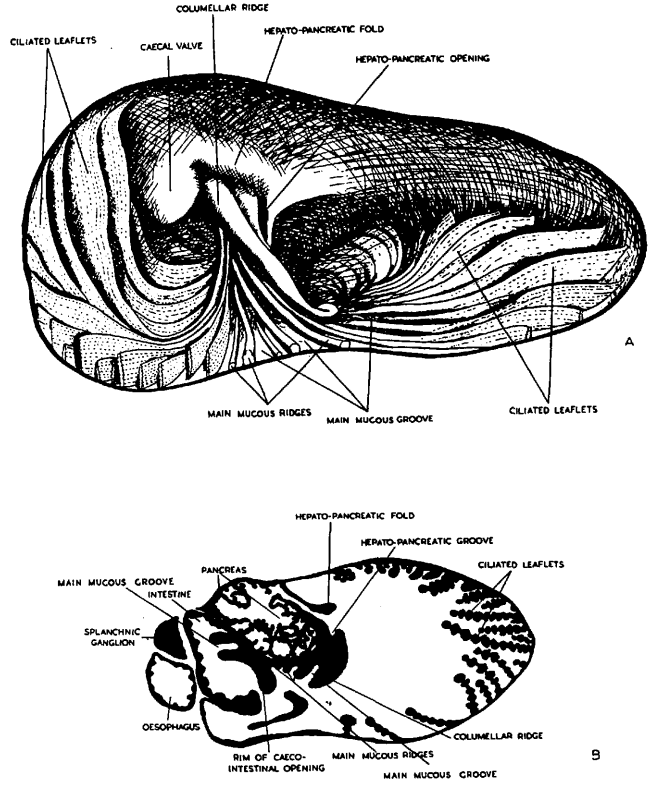
The caeco-intestinal opening is protected by an elaborate closing mechanism: the hepato-pancreatic fold is expanded at the opening into a saucer-shaped lid, and the opening has a thick rim or lip on to which the lid can close (Text-fig. 3). This rim curls round where the columellar ridge passes out of the caecum, and forms with it the main mucous groove in the intestine (p. 27, Text-fig. 4). As a result, the lid shuts down across the columellar ridge and groove, without blocking the latter, which opens freely into the intestine, even when the lid is closed (Text-fig. 3). Moreover, since the lid is continuous with the hepato-pancreatic fold, then when the lid is closed on to the rim and the hepato-pancreatic fold closed down on to the columellar ridge, the hepato-pancreatic groove is shut off from the caecum but communicates freely with the intestine and stomach. It seems probable that the fold and lid act together, so that the hepato-pancreatic duct is only open to the caecum when the caeco-intestinal aperture is also open (cf. p. 39).

The action of the valve and fold thus controls the movements of the caecal contents and the passage of enzymes into the caecum, but not the action of the ciliated organ, nor the passage of the enzymes from the mid-gut gland to the stomach. Its function also appears to be to exclude large food particles from the caecum: masses of debris are often to be found accumulated against the intestinal face of the valve although never found in the caecum (p. 11). It is not easy to envisage how this exclusion takes place, unless contact between solid food and the valve or rim stimulates the valve to close.

Both valve and rim are solid structures, reinforced by cartilage, and the valve is muscular and under nervous control and richly supplied with nerves from the splanchnic ganglion (Alexandrowicz (1928) and personal observations with methylene blue). A sphincter-like muscle runs in the edge of the valve, and can tie it down over the rim, as the cover is tied down over the rim of a jam jar. A valve which had been cut off from the caecum showed slow contractions across the valve and round the rim, showing that the closing action of the valve is due to intrinsic musculature. The valve is attached to the wall of the caecum (Text-fig. 3), so that, when valve and caecal muscles are both relaxed, and the anterior part of the caecum distended, the valve is held widely open, and fluid can pass freely in or out of the caecum. Alternatively it can act as a simple valve, allowing ingress to but not egress from the caecum, or, fastened down over the rim, it can isolate the caecal contents completely. Observations suggest that all three alternatives are employed during the digestive cycle.

Injections of carmine in sea-water into the gut, through the mouth, demon-

strate this complex action. Out of thirty-seven injections, thirty-three entered the caecum readily, sometimes more readily than the stomach. In four animals carmine failed at first to enter the caecum, but entered it



TEXT-FIG. 4. A. Anterior end of caecum of adult *Loligo* viewed from the sac-plane of the section shown in Text-fig. 2A.

B. T.S. of the same region of a young *Loligo*, passing through the caecal valve.

later; this, in view of the successful injections, could not be due to a simple valve-action, but must rather be due to muscular action of the caecal valve. Finally, in a small specimen whose stomach was still actively contracting, the contractions were seen alternately to pump food into the caecum and to suck it back again, which could only take place if the valve were held widely open.

The presence of the valve has been described by Bourquelot (1885, p. 67). Williams (1909, pp. 36–8, Pl. I, fig. 2, Pl. III, figs. 18 and 19) gives a detailed account of it in *L. pealeii*, where the valve is present, but with an extra protection to the hepato-pancreatic opening which it is hoped to discuss in a later paper. According to Bourquelot it is mentioned by Cuvier (1838), but the passage, which was posthumously inserted by Dunervois, appears to refer to the main mucous ridges. Bourquelot's interpretation of the valve in *L. vulgaris* is in direct disagreement with mine. For him the valve opened into the intestine, acted as a simple flap-valve, and could be applied either to the caecal opening to prevent the entry of food passing from the stomach to the intestine, or to the intestinal opening to prevent the entry of digestive juices, passing from the caecum to the stomach. Williams appears to have misinterpreted Bourquelot's account, for he says: 'All these facts lead us to the following conclusion *which agrees with that of Bourquelot* . . . that the food is comminuted and partly digested in the stomach . . . [and] that the partially digested food then passes into the caecum for complete digestion and absorption' (Williams, 1909, p. 38; the italics are mine). Bourquelot's own words are: 'Ainsi donc la digestion se fait tout entière dans l'estomac. . . . Les aliments ne passent pas dans le caecum intestinal: une disposition anatomique spéciale s'y oppose' (Bourquelot, 1885, pp. 68, 71). For Bourquelot the caecum was thus a sort of enormous gall-bladder. In a large number of animals examined by the present writer no trace of the caecal valve has been seen in the intestine from which the whole apparatus is completely invisible.

The caecum further possesses, at the level of the ciliated organ, a triangular pocket, the 'appendix' (Text-fig. 2), which, like the sac, shows great variations in size and shape.

Thus, as already stated, the caecum of *Loligo* consists of two parts: the anterior part, containing the appendix, the ciliated organ, the openings from the mid-gut gland and intestine, and all the complicated structures associated therewith, and the posterior, simple sac. The whole organ is muscular and lined with a ciliated epithelium which, in the ciliated organ, is interspersed with mucous cells.

The caecum of a freshly killed squid is always relaxed and inert. The irritation of dissection and handling the animal, however carefully, often starts contractions of the caecal sac, either gentle waves of contraction, up and down the sac, stirring and mixing the contents, or a more violent pumping driving out the contents, or slow tonic contractions which may ultimately reduce the sac to a tiny, thick-walled, finger-like process (Text-fig. 2c). In trawl-material such a minute sac is not infrequently found, but, as it has never been found in freshly killed material, it should probably be regarded as a purely post-mortem effect, similar to the distorted conditions found in the stomach in the same material (p. 10). In some animals the volume of the sac is somewhat restricted by ripe gonads or by an unusually full stomach, and the appendix may then be greatly enlarged, suggesting that its function is to compensate at times for a constricted sac. Sometimes

the appendix shows active pumping movements, which violently stir its contents.

The sac can be partly cut off from the ciliated organ by a diaphragm-like sphincter (Text-fig. 2c), or by local contractions of the whole anterior region of the sac-wall.

The ciliated organ can contract as a whole, and rhythmic contractions have been seen passing round the spiral. Individual leaflets show slow transverse twitches, which open and close the leaflet grooves and can go on for some hours after the caecum is dissected out. Longitudinal tonic contraction of the leaflets may be due to intrinsic leaflet musculature, or to the general musculature of that part of the caecal wall.

Ciliary Currents of the Caecum

Most of the animals used for observing the ciliary currents had died in the trawl; observations were confirmed wherever possible on decapitated specimens. In general a suspension of finely ground carmine in sea-water was used to determine the currents. The mantle-cavity was opened from the ventral side and the caecum exposed. Carmine was injected through the mouth, until it just appeared in the caecum; a hypodermic syringe was used, with a large needle whose point had been ground away so as to minimize the risk of piercing the thin wall of the oesophagus. The currents were observed through the wall of the caecum, without further dissection. If it was desired to re-examine the animal, after an interval, the whole animal was left under sea-water circulation until required again.

The ciliated organ and caecal sac were also opened and examined independently under the microscope and binocular dissecting microscope, with and without the use of carmine. A dissecting dish was made by lining a flat-bottomed glass dish with paraffin wax, so as to leave a clear space in the middle; and the sac was thus examined by transmitted light.

The currents in material which had died of shock in the trawl (p. 4) were enormously slower than in decapitated material. This had its advantage in the observation of detail, but a disadvantage in the resulting accumulation of mucus, which tended to block the action of the ciliated organ altogether. The cilia of the ciliated organ in 'shock material' were almost invariably inactive when the injection was made, and only began to take effect after 1–3 minutes. This inactivity was never found in decapitated material: it may be related to the fact that trawl-material was inevitably only examined several hours after capture, during which time the cells of the close-set leaflets would become increasingly short of oxygen and under the influence of their own metabolites.

The cilia of the sac act without mucus; particles carried in the currents move freely with respect to each other. Save for a narrow transverse tract, immediately posterior to the ciliated organ, the beat of all the cilia is parallel to the long axis of the sac. In the most usual condition observed, the transverse current gathered particles into a narrow ventral tract which beat into the sac, sweeping particles away from the ciliated organ. The currents of the rest of

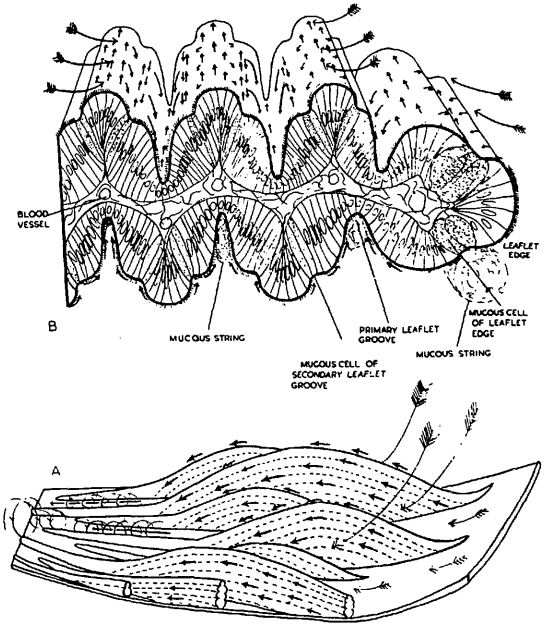
e sac lining were usually directed out of the sac (Text-fig. 2A). In one or two specimens, however, the ventral 'in' current was absent, and in some a corresponding dorsal current was observed (Text-fig. 2B). In one specimen at least, the 'in' and 'out' tracts were of equal width.

These observations must be interpreted as reversals of ciliary current within the sac. Normal individual variations of this magnitude are foreign to all our experience of ciliary tracts; nor can action at a distance, by opposing tracts, on a sheet of mucus, be here in question, since the sac is free of mucus. Again, we cannot postulate here a change of direction effected, as in other molluscan ciliary systems such as the labial palps of lamellibranchs (Kellog, 1915; Yonge, 1926), by opposing tracts on ridges and grooves, whose effect may be controlled by opening or closing the grooves through muscular action. The currents in the sac are all parallel to its long axis, but the sac-wall contracts, not into parallel folds, but into a network of fine wrinkles. The significance of this reversal is hard to find, for in view of the sac's muscular activity (p. 20) the cilia of the sac would seem to function simply as stirrers of the whole contents when the muscles of the sac are completely relaxed; of the layer immediately next the lining epithelium when the muscles are active (a layer which, owing to the friction between it and the epithelium, is but little affected by mass movements of the caecal contents). In either case, any of the systems of tracts described above would appear to be equally effective.

The *ciliated organ* has a mode of action and effect entirely different from that of the sac; its cilia act with mucus, and gather up all particles within range (nutritious and otherwise) into a number of mucus-strings, which are gradually twisted together and passed into the main mucous collecting groove (Text-figs. 3, 4) and so out of the caecum, along the intestine, to the anus. The details of the tracts are shown in Text-fig. 5. The apical cilia of the leaflet ridges combine with cilia of the caecal wall between the tips of the longest leaflets to set up a general drift towards the caecal opening. With the exception of two narrow opposing tracts on the shoulders of each leaflet ridge, the rest of the cilia on the ridges beat down into the 'primary' leaflet grooves, carrying particles from the food and balls of mucus secreted by the cells of the 'secondary' leaflet grooves (p. 16, Pl. II). In the depths of the groove, particles and mucus combine into a mucus-string, which is swiftly carried towards the junction of each leaflet groove with the inter-leaflet groove, i.e. towards the columella of the spiral. The two opposing tracts on the shoulder of the ridges are so narrow that particles often miss them altogether. Sometimes, however, these tracts set up eddies and delay the entry of particles into the grooves. An aggregate of particles, caught in this eddy, will be spread out into a thin line before being swept down into the groove, thus reducing the risk of the groove becoming choked with large masses of particles. The whole action is normally very swift, and carmine appears in the inter-leaflet grooves an astonishingly short time after its injection into the caecum; but, once the leaflet grooves have become choked by a large mucus-string, the cilia of the grooves become powerless.

Especially thick mucus-strings are found in the grooves on each side of the leaflet edges (Text-fig. 5B). The size of these strings is probably due to the specially viscous nature of the mucus which forms them (p. 26).

The collecting-ridge currents are similar to those of the leaflet edges, with which they are continuous. The cilia of the collecting grooves must have a rotating action, as the strings in the larger grooves are found to be complex



TEXT-FIG. 5. Ciliary currents of the caecal leaflets. A. Portion of caecal wall, and attached leaflets. B. Solid section of the edge of a single main leaflet. Water-currents shown by feathered, mucus-currents by plain, arrows.

ropes of many strands, twisted together. The final result is passed out by the main mucous groove of the caecum into that of the intestine (p. 27) and so to the anus.

The caecal valve is covered with a ciliary-mucous-epithelium and particles impinging against its caecal surface are swept in a sheet of mucus round the edge to the intestinal surface.

Those particles which escape the cilia of the leaflet edges are caught into the leaflet grooves, and those which escape the mucus of the leaflets are caught by the collecting ridges and sent down into the collecting grooves, or even into

the main groove itself; and, should any still escape, they are swept against the caecal valve, and become entangled there in a mucus-sheet, in which they are swept round the edge of the valve into the intestine.

There is no sign, in all this elaboration, of any sorting mechanism. There is no evidence that, even in the thicker strings formed at the free edges of the leaflets, larger particles are entangled than those in the leaflet grooves. Further, were it so, there would still be no real sorting, since all strings are ultimately twisted together into the same rope. A sorting mechanism is scarcely to be expected in a mechanism purely active in removing waste and dealing with particles of small average size: only particles 10μ and less in diameter are normally to be found in the mucus-strings: the ciliated organ is purely a collecting mechanism, which removes all solid particles within its range out of the caecum into the intestine. Some food particles are caught into the strings before they can be digested in the sac: this is the only wastage of assimilable food in the whole digestive mechanism (cf. p. 10).

The significance of the leaflet currents would seem to be to clean the apical and latero-frontal regions as quickly as possible, sweeping all particles down into the leaflet grooves where, unless the mucus-strings are abnormally thick, they are deeply buried, so that the upper parts of the ridges are left free for absorption (p. 26, Text-figs. 5B, 6E).

The caecum thus may be regarded as two separate organs which can, in fact, be partially isolated from each other (p. 21). The sac is an organ of final digestion and absorption, its muscles and cilia combine to change and stir its contents; the ciliated organ, hepato-pancreatic groove and fold, and the caecal valve give the anterior part a complexity of structure corresponding to the multiplicity of its function.

The Histology of the Caecum

The arrangement of the muscle layers in the caecal sac is the reverse of that in the oesophagus and intestine: the inner layer is of circular, the outer of longitudinal muscle-fibres. The two muscle layers are separated from each other, and from the lining epithelium and covering peritoneum of the caecum, by layers of delicate connective tissue, set through with connective-tissue fibres. These fibres are particularly plentiful between the circular muscle layer and the lining epithelium of the sac. The middle layer, which lies between the two sets of muscle-fibres, and which has no equivalent in the oesophagus or intestine, contains a rich blood-supply, corresponding to the organ's absorptive activity. The outermost connective-tissue layer contains scattered muscle-fibres running in all directions, as well as connective-tissue fibres, some of which, running radially, penetrate the layer of longitudinal muscle-fibres.

In the ciliated organ the orientation of the muscle-fibres is difficult to trace: there is some evidence that the inner layer runs across, the outer layer round, the spiral. Connective-tissue fibres are particularly well developed immediately below the ciliated epithelium and reinforce the ciliated leaflets, in which Gariaeff (1915, p. 52) has described isolated muscle-fibres. The leaflets

are well supplied with blood-vessels, lying, as in the sac, in a layer of connective tissue in between the two muscle layers.

The caecal valve, columellar ridge, and main mucous ridges are all reinforced with cartilage, apparently indistinguishable from vertebrate cartilage.

The sac of the caecum is lined with a ciliated epithelium, whose cells show, according to the degree of contraction of the surrounding muscular coat, every form from pavement to deep columnar epithelium. The cilia have basal granules, and parallel rootlets penetrating a short distance into the cytoplasm; they are not always present in sections, but their action is always readily demonstrable, even in animals which have been dead for some hours, and at all stages of digestion. The nucleus is oval, with one or more nucleoli, and varies in proportions with the changing proportions of the cell. The cytoplasm appears homogeneous or finely granular. A small acidophil, or faintly basiphil granule occasionally appears between the nucleus and the cell-border, which is well stained with eosin in Prenant-Hollande preparations.

The cells show typical fat-absorption pictures after treatment with osmium tetroxide (Text-fig. 6F), pictures which must represent absorption after complete extracellular digestion. There is no histological evidence of secretion by the lining epithelium of the sac.

The ciliated cells of the ciliated organ are columnar cells, whose individual variations in height assist in the definition of the leaflet ridges and grooves. Alone in all the lining cells of the alimentary canal, these cells do not fluctuate in shape. The cilia are strongly developed, and show basal granules and parallel rootlets, which have been traced half-way between the free edge of the cell and the nucleus (Pl. II). The nucleus, in the middle of the cell, is large and oval, with one or more small nucleoli. The cytoplasm is diffusely granular after all fixatives used, and shows great variation in depth of staining. This is partly, at least, related to the age of the cell: the extrusion of worn-out cells, narrow and very darkly staining, appears not infrequently in sections, but it seems possible that these variations may also represent a rhythm of secretion; the palest cells have a slightly distended appearance, and the cytoplasm distal to the nucleus one consistent with accumulations of diffuse secretion, possibly of mucus-secretion, possibly connected with the enzyme system. The actual secreting surface is much less than that provided by the mid-gut gland, but an activator might be produced by the ciliated leaflets, and this was in fact claimed by Henri (1903) for the leaflets of *Sepia officinalis*, and has been confirmed by Romijn (1935, p. 413). Replacement of the worn-out cells is supplied by cell-divisions, whose mitotic figures have been observed situated close under the cell-border (Text-fig. 6C) as described by Gutheil (1911, Figs. 5–16) and Saguchi (1917, p. 257, Pl. I, fig. 19). A small body appears in the distal half of many cells, chiefly those of the lateral ridges (Text-fig. 6D, Pl. 2). It appears, after fixation with either Helly or Flemming-without-acetic, to consist of an imperfect, basiphil, spherical shell, resembling a unit of the Golgi apparatus, and arising near the nucleus. It apparently moves up the cell, changing as it nears the cell-border into an irregular basiphil mass,

or breaking up into a number of basiphil granules, and is finally extruded. The cells of the ciliated organ show a marked rhythm in regard to this structure: cells of one ridge showing very closely the same phase, and of a whole leaflet, less exactly so; this same rhythm of individual leaflet ridges is also traceable in the occurrence of the mitotic figures. The cilia of the cells of the apical ridges appear to have shorter rootlets than those of the lateral ridges; among the apical rootlets, clusters of basiphil granules may be seen (Pl. II).

The ciliated cells of the ciliated organ do not show the same active absorption shown by those of the sac or of the intestine. The ciliated organ of an animal whose sac and intestine were in active absorption showed fat-droplets chiefly in the cells of a few leaflet ridges near the caeco-intestinal opening; these fat-droplets were very fine and scattered evenly through the upper part of the cell (Text-fig. 6E).

The mucous cells of the ciliated organ are of two types. One, forming the secondary leaflet groove, is a slender cell, staining darkly with haematoxylin in preserved material, readily with methylene blue in life, and extruding a basiphil ball of mucus. The second does not take up methylene blue in life, gives the typical blue stain with Mallory, and green with Prenant-Hollande; the unripe cell is densely granular and darkly staining, the ripe cell greatly swollen, with paler contents (Text-fig. 6 A, B). These cells occur frequently in groups, opening by a common pore, and the extrusion of the sticky secretion can readily be observed in life. They occur thickly all over the caecal valve, columellar ridge, and adjacent main mucous collecting ridges, and line the caecal wall in this region. They are confined, in the leaflets, to the free edges of the largest leaflets (Text-fig. 5A), particularly towards the collecting ridges, although scattered isolated cells of this type appear occasionally on secondary leaflet ridges, close to the mergence of the leaflet grooves with the collecting grooves. Gariaeff (1915, p. 51, Pl. VI, figs. 70, 71) figures both types of cell; he describes the cells of the secondary leaflet ridges as mucous, but not those of the leaflet edges, despite their reactions with Mallory, as he failed to stain them with mucicarmine. Their staining with Mallory and Prenant as well as their appearance and behaviour leave little doubt as to their nature.

Thus, with the exception of the hepato-pancreatic groove, the caecum's whole activity is concerned with the final stages of a complete extracellular digestive breakdown and with the absorption of the results thereof. Absorption takes place principally in the sac, facilitated by the absence of mucus and the resulting stirring action of the sac cilia (p. 22). It may be noted that the blood-supply, though well developed, is not of that great richness to be anticipated in an organ of absorption of this type, and the possibility cannot be excluded that some of the absorbed products pass through the thin layer of the caecal wall directly into the fluids of the perivisceral coelom.

The Intestine and Rectum

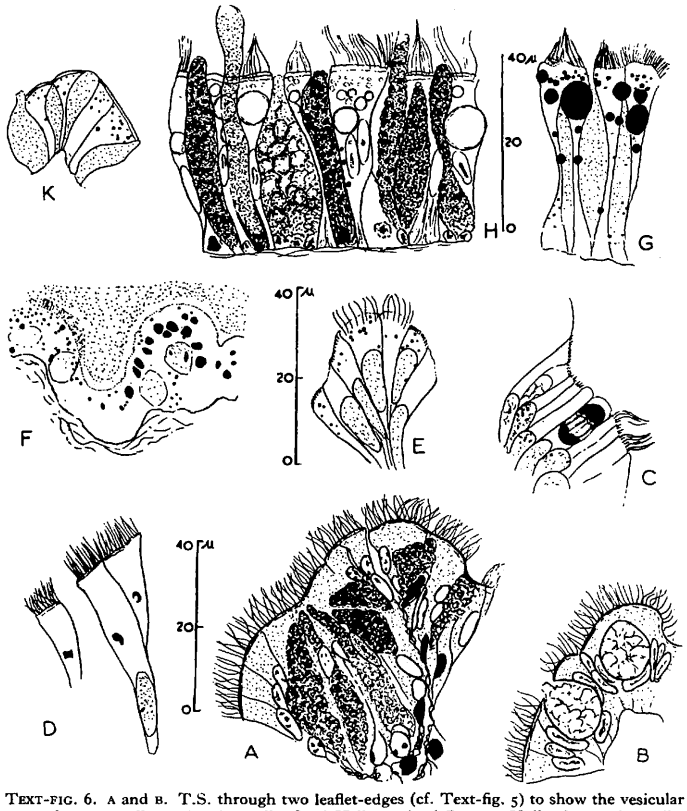
The *intestine* of *Loligo* is a tapering tube, which passes between the two halves of the pancreas and along the ventral surface of the liver, where it leads

into the *rectum*, which opens just behind the funnel (Text-figs. 1, 2). The main mucous groove from the caecum (p. 18) can be distinguished for a short distance along the ventral wall of the intestine (Text-fig. 4B), whose whole inner wall is corrugated with a series of longitudinal folds which, even in the distended organ, are traceable as ridges of connective tissue and longitudinal muscle-fibres. The ridges of the main mucous groove, called by Gariaeff (1915) the 'typhlosole', are further heightened close to the caeco-intestinal opening by underlying ridges of cartilage, similar to the cartilage of the caecal valve (p. 25). The anus is a slit-like opening in a small muscular 'rectal bulb' and is guarded by two 'anal leaflets' (Text-fig. 2). The duct from the ink-sac opens close to the bulb, and the rectum can be closed on each side of this opening. The delimitation of the intestine and rectum is here considered to be a histological one (see p. 29). A substantial outer layer of circular and a thinner, inner layer of longitudinal muscles is present, similar to that of the oesophagus, except that the layer of longitudinal muscles is continuous, and the blood-supply, as might be expected with an absorbing epithelium, much richer, and conspicuous in both the inner and outer layers of connective tissue.

Waves of contraction are frequently to be seen passing along the intestine, continuing for some time after death. In late stages of digestion the intestine may be full of fluid resembling that in the caecum.

The whole of the intestine is lined with a ciliated and mucous epithelium (Text-fig. 6 G, H). As in the ciliated organ, variations in the height of the epithelium accentuate and help to form ridges and grooves, but the mean height here varies greatly with the degree of contraction of the circular muscles; it is also greatest in the widest part of the intestine, near the caeco-intestinal opening, and diminishes steadily towards the rectum. The ciliated cells have long cilia with basal granules, and indications of short, parallel rootlets. The cells have a well-marked cell-border, staining brilliantly with eosin in Prenant-Hollande, differing in this from the cells of either the ciliated organ or caecal sac. The oval nucleus, with one or more nucleoli, is more or less in the middle of the cell, unless displaced by an adjacent ripe mucous cell. The basal portion, often tapering, is markedly fibrillar; the fibrillae, as in the oesophageal and gastric cells, are united with the basement membrane, itself intimately connected with the underlying connective tissue. In late stages of digestion the distal half of the ciliated cell is packed with absorbed fat-droplets. Single basiphil granules are found irregularly in the distal half of these cells. Scattered thickly among the ciliated cells are mucus-secreting goblet cells, which, in their method of secretion and staining, resemble those of the collecting ridges of the ciliated organ, but which always open individually to the intestine. Gariaeff (1915, p. 52) also regarded these cells as mucus-secreting.

Copious supplies of mucus are produced by these cells, and can continue for many hours after death: for an hour or so even in small fragments of intestine. This mucus protects the intestine from the sharp faecal residue, as the gastric cuticle protects the stomach (p. 14).



TEXT-FIG. 6. A and B. T.S. through two leaflet-edges (cf. Text-fig. 5) to show the vesicular type of mucus-cell: A, ripening cells, fixed Helly, stained Prenant-Hollande; B, ripe cells, fixed Zenker, stained Mallory.

C. Mitosis in a ciliated cell of a secondary leaflet groove; D. 'Golgi-like' bodies in leaflet cells, cf. Plate II; C and D fixed Helly, stained iron haematoxylin.

E. Fat-droplets in apical cells of a leaflet-ridge; F. Fat-absorption in the caecal sac; G, H. Fat-absorption and mucus-secretion in the proximal part of the intestine; K. The same in the 'hyaline border' region of the rectum. E, F, G, K, fixed Flemming-without-acetic, unstained; all taken from the same animal. H, fixed Helly, stained Prenant-Hollande. In H the fat-droplets are represented by empty vacuoles.

The currents formed by the ciliated cells are all directed towards the anus. Their function would seem to be like that of the caecal sac: to renew the layer of assimilable food next the absorbing surface. There is no indication of cilia and mucus working together to collect solid particles; indeed the absorbable

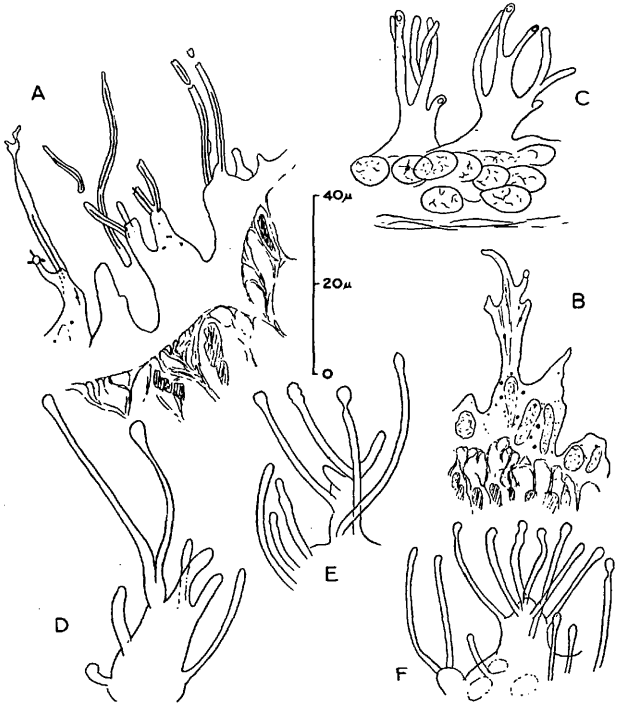
fluid passed to the intestine from the caecum has already been cleared of solid particles which, passed along the intestine in the string of the main mucous groove (p. 23), do not interfere with intestinal absorption.

It seems probable, from the mixture of completely and partially digested residue often found in the stomach, that defaecation does not take place till the meal is ended, so that intestinal absorption is also unimpeded by the passage of large faecal masses. It is curious that intestinal absorption would seem to be just as active and efficient as that of the caecal sac, despite the large number of mucous cells. It is possible that the activity of these cells is discontinuous: the copious supply described above in an excised portion of intestine was associated with repeated strokings of the surface by a needle to free the epithelium of mucus, and the mechanical stimulation of the contact of gastric faeces may be the necessary stimulus for mucus-secretion.

The junction of intestine and rectum may be regarded as marked by the replacement of the ciliated cells by lower cells with a hyaline border (Text-fig. 6K). These contain fine fat-droplets, and are also interspersed with mucous goblet cells. This part of the rectum is short: 2–3 mm. in a fully grown specimen.

The last part of the rectum, including the anal bulb and the opening of the ink-duct, is lined with most remarkable cells forming a cubical epithelium whose cells can put out long retractile processes. The longitudinal grooves of the intestine break up in this region in a fine network, so that the epithelial cells form cushions, from which these processes protrude in bunches, sometimes individually, when the processes can be traced, each one to an individual cell, and may reach a length of over 50μ, sometimes as blunt protrusions from a single mass (Text-fig. 7). A vacuole may be visible in the tip, which is often slightly swollen, especially in fully extended processes, and one or more fine processes may in turn be seen projecting from the terminal knob. Each process has an 'axial filament', faintly visible in unstained sections fixed with Flemming-without-acetic, and staining black with iron haematoxylin after the same fixative. When a process is cut off short in the section, the axial filament may be seen, projecting like a stiff rod from the cut end (Text-fig. 7A). The same fixative shows scattered fine fat-droplets in the cells, and occasionally in the processes. Slow changes of form have been seen in the adult living cell, and in newly hatched larvae the processes are well developed, and wave to and fro in currents of water drawn in and out of the rectum by contractions of the intestine: the appearance in sections of adult material strongly suggests the same flexibility. The cells are described both by Williams for *L. pealeii* (1909, p. 41, Text-fig. 13), who observed them in larva and adult, and by Gariaeff (1915, p. 53, Pl. VII, fig. 93); their accounts agree on the whole with the present description. Williams had observed water being pumped in and out of the larval rectum, and had found that carmine, carried in on a rectal current, was retained in the rectum, he suggested by the action of these processes, when the water was expelled. He also found small bodies, corresponding in size and distribution to the fat-droplets here shown in Text-fig. 7, in the adult processes, and deduced a 'screening' action in the larvae and phagocytosis in

the adult. Gariaeff regarded the terminal vacuoles as secretory, and claimed to have found collapsed processes from which the secretion had been discharged (his figures, however, suggest that this appearance may have been due



TEXT-FIG. 7. Rectal cells of *Loligo*. A and B from T.S. of rectum of the young specimen of *L. vulgaris*, fixed Flemming-without-acetic, whose caecal and intestinal cells are drawn in Fig. 6. A, stained iron haematoxylin, the nuclei invisible; B, unstained. Both show the radiating connective-tissue fibres attached to the epithelium, interspersed with bundles of longitudinal muscle fibres (cross hatched). C from L.S. of rectum of half-grown *L. vulgaris*, fixed Bouin-Duboscq (with ammonium sulphide), stained carmalum. D, E, F, quick sketches of living rectal cells of full-grown *L. forbesii*, dead 1–2 hours.

to imperfect fixation). For him, the cells secrete a coagulent which prevents ink from penetrating into the intestine.

Neither explanation is entirely satisfactory.

The general appearance of the cells does indeed suggest some phagocytic activity, yet it seems meaningless to find phagocytosis in the last few milli-

metres of an alimentary canal along which the food is driven by active peristalsis. Moreover, the solid food which passes the region where these cells are situated is in the form either of relatively large and quite indigestible fragments from the stomach, or of the mucus-string from the ciliated organ of the caecum. It is true (p. 24) that some nutritive particles are entangled on this string, but, were these to be retrieved, such action might be anticipated during the string's relatively slow progress along the intestine, and of this there is no sign. The nutritive liquid, which is passed on to the intestine for absorption, has already been cleared in the caecum by the ciliated organ of all particles suitable for phagocytosis.

If the function of the rectal cells be unrelated to the digestive mechanism, then Gariaeff's association with ink-secretion may be considered. Here again the violence with which the ink is ejected makes it difficult to imagine a part played by the rectal cells. The coagulation of ink which he observed in the intestine may well have been due to the mucus freely secreted by the intestine itself, if not to that which usually appears in a cloud of ejected ink. The extreme dissimilarity between the rectal cells and any known type of secreting cell makes the idea of a secretory activity difficult to accept.

A third possibility is that the reversed peristalsis by which water is drawn into the larval intestine occurs also in the adult, that water is absorbed in the intestine, and that the processes are associated with this activity. It is conceivable that they have a sensory function, testing the indrawn water, and might be associated in this with the anal leaflets, to which no function has been ascribed. It must be admitted, however, that the rectal cells are as unlike any known type of sensory as of secreting cell. It is of interest to note that cells of very similar proportions at the junction of the mid-gut and rectum in *Rhodnius* are described by Wigglesworth (1931, pp. 432, 437, figs. 3, 5), who found them swollen and active during excretion. These cells, however, are 200μ in length, three times the length of the rectal cells of *Loligo*, and, lying side by side, fill the lumen of the rectum completely, so that the whole scale of activity is very different from that of the 60μ processes fringing the last few millimetres of the intestine of a 300 mm. long *Loligo*.

The Mid-gut Gland

With the possible exception of the ciliated leaflets of the caecum (p. 25), there is no evidence to suggest that the alimentary canal or the glands of the fore-gut contribute any digestive enzymes, and we must seek in the mid-gut gland all the enzymes necessary for complete extracellular digestion.

The mid-gut gland consists, as in all dibranchiate cephalopods, of two very unequal parts (Text-figs. 1, 2), for which, for convenience, the names given by early writers of 'liver' and 'pancreas' are retained. The gland arises in development as a pair of finger-like diverticula from the mid-gut. The major, anterior part of each diverticulum becomes the liver, the lesser, posterior part, the hepatic duct and pancreas (Portmann and Bidder, 1928, Figs. 2B, 15B). The duct is non-glandular where it leaves the liver; as it nears the mid-gut, the

walls become elaborated into the spongy pancreas; when the mid-gut becomes differentiated into stomach and caecum the latter, as we have seen, contains the hepato-pancreatic opening. The two glands are thus connected in series and their structural plan is the same: a system of ramifying tubules, opening into a wide central lumen on each side. The tubules are each surrounded by a blood-system which Vigelius (1881, 1883) has shown to be truly capillary. Despite this common plan, the two glands are very different in size, appearance, texture, histology, and mode of action.

The *liver* is a cigar-shaped organ, occupying all the anterior part of the body (Text-fig. 1A). It lies between the retractor muscles of the funnel and abuts against the posterior face of the skull, partly surrounding the poison gland; it is covered by a delicate, transparent muscular sheath. The paired origin of the gland can be traced by the general arrangement of its tubules, and by the passage of the oesophagus and aorta through it (Text-figs. 1, 2). It varies from pale sand-colour to a rich tan-brown, and is of very soft, almost liquid texture. The lumina of the smaller tubules are often obliterated by the swollen ends of the ripe liver cells. The delicate capillary system is generally empty in preserved material.

The non-glandular paired hepatic ducts leave the liver on each side of the oesophagus and aorta. Where the duct leaves the liver a sphincter is formed by a circular layer of muscle-fibres, some of them striated (p. 13), and with a few fibres radially and obliquely arranged.

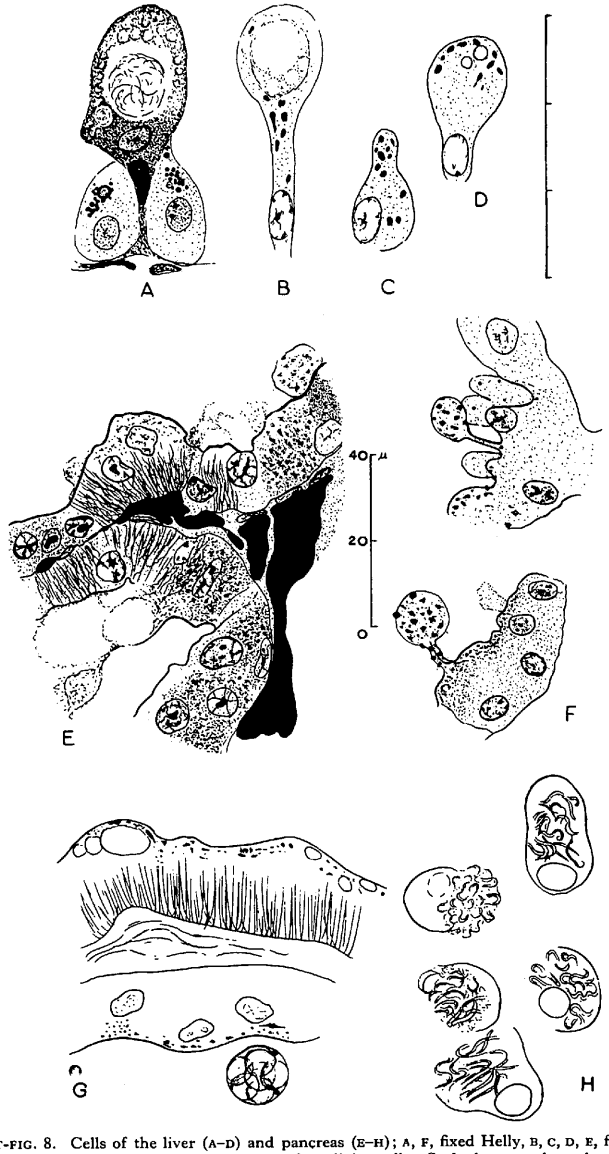
The *pancreas* is a small, wedge-shaped organ, whose volume is about one-tenth that of the liver. It lies completely within the kidney sacs and is covered by a single layer of renal epithelium. It is further in intimate contact with the adjacent kidney tissue, and its blood-supply drains into a pair of large vessels, opening directly into the venae cavae of each side and, like them, covered with renal tissue. The cavity of each hepatic duct expands within the pancreas into a wide lumen, with which the large tubules of the spongy walls communicate freely. The pancreas is sometimes colourless and translucent, sometimes creamy-white and opaque, and is elastic and tough, so that a teased preparation is difficult to make and a smear impossible. This is due to the blood-system, which is reinforced, even in the fine capillaries, with connective-tissue fibres, and is conspicuous in teased or macerated material, or in sections, in which it is usually distended with brightly staining blood. The tubules are wide and their epithelium low (Text-fig. 4B). The opening to the caecum is protected by the hepato-pancreatic fold (Text-figs. 3, 4), but there is no mechanism to prevent the flow of secretion from the gland.

The secreting cells of the two organs show an equally great contrast. The ripe club-shaped liver cells alternate with triangular basal formative stages (Text-fig. 8A). Two elements of secretion are formed in the liver cell, but they mix in the hepatic tubules, and are liberated together. One appears in the basal cells as a single ball which swells when it reaches the swollen distal end of the ripe cell into one large or a group of smaller vacuoles; the second forms a cluster of granules, which also appear first in the basal cells, and follow the

ball into the distal part of the cell, where they usually form an outer layer surrounding the central large vacuole or vacuoles, and then swell to form minute vacuoles or droplets. The large ball is sometimes unstained and yellowish, sometimes lightly basiphil in the unripe cell; the contents of the vacuole into which it ripens are strongly acidophil (green with Prenant-Hollande, blue with Mallory), colourless in the living cell, and staining lightly orange with neutral red as the cell dies, when a small concretion may appear. (The liver cell undergoes rapid alteration after the death of the animal, and only those observations made within a few moments of decapitation can be regarded as related to a normal cell.) The ripe vacuoles sometimes contain droplets of unsaturated lipoid material (giving secondary blackening only with osmium tetroxide, orange with Sudan III, blue with nile blue). This fatty material is present in very varying quantities, sometimes almost filling the vacuoles, sometimes absent, but its occurrence has not been related to the digestive process, since it may be absent in feeding, present in hungering animals. The granules are more basiphil than the vacuoles, staining densely black with iron haematoxylin until they have reached their distal position, where, as they swell into droplets, they become more acidophil (staining strongly with eosin in Prenant-Hollande), while the black colour is first reduced to a shell and then disappears. In the living cell the ripe droplets stain dark red with neutral red but disappear in a matter of seconds as the cell dies. While these granules are passing through the middle part of the cell, they give a strong reaction for iron (p. 6), which is also shown by the shell of the ripening droplet. Two nuclei are often to be found in the middle portion of the cell (Text-fig. 8A); when secretion takes place the whole swollen end of the cell is cast off, often including one of these nuclei; mitotic figures have been seen in the basal regenerating cells.

Two cycles of secretion may be identified. In 'unhurried' secretion the ripe cell-end contains both vacuoles and droplets, fully matured, before it is cast off. In 'short-cut' secretion the cell-ends are cast off before the granules have reached them, and contain the large vacuoles only; a second secretion follows of cell-ends containing granules only (Text-fig. 8 C, D). Secretion may occur before the granules swell into droplets, which then takes place in the lumen of the gland. Short-cut secretion is associated with outbursts of secretion during the meal; unhurried secretion also takes place during the meal, but is characteristic of the gland of a hungering animal, or of one 6 hours after the capture of food, when the whole digestive process is normally completed.

The cells are continuously active; the proportion of basal to club-shaped cells varies, but some basal cells are to be found at all stages of feeding and hunger. The secretion is retained in the gland between meals, and only appears in the caecum about 30 minutes after the capture of food. Storage of secretion has been traced in the liver of living animals which had been fed with nile blue: the liver remained darkly blue for as much as 48 hours in an unfed animal, but lost colour after feeding. The secretion is partly stored in the swollen distal ends of the cells, which then completely fill the smaller



TEXT-FIG. 8. Cells of the liver (A–D) and pancreas (E–H); A, F, fixed Helly, B, C, D, E, fixed Zenker, all stained Prenant-Hollande; G, H from living cells. Scale the same throughout.

tubules, partly in the central lumina, where vacuoles, granules, and discarded cytoplasm all break down to a homogeneous liquid.

The low pancreatic epithelium (Text-fig. 8 E–H) has a well-marked basiphil cell-border, and consists of sub-cubical cells, whose cell-boundaries are usually invisible in section but which are readily separated in teased or crushed material. The broadly oval nuclei (rather larger than those of the liver cells) are usually apical in position; the cytoplasm is conspicuously fibrillar. The fibrillae are easily seen in living tissue, and are visible in isolated living cells as highly refringent threads, bent by the contraction of the free cell into shapes suggesting some elasticity (Text-fig. 8H). As the cell dies the fibrillae appear to break into granules which stain with Höchst's Janus green B. Fibrillae and granules are visible both in preparations made by Regaud's method for mitochondria, and those following Zenker fixation, in which mitochondrial matter had presumably been dissolved away. Vacuoles of secretion appear between the fibrillar cytoplasm and the cell-border, often appearing in preserved material as solid spheres, but in life as vacuoles filled with droplets or lesser vacuoles (Text-fig. 8). Within and among the vacuoles are scattered granules, staining blue with nile blue, red with neutral red, and black, in preserved material, with iron haematoxylin; the whole gland tends to be basiphil in staining. Secretion occurs rhythmically and well-marked resting periods intervene apparently throughout the gland; this may be related to the macroscopical variations in appearance. The rhythm of secretion has not been related to the meal. No mitotic figures have been seen, but degenerate nuclei are frequently found in the lumina of the tubules and within the epithelium. No lipoid material has been found beyond the small granules already mentioned. In the iron saccharate experiments the pancreas was, unfortunately, very badly fixed; no trace of iron was ever found in the gland.

There is but little knowledge of the exact nature of the hepato-pancreatic secretion. Sellier (1910) gives an account of the proteolytic enzymes of the liver and pancreas taken together; Williams (1909, p. 40) states that the extract of the liver is proteolytic, that of the pancreas proteolytic, amylolytic, and lipolytic. Dr. L. E. Bayliss, who kindly made a brief examination of the liver and pancreas of several specimens of *L. forbesii* at Plymouth in 1933, found both proteolytic and lipolytic enzymes in both liver and pancreas. The lipase showed a pH optimum of between 6·6 and 6·8; the protease appeared to have an optimum in the region of 7·2. Dr. Bayliss found the pH of the caecal fluid to be between 5·6 and 5·8. Some earlier observations by the present writer, made on freshly dead trawl-material, gave the pH of the stomach 6·2; caecum 5·6–5·8; pancreas 6·0; liver 6·0. The figures obtained for the enzyme activity of the liver suggested considerable individual variation in the extract, relatable to the physiological state of the gland. The data for the pancreatic extract are of doubtful value, since, as the pancreas is but a glandular elaboration of the hepatic duct, the main pancreatic tubules are liable to be filled with hepatic secretion.

Romijn (1935, pp. 421, 426) found an amylase, but no maltase or saccharase in the 'Magensaft': it is not clear whether this is, in fact, stomach contents, caecal contents, or a mixture (see below). He found a pH optimum of 6·26 for starch digestion and of 5·96 for digestion of glycogen.

None of these observations throws light on the particular role of either liver or pancreas in the digestive process, particularly in gastric as distinct from caecal secretion. Do both glands contribute to each phase, or is one responsible for gastric, the other for caecal digestion?

It is clear that the brown colour which appears in the caecum during meals (p. 9) must be hepatic in origin, and, since only one secretion is produced by the liver, the pancreas must be responsible for the colourless fluid which fills the caecum during hunger (p. 10). The brown hepatic colour has not been detected in the stomach, so that it is tempting to infer that the gastric juice is also pancreatic in origin and that gastric digestion begins under the action of the caecal 'hunger fluid', supplemented, once caecal digestion has begun, by the flow of pancreatic secretion directed to the stomach along the hepato-pancreatic groove, or, more properly, the pancreatic groove. This would be possible (1) if the hepatic sphincter (p. 32) remained shut while the hepato-pancreatic fold closed on to the columellar ridge, thus making the hepato-pancreatic groove a closed tube leading to the stomach (p. 16), and (2) if the sphincter relaxed only when relaxation of the hepato-pancreatic fold gave the hepatic secretion access to the caecum. If the pancreatic extract of *Loligo* be activated by the secretion of the caecal lining (cf. p. 25), as Romijn (1935, p. 421) found to occur in *Sepia officinalis*, then we might suppose that enough of the activator passed into the stomach with the first drive of digestive juice from the caecum. It is, however, possible that the activating system, if present in *Loligo*, acts on hepatic, not pancreatic secretion: there is at present no justification for generalizing from the Sepioidea to the Teuthoidea, even though Romijn found similarities in the starch-splitting enzymes—particularly as it is not clear whence his 'Magensaft' was derived. It seems improbable that, with the large caecum available, juice should be taken from the stomach of *Loligo*, either full of food or contracted and empty; while the hungering stomach of *Sepia* is often distended with fluid. Romijn may thus have compared the stomach juice of *Sepia* with the caecal juice of *Loligo*. Preliminary investigations by the present writer suggest substantial differences between the digestive mechanisms of the two genera—including the use by *Sepia* of the stomach, not the caecum, as a store of 'hunger-secretion'. Caecal activation of hepatic secretion would make possible storage of secretion in the gland.

One of the most striking differences between the liver and pancreas is that the pancreatic secretion is liberated as it is formed (p. 32), accumulating in the caecum between meals and passing to the stomach during gastric digestion, while the hepatic secretion is only liberated during digestion. This control is effected by the hepatic sphincters, whose striated fibres (pp. 13, 32) would effect a rapid closing action; its non-striated fibres control the prolonged closure of the sphincter between meals. The duct is supplied with nerves from

the splanchnic ganglion (Alexandrowicz, 1928), so that nervous co-ordination is possible between the sphincter and the hepato-pancreatic fold, in the manner suggested above. It is less clear what stimulus initiates relaxation of the sphincter and liberation of secretion during digestion. This has been initiated by decapitation or by the subsequent handling and dissection in two animals at very different stages of digestion. Both had been fed with nile blue, one 2¼ hours, the other 17 hours before decapitation. In each the liver when opened was clearly stained; in each the stain had appeared in the caecum and completely vanished from the liver 35–40 minutes after decapitation: despite the very different physiological states of the two animals decapitation in each case stimulated the liberation of hepatic secretion. Further evidence of nervous control was derived from one animal which was captured with the stomach distended with half-digested food, but with no sign of hepatic secretion in the caecum, which only appeared 4 hours after capture, after which digestion was completed at less than the normal speed (cf. p. 10). The shock of capture would here appear to have had an inhibiting effect on the liberation of hepatic secretion.

These observations, while demonstrating clearly the nervous control of the sphincter, throw no light on the immediate stimulus which normally induces it to open.

It may be noted that Sellier (1907, 1910), who investigated a colourless caecal juice, was thus probably examining pancreatic secretion. It is curious that he found no difference between the caecal fluid in hunger or digestion, but, as he describes no difference in colour or turbidity, he probably never examined an animal at the height of hepatic and caecal activity. The present writer never observed more than the faintest hepatic tint in the caecum of trawl-material, in which capture may regularly inhibit liberation of hepatic secretion.

It is clear that careful examination of liver and pancreas extracts is needed, as well as of caecal and gastric juice and caecal extract, all at known stages of digestion.

It seems, however, reasonable to postulate that the pancreas rhythmically liberates the enzymes necessary for the preliminary gastric digestion, while the liver continuously produces those necessary for the final caecal digestion, but only liberates its secretion while digestion is taking place. These two activities do not in themselves account for the differences between the two glands, either in bulk or histology, and it is clear that each must have some other activity.

It is natural to look for an absorptive function in a molluscan mid-gut gland, but, in fact, the evidence is against absorption by either liver or pancreas. The iron experiments were inconclusive on this point (p. 6), but the histology of the liver definitely excludes hepatic absorption: every type of cell can be referred to a cycle of secretion and can be found in an animal which has hungered for many hours (p. 33); further, the only free surface offered by the hepatic epithelium is that of the ripe cell-end, about to be cast off in secretion.

The evidence against pancreatic absorption is more circumstantial. The well-guarded opening makes entry of food into the gland improbable (Text-figs. 3, 4). The almost complete absence of lipoid matter from the pancreas (p. 35) is also strong evidence, since unabsorbed fat is still present when the digested food passes on from the caecum to the intestine (Text-fig. 6), nor does the low pancreatic epithelium with its characteristic apical nuclei resemble most known absorbing tissues. There is no good reason, in fact, for postulating pancreatic absorption and several against doing so. The differences between the two glands must be due to other causes.

The great bulk of the liver must be related to storage of reserves: no other tissue of the body suggests this function. The reserves may be of a protein nature, as suggested by the strongly basiphil staining of the permanent, basal part of the epithelium (Sellier found a high protein content in the liver which he held to be due to hepatic absorption), or may be glycogen, detected by Chaigne (1934) in many tissues, including the liver, of other cephalopods. The presence of iron and the curious and irregular production and liberation of lipoid material, an activity calling for further investigation, also differentiate the liver sharply from the pancreas. An excretory function was attributed to the liver by Cuénot (1907, p. 34), on the basis of the appearance of injected Jodgrün and Echtroth in cells of the liver, as well as in the kidney, 24 hours after injection.

What function, other than that of enzyme secretion, is performed in the pancreas is at present obscure. It may be related to the long 'resting' phase between secretion-production, to the apical nuclei, the well-developed blood-supply, wide tubules, and supporting connective-tissue system, although this last may be only to give strength against the pressure of the hepatic secretion passing through the gland. The wide tubules must result in free contact between the hepatic secretion and the pancreatic cells of all but the smallest tubules, which suggests the possibility of some interaction between the two. This already exists in the larva (Portmann and Bidder, 1928) in which the developing liver surrounds the internal yolk-sac, and the yolk is taken up by the liver cells, passed down to the pancreas, by which it is absorbed into the blood-stream. The intimate connexion between the pancreas and the kidney must also have some functional significance and may be related to the great volume of secretion produced by the pancreas. On the other hand, the histology suggests that the second pancreatic activity may be directed towards the blood-stream, and the possibility of internal secretion cannot be excluded. The difficulties in obtaining pure pancreatic secretion, or of making any experiment involving operation on so highly strung an organism as *Loligo* indicates that the answer to this problem is to be sought by the methods of histochemistry.

Conclusion

The Meal

The digestive mechanism of *Loligo* and *Alloteuthis* which has been outlined in these pages may now thus be summarized:

Food, killed by the action of the poison glands, is bitten into pieces by the jaws (p. 9) and swallowed with the help of the radula and probably of lubricant from the buccal gland (p. 12). In the stomach it encounters colourless pancreatic secretion, which has been stored in the caecum (p. 36). This is driven into the stomach by strong contractions of the caecal sac, the caecal valve being held open by the distension of the anterior part of the caecum (p. 18). Under the influence of the pancreatic enzymes, and through the churning action of the stomach (p. 9), the food is broken down to particulate or liquid form. About half an hour after the capture of food the first consignment of partially digested food is passed on to the caecum (p. 10). When this happens the muscles of the caecal valve, hepato-pancreatic fold, and hepatic sphincter relax so that the caecal valve, acting as a simple valve, allows food to enter the caecum where it is met by an outpouring of hepatic secretion. The caecal valve and hepato-pancreatic fold then close (p. 18), so that the contents of the stomach and caecum are isolated from one another and muscular movements of either will not mix their contents. The hepatic sphincter also closes, so that pure pancreatic secretion passes to the stomach where preliminary digestion proceeds, while, in the caecum, the muscles and cilia of the sac combine to keep the contents gently moving (p. 22) and digestion is completed under the action of hepatic secretion (p. 36). At intervals, further consignments of partially digested food and hepatic secretion meet in the caecum, where the ciliated organ is continuously removing solid particles into the mucous groove of the intestine (p. 22), while absorption begins in the cells lining the caecal sac (p. 25). Gastric digestion lasts (on an average) $1\frac{1}{2}$–2 hours; caecal digestion and absorption are completed after about 4 hours (p. 10). While absorption is still proceeding in the caecum, the caecal valve is opened (p. 19) and some of the caecal contents are driven into the intestine where absorption begins (while caecal absorption is still active) and continues after caecal absorption has ended (p. 27). At an unknown interval after the end of intestinal absorption, sometimes only after a second meal has been captured, digested, and absorbed, the indigestible residue from the stomach is passed out, well wrapped in protective mucus secreted by the intestinal lining (p. 27). The caecal valve, held shut by the muscle in its rim (p. 18), prevents the faeces from entering the caecum.

When digestion is at an end the hepatic sphincter remains closed, the hepato-pancreatic fold and caecal valve relaxed, so that hepatic secretion accumulates in the liver, pancreatic in the caecum (p. 36), while simple valve action of the caecal valve retains the pancreatic secretion within the caecum.

It may be noted that intestinal absorption is probably especially important at sexual maturity. At this time the gonads may occupy almost the whole space generally occupied by the caecal sac. The appendix, even when fully distended, could not compensate fully for the loss either of absorbing surface or of cubic content, and the intestine is probably important at such times, not only for absorption, but for holding unabsorbed food.

Discussion

The most striking facts of digestion in *Loligo* are the speed and completeness of the digestive process, and the complexity of the mechanism by which this is achieved.

The whole of the structure may be interpreted in terms of speed and efficiency. Separation of the mid-gut into stomach and caecum makes possible separation of the digestive process into two phases, a preliminary phase in the stomach, a final in the caecum, which can go on independently and simultaneously under the action of their appropriate enzymes. The division of the mid-gut into two parts, and the complex structure of the hepato-pancreatic fold are closely associated with this division of the digestive process into two phases. The speed of the preliminary gastric digestion is probably due partly to the character of the pancreatic secretion, but largely to the violent churning action of the stomach itself.

Exclusion from the caecum of large masses of undigested food or indigestible residue must greatly facilitate the final stages of both digestion and absorption of the resulting soluble foodstuffs. The development of a large blind sac as an organ of absorption is made possible by the presence of the ciliated organ: this (1) prevents the accumulation of solid particles, which would otherwise increasingly clog and obstruct the lumen, as only the fluid is absorbed, and (2) frees the absorbing epithelium entirely from solid particles for the greater part of the absorptive phase. The ciliated organ has its parallel, and almost certainly its homologue, in the ciliary mechanisms of the primitive gastropod and lamellibranch mid-gut (Graham, 1949). In these groups, however, as frequently in invertebrates, secretion and absorption take place side by side, or successively in the same cell, in a single digestive diverticulum of the mid-gut. By developing a second diverticulum for the absorption of soluble food, completely free from enzyme secretion, *Loligo* is enabled to use its molluscan inheritance to form a digestive mechanism of startling efficiency.

The whole digestive mechanism is admirably adapted to the life of a ceaselessly swimming animal which is a predator on shoaling animals, and itself the prey of active predators. The segregation of gastric and caecal digestion are particularly well fitted to allow of continuous feeding when a shoal is encountered, or of feeding whenever food is available without interfering with the digestion of an earlier meal. The rapid digestive process (4–6 hours, in contrast to 40–60 hours recorded by Dawes (1930, p. 93) for the plaice) reduces to minimal duration loss of activity due to the inertia of repletion. It may be noted that the habit of storing pancreatic secretion in the caecum results in that organ being of more or less constant bulk in feeding and hunger, which should further diminish the effect of feeding on swimming efficiency.

The economy of the digestive process enables the fullest use to be made of any food that is captured. The only assimilable food which is lost is the small amount of fat-droplets and food particles carried out in the mucus-string

from the caecum: compared with the large quantities of utilizable food passed out with the faeces of other carnivorous animals, this wastage may be regarded as negligible.

Especially noteworthy is the delicate interplay of the muscular action of the stomach, caecal sac, oesophageal, intestinal, and hepatic sphincters, and hepato-pancreatic fold and caecal valve. The position of the splanchnic ganglion at the junction of oesophagus and stomach must greatly facilitate the accuracy of this control. It would be of great interest to know exactly what stimuli produce the various conditions allowing food to pass from the stomach to the caecum or from the caecum to the intestine, enzymes from the caecum or pancreas to the stomach or from the liver to the caecum, or faeces from the stomach to the intestine.

The histology of the alimentary canal offers many fascinating problems for further study, not least among which are the pancreatic and rectal cells.

In conclusion, it must be stressed that the picture of the digestive mechanism set forth in these pages is only in part applicable to other Cephalopoda. Even within the Loliginidae, Williams's account, and the present writer's unpublished observation on *L. pealeii*, show that the closing apparatus of the caecum differs markedly within that family, and greater differences are to be found when the comparison is extended to the Cephalopoda as a whole. While the basic plan of structure described for the ciliated organ of the caecum of *Loligo* has been found by the present writer in every cephalopod examined, other features, such as the degree of coiling of the spiral, presence or absence of the sac, and the nature of the closing mechanism of the caecum, show a wide range of variation. Again, while in all described Dibranchiata the mid-gut gland may be divided into 'liver' and 'pancreas', the histology of the liver varies considerably, and the pancreas shows even more marked variation in its relation to the hepatic duct on the one hand and the kidney on the other (Vigelius, 1881, 1883; Castaldi and Musio, 1928). The duration of digestion is also very variable, 18 hours in *Octopus* (Falloise, 1906), 12 in *Sepia* (Gariaeff, 1915): two or three times the length of the digestive process in *Loligo*.

Falloise found in *Octopus*, as the present writer found in *Loligo*, that food did not enter the mid-gut gland, but Gariaeff (1915, p. 90) claimed to have traced the entry of particulate food into the liver of both *Octopus* and *Sepia*, which the present writer, repeating Gariaeff's experiments on *Sepia officinalis*, is at present unable to confirm.

Some of these apparent discrepancies may prove to be due to erroneous observation, but some to the profound difference between the ways of life of the bottom-dwelling, lurking *Octopus* and partially bottom-dwelling *Sepia*, and the perpetually swimming pelagic *Loligo*. Until more information is available it is clearly dangerous to combine, as has too often been done, one observation from *Octopus* with another from *Sepia* under the generalization 'In the Cephalopoda'.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Epithelium lining the oesophagus of *Loligo*, fixed Helly, stained Prenant-Hollande. No formative layer is present between the epithelium and the cuticle.

Fig. 2. The same, fixed Zenker. A narrow formative layer is present.

Figs. 3, 4, 5. Epithelium lining the stomach of *Loligo*, fixed Zenker, stained Mallory.

Fig. 3. The fibrillar formative layer is narrow; masses staining red with Mallory present within the right-hand cell.

Fig. 4. The fibrillar formative layer is wide; the red-staining masses are outside the cell-border, and amongst the fibrillae of the formative layer.

FIG. 5. Active secretion of the formative layer is associated with obliteration of the cell-border.

PLATE II

Transverse section through one ridge of a ciliated leaflet of the caecum, fixed Helly, stained iron haematoxylin. The preparation shows the variations in the staining of the cytoplasm, the 'Golgi-like' bodies of the lateral cells and the scattered granules near the surface of the apical cells; and a newly secreted mucus-ball in one of the secondary leaflet grooves.