The cytology and histochemistry of the digestive gland cells of Helix

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Summary
The digestive gland tubule epithelium of Helix aspersa is made up of 4 cell-types: thin cells, digestive cells, calcium cells, and excretory cells. Thin cells are narrow and undifferentiated. They divide by mitosis and are believed to develop into other cell-types. Digestive cells are highly vacuolated phagocytic and absorptive cells. Food materials are taken in by phagocytosis and are concentrated and digested in the vacuoles in the cell. When digestion is complete, the residual indigestible material in the vacuoles, and excretory material in the form of small granules of lipofuscin, are cast out of the cell surrounded by a portion of cytoplasm. Calcium cells are secretory, with a prominent Golgi apparatus and a high concentration of RNA in the cytoplasm. Most of the cell is occupied by spherules which contain calcium; apically there are protein granules which contain a high concentration of tryptophane. Both these types of inclusion are extruded from the cell. Protein granules may be zymogen granules, but the function of the calcium spherules is not known. Excretory cells are degenerate, and probably derived from calcium cells. They consist chiefly of a large vacuole, surrounded by a little cytoplasm. The vacuole contains one or more granules of lipofuscin. Similar granules can be found in the faeces, and thus they are excretory material.

Introduction
The digestive gland of the snail Helix aspersa consists of much-branched blindly ending tubules (Barfurth 1883; Billett and McGee-Russell, 1955; Krijgsman, 1925), bound together by connective tissue containing blood lacunae (Billett and McGee-Russell 1955). This paper is concerned only with the cells of the epithelium lining the tubules. These cells have frequently been studied in the past (e.g. Barfurth, 1883; Frenzel, 1885; Krijgsman, 1925, 1928; McGee-Russell, 1955; Thiele, 1953), but the descriptions are inadequate, the relationships and functions of the cells are not certainly known, and no histochemical studies have been done on them.

The present paper aims to give a more complete account of the cytology of the cells of the digestive gland tubule epithelium, and describes the histochemistry of these cells.

Material and methods
The digestive gland of the snail H. aspersa was used throughout the present study. Before fixation, the snails were either allowed to feed at will, or starved for various periods up to 132 days. Snails starved for 20 days or less are called short-starved; those starved for longer periods are called long-starved. Hibernating snails were also studied.

For routine cytological study, small portions of digestive gland were fixed in Zenker or Champy, and embedded in paraffin. Bouin, Helly, formalin-alcohol-chloroform (Krijgsman, 1928), Altmann, Regaud, formalin-saline, Lewitsky-saline, Mann, and NH$_4$-Altmann (Baker, 1960) were also used, but were not found to be better than Zenker or Champy. Sections were cut at 6 to 8 μ, and stained with Heidenhain's iron haematoxylin, or with Ehrlich's haematoxylin counterstained with either eosin or light green.

Mitochondria were stained by Metzner's method (Meves, 1911), generally after fixation with Champy's fluid and sectioning at 4 μ. Altmann, NH$_4$-Altmann, Regaud, Mann, and Helly (post-chromed for 48 h) were also tried as mitochondrial fixatives.

The Mann-Kopsch method (Baker, 1933) was used to show the Golgi apparatus. The methods of Kolatchev and of Aoyama were also tried unsuccessfully.

For the study of cell division, staining with crystal violet and iodine (Baker and Jordan 1953) was used, on tissue fixed in Sanfelice's fluid.

The following histochemical methods were used:

For DNA: Feulgen's method (Feulgen and Rossenbeck, 1924), on Zenker-fixed tissue.

For RNA: the P/MG test (Jordan and Baker, 1955) with salivary ribonuclease as a control (Bradbury, 1956).

For carbohydrates: the PA/S test (McManus, 1948), with diastase digestion as a control for the presence of glycogen (Lillie and Greco, 1947), on Zenker-fixed tissue.

For acid muco-substances: metachromasy with toluidine blue, on Zenker-fixed tissue.

For neutral muco-substances: metachromasy with toluidine blue after sulphation with concentrated sulphuric acid for one minute (Lison, 1953), on Zenker-fixed tissue.

For arginine: Sakaguchi's test (Baker, 1947), on Zenker-fixed tissue.

For tryptophane: DMAB/nitrite test (Pearse, 1960), on tissue fixed in formalin-saline.

For lipids: Sudan black, by the method of Baker (1956).

For phospholipids: the acid haematein test, with pyridine extraction as a control (Baker, 1946).

For calcium: the cobalt substitution method; and the calcium red method (McGee-Russell, 1955). Tissue was fixed in formalin-ethanol (1:1) (McGee-Russell, 1955), or formalin-methanol-pyridine (Baker, personal communication).

The following tests for pigments (Pearse, 1960) were used, with tissue fixed in formalin-saline:

Schmorl's test (for lipofuscin, melanin, and reducing substances generally). Chrome-alum haematoxylin (for lipofuscin).

Long Ziehl-Neelsen, with 24 h differentiation (for lipofuscin).
Bleaching with 10% hydrogen peroxide for 48 h (for distinguishing between melanin and lipofuscin).

The methods of Lillie (1956) and of Pearse (1954) for distinguishing between melanin and lipofuscin, with Nile blue, were tried, but did not give satisfactory results.

Results

Four cell-types can be recognized in the digestive gland tubule epithelium: thin cells, digestive cells, calcium cells, and excretory cells. Table 1 gives a list of synonyms found in the literature (see Appendix, p. 189).

The short-starved state. The results of histochemical tests are summarized in table 2, and will only be referred to here when they are of particular interest (see Appendix, p. 190).

Thin cells (fig. 1) are narrow from side to side (2 µ or less in width) and extend to the full height of the epithelium. The nucleus, averaging 10 by 1.5 µ is situated in the basal half of the cell. One or two nucleoli, less than 1 µ in diameter, can usually be seen in it. The cytoplasm is homogeneous and does not contain any special inclusions. Mitochondria are small, averaging 0.7 by 0.5 µ in Champy-fixed tissue, and are sparsely distributed along the cell. The Golgi apparatus consists of an irregular body, 1 to 1.5 µ across, which appears as a ring in optical section. It is immediately apical to the nucleus.

Thin cells are distributed, apparently at random, between the other cells of the epithelium.

Digestive cells (fig. 2) are the most numerous type in the digestive gland epithelium, and are characterized by their highly vacuolated cytoplasm. The nucleus is basal and usually rounded, averaging 8 by 6 µ. There are usually two nucleoli, which average about 1.5 µ in diameter, but up to 5 have been seen.

In the cytoplasm just apical to the nucleus there is usually a variable number of small yellow granules, about 2 µ across, often rather irregular in shape, and sometimes appearing as clumps of smaller granules. These small yellow granules normally appear to lie directly in the cytoplasm. The histochemical tests show that their pigment is mainly, if not entirely, lipofuscin.

Most of the rest of the cytoplasm contains vacuoles, 5 to 6 µ in diameter, which contain the so-called 'green' granules (Krijgsman, 1928) (which stain readily with light green). The green granules average 3.5 by 3 µ and are usually rather irregularly shaped, often with a diffuse outline. The cytoplasm at the apex of the cell contains smaller apical vacuoles, mostly 1.5 µ or less.
in diameter, although those next to the green granule vacuoles may be 3 by 2 \( \mu \) or slightly larger. These vacuoles have no visible contents.

The free surface of a digestive cell is either straight and level with the apices of the other cells of the epithelium, in which case it bears a brush border; or it is hemispherical, protrudes into the lumen of the tubule, and has...
no brush border. When a brush border is present, there is a very thin layer of non-vacuolated cytoplasm immediately below it. Cells with a brush border average 44 μ in height, and those without a brush border 61 μ in height. The average maximum width of digestive cells is about 15 μ.

Mitochondria occur throughout the cytoplasm of digestive cells between the other inclusions. They are evenly distributed except when a brush border is present, when they are concentrated apically. They average 0.8 by 0.6 μ in Champy-fixed tissue. The Golgi apparatus consists of two types of bodies, occurring basally in the cell in very small numbers. Most of the Golgi bodies are ring-shaped in optical section, and 1 to 1.5 μ in diameter. Other Golgi bodies appear as rods, about 1.5 μ long and 0.5 μ wide. Lipid droplets are found throughout the cytoplasm of digestive cells in very variable numbers. They are usually 0.5 to 1.5 μ in diameter, or up to 3 μ basally, where they are often concentrated.

The apical parts of digestive cells with no brush border can often be seen apparently constricting off and being released into the lumen of the tubule. These constricted tips may also be found in the digestive gland ducts.

In two snails only, protein granules have been found in digestive cells. For a description of these granules, see the description of protein granules in calcium cells. The protein granules of digestive cells differ from those of calcium cells in being rather larger, and distributed throughout the cytoplasm. They occur only in very small numbers.

Calcium cells (fig. 3) are characterized by calcium spherules, which occur throughout the cytoplasm except at the apex of the cell. These cells usually occur at the corners of the tubules, normally singly but sometimes in groups of two or three. They are normally triangular in vertical section. The average height of the calcium cells is 37.5 μ, and the average maximum width (at the base) is 33.5 μ.

The nucleus is in the basal half of the cell, and is ovoid, averaging 14.5 by 12 μ. There are usually two nucleoli averaging about 3 μ in diameter, although three have been seen.

The calcium spherules are almost always spherical, and give a positive reaction with tests for calcium. Their average diameter is 3 μ. Different cells have spherules of different sizes, but in any one cell all the spherules are of about the same size. A cell was chosen with spherules of large size; the average diameter of the spherules was 4.8 μ. Another cell was chosen with spherules of small size; the average diameter of the spherules was 1.25 μ. A few calcium spherules show internal structure, although most do not. Some have an inner concentric ring; others contain a small sphere, 0.4 to 1.2 μ in diameter, and with a natural pink colour, in the centre. Free calcium spherules are frequently found in the lumen of the tubules and ducts of the digestive gland.

The apical cytoplasm of calcium cells is free from calcium spherules, and stains strongly, particularly with basic dyes. Usually there is a small number of protein granules, about 4 μ in diameter, lying in the apical cytoplasm;
they stain very strongly with iron haematoxylin, or with acid fuchsin in Metzner's method; they also contain much tryptophane. The free surface of a calcium cell usually has a brush border, with a thin layer of clear cytoplasm below it.

Many calcium cells have a large vacuole in the apical half of the cell, below the apical cytoplasm. This replaces cytoplasm containing calcium spherules, and one cell has been seen where all the cytoplasm containing calcium spherules has been replaced by a vacuole. The vacuoles contain large yellow granules, which react positively to tests for lipofuscins.

Mitochondria occur throughout calcium cells, in between the calcium spherules and the protein granules, and also concentrated apically. They average 1.0 by 0.8 μ. The Golgi apparatus consists of two types of bodies, lying between the calcium spherules in the basal half of the cell (fig. 4). One type of body appears as a black line, slightly bent, and averaging 2.8 by 0.7 μ. The other type appears as a grey component, wholly or partly enclosed by a black line, and averaging 2.2 by 1.5 μ. Basally there are lipid droplets, ranging in diameter from about 1.5 to 5 μ. There may be few droplets, in which case they are all small; or there may be many, even extending into the apical half.
of the cell, in which case the droplets are large. The cytoplasm of calcium cells has a high RNA content.

Excretory cells (fig. 5) consist of one to three large vacuoles, surrounded by a thin layer of cytoplasm. There is usually only one vacuole, which averages 27 by 21 μ, and contains one or more large yellow granules, averaging 5 μ in diameter. Apparently identical granules can be found in the lumen of the intestine and in the faeces. Often very small yellow granules are seen at the periphery of the vacuole. All these yellow granules give positive reactions to tests for lipofuscins. The average height of excretory cells is 38.5 μ, and their average maximum width 23.5 μ. The nucleus is basal, and averages 8 by 6 μ. Up to two nucleoli, 1.5 μ in diameter, can be found, but often none is visible. Feulgen’s test indicates that excretory cell nuclei often have a very low DNA content. There is a layer of cytoplasm apically, which stains strongly, especially with iron haematoxylin. The apical cytoplasm often seems to have a granular or filamentous structure.

Mitochondria are scarce in excretory cells, but they occur apically in at least some cells. A few lipid globules, about 0.8 μ in diameter, can be seen apically; also, when basal lipid droplets are numerous in other cells, they appear to occur in excretory cells. No Golgi apparatus has been found in excretory cells.

The feeding state. No changes have been found in thin cells of feeding snails. However, mitosis of thin cell nuclei occurs very rarely in feeding snails, but has not been seen in starved snails.

The only change from the short-starved state in digestive cells is an increase in the amount of RNA in the basal cytoplasm.

![Fig. 4. Drawings of Golgi bodies in calcium cells, showing the different forms which occur. The scale represents one micron.](image-url)
In calcium cells two changes have been found: a decrease in the number of protein granules, and the appearance throughout the cell of small numbers of glycogen granules, about 1 μ in diameter. In one feeding snail a few calcium cells, smaller than normal, have been found in the tubule epithelium. They average 33.5 μ in height and 7 to 8 μ in width, and are columnar, not triangular, in vertical section. Their nuclei average 7 by 5.5 μ. Apart from the sizes of the cell and nucleus, which are nearer the sizes of digestive cells, these small cells are typical calcium cells.

No changes have been found in excretory cells of feeding snails.

The long-starved state. Many changes occur when snails are starved for longer than 20 days. After 34 days' starvation, the 'green' granules and their vacuoles become larger (table 3 (see Appendix, p. 192) and figs. 6 and 7), a yellow pigment (which reacts negatively to tests for lipofuscins) develops in the 'green' granules, and calcium cell mitochondria become larger (table 3 and fig. 8). After 49 days' starvation the apical surfaces of all cells have become level, none protruding into the lumen of the tubule; also the DNA in the nuclei of all cells has become homogeneously distributed, instead of being in granules throughout the nucleus. After 65 days' starvation, the distinction

![Diagram of an excretory cell.](image-url)
**FIG. 6.** Graph showing increase of size of 'green' granules with length of starvation. The sizes plotted on this graph are the products of the measurements in table 3.

**FIG. 7.** Graph showing increase of size of 'green' granule vacuoles with length of starvation. The sizes plotted on this graph are the products of the measurements in table 3.
between digestive and excretory cells has been lost, and small yellow granules are no longer found in digestive cells. After 82 days' starvation, protein granules have disappeared, and the amount of RNA in calcium cells is greatly reduced.

Basal lipid droplets decrease in size and number as starvation proceeds, and after 82 days' starvation the only remaining lipid is in small droplets throughout the cells, not concentrated basally. Up to 65 days' starvation the apical cytoplasm of digestive and calcium cells may show a positive reaction for phospholipids, but this has disappeared after 82 days' starvation. During prolonged starvation, the Golgi apparatus of calcium cells decreases in size and appears as rings or crescents, 1 to 1.5 µ across, in the basal part of the cell.

The hibernating state. The digestive gland of hibernating snails resembles that of short-starved snails: there are no histochemical differences. There are the following differences in structure, however. In digestive cells, small yellow granules extend almost throughout the cytoplasm, leaving only a small area apically free of yellow granules. Similar yellow granules form a large part of the contents of the intestine of hibernating snails, and appear in the faeces shortly after the snails come out of hibernation. 'Green' granule vacuoles become smaller than in short-starved snails, averaging about 4.5 µ in diameter, although the 'green' granules themselves do not change in size. Calcium cell mitochondria become longer and thinner, averaging 2.0 by 0.5 µ in Champy-fixed tissue; digestive cell mitochondria also appear to be longer and thinner, averaging 1.6 by 0.6 µ in Champy-fixed tissue. The Golgi apparatus of calcium cells appears as small rings, usually 1 to 1.5 µ in diameter, in optical section; there are several of these bodies in each cell. The Golgi
apparatus of digestive cells is similar to that of short-starved snails. There are very few basal lipid droplets in digestive gland cells in hibernating snails.

The calcium cells of hibernating snails characteristically, but not invariably, contain objects here termed rings (fig. 9). They consist of a cortex, about 1.5 μ thick and rich in RNA, surrounding a calcium spherule. The outside diameter of rings averages between 6 and 7 μ.

![Diagram](image)

**Fig. 9.** Rings in calcium cells of hibernating snails. A, drawings of calcium cells showing distribution of rings within them; B, diagram showing the structure of a ring.

**Discussion**

*Relationships of the cell-types.* Barfurth (1883) described three cell-types in the digestive gland of *Helix* and *Arion*. These were Kalkzellen, Leberzellen, and Fermentzellen, equivalent respectively to calcium, digestive, and excretory cells as defined here. Calcium cells have since been found in many other species of pulmonates (Cuénot 1892; MacMunn, 1900; Fretter, 1952; van Weel, 1950; Thiele, 1953; Nakazima, 1956).

Early authors distinguished clearly between digestive and excretory cells. However, beginning with Krijgsman (1928), excretory cells have been widely considered as a variety of digestive cell (e.g. Fretter, 1952; Billett and McGee-Russell, 1955). Thiele (1953) regards excretory cells (which he calls Kalkzellen Stadium III) as degenerating calcium cells (Kalkzellen Stadium II). This is my own view. The relationship with calcium cells is indicated by the strongly staining cytoplasm, especially apically, and by the fact that many calcium cells, as well as excretory cells, possess a large vacuole containing yellow granules. Particularly interesting is a cell (described on p. 178) which has the appearance of a calcium cell except that all the calcium spherules have been lost and replaced by a large vacuole. Abolins-Krogis (1961) describes excretory cells which have protein granules apically; these must be an inter-
mediate stage in the formation of excretory cells from calcium cells. Against these points may be set the fact that excretory cell nuclei are of the same size as digestive cell nuclei, and smaller than calcium cell nuclei. This is probably coincidental, as many excretory cell nuclei have very small quantities of DNA, and Thiele (1953) describes them as strongly shrunken. There is much evidence that they are degenerating (see below). In long-starved snails digestive cells resemble excretory cells as a result of the enlargement of 'green' granule vacuoles. This cannot be taken as an indication of a relationship between the two cell-types. No cells have been found which are intermediate between digestive and excretory cells.

Billett and McGee-Russell (1955) describe two types of digestive cell, one with 'green' granules throughout the cytoplasm and the other with yellow granules basally and 'green' granules apically. There does not appear to be any fundamental difference between these two types of digestive cell; one type has merely accumulated yellow granules while the other has not. In fact, all intermediates can be found between digestive cells with almost no yellow granules and those with very large numbers of yellow granules, as found in hibernating snails.

Thin cells were first described by MacMunn (1900); he called them young cells, presumably implying that they were undifferentiated. Thiele (1953) also believed them to be undifferentiated; he found them between digestive and calcium cells, and believed they could develop into either type. I have not found thin cells restricted to positions between digestive and calcium cells, and they may occur anywhere. I have found no direct evidence that they develop into any other cell-type, although I believe they must do so. The occurrence of mitosis in thin cells indicates, first, that they are undifferentiated (since differentiated cells do not normally divide), and secondly, that they do develop into other cell-types (since they do not appear to increase in number with age). Thiele (1953) claimed that mitosis only occurred in young snails, and that in older snails amitosis occurred. I have not found amitosis in snails of any age. No doubt Thiele overlooked mitotic nuclei in older snails, as they are very rare; I have only found them in feeding snails. Nakazima (1956), working on Japanese snails, found alkaline phosphatase in thin cells. I have found alkaline phosphatase in calcium cells (Sumner, unpublished), which suggests that thin cells develop into calcium cells rather than digestive cells, which only have a little alkaline phosphatase apically, if any.

**Functions of the cell-types.** It has been stated that the function of calcium cells is to store calcium salts (Fretter 1952), although Filhol (1937) believed this to be only one aspect of a general property of storage. Grünbaum (1913) and Nakazima (1956) both found lipid stored in calcium cells, and the latter found that the amount of lipid varied with starvation. I find that the amount of lipid in calcium cells decreases as starvation proceeds, and thus it is clearly a reserve food substance.

There is less certainty about the function of the calcium spherules. Wagge (1951), McGee-Russell (1955), and Abolins-Krogs (1961, 1963b) believed
that the calcium was used for shell repair, whereas Krijgsman (1928) believed it was secreted into the crop-juice and acted as a buffer against changes in pH. I have found that calcium spherules are secreted into the lumen of the digestive gland tubules, and pass down the ducts of the gland. However, there is much evidence that the spherules are not simply balls of calcium salts, but have a much more complicated structure and composition (Grünbaum, 1913; Wagge, 1951; McGee-Russell, 1957; Abolins-Krogis, 1963a, 1963c; Sumner, unpublished); thus their function cannot be considered merely in terms of their calcium content.

The high RNA content and prominent Golgi apparatus of calcium cells indicates strongly that they must have a secretory function. Apart from the calcium spherules, the protein granules are probably passed out of the cell. Although they have not been found in the lumen of the tubules, they decrease greatly in number in feeding snails, and this suggests that they are released into the tubules during feeding. Protein granules have been observed previously in calcium cells, but no suggestions have been made as to their function (Grünbaum, 1913; Filhol, 1937; Abolins-Krogis, 1961).

Calcium cells also have an excretory function; many form vacuoles containing lipofuscin granules, but it is not certain whether the calcium cells are transformed into excretory cells before the lipofuscin granules are released.

Excretory cells were at first believed to secrete enzymes (Barfurth, 1883; Frenzel, 1885; Krijgsman, 1925); other workers believed that they were excretory, and eliminated chlorophyllous pigments taken in with the food (MacMunn, 1883, 1886, 1900; Dastre, 1899; Dhéré and Vegezzi, 1916). Cuénot (1892), Thiele (1953), and McGee-Russell (1955) also believed that excretory cells were excretory, but Krijgsman (1928) was unable to assign any function to them. Abolins-Krogis (1961) thought that the material of the yellow granules was used in shell regeneration. She believed that the main pigments present were urochrome and melanin, although later (1963b) she admitted that lipofuscin might be present. She also found many other substances in yellow granules (Abolins-Krogis, 1961, 1963a). Zacks (1955) appears to be the only previous worker who found lipofuscin in a mollusc, in this case in the amoebocytes and intestinal epithelium of the lamellibranch Venus mercenaria; he believed the pigment to be excretory.

My own work confirms the majority view that excretory cells have an excretory function. Lipofuscin granules similar to those in excretory cells can be found in the intestine and faeces, and thus they are being excreted.

It is clear that excretory cells are degenerating, as Thiele (1953) thought. The nucleus often has a low DNA content, sometimes almost none; the amount of cytoplasm is very small; mitochondria are few; and I have found no Golgi apparatus. Thiele (1953) claimed that he had found a Golgi apparatus, which, however, was degenerating.

The digestive cells are widely believed to secrete, and to absorb soluble food material (Krijgsman, 1925, 1928; Fretter, 1952; Thiele, 1953); therefore they have been named secretory-resorption (SR) cells. The presence of a
brush border, an apical concentration of mitochondria, and a high concentration of phospholipid apically all indicate that absorption occurs in digestive cells. Krijgsman (1928) described a secretory cycle, synchronous in all cells, in which small yellow granules were transformed into ‘green’ granules, which were passed out of the cell; Thiele (1953) supported this hypothesis. However, it is obvious that yellow granules cannot be transformed into ‘green’ granules, because of their histochemical differences. There appears to be no connexion between the two types of granule, except that they occur together in the same cell. The inconspicuous Golgi apparatus and low RNA content of digestive cells show that they do not secrete much. Nevertheless, it is true that both small yellow and ‘green’ granules are secreted in the sense that they are cast out of the cell. The small yellow granules, like the large yellow granules of excretory cells, are excreted. Barfurth (1883) noticed that yellow granules, similar to those of digestive cells, could be found in the faeces; and I have found this particularly obvious in hibernating snails. It seems probable that the secretion of ‘green’ granules is also excretory. Digestive cells have been shown to take up solid food particles, which accumulate in vacuoles (Jordan, 1918; Rosen, 1941). My own experiments (Sumner, unpublished) show that phagocytosed particles accumulate in vacuoles similar to those occupied by ‘green’ granules. Thus it seems that ‘green’ granules represent ingested food material and indigestible residues; this is supported by the fact that they contain muco-substances, which are mixed with the food in the gut before it enters the digestive gland (Graham, 1949; Morton, 1952). No doubt the ‘green’ granules which are extruded from the cell are made up of these indigestible residues.

Other functions which have been attributed to digestive cells are participation in shell regeneration (Abolins-Krogis, 1961), and storage of glycogen and fat (Fretter, 1952). The subject of shell regeneration is not considered here. I have found that digestive cells store lipid, in their basal lipid droplets, and that these decrease in number as starvation proceeds. However, I have found no storage of glycogen; if this does occur, it cannot be of great importance to the snail.

The changes occurring in digestive gland cells of feeding snails, compared with short-starved snails, are few but significant. The increase in RNA in digestive cells is no doubt connected with synthesis of enzymes for intracellular digestion, which is believed to occur (Rosen, 1941). The reduction in number of protein granules is probably caused by the passage of some of them out of the cell (see p. 185).

Most of the changes occurring during prolonged starvation have been found to occur in other organisms. In human tissue culture cells (Cohen and others, 1961) and in Euglena (Malkoff and Buetow, 1964), starvation is accompanied by vacuolization of the cytoplasm, the vacuoles growing larger as starvation proceeds. In Helix vacuoles are already present in the cytoplasm of digestive cells, but they also grow larger as starvation proceeds. In Euglena, starved for more than 8 days, it was found that mitochondria became swollen (Malkoff and Buetow, 1964), as occurs in the calcium cells of Helix. In rat
liver (Lagerstedt, 1949) and in Euglena (Malkoff and Buetow, 1964) starvation results in the DNA becoming diffusely distributed throughout the nucleus; and this also occurs in Helix. However, Helix differs from these other organisms in that it takes several weeks, rather than a few days, for the changes to occur. It seems, as one would expect, that Helix is adapted to going without food for long periods. This is a necessary attribute for an animal which frequently meets adverse conditions and has to stop feeding.

The changes already discussed appear to be typically associated with starvation. Other changes found in digestive gland cells of Helix are more probably connected with inactivity of the cells rather than an absence of food supply. Such changes are the reduction of the amount of RNA in calcium cells, loss of phospholipid in the apical cytoplasm, and reduction in size of the Golgi apparatus of calcium cells. Reduction of the amount of RNA and reduction in the size of the Golgi apparatus are presumably associated with the cessation of secretion, which is no longer necessary when there is no food to digest. Reduction of the amount of RNA during starvation has also been found in rat liver cells (Lagerstedt, 1949). Loss of phospholipid in the apical cytoplasm is probably because absorption and phagocytosis no longer occur.

The changes in the digestive gland cells which occur during hibernation are not similar to those occurring during prolonged starvation. This confirms the view that it is a condition which is specially prepared for (Howes and Wells, 1934), and not merely a prolonged starvation. The most obvious feature of the digestive gland of hibernating snails is the great accumulation of small yellow granules in digestive cells. This was noted by MacMunn (1886), who believed they consisted of enterochlorophyll derived from the food. He thus had difficulty in explaining this increase. It is clear that since no faeces can be passed out during hibernation, excretory material, including yellow granules, must accumulate. In fact, not only the digestive cells of hibernating snails, but also their intestines, are full of small yellow granules. The reduction in size of the Golgi apparatus of calcium cells, which parallels that found in long-starved snails, is probably due to the same cause, that is reduction or cessation of secretion. The small number of basal lipid droplets found in digestive gland cells of hibernating snails is a consequence of using up this food reserve. The elongation of mitochondria might be a result of starvation. However, in hibernation mitochondria become long and thin, while during prolonged starvation they become thicker as well as longer. The other changes in digestive gland cells of hibernating snails cannot be satisfactorily explained at present.

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Sumner—Digestive gland cells of Helix

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Appendix

Table I

Synonyms of names of cells

<table>
<thead>
<tr>
<th>Genus or species</th>
<th>Thin cell</th>
<th>Digestive cell</th>
<th>Calcium cell</th>
<th>Excretory cell</th>
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<td>—</td>
<td>Digestive cell</td>
<td>Calcium cell</td>
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<td>Kalkzell</td>
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<td>Many species</td>
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<td>Fretter, 1952</td>
<td>Agriolimax, Arion, Helix</td>
<td>—</td>
<td>—</td>
<td>Digestive cell</td>
</tr>
<tr>
<td>Krijgsman, 1925</td>
<td>Helix pomatia</td>
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<td>Krijgsman, 1928</td>
<td>Helix pomatia</td>
<td>—</td>
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<td>Granule or Enzyme cell</td>
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<td>McGee-Russell, 1955</td>
<td>Helix aspersa</td>
<td>Young cell</td>
<td>Digestive cell</td>
<td>Calcium cell</td>
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<td>MacMunn, 1900</td>
<td>Arion, Helix, Limax</td>
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<td>Hepatic cell</td>
<td>Lime cell</td>
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<tr>
<td>Nakazima, 1956</td>
<td>Many Japanese species</td>
<td>Narrow cell</td>
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<tr>
<td>Thiele, 1953</td>
<td>Many species</td>
<td>Indifferent cell</td>
<td>S.R. cell</td>
<td>Calcium or Calcareous cell</td>
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<tr>
<td>van Weel, 1950</td>
<td>Achatina</td>
<td>—</td>
<td>p-cell; y-cell</td>
<td>Calcium cell</td>
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189
<table>
<thead>
<tr>
<th>Test</th>
<th>Fixative</th>
<th>Embedding</th>
<th>Thickness of section (μm)</th>
<th>Reference</th>
<th>All cells</th>
<th>Thin cells</th>
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<tr>
<td>Feulgen</td>
<td>Ze</td>
<td>P</td>
<td>8</td>
<td>Feulgen and Rossenbeck, 1924</td>
<td>o</td>
<td>+ + +</td>
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<tr>
<td>Pyronine/methyl green</td>
<td>Ze3</td>
<td>P</td>
<td>8</td>
<td>Jordan and Baker, 1955</td>
<td>o</td>
<td>+ + +</td>
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<tr>
<td>RNAase + P/MG</td>
<td>Ze3</td>
<td>P</td>
<td>8</td>
<td>Bradbury, 1958</td>
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<td>+ +</td>
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<td>PA/S</td>
<td>Ze</td>
<td>P</td>
<td>8</td>
<td>MacManus, 1948</td>
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<td>+</td>
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<td>Diastase + PA/S</td>
<td>Ze</td>
<td>P</td>
<td>8</td>
<td>Lillie and Greco, 1947</td>
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<td>toluidine blue (mstachromasy)</td>
<td>Ze</td>
<td>P</td>
<td>8</td>
<td>Lison, 1953</td>
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<td>sulphation + toluidine blue</td>
<td>Ze</td>
<td>P</td>
<td>16</td>
<td>Baker, 1947</td>
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<td>Sakaguchi</td>
<td>F-Na</td>
<td>G</td>
<td>10</td>
<td>Baker, 1956</td>
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<td>o</td>
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<tr>
<td>DMAB/nitrite</td>
<td>F-Ca/PC</td>
<td>G</td>
<td>10</td>
<td>Baker, 1946</td>
<td>o</td>
<td>o/ +</td>
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<tr>
<td>Sudan black</td>
<td>F-Ca/PC</td>
<td>G</td>
<td>10</td>
<td>Baker, 1946</td>
<td>o</td>
<td>o/ +</td>
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<tr>
<td>acid haematein (AH)</td>
<td>WB/PC</td>
<td>G</td>
<td>10</td>
<td>Baker, 1946</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>pyridine extraction + AH</td>
<td>FE: FMP</td>
<td>P</td>
<td>8</td>
<td>McGee-Russell, 1955</td>
<td>-</td>
<td>-</td>
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<tr>
<td>cobalt method for calcium</td>
<td>FE: FMP</td>
<td>P</td>
<td>8</td>
<td>Pearse, 1960</td>
<td>o</td>
<td>o/ +</td>
</tr>
<tr>
<td>calcium red method</td>
<td>FE: FMP</td>
<td>P</td>
<td>8</td>
<td>Pearse, 1960</td>
<td>o</td>
<td>o/ +</td>
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<tr>
<td>Schmorl</td>
<td>F-Na</td>
<td>P</td>
<td>8</td>
<td>Pearse, 1960</td>
<td>o</td>
<td>+</td>
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<td>chrome-alum haematoxylin</td>
<td>F-Na</td>
<td>P</td>
<td>8</td>
<td>Pearse, 1960</td>
<td>+ +</td>
<td>+ + +</td>
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<td>long Ziehl-Neelsen</td>
<td>F-Na</td>
<td>P</td>
<td>6</td>
<td>Pearse, 1960</td>
<td>*</td>
<td>*</td>
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<tr>
<td>10% hydrogen peroxide (48 h)</td>
<td>F-Na</td>
<td>P</td>
<td>8</td>
<td>Pearse, 1960</td>
<td>*</td>
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</table>
The following abbreviations and symbols are used:
F-Ca/PC, formaldehyde-calcium with post-chroming; FE, formalin-ethanol (1:1); FMP; formalin-methanol-pyridine; F-Na, formalin-saline; Ze, Zenker; Ze3, Zenker for 3 h; o, no reaction; —, no observation; +, weakly positive reaction; ++, moderately positive reaction; ++++, strongly positive reaction; *, not bleached; /, indicates variation; e.g. o/++ indicates a reaction varying between none and moderately positive.
**Sumner—Digestive gland cells of Helix**

**Table 3**

*Changes of size with starvation*

<table>
<thead>
<tr>
<th>Length of starvation</th>
<th>‘Green’ granules</th>
<th>Their vacuoles</th>
<th>Calcium cell mitochondria</th>
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<tbody>
<tr>
<td>feeding</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>short-starved*</td>
<td>3·5×3·0</td>
<td>6·1×5·1</td>
<td>1·0×0·6</td>
</tr>
<tr>
<td>34 days</td>
<td>7·5×6·3</td>
<td>13·1×10·2</td>
<td>1·0×0·8</td>
</tr>
<tr>
<td>49 days</td>
<td>9·1×6·7</td>
<td>12·5×9·5</td>
<td>1·2×0·8</td>
</tr>
<tr>
<td>65 days</td>
<td>12·6×9·6</td>
<td>19·2×15·3</td>
<td>1·3×0·8</td>
</tr>
<tr>
<td>82 days</td>
<td>11·5×9·3</td>
<td>18·0×14·7</td>
<td>1·5×1·0</td>
</tr>
<tr>
<td>96 days</td>
<td>11·2×8·4</td>
<td>17·9×15·5</td>
<td>1·7×1·2</td>
</tr>
<tr>
<td>132 days</td>
<td>12·6×10·2</td>
<td>20·6×15·7</td>
<td>1·7×1·1</td>
</tr>
</tbody>
</table>

All the above measurements are in μ.

* Average of measurements from several snails starved for between 7 and 20 days.