STUDIES IN HISTOPATHOLOGY—CHANGES INDUCED BY A LARVAL MONOSTOME IN THE DIGESTIVE GLAND OF THE SNAIL, *MELANOIDES TUBERCULATUS* (MÜLLER)

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Infection with larval trematodes brings about a modification in the cellular structure of the infected tissues. Although earlier workers (Lebour, 1911; Faust, 1920) have studied the pathology of the infected digestive gland in the molluscs, Agersborg (1924) was probably the first to give a clear picture of the cytophysiological changes in the liver of *Physa gyrina* and *Planorbis trivolvis* due to larval fluke infection. F. G. Rees (1931, 1934) and later W. J. Rees (1936) also made similar observations. Among the more recent work on these lines Cort, Olivier and McMullen (1941), Pratt and Barton (1941), and Pratt and Lindquist (1943) are noteworthy contributions. The present paper gives an account of the changes induced by a larval monostome in the digestive gland of a freshwater snail, *Melanoides tuberculatus* (Müller).

Material and Methods

After isolating the infected snail in the laboratory and identifying the nature of the infection, the digestive gland of the snail was dissected out in normal salt solution. Pieces of the gland, about 2 mm. in size, were fixed in Carnoy's, Zenker's, Gilson's and Bouin's fluid. Following usual dehydration and embedding in paraffin, sections 4–6 μ in thickness were cut and stained with double-staining technique. Pieces of digestive gland from non-infected *Melanoides tuberculatus* (which had been kept under observation in the laboratory and did not shed cercariæ for over two months) were similarly prepared for comparison of structure.

Normal digestive gland.—The normal digestive gland presents a healthy yellowish brown appearance and a smooth outer surface. The histological structure of the digestive gland as revealed in sections is very characteristic. In an uninfected condition the digestive gland is composed of a large number of blindly ending tubules each covered over with a layer of connective tissue. The adjacent layers of the long tubules are separated at places by the blood sinuses and the whole gland is covered over with a smooth external lining

of connective tissue. The nuclei in the cells occupy the basal part. Generally four main types of cells (Fig. 1), very much similar to what Van Weel

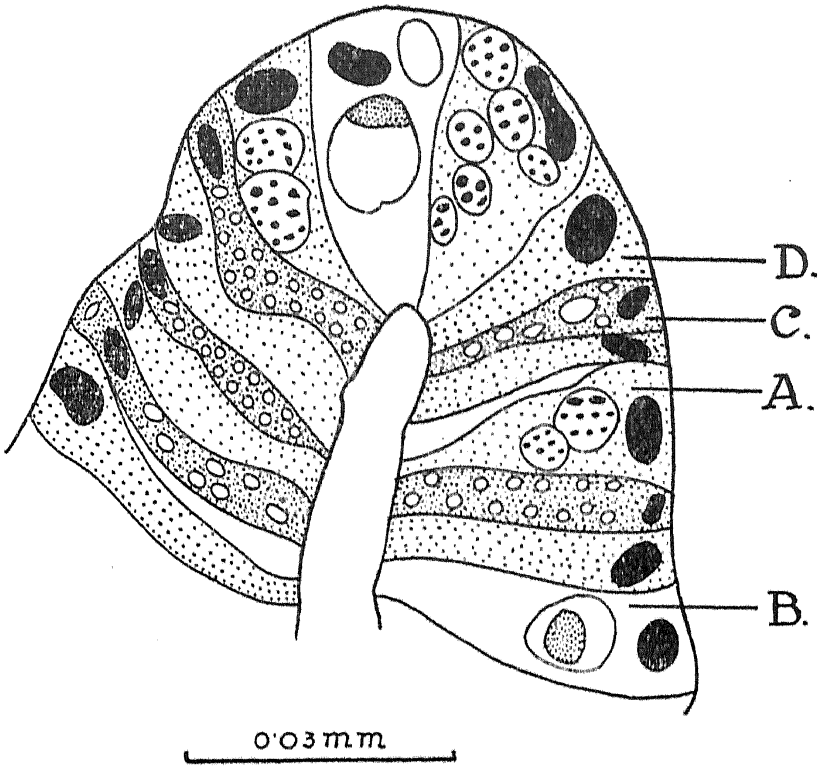

FIG. 1. Part of a section of the normal (uninfected) hepatic tubule of *Melanoides tuberculatus*. A. Triangular cell showing calcium globules. B. Columnar cell with big vacuole containing a cluster of small granules inside the vacuole. C. Columnar cell containing a number of small vacuoles and tiny granules. D. Columnar cell without vacuoles but containing granules.

(1950) described in African Giant Snail, *Achatina fulica* Fer, can be distinguished:

A. Triangular cells lying between the columnar cells and without any vacuoles but containing calcium globules and fine granules = Ca'-cells of Van Weel (1950).

B. Columnar cells with big vacuoles containing clusters of small granules inside them = "b"-cells of Van Weel (1950).

C. Columnar cells containing a number of small vacuoles and tiny granules = "y"-cells of Van Weel (1950).

D. Columnar cells without vacuoles but containing granules = "p"-cells of Van Weel (1950).

Infected digestive gland.—The infected digestive gland appears to assume a pale yellow or slate grey colour. At the place where there is a rapid growth

and multiplication of the larval stages the surface of the digestive gland often shows small projections which may sometimes rupture the outer layer of the gland. Marked internal changes in the cell structure of the infected digestive gland have been noticed. To begin with, at an early stage of infection the columnar cells show a tendency to lay down transverse partitions (Fig. 2) across their length. At the same time the partition walls between different

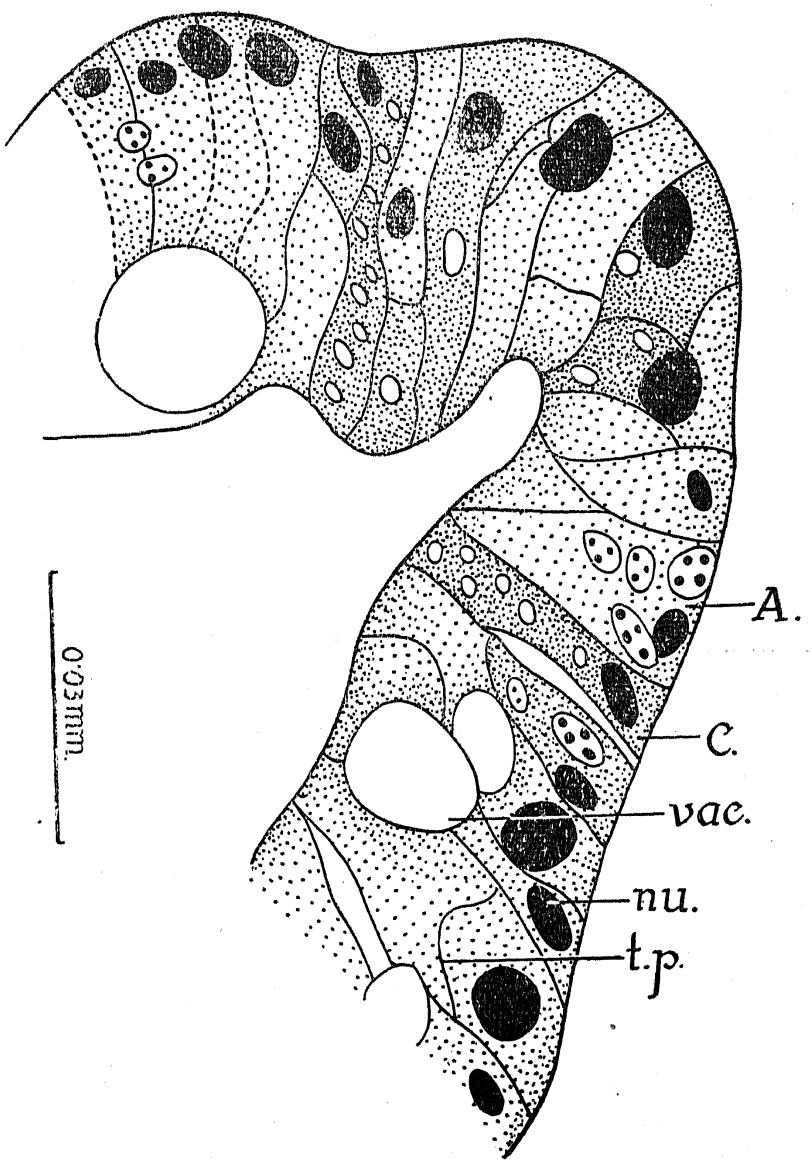

FIG. 2. Part of a section of the infected hepatic tubule of *Melanoides tuberculatus* showing early changes in the cells.

A. Triangular cell showing calcium globules. C. Columnar cell containing a number of small vacuoles and tiny granules.

nu., nucleus; *t.p.*, transverse partition; *vac.*, vacuole.

columnar cells begin to disintegrate. This results in an aggregation of a number of small squarish cells simulating squamous epithelium (Fig. 3).

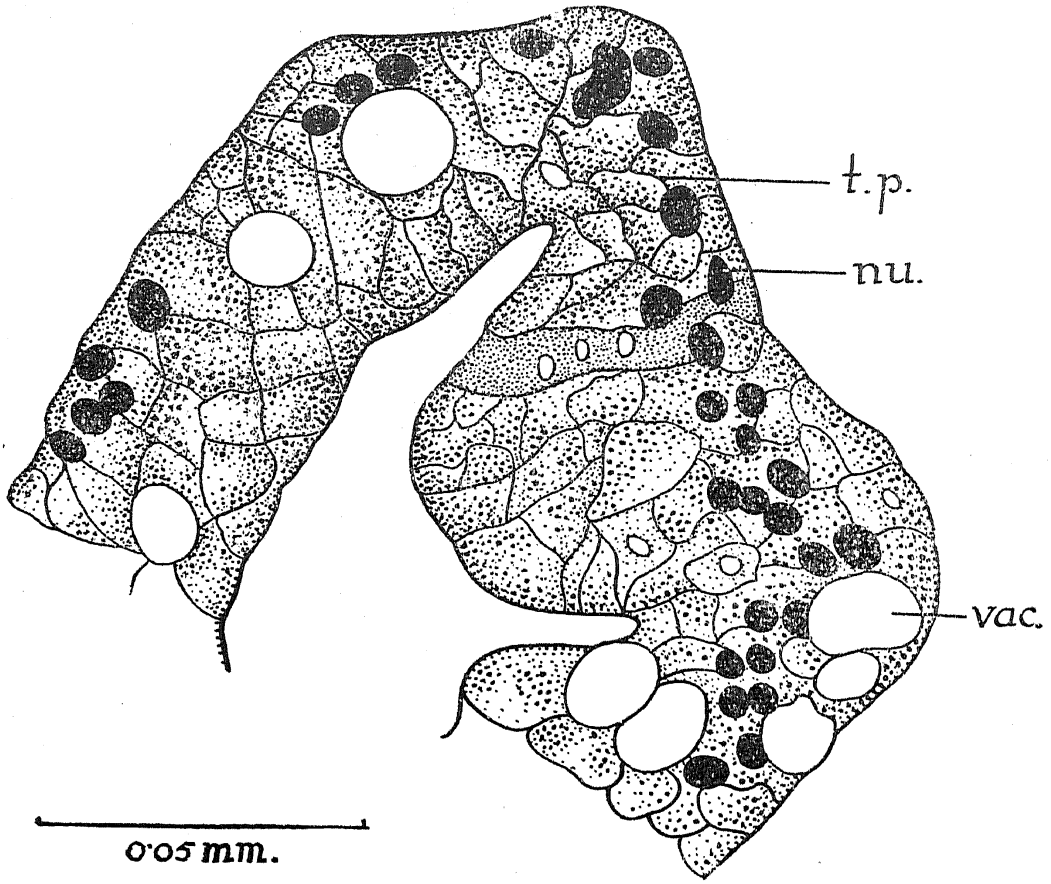

FIG. 3. Part of a section of the infected hepatic tubule of *Melanoides tuberculatus* showing marked changes in tne cells.

nu., nucleus; *t.p.*, transverse partition; *vac.*, vacuole.

With the further disintegration of the cell walls, a syncitial nucleated mass results (Fig. 4). The nuclei which usually occupy the base of the cells in the normal gland begin to migrate from the base towards the apex and in those cases where normal columnar cells are replaced by an aggregation of a number of squarish cells, the nuclei even tend to disintegrate. The vacuoles and the granules seen normally in the different types of columnar cells of the digestive gland also undergo modifications. With the disappearance of the cell outlines, typical shape of the various cells is lost. In fact the arrangement into the four types of cells as seen in the normal uninfected gland becomes less apparent or may entirely disappear. The triangular calcium cells lose their calcium globules which form such a distinguishing feature of these cells in the normal gland. Besides these changes all the cells appear to be full of dark pigment. Lastly, instead of the numerous small vacuoles seen

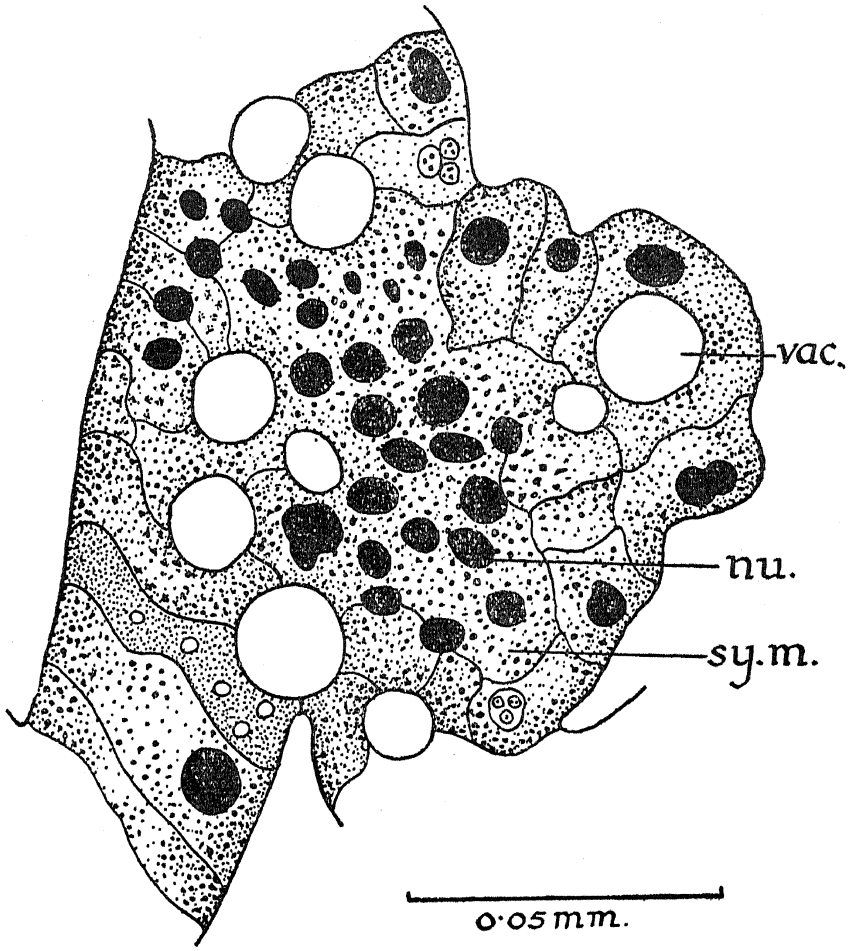

FIG. 4. Part of a section of the infected hepatic tubule of *Melanoides tuberculatus* showing disintegration of the cells and formation of the syncitial mass.

nu., nucleus; *sy. m.*, syncitial mass; *vac.*, vacuole.

in the columnar cells of an uninfected gland, only a few large vacuoles of globular shape are seen in the infected gland. At places where infection is very heavy, there is a total necrosis of the gland tissue (Fig. 5) which is practically replaced by these larval trematodes.

DISCUSSION

Various degrees of cell disintegration have been noticed in the digestive gland of *Melanoides tuberculatus* (Müller). The disappearance of the calcium globules from the triangular cell (= Calcium cell of Van Weel, 1950) produces an adverse effect on the calcium metabolism of the snail. It is said that the shell of snail which contains calcium salts helps in buffering the acidic products of anaerobiosis (Culbreth, 1941; Robertson, 1941). Also he acidity of the hepatic tubules in the digestive gland is neutralized by the

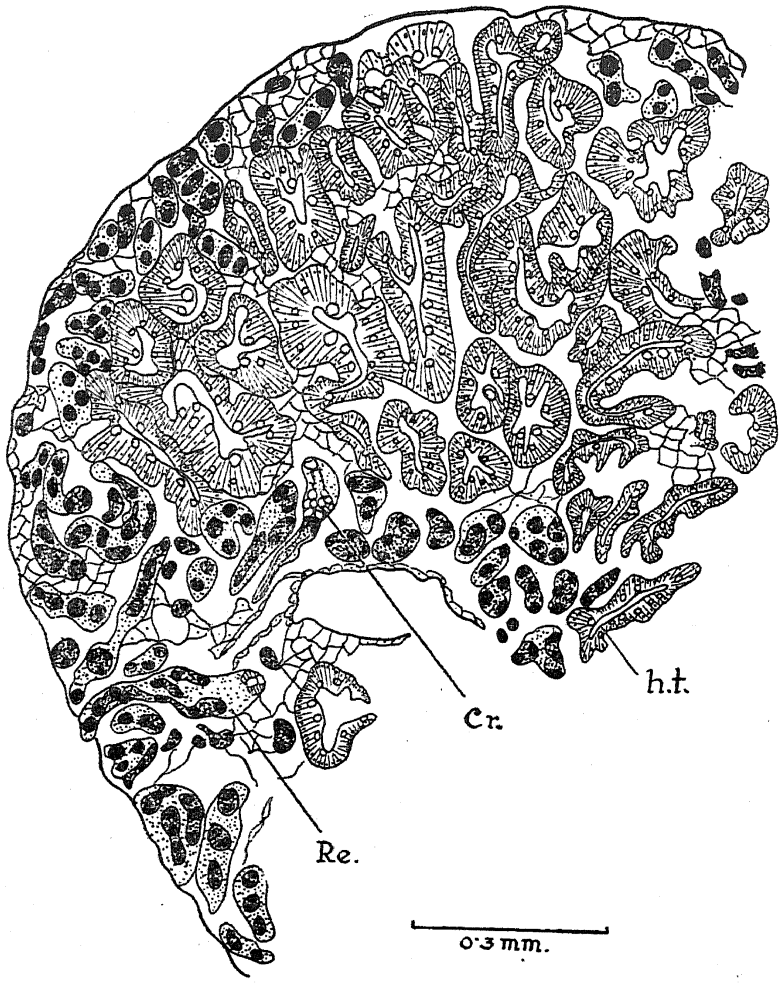

FIG. 5. Section of the infective digestive gland of *Melanoides tuberculatus* showing necrosis of the gland tissue (hepatic tubules) and replacement by the parasites.

Cr., cercaria; *h.t.*, hepatic tubule; *Re.*, redia.

calcium globules contained within the triangular cells. This helps in maintaining a constant pH in the internal medium of the snail. In case of such infection where the loss of calcium globules is enormous the snail would not be able to meet its normal requirements of calcium. Whether the deposit of calcium in cells of the infected gland is interfered with or the calcium globules are used up in neutralizing the acidic secretions of the larval trematodes is not clear. The disintegration of the cell structure would interfere with the normal digestion and absorption in the infected digestive gland. There would be no production of the enzymes; in fact with the disappearance of cell granules the secretory activities of the cells would be greatly diminished.

SUMMARY

An account is given of the effects of infection in the digestive gland of *Melanoides tuberculatus* (Müller) by a monostome larval trematode. The distinction into four types of cells of the tubules of digestive gland as seen in the normal uninfected gland is lost due to this infection. The columnar cells become squarish and may even change into a nucleated syncitial mass. Loss of calcium globules and cell granules together with the disintegration of the cell would impair the normal metabolic activity of the snail.

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