

5-HYDROXYTRYPTAMINE AND ENTEROCHROMAFFIN CELLS IN THE OVINE BILIARY MUCOSA DURING FASCIOLIASIS

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In fascioliasis, the mature liver-flukes reside in the biliary tract. The infested bile ducts have an enlarged lumen, a proliferated mucosa and a fibrous thickening in the outer wall. The caeca of the liver flukes contain both biliary mucosal cells and blood (Dawes, 1963).

Erspamer (1936) noted the presence of enterochromaffin cells in the intrahepatic bile ducts of three bovines infected with *Fasciola hepatica*. In uninfested cattle enterochromaffin cells were not found in bile ducts but were present in the gallbladder. The enterochromaffin cells probably contain 5-hydroxytryptamine (5-HT) (Erspamer & Asero, 1952; Barter & Pearse, 1955), which stimulates the carbohydrate metabolism and contractility of *Fasciola hepatica* (Mansour, 1957, 1962). In the present experiments ovine hepatic 5-HT was found to be raised during fascioliasis, a very high 5-HT content and numerous enterochromaffin cells being found in the mucosa of infested bile ducts.

METHODS

Small blocks of tissue (3-5 mm thick) were fixed in a solution containing 1 part formalin and 9 parts 0.9% w/v aqueous NaCl, or 1 part formalin and 9 parts 5% potassium bichromate-chromate solution (Falck, Hillarp & Torp, 1959) and were embedded in paraffin wax or in carbowax (Zugibe, Kopaczky, Cape & Last, 1958). Sections (7 μ) were stained with the diazotate of 5-nitroanisidine (Fast Red salt B, I.C.I. Ltd.), 2:6-dichloroquinone-chloroimide (Gibbs' reagent) or alkaline silver nitrate (Fontana's silver reagent) as described by Pearse (1953), or with astrablau or toluidine blue (Bloom & Kelly, 1960).

Specimens for the estimation of 5-HT (Bartlet, 1965) were homogenized in 0.1 N HCL within 40 min of collecting the livers from the slaughter house. Parenchyma was dissected from the margin of the caudate lobe of the liver and from the gallbladder fossa as it was thought that the hepatic 5-HT concentration might vary in different regions of the organ. Three of the fluke-infested livers were from lambs.

RESULTS

Enterochromaffin cells

The enterochromaffin cells of the ovine biliary tract were stained an orange-brown colour by the diazotate of 5-nitroanisidine, grey-black by Gibbs' reagent and black by Fontana's silver reagent, but the cells were not stained by astrablau or toluidine blue.

Pieces of gallbladder, hepatic or cystic bile ducts, intrahepatic bile duct and liver from three control and four fluke-infested livers were embedded in paraffin wax, and five sections from each block of tissue were stained with the diazotate of 5-nitroanisidine and examined for the presence of enterochromaffin cells. In the controls, each of the sections made from one gallbladder contained one to three enterochromaffin cells and a single

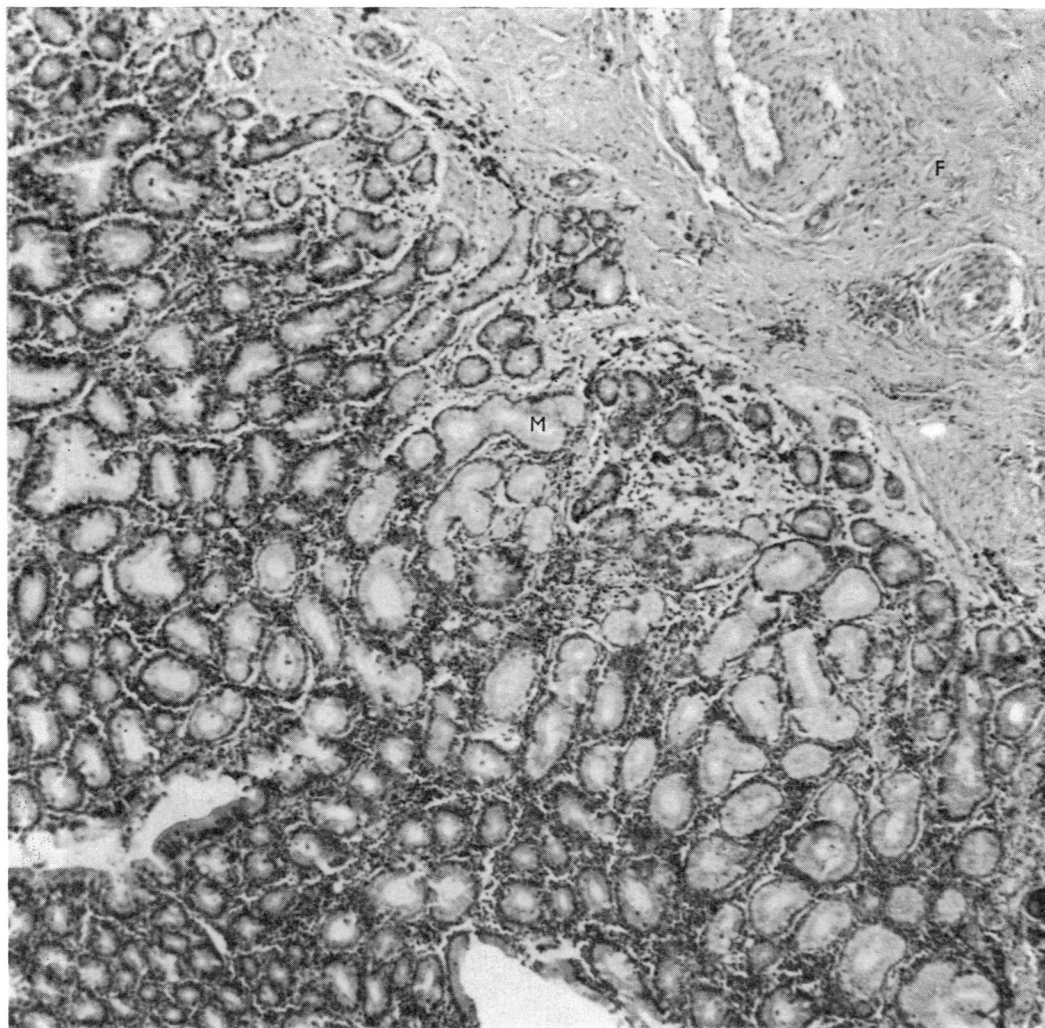
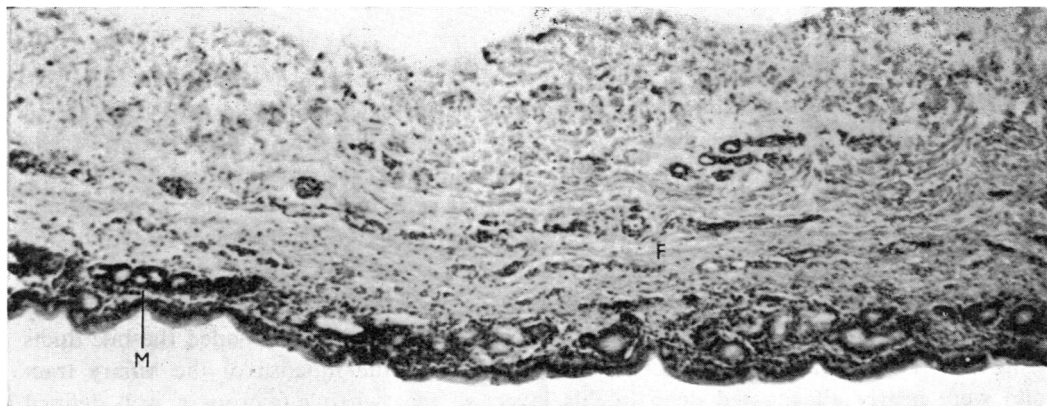


Fig. 1. Transverse sections of sheep cystic bile ducts, control (above) and infested (below). Infestation produced a thickening of the outer fibrous layer (F) and a proliferation of the mucosa (M). Haematoxylin, $\times 130$.

enterochromaffin cell was found in one section of an external bile duct. In three of the fluke-infested livers, enterochromaffin cells were found in all the sections of the biliary tract but were absent from the liver parenchyma. In some of these sections the enterochromaffin cells were very numerous, several cells often being present in a single acinus of the mucosal glands. No enterochromaffin cells were, however, found in the fourth fluke-infested liver.

In all sections made from fluke-infested material, the glandular mucosa of the biliary tract was greatly proliferated and a thick layer of fibrous tissue surrounded the bile ducts (Fig. 1). The enterochromaffin cells were confined to the mucosa of the biliary tract and were nearly all situated deep in this layer, so that when numerous a well defined layer of enterochromaffin cells was present in the deep mucosa adjoining the fibrous tissue (Fig. 2). The enterochromaffin cells were nearly always situated among the cells lining the mucosal glands (Fig. 3).

5-Hydroxytryptamine

The 5-HT concentrations found in a lightly infested lamb liver and in the infested sheep liver without demonstrable enterochromaffin cells were no greater than the values of the controls; however, the concentrations of 5-HT were raised above control values in the five sheep and two lamb livers which were more heavily infested with liver-flukes. The 5-HT concentrations found in all the fluke-infested livers have been combined and compared with the control values (Table 1).

The hepatic 5-HT concentration was raised during fascioliasis. Whether or not the rise in the 5-HT concentration was confined to the biliary tract remains uncertain, because although the 5-HT content of the liver in the gallbladder fossa was raised during

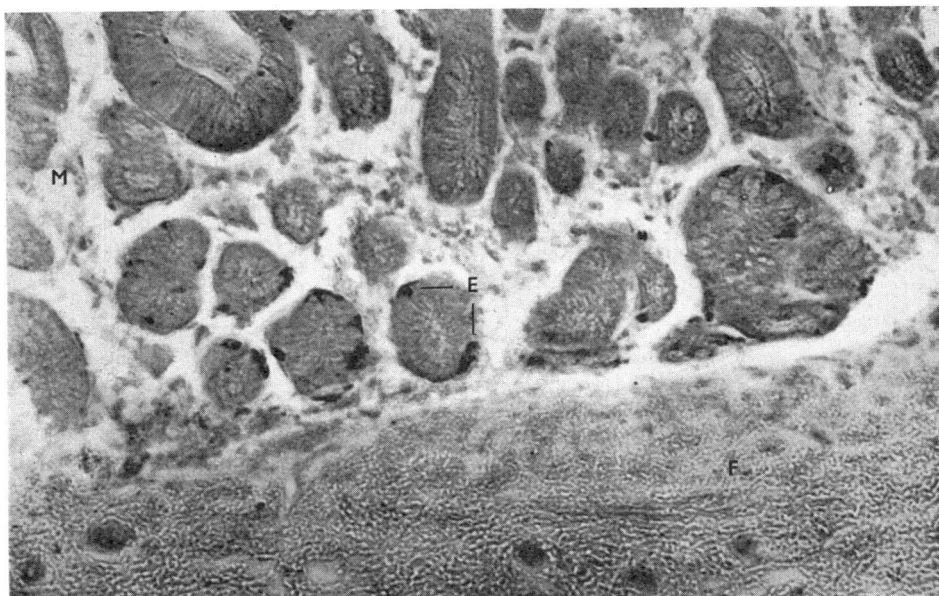


Fig. 2. Transverse section of an infested intrahepatic bile duct. Enterochromaffin cells (E) are numerous at the base of the mucosa (M) and near the fibrous layer (F). Gibbs' reagent, $\times 180$.

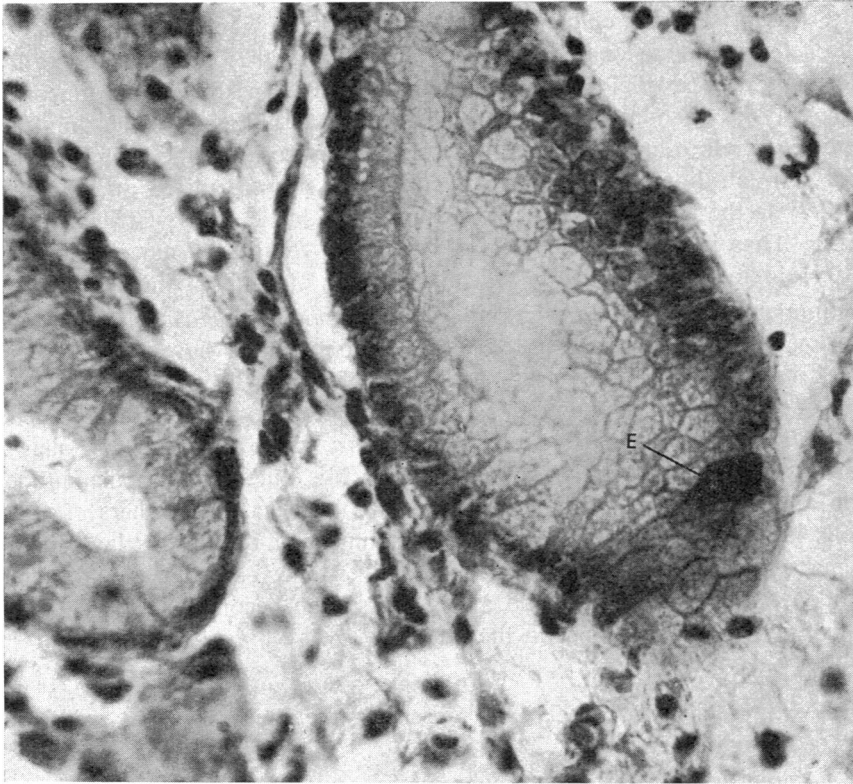


Fig. 3. Transverse section of the mucosa of a cystic bile duct infested with *Fasciola hepatica*. An acinus of a mucus secreting gland contains an enterochromaffin cell (E). Diazo coupling reaction, $\times 900$.

fascioliasis, this may have been due to the presence of small bile ducts. The rise in 5-HT concentration was most marked in the bile ducts, where it seemed to be confined to the mucosa. In ten experiments, the concentration of 5-HT in the mucosa of fluke-infested bile ducts was $3.26 \pm 0.53 \mu\text{g/g}$, whereas in three pieces of fibrous tissue from infested bile ducts, which were microscopically free of mucosa, 5-HT was not detectable.

TABLE 1
EFFECT OF FASCIOLIASIS ON SHEEP LIVER 5-HT CONCENTRATION

The number of experiments is given in parentheses.

		Mean 5-HT concentration ($\mu\text{g/g} \pm \text{s.e.}$)		P
		Control	Fascioliasis	
Liver	{ Caudate lobe	0.50 ± 0.03 (6)	1.29 ± 0.43 (6)	>0.05
	{ Gallbladder fossa	0.49 ± 0.04 (6)	1.53 ± 0.42 (6)	<0.05
Gallbladder		0.29 ± 0.06 (9)	0.48 ± 0.12 (6)	>0.10
Hepatic and cystic bile ducts		0.41 ± 0.09 (9)	1.60 ± 0.49 (9)	<0.05
Intrahepatic bile ducts		0.43 ± 0.10 (8)	2.94 ± 0.69 (9)	<0.01

DISCUSSION

Enterochromaffin cells were identified in the ovine biliary mucosa by positive argentaffin, diazonium and Gibbs' reactions. Astrablau and toluidine blue, which stain

most cells, did not stain the enterochromaffin cells of the ovine biliary mucosa. Enterochromaffin cells were occasionally found in the mucosa of the bile ducts and gallbladders of uninfested sheep, so their more frequent occurrence in the biliary mucosa during fascioliasis may be an outcome of the proliferation of this layer. The uninfested bile ducts were too small to allow collection of sufficient mucosa for the estimation of 5-HT. The 5-HT concentrations of whole-bile ducts and liver were raised during fascioliasis, however, despite the thick fibrous tissue of the infested bile ducts which contained very little 5-HT. Thus it seems that the 5-HT content of the biliary mucosa was increased both by a proliferation and a rise in the concentration of 5-HT of this layer.

5-HT stimulates the metabolism and motility of *Fasciola hepatica*; thus the high 5-HT concentration found in the proliferated biliary mucosa might have a marked influence on mature liver-flukes in bile ducts. Whether 5-HT expels liver-flukes from the biliary tract may depend on the amount of 5-HT they ingest. 5-HT is concentrated in the enterochromaffin cells which are deep in the biliary mucosa during fascioliasis. The development of the fibrous tissue surrounding the bile ducts and the magnitude of the infestation are two of the factors which are likely to affect the numbers of enterochromaffin cells ingested by liver-flukes. The development of the fibrous layer makes liver parenchyma and blood less accessible to the liver-flukes so that they become more dependent on the biliary mucosa, and as the infestation becomes heavier it is probable that the liver-flukes forage deeper in the mucosa.

SUMMARY

1. A few enterochromaffin cells are present in the mucosa of the gallbladder and bile ducts of normal sheep.
2. In fascioliasis, numerous enterochromaffin cells occur deep in the proliferated biliary mucosa, and the concentration of 5-hydroxytryptamine in the mucosa is raised.

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