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13.5 Endosymbiosis

Hirudo medicinalis has virtually an obligatory symbiotic relationship with the bacterium *Aeromonas hydrophila* (= *Pseudomonas hirudinis)*, the only species of bacteria which lives in its gut (Zirpolo 1923; Lehmensick 1941, 1942; Hornbostel 1941; Büsing 1951; Büsing *et al*. 1953; Wilde 1976; Jennings and Van der Lande 1967; Whitlock, O'Hare, Saunders and Morrow 1983). *Aeromonas hydrophila* is important in the life of *H. medicinalis* in several respects.

1. It secretes an antibiotic which prevents the growth of other bacteria and accordingly retards putrefaction so that blood can be stored for long periods.

2. It contributes enzymes which play a major role in digestion (see Digestion).

3. A presumed role for the bacterium, and probably the context in which endosymbiosis evolved in leeches in the first place, is production of deficient vitamins. This is an inference based on the observation that animals which live exclusively on blood generally have diets deficient in certain necessary compounds, including some of the B complex vitamins (Wigglesworth 1965; Buchner 1965). Inexplicably, dietary deficiency in exclusively bloodsucking leeches has never been investigated.

13.6 Digestive physiology

Digestion and absorption take place predominantly in the leech intestine, food being passed into this region a little at a time from the crop (Büsing *et al*. 1953; Jennings and Van Der Lande 1972; Garcia-Mas 1979ff; Redondo, Garcia-Mas, Beltram and Moreno 1980; see also Gooding 1972). In most species the crop functions primarily, if not exclusively, for food storage, but some digestion takes place in the crop of certain, mainly non-bloodsucking species, eg. *Glossiphonia complanata* and *Helobdella stagnalis* (Damas 1962; Jennings and Van Der Lande 1967).

For the most part secretions from leech salivary cells are not involved in digestion. However, the functional significance of a salivary protease in *Hirudo medicinalis* (Damas 1974a) and of esterases in *Poecilobdella granulosa* (Dev and Mishra 1971, 1972a; Mishra and Dev 1976) and in *Piscicola geometra* (Van der Lande 1968) remains unsolved. Interestingly, the latter species appears to be exceptional among haematophagous leeches studied in some digestion, probably aided by endopeptidases (i.e. esterases), takes place in the crop (Jennings and Van Der Lande 1967). The rate of digestion is also very rapid in this species, on the order of ten days.

The mid-gut of leeches is characterized by a great reduction or absence of endogenous carbohydrase, lipases, and most proteases, particularly endopeptidases (Diwany 1925; Graetz and Autrum 1935; Damas 1962; Jennings and Van Der Lande 1967; Van Der Lande 1968, 1972). An important exception is the presence of endogenous exopeptidases (arylamidases) which have been found in the intestine of all leeches studied.

Exopeptidases are especially prominent in the non-bloodsucking species *Erpobdella octoculata.* In this species these enzymes are distributed evenly and uniformly through the intestine, being especially rich at the brush border (microvilli) of the endothelium. The presence of exopeptidases is independent of the animal's state of nutrition and age. Activity is optimal at about pH 7.0 but is still present in the range pH 5.0-8.7 suggesting the possibility that at least two enzymes are present. At least one of these is activated by metal ions (CO^{2+} and Mn^{2+}), a characteristic of many exopeptidases. Similarity in activity of exopeptidases from a diversity of leech species suggests that leeches all share the same endogenous exopeptic enzymes. However, the degree of activity varies somewhat with species, being weakest in *Haemopis sanguisuga* and *Pontobdella muricata* among species studied.

Deficiency of digestive enzymes (except exopeptidases) in leeches is compensated by enzymes produced by endosymbiotic microflora (see Endosymbiosis) (Table 13.5). In *Hirudo medicinalis* these supplementary enzymes are produced by the bacterium *Aeromonas hydrophila* discussed elsewhere (Lehmensick 1941, 1942; Hornbostel 1941; Büsing 1951; Büsing *et al.* 1953). Tests on isolated cultures of this bacterium from both the crop and intestine demonstrate the presence of potent versatile proteases, lipases, and amylases (Jennings and Van Der Lande 1967). Lecithinase is a lipase possibly involved in the breakdown of the erythrocyte wall. Haemolysin is a protease probably responsible for the degradation of haemoglobin (Büsing *et al. 1953;* Jennings and Van Der Lande 1967). The haemolytic properties of the bacterium is inhibited *in vivo* when the gut of *Hirudo medicinalis* is exposed to the antibiotic chloromycetin. Interestingly, the same species of *Aeromonas* plays a similar role in the gut of the vampire bat, *Desmodus rotundus* (Müller, Pinus and Schmidt 1980; Pinus and Müller 1980).

Other haematophagous leech species also depend on endosymbiotic bacteria for digestion but the bacterial species involved and its relative contribution in digestion is species-specific (Table 13.6). Haematophagous leeches harbour only one species of endosymbiotic bacterium. Non-haematophagous leeches, such as *Erpobdella octoculata*, harbour more than one bacterial symbiont, but dependence on them can be considerable.

Many leech species feed more or less exclusively on vertebrate blood, a highly efficient source of food, especially proteins (Andrew 1965). The primary energy source is haemoglobin which comprises about 33 per cent of weight of erythrocytes and is the major (> 95 per cent) erythrocyte protein. Erythrocytes themselves comprise about 45 per cent by weight of vertebrate blood.

Chemical composition of blood is similar for the respective vertebrate classes, but there are marked differences in the density of erythrocytes, being much more numerous in mammals (5,000,000-7,000,000 cm^3) than in other classes (e.g. 400,000-500,000 in frogs and turtles). Thus, it is not a biological accident that erythrocyte-rich mammals play a disproportionate role in the feeding biology of bloodsucking leeches. A phylogenetic trend toward parasitism of mammals is particularly striking in the Glossophoniidae.

Upon entering the crop ingested blood rapidly loses excess water and electrolytes with assistance of the nephridia (See Excretory system). Even while still feeding liquid droplets can be seen emerging from the nephridiopores. Most of the ingested liquid is removed within a couple of days (Fig. 2.22). Interestingly, the painful bite of the 'stinging' land leech *Haemadipsa ornata* (Moore 1927) is reportedly owing to such excreted liquids from the first pair of nephridia onto the oral sucker while feeding.

Haemolysis

After removal of excess fluid the condensed blood is stored in the crop for a long period, depending on the age of the leech and ambient temperature. The stored blood undergoes extremely slow changes, involving gradual breakdown (haemolysis) of erythrocytes. In some cases structural integrity of erythrocytes can persist for up to 18 months (Pütter 1907).

Haemolysis of blood stored in the crop of *Hirudo medicinalis* has been followed by several investigators (Stirling and Brito 1882; Korzhuev and Khudailberdiev 1937a,b; Despotov 1966; Gondko, Leyko, Majdak and Wojtas 1979; see also Van Der Lande 1983). For the first few days the blood undergoes little change, but after about two weeks the blood, still structurally intact, takes on a very deep dark-red colour and a jelly-like consistency. The colour is due to the reduction of oxyhaemoglobin to reduced haemoglobin, and the consistency apparently comes from admixture with mucus. Cellular membranes of the erythrocytes begin to dissolve an haemoglobin is released into solution. With time numerous crystals, presumably haemoglobin but possibly artefactual, becomes evident in the crop. After several months most of the erythrocytes are haemolysed in this manner. Interestingly, leucocytes and nuclei of erythrocytes (when present) greatly resist digestion.

In *Hirudo medicinalis* haemolysis of erythrocytes is due to haemolytic properties of the endosymbiotic bacterium *Aeromonas hydrophila* discussed elsewhere (Büsing *et al.* 1953; Jennings and Van Der Lande 1967). The exact mechanism is not known but, as with other haemolytic bacteria, haemolysis is probably effected by an enzyme, haemolysin. The same bacterium is probably also responsible for the prolonged preservation of the intact erythrocytes by secreting an antibiotic which prevents putrefaction of the stored blood by other micro-organisms.

Crop contents with its haemoglobin now in solution slowly pass a little at a time into the intestine where proteolysis of the haemoglobin takes place. Passage into the intestine is regulated by a sphincter muscle at XIX/XX which demarcates the crop from the intestine.

Proteolysis

All vertebrate haemoglobins, with the exception of the monomeric haemoglobins of lampreys, are tetramers with molecular weights of about 64 000. In humans the tetramers consist of two identical and two identical chains; each chain is associated with an oxygen-binding porphyrin, haem, containing a single iron molecule, and is very similar to myoglobin in amino-acid residues and the chain of 146 residues. In lower vertebrates similar chains are present in the tetramer.

The faeces of bloodsucking leeches are virtually pure haem (Fukui 1926; Windsor 1970) from which we can conclude that haemoglobin is split into globin, which is totally digested, and haem which is for the most part eliminated.

Leeches lack endopeptidases so that the mechanism of proteolysis of globin cannot follow the same course as it would in other animals where endopeptidases and exopeptidases act in sequence. In leeches the endogenous exopeptidases (aided by proteases from endosymbiotic bacteria) (Table 13.5) in the intestine slowly degrade globin chains by progressive and terminal removal of the 300 or so amino acids.

To what extent degradation of haemoglobin is intracellular or extracellular is unclear. That some intracellular digestion takes
place is supported by the occasional presence of haem compounds within the intestinal endothelium (Bradbury 1959; Jennings and Van Der Lande 1967). Extracellular digestion is also likely since some exopeptidase activity is occasionally present in the intestinal lumen. Some activity may also occur in the contents of the crop but since the crop endothelium lacks exopeptidases the source of the enzyme is probably intestinal.