

## The effect of helminthic infection on the protein metabolism of the host

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It is usually assumed that gastro-intestinal parasites cause some derangement of function of the gut, which may in turn lead to a more widespread metabolic impairment and the poorer productivity of the parasitized animal. The primary disturbances most likely to arise from a gastro-intestinal helminth burden are any or all of the following: loss of appetite, impaired digestion, malabsorption and abnormal losses of endogenous metabolites into the gut. It is unfortunate that to date this whole range has not been examined simultaneously in any single host-parasite system. Most workers have tended to select one aspect and to study it in several different helminth infections. This state of affairs is due not so much to narrowness of outlook but to the fact that, apart from anorexia, quantification of the other disturbances requires specialized techniques and a single worker or a small group is unlikely to be proficient in them all.

The present paper is also subject to this general criticism. It deals with the effects of certain gastro-intestinal parasites on the protein metabolism of the host, particularly those arising from abnormal losses of plasma protein into the gut. These problems have much in common with the so-called 'protein-losing gastroenteropathies' in man (see Jarnum, 1963) and require many of the same experimental techniques. The methods which have so far proved most useful in animal parasitology are outlined and the results of their application to several economically important parasitic diseases are discussed. Some results on fascioliasis in sheep and rabbits are included. Although *Fasciola hepatica* is not an intestinal parasite it does bring about substantial losses of whole blood and additional plasma into the gut, and the general situation is therefore not dissimilar to that arising from some purely intestinal parasitic infections.

### *Experimental methods*

The hypoproteinaemias, particularly hypoalbuminaemias which are a common feature of many parasitic diseases, can arise through interference with protein synthesis, abnormal breakdown of protein, or both. Catabolism of plasma protein is much easier to follow than synthesis, and for this and other reasons most of the work on parasitized animals has centered around the measurement of catabolic rates. These can be measured most easily by the use of plasma proteins labelled with the isotopes of iodine ( $^{131}\text{I}$  or  $^{125}\text{I}$ ).

*Albumin labelled with isotopic I in metabolic studies.* A succinct account of the various considerations surrounding the use of albumin labelled with isotopic I in

catabolic measurements is given by Jarnum (1963). The methods for determining catabolic rate fall essentially under two headings:

- (a) methods which depend entirely on analysis of the plasma disappearance curve after intravenous injection of the tagged albumin (e.g. Matthews, 1957);
- (b) methods which depend on the measurement of excreted activity as well as plasma activity (e.g. Campbell, Cuthbertson, Matthews & McFarlane, 1956).

When these measurements are combined with a determination of plasma albumin one can obtain values for:

CA, the intravascular pool of albumin,

TA, the total body pool of albumin,

EA, the extravascular pool,

F(CA), the 'fractional catabolic rate' or fraction of the intravascular pool broken down each 24 h.

Of these measurements the least reliable is likely to be EA. For the determination of F(CA) the method of Campbell *et al.* (1956) is probably preferable to that of Matthews (1957) in that it is more direct. On the other hand for farm animals, in certain circumstances, there are attractions about a method which does not require complete collection of urine and faeces.

For complete validity both techniques require the existence of 'steady state' conditions which may not apply in some of the measurements on infected animals. In such instances, although absolute significance cannot be attached to the figures obtained, the comparative measurements in infected and control animals can be very significant.

The results of the application of the above procedures to Type II ostertagiasis of cattle (Halliday, Mulligan & Dalton, 1968), ostertagia infection in sheep (Holmes & MacLean, 1971), and fascioliasis in rabbits (Dargie, Holmes, MacLean & Mulligan, 1968) and sheep (Holmes, 1969) are summarized in Table 1.

Table 1. *Albumin metabolism in some helminthic infections*

(Mean values and standard deviations)

Host: parasite system	Group	No. of animals	Plasma albumin (g/100 ml)	Intravascular pool g/kg	Fractional catabolic rate
Cattle: type II ostertagiasis (field cases)	Control	7	2.67 ± 0.28	0.98 ± 0.147	0.067 ± 0.008
	Infected	8	1.44 ± 0.50	0.77 ± 0.24	0.104 ± 0.030
Sheep: ostertagiasis: Experimental infection (3 weeks)	Control	6	3.36 ± 0.39	1.46 ± 0.28	0.062 ± 0.011
	Infected	3	2.53 ± 0.31	1.27 ± 0.16	0.142 ± 0.062
Rabbits: fascioliasis: Experimental infection (9 weeks)	Control	4	3.38 ± 0.55	1.26 ± 0.26	0.201 ± 0.071
	Infected	4	2.54 ± 0.25	0.99 ± 0.14	0.431 ± 0.082
Sheep: fascioliasis: Experimental infection (12 weeks)	Control	6	2.57 ± 0.25	1.01 ± 0.26	0.081 ± 0.017
	Infected	6	1.73 ± 0.32	0.84 ± 0.13	0.202 ± 0.100

In the examples shown in Table 1, the parasitized animals all show apparent reductions in plasma albumin concentrations and in the size of the intravascular pools of albumin, and very marked increases in albumin catabolic rate. The disturbances could therefore all be classified as 'hypercatabolic hypoalbuminaemias'. To demonstrate that this hypercatabolism is due to abnormal losses of plasma into the gut requires the use of the special markers described below.

*Measurement of gastro-intestinal protein loss.* In the experiments with albumin labelled with isotopic I discussed above, some indication that the increased catabolism in infected animals is due to abnormal losses of plasma into the gut can be obtained by comparing faecal radioactivities of parasitized and control animals. Such measurements are of course far from quantitative in that substantial amounts of the plasma proteins are degraded in the gut and the label reabsorbed. What is required for proper quantification is some marker for plasma macromolecules, which is not subject to enzymic attack in the gut, or a label for protein which is not significantly reabsorbed. There is in fact no completely satisfactory material for this purpose, but very useful results have been obtained from the following: polyvinylpyrrolidone (PVP) labelled with isotopic I; plasma protein labelled with chromium 51; plasma protein labelled with niobium 95.

The first of these is a synthetic polymer introduced by Gordon (1959) for the study of 'idiopathic hypercatabolic hypoproteinaemia' in man. A preparation of average mol.wt 30 000-40 000 is usually employed; although the mol.wt is much lower than that of any of the plasma proteins, such a preparation has, because of the different molecular shape, a diffusion coefficient not dissimilar from albumin. Infected and control animals are injected intravenously with PVP labelled with isotopic I, plasma samples are withdrawn daily and 24 h samples of faeces are collected and assayed for radioactivity. Results are expressed as a percentage of the injected dose appearing in the faeces over a standard collection period or as a 'faecal clearance', the latter representing the volume of 'plasma' which would have to leak into the gut each 24 h to account for the faecal radioactivity.

<sup>51</sup>Cr-albumin was recommended by Waldmann (1961) as a test substance for gastro-intestinal protein loss and it was at one time regarded as much preferable to PVP labelled with isotopic I for the measurement of albumin leak. However, it has been demonstrated (van Tongeren & Majoor, 1966) that the isotope's attachment to

Table 2. *Gastro-intestinal plasma leak in sheep 2 weeks after a single infection with Ostertagia circumcincta*

(Mean values and standard deviations)

Group	No. of sheep	Plasma pepsinogen (M units tyrosine)	Faecal 'plasma' clearance (ml/d)
Control	8	388 ± 118	28.8 ± 9.5
Infected (300 000 larvae)	3	4800 ± 800	82.5 ± 29.5
Infected (900 000 larvae)	3	7900 ± 2900	92.3 ± 46.5

albumin is labile and that shortly after injection it has become attached to other plasma proteins. For the estimation of general plasma protein leak it is satisfactory to inject, intravenously, high specific activity  $^{51}\text{CrCl}_3$  and allow the  $^{51}\text{Cr}$  to bind to plasma proteins in vivo.

Table 2 summarizes the results of some measurements with  $^{51}\text{Cr}$  of the gastro-intestinal plasma leak in sheep infected with *Ostertagia circumcincta*.

In all the host:parasite systems mentioned in Table 1 it has been possible to demonstrate, using PVP labelled with isotopic I,  $^{51}\text{CrCl}_3$  and in some instances  $^{95}\text{Nb}$ , a substantially greater leak of plasma into the gut in infected as compared to control animals.

It is now generally accepted that the liver fluke is haematophagic, hence some loss of plasma into the gut via the bile ducts is to be expected. However, simultaneous experiments with  $^{95}\text{Nb}$ -albumin and  $^{51}\text{Cr}$ -tagged red cells have demonstrated that the plasma loss is considerably greater than can be accounted for by the loss of whole blood, that is apart from straight blood loss there is an additional leak of plasma (MacLean, Holmes, Dargie & Mulligan, 1968).

#### *Mechanism of increased gastro-intestinal plasma leak in parasitized animals*

Studies by light and electron microscopy of the gut wall in bovine ostertagiasis have demonstrated a breach in parasitized mucosae involving the breakdown of junctional complexes between cells (Murray, Jarrett, Jennings & Miller, 1971). Similar structural changes have been observed in the biliary mucosa of animals infected with *F. hepatica* and these would explain the additional plasma leak in fascioliasis.

There is some evidence that the increased permeability to macromolecules can operate in both directions, for example in ostertagiasis of sheep and cattle, pepsinogen appears to leak from the abomasum back into the circulation and a characteristic feature of these diseases is a high level of plasma pepsinogen (see Table 2).

#### *Consequences to the host of increased gastro-intestinal plasma leak*

The difficulties in normal animals of assessing the importance of endogenous losses of nutrients via the alimentary tract have been stressed by Phillipson (1971). The position is a good deal more complicated in the parasitized animal. There is no doubt that substantial amounts of amino acid liberated in the gut from degraded plasma can be reabsorbed and reutilized. The host does however have to manufacture plasma protein at a greater rate than normal and it will therefore be more vulnerable to stresses that may interfere with normal protein synthesis.

It should be pointed out that if the hyperproteinaemia is severe the absolute amount of plasma protein degraded per d by the infected animal may not be in excess of normal. There may be in effect a more rapid turnover of a smaller pool.

Although it has not been specifically demonstrated in all instances it seems likely that all of the plasma proteins are involved in the increased leak. This can have important immunological consequences in that when immunoglobulins are degraded in the gut there is a loss of specific configurations. It has already been demonstrated

that puppies born with a hook-worm infection lose their maternal immunity to distemper much more rapidly than normal puppies (T. A. Miller, personal communication).

There is little information in the literature on the effects of intestinal plasma leak on nitrogen balance. Some preliminary studies (P. H. Holmes and J. J. Parkins, personal communication) on lambs infected with *Ostertagia circumcincta* would appear to demonstrate a deleterious effect of infection on nitrogen balance. The problem is somewhat complicated by the fact that this parasite causes a period of marked but transient anorexia so that parallel studies have to be carried out on pair-fed controls for each of the parasitized animals and both groups go into negative nitrogen balance for a period of about 2 weeks during the acute stage of the disease. However, it was found that the infected animals showed a greater output of urinary nitrogen and an elevated blood urea over this period. This could be interpreted as due to an increased catabolism of amino acids resulting from a shift in the position of equilibrium between body protein and amino acid arising in turn from the increased degradation of endogenous protein in the gut. Further experiments to elucidate this situation are required.

## REFERENCES

- Campbell, R. M., Cuthbertson, D. P., Matthews, C. M. E. & McFarlane, A. S. (1956). *Int. J. appl. Radiat. Isotopes* **1**, 66.
- Dargie, J. D., Holmes, P. H., MacLean, J. M. & Mulligan, W. (1968). *J. comp. Path. Ther.* **78**, 101.
- Gordon, R. S. Jr (1959). *Lancet* **i**, 325.
- Halliday, G. J., Mulligan, W. & Dalton, R. G. (1968). *Res. vet. Sci.* **9**, 224.
- Holmes, P. H. (1969). Studies on the pathophysiology of chronic ovine fascioliasis. PhD Thesis, University of Glasgow.
- Holmes, P. H. & MacLean, J. M. (1971). *Res. vet. Sci.* **12**, 265.
- Jarnum, S. (1963). *Protein-losing Gastroenteropathy* 1st ed. Oxford: Blackwell Scientific Publications.
- MacLean, J. M., Holmes, P. H., Dargie, J. D. & Mulligan, W. (1968). In *Isotopes and Radiation in Parasitology* Vol. 1, p. 117. New York: Unipub Inc.
- Matthews, C. M. E. (1957). *Physics Med. Biol.* **2**, 36.
- Murray, M., Jarrett, W. F. H., Jennings, F. W. & Miller, H. R. P. (1971). In *Pathology of Parasitic Diseases* p. 197 [S. M. Gaafar, editor]. Lafayette, Indiana: Purdue University Studies.
- Phillipson, A. T. (1971). *Proc. Nutr. Soc.* **30**, 61.
- van Tongeren, H. J. M. & Majoor, C. L. H. (1966). *Clinica chim. Acta.* **14**, 31.
- Waldmann, T. A. (1961). *Lancet* **ii**, 121.