Sikes D, Fletcher O J & Jones T J
(1970) *American Journal of Veterinary Research* **31**, 2191–2195
Sikes D, Neher G M & Doyle L P (1956) *Journal of the American Veterinary Medical Association* **128**, 283–286
Sokoloff L (1966) In: Arthritis and Allied Conditions. Ed. J L Hollander. 7th ed. Kimpton, London; p 194
Timoney J (1971) *Journal of Comparative Pathology* **81**, 243–248
Uruchurtu A & Ajmal M
(1970) *Journal of Comparative Pathology* **80**, 607–611
Veterinary Record
(1959) 71, 777–786
(1960) 72, 1240–1247
Waksman B H & Wennersten C (1963) *International Archives of Allergy and Applied Immunology* **23**, 129–139
Weissman G, Becher B & Thomas L
(1964) *Journal of Cell Biology* **22**, 115–126
Weissmann G, Becher B, Wiedermann G & Bernheimer A W
(1965) *American Journal of Pathology* **46**, 129–147
Weissmann G, Keiser H & Bernheimer A W
(1963) *Journal of Experimental Medicine* **118**, 205–222
White T G, Puls J L & Mirikitani F K
(1971) *Infection and Immunity* **3**, 715–722

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Chronic Mycobacterial Enteritis in Ruminants as a Model of Crohn's Disease

In 1895, Johne & Frothingham observed an unusual form of intestinal tuberculosis in cattle and Bang (1906) described this condition as chronic pseudotuberculosis. Nowadays it is termed paratuberculosis, or Johne's disease, and results from oral infection with *Mycobacterium johnei*. The disease may affect any ruminant species and terminally the subject is often markedly cachectic. This may be the first recognizable or even the sole clinical sign. In its early confusion with intestinal tuberculosis and in its chronic onset with few symptoms, it resembles Crohn's disease in man. There are many other similarities, as shown in Table 1.

Crohn's disease is a chronic inflammatory disorder affecting most parts of the intestinal tract, with cellular changes characteristic of a granulomatous hypersensitivity reaction (Lennard-Jones 1970) but, unlike Johne's disease, no specific infectious or sensitizing agent has been identified. Because of the strikingly similar manifestations, our study of the experimentally reproducible animal disease seems to provide a suitable analogue for increasing our understanding of some aspects of Crohn's disease in man. Our investigations fall under three headings: (1) pathological chemistry, (2) changes in protein metabolism and (3) biochemical parameters and allergic tests as diagnostic tools.

Pathological Chemistry

Measurements of the plasma levels of 24 enzymes in clinical cases of the disease in cattle showed that despite longstanding enteritis and severe muscular wasting there was virtually no enzyme abnormality. Nevertheless, because of the chronic association between the infecting mycobacteria and intestinal macrophages it was considered that, if nothing else, acid hydrolase activities would be raised: these enzymes originate in lysosomes and are implicated in phagocytosis. In fact, acid phosphatase, aryl sulphatase and β-glucuronidase activities in plasma from groups of field cases were all slightly increased but only the elevation of β-glucuronidase activity was statistically significant (Patterson, Allen & Berrett 1967). Later, in experimentally infected calves and lambs it was confirmed that raised levels of β-glucuronidase activity appeared shortly after oral infection and this possibly reflected active phagocytosis of the invading organisms (Patterson *et al.* 1969, Allen 1971).

Serum glycoprotein concentrations were also raised in clinical Johne's disease (protein-bound hexose >115 mg/100 ml; Patterson & Sweasey 1966) but because of a generalized hypoproteinæmia this was not a consistent finding. In the terminal stages of the disease values were about 140 mg/100 ml, which are considerably lower than those encountered in active tuberculosis in man. Between 40% and 50% of protein-bound carbohydrate in serum of the bovine animal (Patterson & Sweasey 1968) has the electrophoretic mobility of α-globulins; compared with healthy cattle,

Table 1

A comparison of Crohn's disease in man with chronic mycobacterial enteritis of ruminants

	Crohn's disease	*Johne's disease*
Early confusion with intestinal tuberculosis	Yes	Yes
Incidence	Familial	Breed susceptibility (e.g. Channel Island breeds)
Chronic diarrhœa and wasting	Yes	Yes
Acute episodes induced by 'stress'	No	Yes
'Regional ileitis' but other sites may be affected	Yes	Yes
Adaptation – subject may be clinically healthy when gut is severely affected	Yes	Yes
'Cobblestone' appearance of gut due to submucosal thickening	Yes	Yes
Ulceration and fistula formation	Yes	Not seen
Caseation necrosis	No	Occasionally in sheep, not in cattle
Aggregation of epithelioid cells into granulomatous lesions	Yes	Yes
Etiology	?Hypersensitivity state caused by unknown infective or sensitizing agent	Hypersensitivity reaction to specific infection by *Myco. johnei*

clinically affected animals had lower concentrations of all other serum protein fractions, demonstrating that there was a relative increase in serum α-glycoproteins (Patterson, Allen, Berrett, Ivins & Sweasey 1968).

The reduction in serum protein and especially the albumin content was associated with a slightly expanded extracellular water compartment and a decrease in plasma volume. This was consistent with the clinical picture of regional œdema in affected cattle. Total body water (as a percentage of body weight) was increased and this was paralleled by sodium retention. Chronic potassium loss culminated in a precipitous fall in plasma potassium levels during 3 or 4 days of terminal illness to about 60% the normal concentration without consistent alterations to the electrocardiogram. Animals dying suddenly in this condition were characteristically hypokalæmic and hypochloræmic without signs of acidosis or alkalosis.

Changes in muscle composition were those expected of a cachectic animal. There was a slight increase in total water content accompanied by a fall in extracellular potassium concentration of some 20%. The total potassium content and sodium and chloride concentrations were unaltered. Muscle protein content was lowered while non-protein nitrogen, collagen and DNA contents were raised. Using DNA as an index of cellularity, the mean cell mass was 1.16 mg/μg DNA in wasted muscle (control 2.96 mg/μg DNA). The adenosine triphosphate (ATP) content was approximately one third that of control muscle and there was a parallel decrease in creatine phosphokinase (CPK) activity. This suggested that the depletion of muscle protein was exacerbated by the decreased availability of ATP via the CPK reaction (Patterson, Allen, Berrett, Sweasey & Slater 1968). To some extent the existence of such a metabolic deficiency was confirmed when prolonged anabolic steroid therapy failed to put back wasted muscle in affected cows (Allen *et al.* 1968).

The only significant alterations in the chemical composition of the liver in clinical Johne's disease of cattle were a small rise in lipid content and a marked increase in tissue hydroxyproline. The latter result indicated an increase in the proportion of connective tissue suggestive of early fibrosis (Patterson, Allen, Berrett & Sweasey 1968).

In mucosal scrapings from the lesion itself, increased water and decreased protein contents paralleled the œdematous appearance of the intestine. The mean thickness of the mucosal layer in the ileum of clinically affected cows (0.85 mm) did not differ significantly from that of normal gut samples and this confirmed the impression that the gross thickening of the intestine in Johne's disease is due to submucosal infiltration by epithelioid cells (Jubb & Kennedy 1963). The overall thickening of the intestine can be judged from the fact that in a group of clinically affected cows, the mean weight of ileum per unit length was 375.1 g/m (control 99.8 g/m).

Apart from specific effects on the intestine these biochemical changes resembled closely those of human protein calorie malnutrition (Whitehead & Alleyne 1972).

Protein Metabolism

The interaction between disturbed gut function and liver protein metabolism was investigated in clinical cases of Johne's disease in cattle and in experimentally infected sheep by (1) measuring the leakage of protein across the intestinal wall, (2) estimating the degree of amino acid malabsorption, and (3) observing effects on liver protein synthesis.

Exudative loss of plasma proteins was measured after intravascular labelling with $^{51}CrCl_3$ (van Tongeren & Reichert 1966). The label was taken up preferentially by the β-globulins and this fraction was thus the effective marker of protein diathesis. There was a massive loss of plasma proteins across the gut wall in cattle and sheep (Patterson, Allen & Lloyd 1967, Allen 1971) sufficient in either case to account for most of the body weight loss.

Amino acid malabsorption was demonstrated *in vitro* when rings of gut or mucosal scrapings were allowed to accumulate L-histidine under standard incubation conditions (Patterson & Berrett 1968). Comparisons were made between normal and affected animals using the same anatomically defined portion of the intestine whether or not gross lesions were exhibited. In cattle there was a marked depression of amino acid uptake by ileal tissues but in sheep this effect was observed in the jejunum, corresponding with the usual sites of gross lesions in the two species. In the case of sheep, a compensatory increase in amino acid uptake was observed in the ileum.

Protein liver synthesis was assayed using slices of liver tissue incubated with ^{14}C-L-leucine. Both cattle and sheep appeared to respond to the deficiency of dietary amino acids by increasing the rate of protein synthesis, presumably utilizing substrates from wasted muscle in the live animal. Because levels of certain liver enzyme activities were lowered, enhanced protein synthesis was probably selective rather than generalized. The increase in protein turnover caused by infection was apparent when cycloheximide was administered to a small group of sheep. Protein synthesis was temporarily arrested in both infected and control sheep but while the maximum depression of serum protein concentration observed 8 hours after an

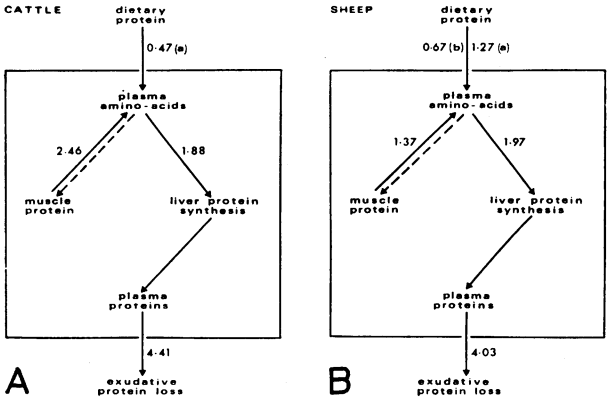

Fig 1 *Protein metabolism in clinically wasted cattle* (A) *and sheep* (B). *The numerical factors represent comparative rates for the stated reactions measured in groups of affected and control animals matched for age and breed. Rates of muscle wasting are derived from body weight data but intestinal amino-acid uptake* ((a) *in the ileum,* (b) *in the jejunum), liver protein synthesis and protein leakage were assayed directly*

intravenous dose (1 mg/kg body weight) was only 7% in control sheep it was about 19% in infected sheep.

Thus, while infection with *Myco. johnei* has its effect primarily on the intestine, inducing excessive plasma protein leakage and malabsorption, it appears to induce a secondary and perhaps selective response in the liver, protein synthesis and turnover being enhanced. In cattle 30% losses in body weight are not uncommon, but the sheep appears better able to compensate for protein and weight loss, and compensatory amino acid uptake lower down the intestine may be an important factor in the sheep's adaptive mechanism. The situation is summarized in Fig 1.

Diagnostic Tests

The intradermal tuberculin test has been widely used for diagnosing and eradicating tuberculosis in cattle and an analogous purified protein, derived from culture filtrates, and known as johnin, has been used to identify cattle infected with *Myco. johnei*. The test for complement fixing antibodies to the Johne's bacillus (Hole 1958) is somewhat more reliable.

The fact that allergic and serological tests for mycobacterial infection are relatively nonspecific stimulated our interest first in the chemical pathology of Johne's disease and then in the physiological responses of infected animals to johnin inoculation. Of the biochemical changes reviewed above, only the elevated concentration of serum glycoproteins appeared to form the basis of a test. It is an entirely nonspecific test since a positive response may reflect acute or chronic inflammation, neoplasia or even general malaise but, given the clinical history of chronic diarrhœa in a mature animal, it seemed to be potentially useful. In calves infected experimentally with *Myco. johnei* but not clinically ill, the determination of serum glycoprotein was as useful in identifying affected animals as the complement fixation test, with a success rate of about 66% (Patterson *et al.* 1969). Our experience of its application to clinical field cases of the disease in cattle suggests that, in fact, it was a better guide to prognosis than to diagnosis. High values for protein-bound hexose of about 140 mg/100 ml identified animals in terminal stages of Johne's disease and death followed in a day or two.

It was not possible to quantify the hypersensitivity reaction to johnin administration in biochemical terms but a rise in rectal body temperature and a reversal of lymphocyte to neutrophil ratio in circulating blood were found to be useful systemic parameters. These responses provided the means of assessing relative merits of the intradermal and intravenous routes of johnin administration and while the latter test was not without risk to chronically sick animals, it was more sensitive and gave fewer false positives than the intradermal test (Allen *et al.* 1967, 1970, Larsen & Kopecky 1965). A sensitive immunofluorescence test has recently been introduced by Gilmour & Gardiner (1969) to identify experimentally infected animals with a high degree of accuracy, but like all serological tests in this field it suffers from the basic defect of poor specificity.

Conclusion

Infection of ruminants with *Myco. johnei* is characterized by the persistence or even multiplication of organisms in the submucosal macrophages and lymph nodes. The viability of bacilli seems to point to interference with the normal events of phagocytosis, perhaps by some unidenti-

fied mycobacterial constituent or metabolite. This is almost certainly fundamental to the pathogenesis of the intestinal lesions but so far has received little attention from research workers. *Myco. johnei*, or indeed any specific organism, has never been implicated in the etiology of Crohn's disease but this possibility appears continually to be under consideration (e.g. *see* Golde 1968). In this connexion it may be mentioned that even in animals experimentally infected with *Myco. johnei* it is sometimes quite difficult to demonstrate bacilli or acid-fast debris in the intestinal tissues (Allen 1971).

During these studies it became evident that conclusions could not readily be transferred from one species to another. Thus, the sheep adapts better to *Myco. johnei* infection than the cow, not only in compensating for disturbances to protein metabolism so that little wasting is generally observed, but also by absorbing water more efficiently from the colon so that diarrhœa is an uncommon feature of Johne's disease in sheep. Because we recognize these differences amongst the ruminants perhaps we should be even more cautious in applying the present observations on chronic ruminant enteritis to the problem of Crohn's disease in man, but they may serve to indicate profitable areas for investigation in the human disease.

REFERENCES
Allen W M (1971) PhD Thesis, University of London
Allen W M, Berrett S & Patterson D S P (1967) *Journal of Comparative Pathology and Therapeutics* **77**, 71
(970) *Journal of Comparative Pathology and Therapeutics* **80**, 267
Allen W M, Saba N S & Patterson D S P (1968) *Veterinary Record* **82**, 562
Bang B (1906) *Berliner und Münchener tierärztliche Wochenschrift* **22**, 759
Gilmour N J L & Gardiner A C (1969) *Journal of Comparative Pathology and Therapeutics* **79**, 71
Golde D W (1968) *Lancet* i, 1144
Hole N (1958) *Advances in Veterinary Science* **4**, 341
Johne H A & Frothingham L (1895) *Deutsche Zeitschrift für Thiermedicin und vergleichende Pathologie* **21**, 438
Jubb K V F & Kennedy P C (1963) Pathology of Domestic Animals. Academic Press, New York; **2**, 121
Larsen A B & Kopecky K E (1965) *American Journal of Veterinary Research* **26**, 673
Lennard-Jones J E (1970) In: Modern Trends in Gastroenterology Ed. W I Card & B Creamer. Butterworths, London; **4**, 273
Patterson D S P, Allen W M & Berrett S (1967) *Veterinary Record* **81**, 435
Patterson D S P, Allen W M, Berrett S, Ivins L N & Sweasey D (1968) *Research in Veterinary Science* **9**, 117
Patterson D S P, Allen W M, Berrett S & Sweasey D (1968) *Journal of Medical Microbiology* **1**, 137
Patterson D S P, Allen W M, Berrett S, Sweasey D & Slater T F (1968) *Journal of Medical Microbiology* **1**, 127
Patterson D S P, Allen W M & Lloyd M K (1967) *Veterinary Record* **81**, 717
Patterson D S P & Berrett S (1968) *Journal of Medical Microbiology* **2**, 327
Patterson D S P, Berrett S, Sweasey D & Gilmour N J L (1969) *Journal of Comparative Pathology and Therapeutics* **79**, 79
Patterson D S P & Sweasey D (1966) *Veterinary Record* **78**, 364
(1968) *Research in Veterinary Science* **9**, 319
van Tongeren J H M & Reichert W J (1966) *Clinica chimica acta* **14**, 42
Whitehead R G & Alleyne G A O (1972) *British Medical Bulletin* **28**, 72

Meeting April 19 1972

Toxoplasmosis [*Abridged*]

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Cats as a Source of Toxoplasmosis

Toxoplasma has been discovered to be a coccidian, with schizogony and gametogony occurring in the intestine of the domestic cat and other Felidæ (as yet, no other family has been implicated). Oocysts produced can survive for 17 months. When ingested by warm-blooded animals they give rise to the extraintestinal proliferative and chronic forms (endozoites and cystozoites) which, until recently, were the only stages of the parasite known. The fact that chimpanzees (as well as mice, rats, sheep, pigs, chickens, dogs, and, of course, cats) have been successfully infected experimentally by the administration of oocysts strongly suggests that man is also infected from this source. The infective cystozoite form, often present in raw meat, is destroyed by mild heat (47°C) and is probably less of a hazard than the oocyst. Toxoplasmosis is not usually a serious disease in man except under two conditions. (1) Persons harbouring chronic infections may succumb when subjected to immunosuppressive treatment which allows latent infections to become active and develop unchecked. This problem is one associated with transplant surgery. (2) A seronegative pregnant woman, if infected, can transmit endozoites across the placenta to the fœtus. This can result in a range of serious effects, from abortion to greater or lesser neural damage in survivors to term. In view of this, seronegative women likely to become pregnant should be particularly careful in handling raw meat, and should avoid contact with cats, especially those fed on raw meat or of a hunting habit.