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# Serological comparison and haemagglutinating activity of *Mycoplasma dispar*

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SUMMARY

A comparison of twelve strains of *Mycoplasma dispar* by the metabolism inhibition and indirect haemagglutination tests has shown them to form a serologically homogeneous group of micro-organisms. The twelve strains vary in their haemagglutinating activity against erythrocytes from different animal species, and certain of the strains can be distinguished by the erythrocytes they agglutinate. Haemagglutination may thus provide a method by which certain strains can be typed. The erythrocyte receptor site does not appear to contain sialic acid and is not sensitive to proteolytic enzymes. On the mycoplasma cell two attachment sites have been demonstrated. One, by which it attaches to sheep and bovine erythrocytes, is a protein or contains a protein moiety. The chemical nature of the other attachment site, by which *M. dispar* attaches to rabbit erythrocytes, is unknown.

## INTRODUCTION

*Mycoplasma dispar* was first isolated from pneumonic calf lungs (Gourlay, 1969; Gourlay & Leach, 1970) and subsequently from the respiratory tract of non-pneumonic calves (Thomas & Smith, 1972). Its role in calf pneumonia remains to be determined. However, the preliminary experiments of Gourlay & Thomas (1969) and St George, Horsfall & Sullivan (1973), who found lesions in the lungs of calves after endobronchial or intratracheal inoculation of broth cultures but not sterile broth, together with the observation that, of the mycoplasmas commonly found in the respiratory tract of calves in England, *M. dispar*, *M. bovirhinis*, *Acholeplasma laidlawii* and T-mycoplasma (Gourlay, Mackenzie & Cooper, 1970), only *M. dispar* had any cytopathic effect on bovine fetal tracheal organ cultures (Thomas & Howard, 1974) and the finding that certain strains of *M. dispar* cause clinical mastitis in cows experimentally inoculated (J. Brownlie, R. N. Gourlay & C. J. Howard – to be published) supports the hypothesis that this micro-organism is pathogenic for cattle, perhaps with a particular propensity for the respiratory tract.

Comparisons within certain mycoplasma species, e.g. *M. hominis* (Purcell *et al.* 1967) *M. gallisepticum* (Taylor-Robinson & Berry, 1969) and *M. pulmonis* (Forshaw & Fallon, 1972) have shown these species to be serologically heterogeneous and to contain various subtypes. No serological comparison of a group of *M. dispar*

strains has been made except for that of Gourlay & Leach (1970) who found that antiserum to strain 462/2 inhibited the growth on agar of 23 out of 36 strains examined. As little information is available on the serological heterogeneity or homogeneity of the species the first part of this study deals with this aspect of *M. dispar*.

Attachment to mucosal epithelium is considered to be the first step in the production of disease by many micro-organisms (Savage, 1972). The attachment of mycoplasmas to cells is well documented and differences between strains in their ability to attach to cells has been observed (Manchee & Taylor-Robinson, 1969*a*, *b*). The intimate association between mycoplasmas and cells has been proposed as a mechanism by which high local concentrations of toxic metabolites e.g. $H_2O_2$, are built up which may then cause cell damage (Sobeslavsky, Prescott & Chanock, 1968). In the second part of this paper the demonstration of attachment sites on *M. dispar* for erythrocytes is reported, and these attachment sites may represent the mechanism by which this micro-organism maintains itself in the bovine respiratory tract. Besides this, a study of haemagglutinating activity of *M. dispar* provides information on its surface structure and also forms the basis of a method useful for strain identification.

## METHODS

### *Media*

Mycoplasmas were grown in glucose calf-serum (GS) broth (Gourlay & Leach, 1970) containing ampicillin (Andrews, Leach, Gourlay & Howard, 1973) except when organisms were grown for injection into rabbits, in which case rabbit digest broth and rabbit serum, heated at 56° C. for 30 min. were substituted for Hartley's digest broth and fetal calf serum.

### *M. dispar strains*

All strains were from the lungs of calves with pneumonia. In order that we should not select serologically similar strains the criteria used to identify organisms as *M. dispar*, in the first instance, were production of acid from glucose in GS broth and colony morphology. *M. dispar* forms atypical centreless colonies on primary isolation (Gourlay & Leach, 1970). The serological studies confirmed the identity of the strains used subsequently for haemagglutination. Antisera were prepared as described by Howard & Gourlay (1972). All strains were cloned by filtering broth cultures through 650 nm. Millipore filters, and propagation of single colonies on three successive occasions (Sub-committee on the Taxonomy of *Mycoplasmatales*, 1972).

### *Influenza A virus*

Strain SIWG/1/57 was kindly provided by Dr A. S. Beare (Common Cold Unit, Salisbury) and stored at −70° C.

*Serological tests*

The metabolism inhibition test was performed according to Taylor-Robinson, Purcell, Wong & Chanock (1966). The indirect haemagglutination test was performed according to Herbert (1967) using a microtitre system (Cooke Engineering Co., Flow Laboratories, Irvine, Scotland), sonicated *M. dispar* as antigen and tanned sheep erythrocytes that had been stored in Alsevers solution (Wellcome Laboratories, Beckenham, Kent).

*Haemagglutination*

The various strains of *M. dispar* used were grown in GS broth at 37° C., harvested by centrifugation at 15,000 ***g***, washed and resuspended in 0·15 M-NaCl and stored at −70° C. The optical density (OD) of mycoplasma suspensions at 500 nm. was measured with a 10 mm. light path cell in a Unicam SP 800 and haemagglutination titres are given for standard suspensions with an OD of 10.

Rabbit, rat, hamster, chicken, calf and guinea-pig red cells were collected and stored in Alsevers solution. Sheep and horse red cells in Alsevers solution were obtained from the Wellcome Laboratories and human group O citrated packed cells from the Regional Transfusion Centre, Oxford. All red cells were stored at 4° C. Before use they were washed three times with 0·15 M-NaCl and made up to the required concentration by volume.

Doubling dilutions of antigen were made in 25 $\mu$l. volumes of 0·15 M-NaCl using a microtitre system and U trays (Cooke Engineering Co.) and the red cells, 25 $\mu$l. of a 1 % v/v suspension, were added to the dilutions of antigens. The results were read after overnight incubation at 4° C. All haemagglutination titres are given as the reciprocal of the highest dilution of mycoplasma suspension that caused agglutination.

*Treatment applied to erythrocytes*

The methods followed were mainly those of Buckland & Tyrrell (1963). Receptor-destroying enzyme (Wellcome Laboratories, from *Vibrio cholerae*) diluted 1/4 in 0·15 M-NaCl was mixed with an equal volume of 5 % v/v erythrocytes. Equal volumes of the other reagents and 10 % v/v erythrocytes were incubated together. Trypsin (bovine pancreas type III) and chymotrypsin (bovine pancreas type I) were obtained from Sigma (Kingston upon Thames). Pronase (B.D.H., Poole) and erythrocytes were incubated together for 30 min. at 37° C. After the various treatments the cells were washed once with 0·15 M-NaCl, resuspended to give a 1 % suspension, and 25 $\mu$l. volumes were added to the doubling dilutions of the mycoplasma suspension.

*Treatment applied to mycoplasmas*

Equal volumes of mycoplasma suspension and the reagents listed in Table 5 were incubated together. The temperatures and times of incubation were as used by Buckland & Tyrrell (1963) for treating viruses. Pronase and mycoplasmas were incubated together at 37° C. for 30 min. After incubation the mycoplasmas were centrifuged and made up to their original volume.

Table 1. *Metabolism inhibition titres* of antisera to* M. dispar *strains*

| Strain tested against: | Antiserum prepared against: F370 | 462/2 | Vic12 | Vic13 | Gri 226 | D2 | D44 | Vic7 | Gri 250 | Mmb 177 | D52 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| F370 | **2560** | 1280 | 2560 | 640 | 2560 | 1280 | 2560 | 2560 | 1280 | 1280 | 1280 |
| 462/2 | 1280 | **640** | 640 | 640 | 160 | 80 | 160 | 40 | 80 | 160 | 320 |
| Vic12 | 1280 | 640 | **1280** | 320 | 640 | 320 | 640 | 160 | 320 | 320 | 640 |
| Vic13 | 1280 | 2560 | 1280 | **640** | 1280 | 640 | 1280 | 1280 | 640 | 640 | 640 |
| Gri226 | 1280 | 640 | 1280 | 640 | **320** | 320 | 320 | 320 | 320 | 320 | 160 |
| D2 | 2560 | 640 | 1280 | 640 | 1280 | **1280** | 2560 | 1280 | 1280 | 640 | 1280 |
| D44 | 1280 | 1280 | 1280 | 1280 | 1280 | 1280 | **1280** | 1280 | 1280 | 640 | 640 |
| Vic7 | 2560 | 1280 | 1280 | 1280 | 1280 | 1280 | 1280 | **1280** | 640 | 640 | 640 |
| Gri250 | 1280 | 640 | 640 | 320 | 640 | 160 | 160 | 320 | **160** | 320 | 320 |
| Mmb177 | 1280 | 1280 | 1280 | 640 | 640 | 1280 | 320 | 640 | 640 | **640** | 640 |
| D52 | 2560 | 1280 | 1280 | 1280 | 1280 | 1280 | 1280 | 640 | 640 | 640 | **1280** |
| Gri221 | 640 | 1280 | 640 | 160 | 320 | 160 | 80 | 160 | 160 | 160 | 160 |

* Reciprocal of highest dilution of antiserum inhibiting the production of acid from glucose.

*Electron microscopy*

*M. dispar* and erythrocytes were examined by electronmicroscopy after staining with ruthenium red (Springer & Roth, 1973) as previously described (Howard & Gourlay, 1974).

## RESULTS

*Serological comparison of twelve* M. dispar *strains*

The results of the metabolism inhibition and indirect haemagglutination tests (Tables, 1, 2) confirmed that the strains, identified primarily on the basis of colony morphology and ability to produce acid from glucose, were *M. dispar*. The twelve strains studied form a serologically homogeneous group, only minor differences in antigenicity being indicated by the two tests. It was considered that these differences might be largely due to the variation in potencies of the different antisera, the state of growth of antigen in the metabolism inhibition test and the antigen concentration used to sensitize the red cells in the indirect haemagglutination test. Normal rabbit serum had titres of $< 20$ in the metabolism inhibition and indirect haemagglutination tests. No factor was observed analogous to that in rabbit sera which prevents the growth of human T-mycoplasmas (Howard & Gourlay, 1973).

Antisera were raised against organisms grown in medium containing rabbit digest broth and serum. However, in order to ensure that antibodies to bovine serum were not being detected in the indirect haemagglutination test, the dilutions of antisera and the final suspensions of tanned–sensitized sheep red cells were made in phosphate buffered saline containing 5% v/v fetal calf serum. Besides this, absorption of antiserum with bovine erythrocytes had no effect on the titre of antisera for sensitized red cells.

Table 2. *Titres* in indirect haemagglutination test of antisera to* M. dispar *strains*

| Strain used as antigen | Antiserum prepared against: F370 | 462/2 | Vic12 | Vic13 | Gri226 | D2 | D44 | Vic7 | Gri250 | Mmb177 | D52 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| F370 | **5120** | 5120 | 5120 | 5120 | 1280 | 2560 | 1280 | 640 | 640 | 640 | 320 |
| 462/2 | 10240 | **5120** | 320 | 1280 | 1280 | 320 | 640 | 80 | 160 | 640 | 320 |
| Vic12 | 5120 | 2560 | **10240** | 20480 | 2560 | 2560 | 2560 | 1280 | 2560 | 640 | 1280 |
| Vic13 | 10240 | 5120 | 2560 | **5120** | 5120 | 2560 | 640 | 640 | 320 | 2560 | 640 |
| Gri226 | 20480 | 10240 | 5120 | 10240 | **10240** | 2560 | 2560 | 2560 | 1280 | 2560 | 1280 |
| D2 | 20480 | 20480 | 5120 | 20480 | 5120 | **20480** | 10240 | 10240 | 5120 | 2560 | 1280 |
| D44 | 10240 | 2560 | 1280 | 5120 | 1280 | 2560 | **1280** | 640 | 160 | 1280 | 320 |
| Vic7 | 10240 | 5120 | 5120 | 10240 | 2560 | 5120 | 2560 | **2560** | 2560 | 1280 | 640 |
| Gri250 | 40960 | 20480 | 10240 | 20480 | 10240 | 10240 | 10240 | 2560 | **2560** | 1280 | 1280 |
| Mmb177 | 40960 | 40960 | 20480 | 40960 | 10240 | 10240 | 10240 | 10240 | 5120 | **5120** | 2560 |
| D52 | 20480 | 5120 | 10240 | 20480 | 20480 | 5120 | 10240 | 10240 | 2560 | 2560 | **2560** |
| Gri221 | 20480 | 20480 | 20480 | 20480 | 10240 | 5120 | 5120 | 10240 | 2560 | 1280 | 640 |

* Reciprocal of highest dilution of antiserum causing agglutination of sensitized erythrocytes.

*Haemagglutination by* M. dispar

Strains 462/2, Gri226, Gri221, F370, Mmb177 and Vic13 were tested for haemagglutinating activity against sheep, rat, rabbit, human, guinea-pig, hamster, chicken, horse and calf erythrocytes at 4°, 22°, and 37° C. There was little variation in agglutination titres at the different temperatures. However, the more concentrated mycoplasma suspensions caused haemolysis at 37° and 22° C. and subsequent tests were therefore performed at 4° C.

The growth of *M. dispar* strains F370 and Vic12 was studied over a 7-day period, the number of viable organisms per ml. measured (Gourlay & Leach, 1970) and the pH of the medium recorded. No difference was found in haemagglutinating activity of standard suspensions of these organisms measured after 2, 3, 4 and 7 days' growth.

The haemagglutinating activity of twelve strains for erythrocytes from nine animal species was measured. Samples from at least three animals of each species were tested on at least two occasions and the average haemagglutinating titre is shown in Table 3. The titre range was within one doubling dilution of the average given. The types of erythrocytes agglutinated by strain Mmb177 distinguished it from the eleven other strains examined. These other eleven strains showed a varied haemagglutinating activity. Strain Gri250 appeared the least active while, at the other end of the range strain Vic12 was the most active. Some of these eleven strains appeared rather similar but others could be distinguished on the basis of their haemagglutinating activity.

*Effects of various treatments of red blood cells on their susceptibility to agglutination by* M. dispar

Various treatments of red cells were carried out in an attempt to characterize the receptor site on the red cell surface. Strains F370 and Vic12 were chosen because they gave high haemagglutination titres and because their haemagglutinating

Table 3. *Haemagglutination titre* of* M. dispar *strains with erythrocytes from various animal species*

| *M. dispar* strain | Source of erythrocytes: Rabbit | Sheep | Hamster | Calf | Horse | Chicken | Rat | Human | Guinea-pig |
|---|---|---|---|---|---|---|---|---|---|
| Vic12 | 32 | 16 | 16 | 8 | 16 | 16 | 8 | 16 | 8 |
| D44 | 64 | 16 | 8 | 8 | 4 | 8 | 8 | 8 | 8 |
| Vic13 | 32 | 4 | 4 | 8 | 2 | 2 | 1 | 2 | 1 |
| F370 | 64 | 16 | 8 | 8 | 8 | 8 | — | — | — |
| Vic7 | 32 | 8 | 4 | 4 | 1 | 1 | 1 | 1 | 1 |
| D52 | 16 | 8 | 4 | 2 | 2 | — | 1 | — | — |
| 462/2 | 32 | 4 | 4 | 2 | 2 | — | 1 | — | — |
| D2 | 32 | 4 | 4 | 1 | 1 | 1 | — | — | — |
| Gri226 | 32 | 8 | 2 | — | 2 | — | — | — | — |
| Gri221 | 32 | 2 | — | — | — | — | — | — | — |
| Gri250 | 8 | 1 | — | — | — | — | — | — | — |
| Mmb177 | — | 32 | — | 64 | 1 | — | — | — | — |

* Reciprocal of highest dilution of standard suspensions of *M. dispar* strains which agglutinated erythocytes.

Table 4. *Effects of various treatments of erythrocytes on the haemagglutination titre of* M. dispar *strain F*370

| Treatment | Titre* against erythrocytes from: Rabbit | Calf | Sheep |
|---|---|---|---|
| Phosphate-buffered saline | 64 | 16 | 32 |
| Tris buffer | 64 | 16 | 16 |
| Pronase (0·02 %) | 128 | 64 | 64 |
| Trypsin (0·01 %) | 128 | 64 | 32 |
| Chymotrypsin (0·01 %) | 64 | 32 | 32 |
| Periodate (0·02 %) | 32 | 16 | 8 |
| Bisulphite (0·5 %) | 64 | 16 | 32 |
| RDE† | 32 | 16 | 16 |

* Reciprocal of highest dilution of mycoplasma suspension causing haemagglutination.
† Receptor-destroying enzyme.

activity was representative of the majority of strains. The results obtained with both strains were essentially the same and those for strain F370 are given in Table 4. Erythrocytes from sheep, calves and rabbits were affected in essentially the same way by the reagents. Pronase and trypsin caused some red cells to become slightly more susceptible to agglutination. The other reagents had very little effect. The change with receptor-destroying enzyme was negligible compared with the change in susceptibility of erythrocytes to the stock suspension of influenza A virus, which had haemagglutination titres of $< 4$, 32 and 256 against calf, rabbit and sheep erythrocytes respectively before receptor-destroying-enzyme treatment and $< 4$ against all three types of erythrocytes subsequently.

Table 5. *Effects of various treatments of* Mycoplasma dispar *F370 on haemagglutinating activity*

| Treatment | Titre* against erythrocytes from: Rabbit | Calf | Sheep |
|---|---|---|---|
| Phosphate-buffered saline | 32 | 8 | 16 |
| Tris buffer | 64 | 8 | 16 |
| Pronase (0·02 %) | 64 | < 2 | 2 |
| Trypsin (0·01 %) | 64 | < 2 | < 2 |
| Chymotrypsin (0·01 %) | 64 | 8 | 16 |
| Periodate (0·1 %) | 16 | 8 | 16 |
| Bisulphite (1·0 %) | 32 | 8 | 4 |

* Reciprocal of the highest dilution of mycoplasma suspension causing haemagglutination.

*Effect of various treatments of mycoplasmas on haemagglutinating activity*

In an attempt to characterize the attachment site on *M. dispar* two strains, F370 and Vic12, were treated with various compounds. The treatments affected both in essentially the same way and the results obtained for strain F370 are given in Table 5. The effect of treatments on the agglutination of bovine and sheep erythrocytes seemed similar but was different from the effect of the treatments on the agglutination of rabbit erythrocytes. Pronase and trypsin reduced the haemagglutination titre for bovine and sheep erythrocytes, but not for rabbit erythrocytes. On the other hand periodate, bisulphite and chymotrypsin had little effect.

*Electron-microscopy*

*M. dispar* strain F370 was incubated with sheep and rabbit erythrocytes at 4° C. for 1 hr. The mixture was then examined by electron-microscopy. The capsular material previously described by Howard & Gourlay (1974) can be seen outside the membrane (Plate 1). Fine threads of material seem to bridge the gap between the dense extramembranous material and the erythrocyte membrane.

## DISCUSSION

The twelve strains examined formed a serologically homogeneous group and no serological heterogeneity was observed to the extent that exists amongst certain other mycoplasma species, e.g. *M. hominis* (Purcell *et al.* 1967), *M. pulmonis* (Forshaw & Fallon, 1972) and *M. gallisepticum* (Taylor-Robinson & Berry, 1969). The metabolism inhibition test was chosen because of its sensitivity and specificity, and indirect haemagglutination because of its sensitivity (Purcell, Chanock & Taylor-Robinson, 1969).

Considerable strain variation in haemagglutinating activity was observed. Strain Mmb177 appeared distinct from the other eleven strains examined. The other strains exhibited a graded haemagglutinating potency. Although serological methods do not appear to be useful for distinguishing strains haemagglutination may be. Haemagglutinating activity appears unrelated to serological structure for *M. dispar* as is the case with *M. gallisepticum* (Manchee & Taylor-Robinson, 1969*b*).

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In contrast to certain other mycoplasmas (Manchee & Taylor-Robinson, 1968) neither the incubation temperature for the haemagglutination reaction nor the final pH of the growth medium appear to affect the haemagglutinating potency of *M. dispar*.

*M. dispar* strain 462/2 has been reported to have no haemagglutinating activity for bovine and guinea-pig erythrocytes (Erno & Stipkovits, 1973). The slight difference between the previously reported results and ours for this strain probably does no more than reflect the use of different methods. The variation observed between strains of the same species of mycoplasma emphasizes the necessity to study more than one representative before comparisons between different mycoplasma species are made.

The nature of the receptor site for mycoplasmas on erythrocytes and other cells and the nature of the attachment site on mycoplasmas have been investigated by other workers using haemagglutination and haemadsorption. However, it should be noted that haemagglutination and haemadsorption are not necessarily correlated (Manchee & Taylor-Robinson, 1968). Strains of *M. dispar* rarely form centres (Gourlay & Leach, 1970). It is therefore difficult to perform haemadsorption experiments because colonies wash off the agar.

*M. gallisepticum*, *M. synoviae* and *M. pneumoniae* have been shown to attach to various cells by means of sialic-acid-containing cell receptor sites (Gesner & Thomas, 1966; Manchee & Taylor-Robinson, 1969*a*, *b*). It has been suggested that in the case of *M. hominis* and *M. salivarium* the receptor sites are protein (Manchee & Taylor-Robinson, 1969*a*).

The experiments performed here to determine the nature of the receptor sites on erythrocytes for *M. dispar* indicate that they do not contain sialic acid since they are resistant to receptor-destroying enzyme (cf. influenza A virus). Furthermore the observation that bovine red cells are not agglutinated by influenza A virus but are agglutinated by *M. dispar* supports the hypothesis that *M. dispar* does not attach to a similar sialic-acid-containing erythrocyte receptor. The erythrocyte receptor site is resistant to bisulphite and periodate at the concentrations used. Treatment with pronase and trypsin perhaps makes erythrocytes slightly more susceptible to agglutination by *M. dispar*, possibly by altering the surface charge on the cell, although there is no relation between either the zeta potential or electrophoretic mobility of red cells from different animal species (values taken from Spector, 1956) and susceptibility to agglutination by *M. dispar*.

The results reported by Sobeslavsky *et al.* (1968) indicate that the attachment site of *M. pneumoniae* contains a lipid or glycerophosphate hapten. In contrast *M. salivarium* and *M. hominis* have been reported to have protein attachment sites (Manchee & Taylor-Robinson, 1969*a*; Hollingdale and Manchee, 1972). The attachment site on *M. dispar* for bovine and sheep erythrocytes is sensitive to pronase and trypsin. Since neither of these proteolytic enzymes destroys the mycoplasma attachment site for rabbit erythrocytes at least two attachment mechanisms must exist on the mycoplasma. The attachment site for bovine and sheep erythrocytes is therefore either a protein or contains a protein moiety. The chemical nature of the attachment site for rabbit erythrocytes is unknown.

Savage (1972) suggested that surface components similar to capsules may be involved in cell attachment by lactobacilli and the possibility exists that the capsule of *M. dispar* observed by electron-microscopy after staining with ruthenium red (Howard & Gourlay, 1974) may take part in cell attachment.

As has been stated by Manchee & Taylor-Robinson (1969*a*) the ability to attach to cells should not be regarded as necessarily indicating pathogenicity *in vivo*, but as a valuable property for an invading mycoplasma which might assist in the disease process. Thus the demonstration of attachment sites on *M. dispar* for erythrocytes may indicate the mechanism by which this micro-organism maintains itself in the bovine respiratory tract.

We would like to thank Mrs P. Bland for the electron-micrograph.

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## EXPLANATION OF PLATE

Electron-micrograph of *M. dispar* following incubation with rabbit erythrocytes. Mm, *M. dispar* membrane; Em, erythrocyte membrane; C, capsular material; bar marker, 100 nm.

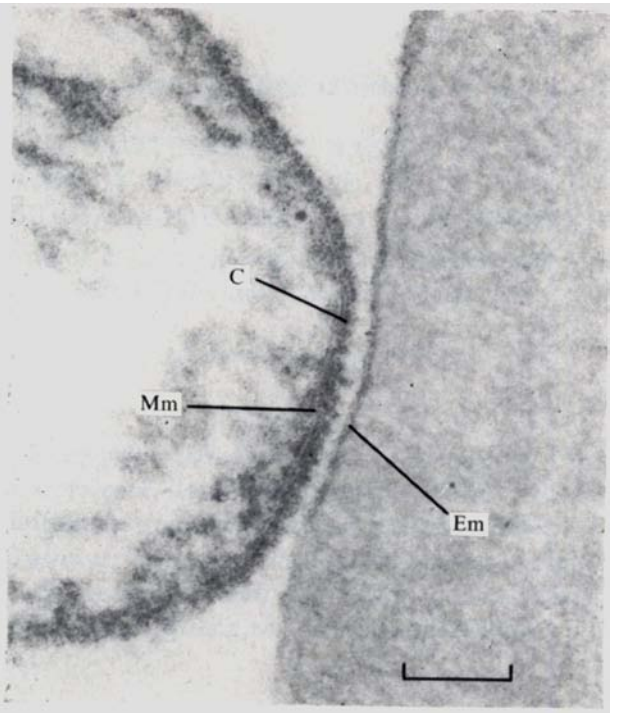


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Table 1. *Numbers of strains examined and serotyped from different specimens*

| Source of strains | No. of specimens | No. of colonies examined | No. of different serotypes isolated from each source |
|---|---|---|---|
| Animals | | | |
| Cattle | 38 | 274 | 139 |
| Chickens | 18 | 87 | 76 |
| Pigs | 11 | 65 | 60 |
| Meat | | | |
| Beef and veal | 25 | 52 | 36 |
| Poultry | 17 | 71 | 63 |
| Pork | 6 | 21 | 13 |
| Persons | | | |
| Faeces | 55 | 506 | 116 |

Table 2. *Distribution of O typable, non-typable and rough strains*

| | Sources of strains | | | | | | |
|---|---|---|---|---|---|---|---|
| | Animals | | | Meat | | | Humans |
| Types of strains | Cattle | Chickens | Pigs | Beef or veal | Poultry | Pork | Faeces |
| No. typable (150 O sera) | 42 | 32 | 17 | 15 | 34 | 3 | 77 |
| No. smooth but not O typable | 66 | 34 | 33 | 18 | 24 | 4 | 14 |
| No. O rough | 31 | 10 | 10 | 3 | 5 | 6 | 25 |
| Total | 139 | 76 | 60 | 36 | 63 | 13 | 116 |

Table 3. *Distribution of H typable, non-typable and non-motile strains*

| | Sources of strains | | | | | | |
|---|---|---|---|---|---|---|---|
| | Animals | | | Meat | | | Humans |
| Types of strains | Cattle | Chickens | Pigs | Beef or veal | Poultry | Pork | Faeces |
| No. of typable (51 H sera) | 126 | 57 | 41 | 25 | 43 | 7 | 71 |
| No. motile but not typable | 5 | 3 | 11 | 9 | 11 | 6 | 5 |
| No. non-motile | 8 | 16 | 8 | 2 | 9 | 0 | 40 |
| Total | 139 | 76 | 60 | 36 | 63 | 13 | 116 |

by tube agglutination based on methods previously described (Bettelheim & Taylor, 1969).

## RESULTS

The number of specimens obtained, of *E. coli* examined and of the different serotypes found are given in Table 1.

The distribution of the typable, non-typable, rough and non-motile strains is given in Tables 2 and 3, and the distribution of common urinary infecting serotypes in Table 4.

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Table 4. *Occurrence of common urinary serotypes in strains from the various sources*

| | Sources of strains | | | | | | |
|---|---|---|---|---|---|---|---|
| | Animals | | | Meat | | | Humans |
| Types of strains | Cattle | Chickens | Pigs | Beef or veal | Poultry | Pork | Faeces |
| No. of smooth strains | 108 | 66 | 50 | 33 | 58 | 7 | 91 |
| No. of common urinary serotypes (O1, O2, O4, O6, O7, O11, O18, O39 and O75) | 4 | 3 | 1 | 8 | 9 | 1 | 26 |
| % common urinary types of smooth strains | 4 | 5 | 2 | 24 | 16 | 14 | 28 |

Table 5. *Source of O serotypes commonly associated with urinary tract infection*

| Serotype | Source | Serotype | Source |
|---|---|---|---|
| O1:H6 | cattle | O6:H1 | humans |
| O1:H7 | cattle, humans | O6:H16 | chickens, poultry |
| O1:H45 | poultry | O6:H45 | poultry |
| O1:Hnt* | poultry | O6:H− | humans |
| O1:H− | chickens, humans | O7:H24 | beef |
| O2:H5 | beef | O7:H− | humans |
| O2:H6 | humans | O11:H4 | humans |
| O2:H7 | cattle | O11:H16 | poultry |
| O2:H8 | cattle | O18:H7 | humans |
| O2:H27 | beef | O18:H14 | cattle, beef, humans |
| O2:H− | beef, poultry | O18:H− | pigs, humans |
| O4:H1 | humans | O39:Hnt* | humans |
| O4:H5 | humans | O75:H55 | humans |
| O4:H16 | poultry | O75:H− | beef, pork, humans |
| O4:H42 | poultry | | |
| O4:H− | humans | | |

* nt, non-typable.

## DISCUSSION

The number of strains which were O serotypable with the antisera used was much less among the animal population than among the human strains, with the meat strains resembling the animal strains. It was also noted that of the serotypes found, those commonly associated with urinary tract infections of humans and probably derived from the normal human faecal flora (Gruneberg, Leigh & Brumfitt, 1968) were found in relatively large numbers in the strains of human origin but rarely in the animal strains, with the meat strains falling between them (Table 4).

We have previously suggested that animal strains of *E. coli* reach the human bowel and can implant (Shooter *et al.* 1970). This is significant because of the widespread use of antibiotics in animal husbandry. It has been shown by Cooke, Hettiaratchy & Buck (1971) that the ingestion by volunteers of cultures of *E. coli*

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of animal origin can result in these strains implanting in the bowel for long periods, although other workers have obtained rather different results (Williams Smith, 1969).

The difference found in the serotypes from animals and man with those from meat falling between them may reflect geographical variation in serotype distribution which has been shown to occur over quite small areas (Gruneberg & Bettelheim, 1969). All the human specimens came from people in the London area but it is possible that the results obtained with the abattoir specimens may reflect the distribution in animals from a limited number of environments. Nevertheless, the indications from this work are that the feed-through of *E. coli* from animals to man may be limited in the general population and that either serotypes from animals are generally not reaching the human population to any great extent, or they may not establish well in the human bowel. Most of the persons in this survey at the time of the study were eating mainly home-cooked food, that our own unpublished studies have shown usually to contain fewer *E. coli* than food served in hospitals and canteens (Shooter *et al.* 1971). The fact that a larger percentage of what are generally regarded as typically human serotypes were found in the meat strains would indicate contamination with strains of human origin during handling or selective survival of certain serotypes.

A difficulty in this work is the small amount of published material on the normal faecal flora of man and domestic animals. A large percentage of the results which are available have been obtained with limited numbers of antisera, because the authors were only looking for certain serotypes, such as the common enteropathogenic or urinary-tract-infecting serotypes. Unless a full range of O antisera is used and the numerous cross-reactions between various *E. coli* O types taken into account, an accurate interpretation of the results cannot be made because a number of O types might be grouped together.

The use of H antigen typing also indicated certain differences. Thus, on three occasions O6:H1 was isolated from human faeces but not from any of the other specimens. The only representatives of O group 6 found among the animal or meat strains were one strain of type O6:H16 from a chicken in the poultry packing station and two from chickens on arrival at the hospital kitchen.

It was also seen that for most O groups which are found in a number of different types of specimens, different H antigens appear associated with different sources. The sources of the O types which are commonly associated with urinary tract infections and their associated H antigens are shown in Table 5.

Three enteropathogenic serotypes were found: O86:H21, O114:H− and O128:H35. Two of these were found in the human faeces and the O114:H− serotype was found in a poultry packing station.

The results generally appear to indicate that there are obvious differences in serotype distribution of *E. coli* in man and animals. More work is required, particularly studying different geographical areas and *E. coli* serotype distribution in hospital patients.

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