PROPERTIES OF ESCHERICHIA COLI IN RECURRENT URINARY TRACT INFECTION.

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by

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ABSTRACT

Properties of Escherichia coli considered to be of importance in overcoming host defence mechanisms against urinary tract infection (UTI) were investigated. These were:

1) O and H serotype
2) K antigen content
3) Sensitivity to the bactericidal activity of human serum
4) Haemolysin production
5) Fimbriae production
6) Fermentation of sucrose, salicin and dulcitol
7) Sensitivity to serine, spermine and urea
8) Growth requirements
9) Mucinase production

E. coli strains isolated from normal subjects and patients attending the Nephrourological Clinic at St. Bartholomew's Hospital because of known or suspected UTI, were studied.

Strains isolated from urines more frequently belonged to O serogroups 2, 4, 6, 8, 18ab and 75, had high K antigen titres, were haemolytic and fimbriate, and fermented salicin than periurethral strains from normal subjects. These findings support the concept of "special pathogenicity", that certain strains are more invasive for the urinary tract than others. Strains rich in these "pathogenic properties" were rarely isolated from normal subjects but were significantly more frequehtly isolated from periurethral swabs of patients. Periurethral strains from symptomatic, abacteriuric (urethral syndrome) patients were similar to those from bacteriuric patients when they were between infections. Previous work has not

implicated bacteria in the aetiology of most cases of this disease and this finding remains unexplained. Strains isolated from the upper tracts of patients undergoing localisation tests more frequently exhibited pathogenic properties than those isolated from only the lower tract, and this was considered to reflect the superior ability of these strains to reach the upper tract or better combat host defence mechanisms.

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CONTENTS

Page No.

PART I. INTRODUCTION

SECTION A: AIMS OF STUDY 13-14

SECTION B: EPIDEMIOLOGY AND PATHOGENESIS OF URINARY TRACT INFECTION 15-18

1) Morbidity and mortality associated with UTI 16

2) Incidence of UTI in different population groups 16

3) Bacterial flora of UTI 17

4) Routes of infection 17

5) Host-parasite relationship 18

SECTION C: HOST DEFENCE MECHANISMS 19-41

1) Initiation of urinary tract infection 20-28

a) Introital colonisation 20

b) Introital carriage 21

c) Urethral ascent 26

d) Antibacterial activity of the urethra 27

2) Lower tract defence 28-38

a) Resistance of the normal bladder 28

b) Hydrodynamic factors 29

c) Antibacterial activity of urine 30

i) Chemistry of urine 30

ii) Growth of various bacterial species 32

iii) Population differences 33

Page No.

d) Antibacterial activity of the bladder wall 34

e) Immunoglobulin secretion 35

i) Normal urine 35

ii) Effect of immunisation 36

3) Upper tract defence 38-41

a) Resistance of the normal kidney 38

b) Urine flow 38

c) Immunological mechanisms 40

d) Role of antibody 40

e) ?Antibacterial secretions 41

SECTION D: FACTORS AFFECTING BACTERIAL PATHOGENICITY 42-61

1) O serotype 43-48

a) O types associated with UTI 43

b) Distribution of O types 46

c) Relation to severity of disease 47

d) Virulence in animal models 48

2) H serotype 48-49

3) K antigen 49-52

a) Possession of K antigen 49

b) K content and type 50

c) Antigenicity 51

4) Serum sensitivity 52-53

a) Relation to virulence 52

b) Occurrence of resistant strains 53

	Page No.
5) Toxin production	54-59
a) Haemolysin production	54-56
i) Cytoxicity	54
ii) Animal toxicity	54
iii) Nephropathogenicity	55
iv) Occurrence	56
b) Endotoxin	56-58
i) Effect on phagocytosis	57
ii) Effect on ureteral peristalsis	57
iii) Effect on renal function	58
c) Lipoprotein toxin	58
6) Fimbriae production	59-60
a) Function	59
b) Occurrence	60
7) Sugar fermentation	60
8) Other properties	61
SUMMARY	62-63

Page No.

PART II: EXPERIMENTAL WORK

SECTION A: ORIGIN, IDENTIFICATION AND MAINTENANCE OF E. COLI STRAINS 64-72

1) Origin 65-70

a) Patients studied 65

b) Sampling techniques 65

i) Urines 65

ii) Periurethral swabs 66

iii) Localisation tests 66

c) Grouping of strains 67-70

i) Urinary strains 67

1) Bacteriuric group-BU

2) Upper tract group-UT

3) Lower tract group-LT

ii) Periurethral strains 67

4) Bacteriuric patients between infections group-BP

5) Abacteriuric group-AP

6) Control group-CP

2) Identification and maintenance 71-72

a) Identification of strains 71

b) Maintenance of cultures 72

SECTION B: STATISTICAL TESTS 73-74

	Page No.
SECTION C: PROPERTIES OF E. COLI	75-163
1) O and H serotype	76-95
a) Introduction	76
b) Materials and methods	77
i) O typing	77
ii) H typing	78
c) Results	79-95
i) O typing	79
ii) H typing	86
iii) Motility	92
iv) O/H combinations	94
2) K antigen content	96-103
a) Introduction	96
b) Materials and methods	96
c) Results	99
3) Serum sensitivity	104-115
a) Introduction	104
b) Materials and methods	104
c) Results	107
i) Serum sensitivity	107
ii) Relation of O type, K antigen and serum sensitivity	112
4) Haemolysin production	116-124
a) Introduction	116
b) Materials and methods	116
c) Results	117
i) Haemolysin production	117
ii) Relation of haemolysin production to O type	122

	Page No.
5) Fimbriae production	125-131
a) Introduction	125
b) Materials and methods	125
c) Results	126
i) Fimbriae production	126
ii) Relation of fimbriae production to O type	129
6) Fermentation of sucrose, salicin and dulcitol	132-138
a) Materials and methods	132
b) Results	132
7) Sensitivity to serine, spermine and urea	139-153
a) Introduction	139
b) Materials and methods	140
c) Results	142
8) Growth requirements	154-158
a) Materials and methods	154
b) Results	155
9) Mucinase production	159-163
a) Introduction	159
b) Materials and methods	159
c) Results	161
SECTION D: COMBINED PROPERTIES OF E. COLI	164-170
a) Introduction and methods	165
b) Results	166

Page No.

PART III: DISCUSSION

SECTION A: PROPERTIES OF E. COLI 171-195

1) O and H serotype 172-176

a) O serotype 172

b) H serotype 175

c) O and H type combinations 176

2) K antigen content 176-179

3) Serum sensitivity 179-184

a) Relation of O and K antigen to serum sensitivity 180

b) Relation of O type to K antigen and serum sensitivity 181

4) Haemolysin production 182

a) Relation of haemolysin production to O type 184

5) Fimbriae production 184-185

6) Sugar fermentation 186

7) Serine, spermine and urea sensitivity 186-189

a) Serine sensitivity 186

b) Spermine sensitivity 187

c) Urea sensitivity 188

8) Growth requirements 189

9) Mucinase production 189-190

SECTION B: COMBINED PROPERTIES OF E. COLI 191-196

1) Strains isolated from urines 192

2) Periurethral strains from bacteriuric patients 192

Page No.

3) Periurethral strains from abacteriuric patients 193

4) Periurethral strains from normal subjects 195

5) Upper and lower tract strains 195

CONCLUSION 196-199

APPENDICES: 200-205

1) Radiological abnormalities of bacteriuric patients 201

2) Instructions for:

a) Use of filter strips
b) Introital (periurethral) swab technique } 202

3) Table headings and figure legends 203-205

REFERENCES 206-245

PART I. INTRODUCTION

SECTION A. AIMS OF STUDY

The intention of this study was to investigate factors affecting the pathogenicity of Escherichia coli in recurrent urinary tract infection (UTI), in adult females. An attempt is made to answer the following questions:

1) Are all strains of E. coli equally capable of causing UTI?

2) What are the specific properties which enable them to overcome host defence mechanisms?

Section B of the introduction deals briefly with relevant aspects of the pathogenesis and epidemiology of UTI. Section C is concerned with the nature of normal host defence mechanisms, and section D with factors affecting the pathogenicity of E. coli with special reference to UTI.

Part II is concerned with experimental work designed to answer these questions and the rationale of the experimental models used.

Part III, the discussion, attempts to relate previous findings with the results of the experimental work, to draw conclusions and bring out interesting and unexpected results.

SECTION B: EPIDEMIOLOGY AND PATHOGENESIS OF URINARY TRACT INFECTION

1) Morbidity and mortality associated with UTI

2) Incidence of UTI in different population groups

3) Bacterial flora of UTI

4) Routes of infection

5) Host-parasite relationship

1) MORBIDITY AND MORTALITY ASSOCIATED WITH UTI

Morbidity associated with UTI is considered to be a major problem. It is estimated that 1-2% of general practice consultations are for symptoms of UTI, and chronic pyelonephritis is a cause of renal failure in about 20% of patients attending European dialysis units (Asscher 1975).

Infection in childhood is frequently associated with reflux and may result in stunted growth and scarring of the affected kidney (Kunin 1972). Impairment of renal function and chronic renal failure often follow (Smellie and Normand 1968). It is generally accepted that, in the absence of obstructive disease, pyelonephritis does not lead to renal failure in adults (Rocha 1972b). However, pyelonephritis is a common complication of pregnancy and is associated with increased risk of foetal mortality and prematurity (Condie, Williams, Reeves and Brumfitt 1968).

The distressing symptoms of frequency and dysuria are a common accompaniment to bacteriuria and may even occur in its absence. The aetiology of the latter disease, commonly termed the "urethral syndrome", is unknown. O'Grady et al (1970) found 43% of women presenting with recurrent UTI symptoms to be persistently abacteriuric. The recent formation of an association (the U and I Club) specifically for individuals suffering from UTI is perhaps an indication of the frequency of this group of diseases.

2) INCIDENCE OF UTI IN DIFFERENT POPULATION GROUPS

The incidence of UTI is much greater in females than males up to the age of about 50 years. Thereafter it rises rapidly in males,

probably reflecting the increase in occurrence of prostatic hypertrophy, urolithiasis and instrumentation of the urinary tract (Rocha 1972b). According to the studies of Kass et al (1965), the incidence of bacteriuria in schoolgirls is approximately 1%, rising to 4% in adulthood.

The prevalence of bacteriuria in hospitalised patients is associated with instrumentation of the urinary tract, general debility due to underlying disease and in some cases the use of immunosuppressants (Sexton 1961; Rocha 1972b). This type of infection is often caused by nosocomial strains of exogenous origin.

3) BACTERIAL FLORA OF UTI

Escherichia coli is the most frequently isolated pathogen, especially in cases of primary infection. Gould (1968) isolated E coli from 91% of patients presenting with bacteriuria for the first time, and from 50% of patients with reinfection. Blazevic et al (1972) isolated E. coli from only a third of a large series of infected urines from hospitalised patients, but it was still the single commonest pathogen. Approximately 70% of urinary infections in patients attending the Nephrourological Clinic at St. Bartholomew's Hospital were due to E. coli (unpublished work).

4) ROUTES OF INFECTION

The early experimental work of Alberren and Hallé (1888) indicated that infection of the urinary tract occurred by the ascending route; that is via the urethra, bladder and ureters. The discovery that intravenous injection of bacteria resulted in bacteriuria led to the acceptance of the haematogenous mode of spread (Sherrington 1893; Bazy 1893). Cabot and Crabtree (1916) believed that bacteria in the bladder

entered the bloodstream via the perivesical lymphatics, and localised in the kidney during glomerular filtration.

It is now accepted that haematogenous infection plays a minor role in the pathogenesis of UTI and that organisms residing in the bowel give rise to infection of the lower tract by ascending the urethra, and the upper tract by ascending the ureters (Rocha 1972a).

5) HOST-PARASITE RELATIONSHIP

The establishment of UTI depends on two factors:

1) Pathogenicity of the invading strains.
2) Status of the host defences.

The relationship of these two factors may be said to govern the occurrence of any microbial disease. Organisms of low pathogenicity are usually associated with infection in immunologically compromised patients but rarely in basically healthy individuals. A large variety of microbes, including saprophytic fungi are associated with disease in immunosuppressed patients. On the other hand organisms such as Corynebacterium diphtheriae and Neisseria gonorrhoeæ can give rise to disease in otherwise normal individuals.

SECTION C: HOST DEFENCE MECHANISMS

1) Initiation of urinary tract infection

2) Lower tract defence

3) Upper tract defence

As the normal urinary bladder is sterile, there must be innate defences against invading micro-organisms. Incompetence of these mechanisms predisposes the host to UTI, and once infection is established a secondary response occurs which may be regarded both as a reaction to toxic agents and an effort by the host to limit the disease.

1) INITIATION OF URINARY TRACT INFECTION

a) INTROITAL COLONISATION

O'Grady *et al* (1970) suggested UTI arises from the following sequence of events:

1) In some women faecal organisms colonise the introitus.
2) In a proportion of these, colonisation extends to the urethra.
3) In a proportion of these, urethral organisms enter the bladder during micturition.
4) In a proportion of these, imperfect defence mechanisms allow the organisms to grow.

That bacteriuria is associated with introital colonisation was demonstrated by Stamey *et al* (1971) and Stamey (1973), who found that enterobacteria could be recovered from the vaginal vestibule of most women prior to infection. Bailey *et al* (1973) isolated enterobacteria from periurethral and vaginal swabs from the majority of bacteriuric female at the time of infection. The enterobacterial carriage rate in these patients fell to 19% immediately after treatment. Elimination of introital organisms during long term, low dose co-trimoxazole treatment is thought to play a major part in the success of this form of therapy in controlling recurrent UTI (Cattell, McSherry, Brooks, and O'Grady 1976).

However, introital colonisation does not necessarily lead to bacteriuria in the susceptible patient who is without the protection of antibiotic therapy. Cattell et al (1974) and Marsh et al (1972) recovered enterobacteria from introital swabs of more than half their patients when they were between episodes of infection, and were not taking antibiotics. Furthermore, strains isolated during uninfected intervals are not necessarily responsible for the subsequent bacteriuric episode (see Part II, section A). This may reflect the inability of the strain to invade the urinary tract, or a temporary change in the status of the host defence mechanisms, or a combination of these factors.

b) INTROITAL CARRIAGE

Many reports have been published concerning introital carriage of enterobacteria in women suffering from recurrent UTI. The results of these studies are summarised in table I. In contrast to other reports, Fair et al (1970)observed that normal (ie.asymptomatic, abacteriuric) women rarely carried enterobacteria. Carriage rates of between 21% and 44% in normal women, and 25% and 63% in bacteriuric women when they were between infections, have been reported by other workers. Significantly higher carriage rates in bacteriuric compared to abacteriuric patients and normal subjects have been observed in individual studies, but Bailey et al (1973), Cattell et al (1974) and O'Grady et al (1970) found no such differences, although the latter study indicated that persistant carriers were more likely to become infected. Variations in the outcome of these studies are probably due to differences in sampling sites, the time of sampling in relation to bacteriuric episodes, the population studied and bacteriological techniques. Allowing that these studies are not strictly comparable, some overlap of the different groups of patients can be demonstrated (fig. 1).

Whilst the presence of enterobacteria on the introitus indicates a ready source of urinary pathogens, this does not necessarily result in bacteriuria in the susceptible individuals. However, UTI is usually associated with introital colonisation. The enterobacterial carriage rate in bacteriuric women, when they are between infections, may be slightly higher than in normal women, but does not differ greatly. It seems unlikely that colonisation of the introitus represents a deficiency in the host defence mechanisms aginst UTI.

Fig.1 URETHRAL AND INTROITAL CARRIAGE OF *E. COLI* AND ENTEROBACTERIA

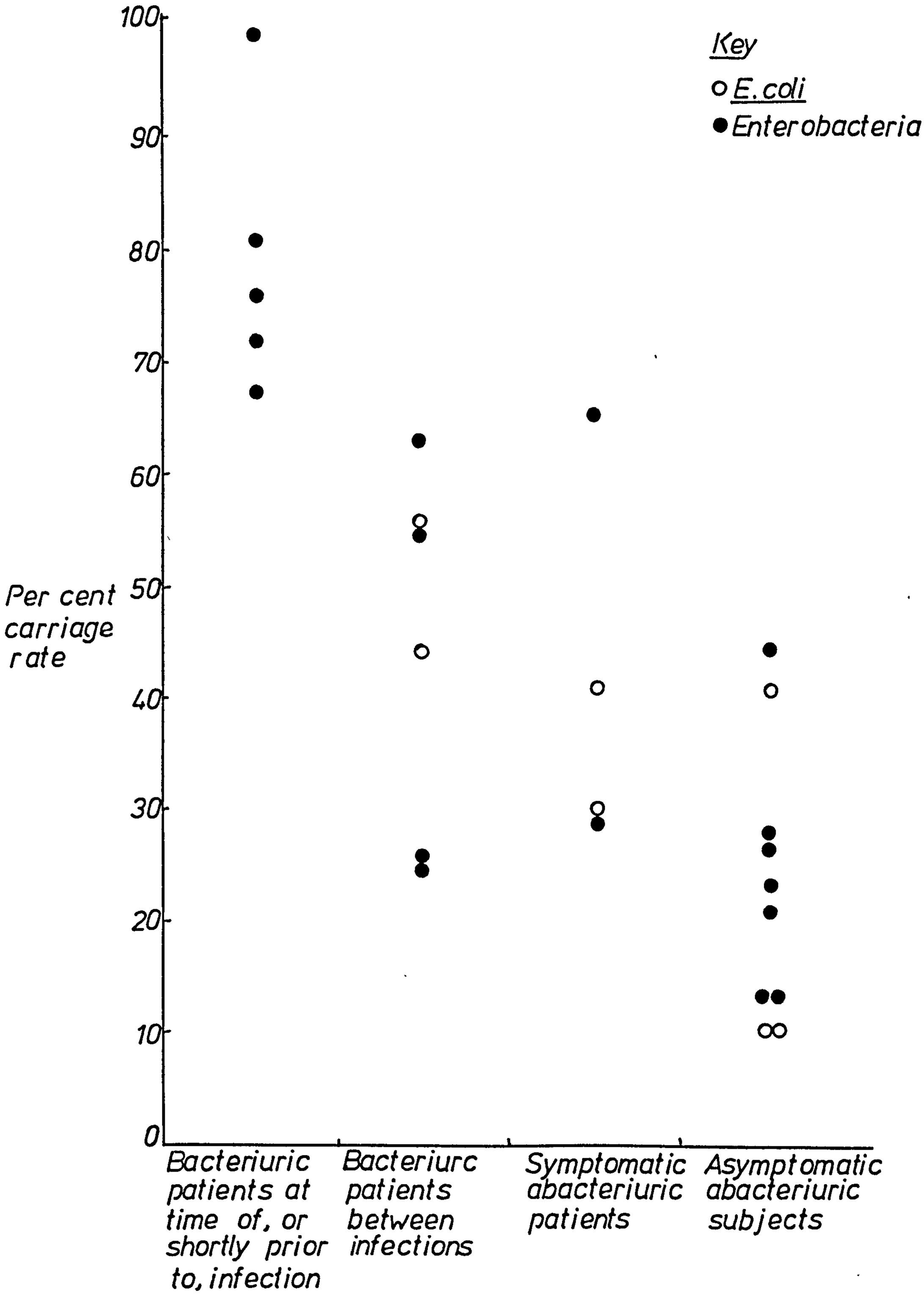


TABLE I: URETHRAL AND INTROITAL CARRIAGE OF E. COLI AND ENTEROBACTERIA

AUTHORS	SUBJECTS STUDIED	SITES SAMPLED	ENTEROBACTERIAL CARRIAGE % positive for: ENTEROBACTERIA	E. COLI
Cox (1966)	Asymptomatic, abacteriuric patients	Urethra: distal	26.9%	
		proximal	21.8%	
Cox et al (1968)	Bacteriuric patients (between infections)	Urethra	54%	
Marsh et al (1972)	Symptomatic abacteriuric patients	Vaginal introitus		30%
	Bacteriuric patients (between infections)			56%
Fair et al (1970)	Asymptomatic, abacteriuric subjects	Vaginal vestibule		10%
		Urethral urine		10%
Stamey (1973)	Bacteriuric patients (shortly before infection)	Vaginal vestibule	67%	
Stamey et al (1971)	Asymptomatic, abacteriuric subjects	Vaginal vestibule	13%	
	Bacteriuric patients (shortly before infection)	Vaginal vestibule	99%	
O'Grady et al (1970)	Asymptomatic, abacteriuric subjects	Introital (periurethral)	23%	
	Symptomatic, abacteriuric patients	"	28%	
	Bacteriuric patients (between infections)	"	26%	
	" " (at time of infection)	"	81%	

AUTHORS	SUBJECTS STUDIED	SITES SAMPLED	ENTEROBACTERIAL CARRIAGE % positive for: ENTEROBACTERIA	E. COLI
Cattell et al (1974)	Asymptomatic, abacteriuric subjects	Introitus (periurethral)	44%	41%
	Bacteriuric patients (between infections)	"	63%	44%
	Symptomatic, abacteriuric patients	"	65%	41%
	Asymptomatic, abacteriuric patients	"	70%	53%
Bailey et al (1973)	Asymptomatic, abacteriuric subjects	Introitus (periurethral)		
	pre-menopausal		13%	
	post-menopausal		26%	
	Bacteriuric patients (between infections)	"	25%	
	Asymptomatic, bacteriuric subjects (at time of infection)	"	76%	
	Symptomatic patients: at time of infection	"	72%	
	post-treatment		19%	
	1-4 weeks post-infection		35%	

c) URETHRAL ASCENT

Cox (1966) recovered enterobacteria from the proximal 2cm of urethra in a substantial proportion (21%) of normal women indicating that it is not the total absence of bacteria in the urethra which alone renders the normal individual free from infection. Bacteria were less often recovered from the proximal, compared to the distal, urethra and were less in number.

Corrière et al (1972) demonstrated that particles introduced into the distal urethra of dogs could be recovered from the bladder after spontaneous, but not passive, micturition. In addition, a study of 15 normal women revealed that intravesical particles were not completely eliminated from the bladder after voiding; they could also be deposited in the urethra and thus transmitted back to the bladder during subsequent micturition. Reflux was ascribed to a mid-urethral high pressure zone by Mayo and Hinman (1973). They found that radioactively labelled E. coli introduced into the mid-urethra of dogs were usually recovered from the bladder whereas organisms introduced into the distal end were recovered from the bladder in only one quarter of the dogs. Bacterial motility and colonisation were not implicated in urethral ascent in this study, but cannot be excluded as factors effecting transfer to or beyond the high pressure zone; although abnormal voiding has been suggested as the general mechanism.

Mayo and Hinman also mention that only 1-5% of the original urethral inoculum was recovered from the urethra at the end of the experiments, the rest being recovered from vaginal swabs inserted at the beginning of the experiment. This suggests that a flushing mechanism operates during voiding. The scanning electron microscope studies of Lloyd-Davies et al (1971a) showed the urethral mucosa to be intricately

folded in its resting state, but smooth during dilatation favouring bacterial washout during voiding. Burdon (1971) investigated the immunoglobulins of normal human urine and urethral secretions. He reported significant levels of secretory IgA in urine, about half of which originated in the urethra. Secretory IgA is the predominant class of antibody in secretions bathing mucous membranes and has been shown to inhibit adherence of bacteria to buccal epithelium (Williams and Gibbons 1972). It is possible that sIgA complements the flushing action of voiding in the normal individual preventing colonisation at or above the high pressure zone.

d) ANTIBACTERIAL ACTIVITY OF THE URETHRA

In addition to mechanical factors, various antibacterial properties have been ascribed to urethral mucosa. Kunii and Kass (1966) demonstrated that radio-labelled E. coli instilled into intact rabbit bladders were rapidly killed. The radio-label could be detected in the surface layers indicating that the organisms had been phagocytosed.

Lloyd-Davies et al (1971b) reported the presence of pockets or holes on the surface of urethral mucosa cells which they believed represented secretory pores - the granules and amorphous intracellular substances seen in these cells being released into the lumen as an antibacterial secretion. The existence of such a secretion is a popular hypothesis. Moore and Hira (1965) described periurethral glands and ducts in the female as the analogue of the prostate. Prostatic fluid has been shown to be markedly inhibitory for many bacterial species (Stamey, Fair, Timothy & Chung 1968) but no analogous substance has yet been isolated from the female urethra. Alternatively, antibody could be responsible for urethral antibacterial activity. Secretory IgA is bactericidal independent of complement provided it is present in

sufficient quantity. With regard to local antibody production, it is of interest to note Stamey's finding that bacteria disappeared from the vaginal vestibule just before an episode of bacteriuria in some patients (Stamey, Timothy, Millar & Mihara 1971). This he ascribed to stimulation of local antibody production by bacteriuria.

Urethro-vesical reflux probably occurs even in the absence of structural or functional abnormalities. If organisms are present in the proximal urethra they must be frequently entering the bladder, and if in large numbers may be more difficult to eliminate. Although evidence for the existance of a protective urethral secretion is rather circumstantial, it may be of value as a first-line defence against the establishment of bacteriuria. Bacterial strains insensitive to this secretion or able to resist the effect of wash-out are more likely to be associated with UTI.

2) LOWER TRACT DEFENCE

a) RESISTANCE OF THE NORMAL BLADDER

Bacteria residing in the urethra may be introduced into the bladder by reflux during micturition or possibly during intercourse (Fair 1971, Asscher 1975). Resistance of the normal bladder to infection was demonstrated by Cox and Hinman (1961). They introduced large numbers of E. coli into the bladders of healthy volunteers and subsequently examined voided specimens for the presence of bacteria. Six hours after inoculation counts were considerably reduced, and all subjects were free from bacteriuria after 72 hours.

The mechanisms of self-sterilisation are not fully understood, but hydrodynamic and intrinsic antibacterial factors appear to be of

great importance. The relationship between urinary infection and the ability of urine to support microbial life is more tenuous.

b) HYDRODYNAMIC FACTORS

In principle, the growth of organisms in the bladder depends in part on the urine flow rate. In the normal bladder the volume of residual urine is small and any organisms remaining after micturition will be diluted by uncontaminated ureteral urine, although this will be partly offset by the availability of fresh nutrients. If frequent voiding is maintained, the organisms should eventually be eliminated (O'Grady and Cattell 1966a). Conversely, if the residual urine volume is sufficient to retain a large number of organisms wash-out will not occur. In accordance with these suppositions Cattell et al (1970) demonstrated a positive correlation between residual urine volume and the inability of patients to eliminate bacteria by frequent voiding. Shand et al (1970) observed that bacteriuric patients had significantly greater residuals than normal women; and those with disease refractory to treatment had significantly greater residuals than patients who were easily treated.

It is generally accepted that abnormalities of micturition which disturb normal vesical emptying predispose to infection (Hinman 1965). As early as 1888, it was found that bladder infection in animal models could only be induced if urethral ligation followed intravesical inoculation (Albarren and Hallé 1888). Since then obstruction of urinary flow has become a popular technique in the study of UTI in animal models (Cabot and Crabtree 1916; Fiveash, Foster and Paquin 1965).

c) ANTIBACTERIAL ACTIVITY OF URINE

Human urine was observed by Pasteur (1863) readily to support bacterial growth. Kass (1956) considered urine to support growth equally as well as nutrient broth; variations in composition within normal limits exerting but slight effect. In contrast, other authors have reported urine to be frequently inhibitory even bactericidal. The relevance of urine composition to the pathogenesis of UTI is not altogether clear, and conflicting reports are not uncommon.

(i) Chemistry of urine

Urine is a complex secretion containing the waste products of metabolism as well as inorganic salts, water and various organic substances such as amino acids and hormones.

Individual urine samples appear to differ markedly in their ability to support bacterial growth according to the nature and concentration of their constituents. The comprehensive studies of Kay (1968), Mulholland *et al* (1969), Asscher *et al* (1968) and De Geus (1970) are reviewed below in conjunction with other relevant publications.

Urea

In vitro studies have shown urea to inhibit bacterial growth (Weinstein and McDonald 1945; Schlegel, Cuellar and O'Dell 1961). It is generally agreed that urine becomes less favourable to bacterial growth as urea concentration increases, but under normal conditions does not reach bactericidal levels.

Glucose

Asscher *et al* estimated the average glucose content of normal urine to be about 60mg/L and Fine (1965) found only two out of a series

of 700 urines to contain less than 10mg/L. Roberts et al (1968) showed variation in glucose concentration in vitro, over a range of 5-500mg/L, to have little effect on growth of E. coli, and considered the amount of glucose normally present in urine sufficient to support maximal growth. In contrast, Weiser et al (1969) demonstrated that competative inhibition of glucose utilisation by inert analogues reduced mean generation time and maximum population size by as much as 75%.

Organic acids

At acid pH, organic acids are known to exhibit antibacterial activity due to the ability of undissociated molecules to enter living cells. Hippuric acid, a common constituent of urine, has been shown to inhibit bacterial growth if present in sufficient concentration. Its use as a urinary antiseptic was proposed by Bodel et al (1959) and Kass and Zangwill (1960), but practical ways of raising excretory levels have not yet been found. B-hydroxy butyric acid has also been found to be inhibitory (Jackson and Grieble 1957). Other as yet unspecified organic acids may contribute to antibacterial activity. Kaye reduced the inhibitory action of urine samples by extracting up to 50% of the organic acid content.

Amino acids

Roberts et al (1968) determined the effect of various amino acids on the growth of E. coli using defined culture media. Several amino acids were found to be bacteriostatic, but apart from serine their action could be minimised by the addition of other amino acids.

Osmolality and pH

Osmolality and pH appear to be intimately concerned with the growth supporting ability of urine. Inhibitory activity is most

marked below pH6 although urinary pathogens will usually grow at much lower pH *in vitro*. Combination of acid pH and high osmolality has been found to depress bacterial growth. There is some disagreement as to whether inhibition produced by increasing the urea content of urine merely reflects an increase in osmolality.

Truly alkaline conditions have been shown to be inhibitory, but normal urine is invariably acid. Dilution of urine decreases inhibitory activity until the reduction in nutrient concentration becomes a limiting factor (De Geus 1970).

Whilst the glucose, organic and amino acid content of urine may influence growth supporting ability, most authors feel that pH, osmolality and possibly urea concentration are the most important variables. The marked variation between individual samples suggest that urine composition is not a prime defence mechanism. However, highly concentrated acid urine may have protective value at night when hydrodynamic mechanisms are inoperative.

Strains of bacteria more able to withstand these inhibitory factors and multiply in the often hostile urinary environment will have advantages over strains less suited to this particular milieu. It follows that such strains should be more frequently associated with UTI.

(ii) *Growth of various bacterial species*

Surprisingly, Schlegel *et al* (1961) found *E. coli* to be more susceptible to urea than other urinary pathogens; *Staphylococcus albus*, which is less frequently associated with UTI was the most

resistant. Mulholland et al (1969) frequently found rabbit urines unfavourable or even bactericidal for E. coli whilst Streptococcus faecalis grew well in nearly all specimens. These results conflict with epidemiological findings and indicate that either the experimental models used were inappropriate or E. coli possesses other properties which especially suit it for life in the urinary tract.

De Geus (1970) noted that non-urinary pathogens such as Haemophilus sp and beta haemolytic streptococci were unable to multiply in urine. However, diphtheroids, which have rarely been implicated in UTI were able to grow in urine. Thus, ability to survive and multiply in urine does not appear to be a major factor determining pathogenicity.

(iii) Population differences

Asscher et al (1966) and Waters (1967) investigated the growth supporting ability of urines from three communities in South Wales. They measured pH, osmolality and ability to support growth. They found urines from pregnant women were more favourable for bacterial multiplication than those from a matched sample of normal women. Roberts and Beard (1965) reported similar observations. Kass (1960) reported an increased incidence of bacteriuria in pregnant women, but Condie et al (1968) conclude that the incidence is no greater in these women than in the population at large.

In both sexes Asscher et al found the osmolality of early morning specimens decreased with age; this may be related to the increased incidence of UTI in old age. In spite of the much lower incidence of UTI in males, differences between the sexes were small.

Buranský et al (1974) studied growth of E. coli, Proteus vulgaris and Pseudomonas aeruginosa in urine of patients with cystitis, pyelonephritis, glomerular nephritis, diabetes mellitus and in healthy subjects. Urines from diabetic and normal subjects were the best supporters of growth in vitro.

At the present time there is little evidence that certain populations are more susceptible to UTI because of differences in urine composition. However, there are but few publications on this subject. There is some evidence that urines from pregnant women support growth better than those from non-pregnant women, but it is not clear whether the incidence of UTI is greater in this group than in the general population.

d) ANTIBACTERIAL ACTIVITY OF THE BLADDER WALL

It is believed that after normal voiding the small amount of remaining urine is thinly spread over the surface allowing maximal antibacterial activity of the bladder mucosa (Hinman 1965). Experimental investigations of this activity have usually involved isolation of the bladder from urine flow, and determination of the fate of bacteria inoculated onto the mucosa. The use of different animal models, techniques of isolation and undefined bacterial strains probably account for at least some of the discrepancies in results reported by various authors.

Mulholland et al (1966) were unable to demonstrate antibacterial activity in the isolated rabbit bladder against E. coli, Strep. faecalis or Proteus mirabilis. Norden et al (1968) observed that E. coli and P. mirabilis were rapidly killed in the isolated

rabbit bladder whilst Staph. aureus was rather more resistant. There was no evidence to suggest that phagocytosis was involved and they postulated the existence of a bactericidal bladder secretion. Vivaldi et al (1965) observed similar rapid killing of a radiolabelled E. coli strain by isolated rabbit bladder flap. However, the radio-label persisted in the mucosa indicating that phagocytosis had taken place. A much slower clearance rate was reported by Cobbs and Kaye (1966 & 1967) using isolated rat bladders, and they present convincing evidence that phagocytic mechanisms were involved. It is of particular interest to note that in this study about half the initial inoculum appeared to be excreted through the urethra despite the abolition of urine flow. Furthermore, ligation of the urethra or bladder base abolished antibacterial activity.

The scanning electron microscope studies of Skoluda et al (1974) on normal rabbit bladder mucosa revealed introcellular fissures, and it was postulated that antibacterial secretions are discharged through them. Walker (1960) noted that the surface cells of mouse bladder contain many compressed vesicles which were most numerous at the free cell surface and often connected with the cell membrane. He considered them to have an excretory function, probably the removal of excess fluid, but it is possible that these vesicles may discharge an antibacterial substance into the bladder.

e) IMMUNOGLOBULIN SECRETION

(i) Normal urine

Normal human urine has been shown to contain IgA, secretory IgA and IgG (Uehling 1973); IgD could be demonstrated in highly concentrated urine only, and IgM was usually absent (Burdon 1971). Tourville et al (1968) demonstrated antibody against E. coli in

normal human urine. IgG, IgA and secretory piece active against E. coli were detected. The origin and significance of these immunoglobulins is uncertain. Burdon (1972) considered IgG to enter the bladder by glomerular filtration thus reflecting serum levels. As complement is absent and phagocytes may be unable to operate in urine (Chernew and Braude 1962), he considered IgG had little protective effect. Tourville et al suggest that IgA passes through the glomeruli and combines with secretory piece in the urine. Alternatively, a secretory mechanism comparable to that observed at other mucosal surfaces may exist in the bladder wall. Both serum and secretory IgA are bactericidal independent of complement, but under normal circumstances effective urine levels are not reached (Burdon 1972). However, it is not inconceivable that sIgA may be of value in deterring adhesion of bacteria to the bladder surface; a process requiring only low sIgA levels.

(ii) Effect of immunisation

If antibodies exert a protective effect, stimulation of their production should enhance the antibacterial activity of the bladder. Norden et al (1968) were unable to improve the bactericidal activity of the excised guinea-pig bladder by prior immunisation, although high serum titres were obtained. Similarly, Mulholland et al (1969) were unable to increase the antibacterial activity of rabbit urine by immunisation with the homologous strains. Williamson et al (1964) found no correlation between initial serum titres and frequency of subsequent infection in UTI patients. In contrast Spencer and Fairhead (1972) were able to show prior immunisation influenced the course of experimental infection in rats. Intravesical immunisation elicited local IgG and IgA response with rapid elimination

of the infecting strains.

Whilst circulating antibody has not been implicated in lower tract defence, evidence suggests that locally produced antibody may be of importance. However, Kaye (1968) was unable to demonstrate antibacterial activity in the protein fraction of urine.

Taken as a whole, existing evidence indicates that the bladder wall possesses antibacterial activity of some sort. The seemingly discrepant results obtained with isolated animal bladders suggest that more than one mechanism is active. Phagocytic processes may have been responsible for the slow clearance rate noted by some authors, whilst rapid bactericidal activity may have been due to an antibacterial secretion; IgA could be a likely candidate.

It follows that incompetence of these mechanisms predisposes the individual infection. Besides intrinsic immunological deficiency, significant residual urine volume may adversely affect bladder wall defense mechanisms. Conditions of low pH, high osmolality and the presence of urea have been shown to inhibit phagocytosis (Chernew and Braude 1962). Thus, whilst phagocytic cells may be able to scavenge the surface of the empty bladder, they may be ineffective in the urine residual. Any antibacterial secretions produced by the bladder wall be diluted in urine residue, perhaps beyond bactericidal level.

Strains of bacteria resistant to phagocytosis or the proposed antibacterial secretions should be more often associated with bacteriuria by virtue of their superior ability to overcome the hosts defence mechanisms. The apparently contrary finding of Norden that the commonest urinary pathogens, E. coli and P. mirabilis were

rapidly killed by isolated bladder wall, whilst Staph aureus, a rare pathogen in uncomplicated UTI, was more resistant may reflect introital distribution. Staph aureus is almost never found on the introitus of non-hospitalised patients and therefore is unlikely to be spontaneously introduced into the bladder.

3) UPPER TRACT DEFENCE

Bacteria usually infect the kidney by an ascending route. Thus, the normal sterile bladder is a barrier between organisms colonising the perineum and urethra, and the upper tract. Once infection is established in the lower tract the invading strain becomes a potential upper tract pathogen.

a) RESISTANCE OF THE NORMAL KIDNEY

Spread of bacteria from the lower to the upper tract does not occur in all patients with demonstrable bacteriuria. Less than half the patients undergoing localisation tests in the series studied by Cattell et al (1972) had upper tract infection, and in many of these infection was confined to one side only.

In experimental studies, renal infection cannot usually be induced without manipulation or obstruction of the upper tract (Lepper 1921; McCabe and Jackson 1961). E. coli inoculated intravenously into animals are destroyed by the normal kidney, although at a slower rate than in other organs (Guze and Beeson 1956).

b) URINE FLOW

The one-way movement of urine from the kidney to the bladder and the presence of valves at the lower end of the ureters normally hinder the ascent of bacteria from the bladder. These valves prevent

reflux of urine when intravesical pressure is raised during micturition (Brumfitt and Percival 1965). Ureteric Peristalsis and adaptation of activity according to flow rate has a net wash-out effect (Hinman 1968).

As would be expected, reflux is often associated with chronic pyelonephritis in otherwise normal tracts (Rosenheim 1965; Hutch, Miller, and Hinman 1965). The relationship between reflux and infection is not a simple one. Reflux cannot be demonstrated in all cases of upper tract infection, whilst sterile reflux is a common occurrence especially in children (Asscher 1975).

If bacteria reach the kidney, the constant exchange of urine probably produces a wash-out effect similar to that of the bladder. O'Grady and Cattell (1966b) equate the upper tract to a continuous cultivation system, organisms multiplying in an infected nephron being constantly diluted by fresh urine. The rate of change of urine will be determined by rate of flow and the volume of the system. Abnormalities of the tubules or obstructions which interfere with urine flow are considered to predispose the kidney to infection (Hinman 1968; Rocha 1972b).

The importance of urine flow is readily demonstrated in animal models whatever the route of infection. Ureteral ligation has been shown to give rise to kidney lesions when the inoculation route is intravenous (Lepper 1921; Brumfitt and Heptinstall 1960) or ascending (Heptinstall 1964). However, it is probable that other effects, besides stagnation of urine render the kidney susceptible to infection (Rocha 1972a).

c) IMMUNOLOGICAL MECHANISMS

The unusual metabolic milieu of the kidney is believed to increase its susceptibility to infection by interfering with the normal humoral and cell-mediated immune mechanisms. Kidney tissue has been found to exert a strong anti-complementary effect, believed to be due to ammonia production by renal glutaminase (Freedman 1960). Beeson and Rowley (1959) demonstrated the renal medulla to be the major site of ammonia production and indeed it is accepted that the medulla is more susceptible to infection than the cortex. The normal bactericidal activity of serum is probably reduced in the kidney; Hubert *et al* (1967) and Acquatella *et al* (1967) were able to inhibit serum activity by increasing osmolality, acidification, or adding urea. In spite of the availability of circulating antibody, phagocytes may be unable to operate effectively in the kidney due to the presence of urine (p.36).

In addition there is some evidence that patients with chronic pyelonephritis have a factor in their serum, probably an antibody, which reduces bactericidal activity (Waisbren and Brown 1966; Kalmanson, Hubert and Guze 1965). Whether this factor is a cause or effect of infection is not known.

d) ROLE OF ANTIBODY

The role of antibody in the prevention of upper tract infection has been partially elucidated through investigations into the efficacy of immunisation. Kaijser and Olling (1973) induced some protection against renal infection in rabbits by immunisation. Stimulation of antibodies against the O and K, but not the H, antigens of *E. coli* significantly decreased the rate of infection when the animals were challenged with the homologous strain. However, infection was induced by the intravenous route in this study, and the high levels of circulating

antibody may have prevented bacteria from reaching the kidney. Montgomerie et al (1972) failed to protect diuresing mice against ascending infection by immunisation, although serum antibody response was evident. Spencer and Fairhead (1972) provoked a general and local antibody response in rats by immunisation; intravesical or intrapelvic lymphatic routes were particularly effective. The immune response was characterised by increased local IgG and sIgA production, and bacterial clearance was rapid when the animals were challenged with the homologous strains by the ascending route.

The variation in results of these studies are probably due to different methods of immunisation and route and technique of inducing infection. The work of Spencer and Fairhead seems to be the most pertinant to the situation in man, where the route of renal infection is usually ascending. They consider the sIgA response to be responsible for the resistance of immunised animals to infection.

e) ?ANTIBACTERIAL SECRETIONS

If the bladder wall is capable of secreting an antibacterial substance, then it seems likely that the transitional epithelium of the ureters, which is continuous with that of the bladder, functions similarly. In her study on the fine structure of the transitional epithelium of rat ureter, Hicks (1965) noted the presence of cytoplasmic vesicles. These were numerous and angular and similar to those described in the bladder by Walker (1960). In addition, "Bundle Cells" were observed which contained aggregates of tubular crystallites and which appeared to be discharging their contents into extracellular spaces and the lumen of the ureter. It is not inconceivable that one or both these types of cells are secreting an antibacterial substance.

SECTION D: FACTORS AFFECTING BACTERIAL PATHOGENICITY

1) O serotype

2) H serotype

3) K antigen

4) Serum sensitivity

5) Toxin production

6) Fimbriae production

7) Sugar fermentation

8) Toxins

Organisms belonging to the species E. coli have a special predilection for the urinary tract. Other faecal organisms, apart from coliforms and Streptococcus faecalis, have rarely been implicated in UTI in spite of their availability. Anaerobes form the bulk of the gut flora (Rosebury 1962) but Headington and Beyerlin (1966) were unable unequivocally to attribute a single case of UTI to anaerobic bacteria out of over 15,000 urines routinely screened for anaerobes. Neither is E. coli found in large numbers in the urethra; the commensal flora is profuse and largely consists of microaerophilic and anaerobic lactobacilli, and bacteria of the Haemophilus vaginalis type (Gould 1968). E. coli are notoriously fast, non-fastidious growers, able to replicate in conditions unfavourable to many other species and this is of obvious importance in a hydrodynamic system such as the urinary tract where a lengthy lag phase would result in wash-out.

There are numerous publications in the literature attempting to establish whether some strains of E.coli are more likely to cause UTI than others. These have mainly employed serotyping techniques. Much less attention has been paid to characteristics which may enable strains to combat host defence mechanisms, their frequency in strains isolated from pathological material, and the relative ability of strains to produce experimental infection.

1) O SEROTYPE

a) O TYPES ASSOCIATED WITH UTI

Certain serotypes of E. coli are known to be enteropathogenic, and many studies have attempted to prove the existence of "uropathogenic" types. It was found that a large number of O types could be isolated from cases of UTI, but that a small number of types were responsible for a great proportion of infections. Table II summarises some previous publications; O types listed as predominant each account for 7% of

smooth typable strains (where this information was published). Overall, types 1,2,4,6,7 and 75 appear to be most frequently responsible for infection. Discrepancies between studies may reflect geographical differences. World-wide and local variation in serotype have been shown to occur (Grüneberg, Leigh and Brumfitt 1968). In addition, many of the populations studied were hospitalised and the types most frequently isolated may have been nosocomial in origin. Turck et al (1969) found types 04, 06 and 075 were more prevalent in the gut of hospitalised patients than in the general community. Kennedy et al (1965) found types 04, 06 and 075 almost exclusively in the stools of hospitalised patients, whilst they were relatively infrequent in the general community. Both observed that carriage of these types increase with length of stay in hospital. Nevertheless, types 02, 04, 06 and 075 were also amongst the commonest isolated from the urinary tract of domicilliary patients.

TABLE II: PREDOMINANT O-TYPES IN UTI

AUTHORS		COUNTRY	SOURCE OF STRAINS	COMMON O-TYPES	% OF SMOOTH TYPABLE STRAINS
KAWADA	1971	JAPAN	–	2,4,6,16,80	73%
PRYLES,GLAGOVSKY	1965	USA	Children	1,4,6,7,75	57%
JACKSON,KOZIJ,JAO	1965	USA	Diagnostic laboratory	1,4,6,75	56%
MAIZTEGUI,KASS	1965	USA	Pregnant out-patients	4,6,62,75	-
EWING,DAVIS	1961	USA	Public Health Laboratory	2,4,6,7,75	57%
RANTZ	1962	USA	Diagnostic laboratory	4,6,75	56%
KUNIN,HALMAGYI	1962	USA	School-children	1,2,6,7,30,75	57%
KUNIN,DEUTSCHER,PAQUIN	1964	USA	School-girls with recurrent UTI	1,6,7,50,75 primary infection	58%
				1,2,4,6,7,75 recurrent infection	69%
TURCK,PETERSDORF	1962	USA	Hospitalised patients	1,4,6,75	57%
VOSTI,GOLDBERG,MONTO,RANTZ	1964	USA	Out-patients with recurrent UTI	4,6,25,50	67%
NETER	1975	USA	Children	1,4,6,7,75	56%
DOOTSON,MACLAREN,TITCOMBE	1972	UK	Out-patients	2,4,6,7,18,75	82%
GRÜNEBERG,LEIGH,BRUMFITT	1968	UK	Domicilliary patients	2,4,6,7,75	60%
McGEACHIE	1965	UK	Diagnostic laboratory	1,2,4,6,18,75	87%*
GANGULI	1970	UK	Out-patients asymptomatic bacteriuria of pregnancy	2,4,6,75	65%

*Limited O antisera used.

b) DISTRIBUTION OF O TYPES

Various authors have attempted to correlate the frequency of O types in UTI with their prevalence in the environment. Rantz (1962) recovered types O2, O4, O6 and O75 from half the infected urines received in a diagnostic laboratory, but from only a fifth of stools. Kennedy et al (1965) considered O4, O6, O75 and O117 to be responsible for more cases of UTI in hospitalised patients than would be expected from the faecal carriage rate.

In contrast, Jackson et al (1965) found no significant difference in the distribution of O1, O4, O6 and O75 in urines, stools and other extra-intestinal infections. The rarer types were found to be less frequently associated with UTI compared to other sites of infection. Turck andPetersdorf (1962) were also unable to demonstrate any difference in the distribution of O types from UTI, other extra-intestinal infections, urethral swabs and stools of hospitalised patients. McGeachie (1965) found no difference in the distribution of serological type from infected and uninfected urines.

With one exception, Grüneberg et al (1968) were unable to demonstrate significant differences in the distribution of individual serotypes of faecal isolates from normal controls and urinary strains from domiciliary patients. Only O type 6 was more frequently associated with infection. However, together types O2, O4, O6, O7 and O75 accounted for 60% of all smooth, typable strains from UTI but only 29% of those isolated from normal controls. In the series studied by Vosti et al (1964) O4, O6, O25, and O50 accounted for 67% of recurrent UTI, but occurred in only 16% of stools from out-patients. Turck et al (1969) found over half the urinary infections in domiciliary patients were due to O4, O6 or O75 whilst faecal carriage rate in normal controls

was considerably lower. Similarly, Dootson et al (1972) found O2, O6, and O75 accounted for over half the UTI flora of out-patients, yet only 12% of stools.

Opinion on the special pathogenicity theory - that some serotypes are more virulent for the urinary tract than others - is divided. Previous work indicates that the overall frequency of O types in UTI is slightly different than would be expected from faecal carriage rates, although differences in the distribution of individual types is less striking. The types common in UTI also appear to be common in the environment, but to a lesser extent. As Turck and Petersdorf (1962) point out "virulence and prevalence may well be related and inseparable factors".

c) RELATION TO SEVERITY OF DISEASE

Rantz (1962) noted that 72% of infections with O4 and O6 were associated with clinical signs of pyelonephritis. Vosti et al (1964 and 1965) found groups O7, O16/62 and O50 were disproportionately associated with acute pyelonephritis, but considered it possible that this merely reflected population differences. They could find no correlation between clinical chronic pyelonephritis and any particular serotype.

Kunin and Halmagyi (1962) did not find that types O4 and O6 were responsible for more clinically apparent disease. Pryles and Glagovsky (1965), in their study of children, could not correlate O type with symptomatology or radiological abnormality. Kunin et al (1964) noted that O groups were distributed similarly in initial and recurrent infections, and no O types were associated with any particular symptomatology.

d) VIRULENCE IN ANIMAL MODELS

Kimball et al (1964) were unable to correlate ability to produce experimental pyelonephritis in rats after ureteral ligation with O type. Enteropathogenic serotypes and types infrequently associated with UTI were often no less virulent than those types commonly isolated from UTI. Similarly, Prát et al (1964) found no correlation between O type and ability to cause infection in non-obstructed rabbit kidney. The incidence of infection was low and enteropathogenic O types were no less virulent than those usually associated with UTI.

Support for the theory that "nephropathogenic" O serotypes exist or that some serotypes are capable of causing a more clinically severe form of disease is tenuous. The disproportionate isolation of some types from cases of pyelonephritis may be fortuitous; the O types associated with pyelonephritis are different in each of the studies mentioned.

There is no evidence that some types are more able to cause pyelonephritis in animal models, although differences in host susceptibility may obscure the true relationship in man. Infection was by the haematogenous route and thus these studies may have been a measure of ability of strains to survive the bactericidal activity of serum, not the ability to produce pyelonephritis.

2) H SEROTYPE

Studies concerned with the H type of strains causing UTI are few. Maiztegui and Kass (1965) found types H1, H4, H5 and H6 were predominant in UTI of pregnancy. Pryles and Glagovsky (1965) reported that H1, H4, H5, H7 and H28 were the commonest types in

children with UTI. Kunin et al (1964) isolated H1 and H5 most frequently from infected urines of schoolgirls.

All found that only 30% to 55% of strains were motile. Kimball et al (1964) could not correlate virulence for rat kidney with motility, and there was no consistent relation between H serotype and infectivity, although overall H5 strains had the highest infectivity rate.

Motility does not appear to be a special feature of strains causing UTI in spite of the potential advantage this property endows in allowing strains actively to transverse the urinary tract. The dearth of investigations on the relation of H type to virulence does not allow any satisfactory conclusions to be drawn.

3) K ANTIGEN

Usually an acidic polysaccharide, the K antigen is the outermost layer of the bacterial cell and lies outside the cell wall. Not all E. coli strains possess K antigen which appears to have a profound protective effect against host defence mechanisms.

a) POSSESSION OF K ANTIGEN

Capsule production (one type of K antigen) was first related to virulence by Smith and Little (1927). E. coli strains associated with scours in calves were found to differ from commensal strains in motility, fermentative capacity and capsule formation. Smith and Bryant (1927) demonstrated the protective capacity of capsules. They found that acapsular variants were more rapidly phagocytosed than wild-type strains.

Wolberg (1969) prepared a K deficient mutant from a strain isolated from a case of septacaemia. Unlike the wild-type it was avirulent for mice when injected intracerebrally, and could be phagocytosed in the absence of opsonins. Sjöstedt (1946) also observed that acapsular forms underwent phagocytosis more readily than capsular forms in normal human blood.

b) K CONTENT AND TYPE

Glynn and Howard (1970) measured K antigen quantitatively by measuring total carbohydrate content and haemagglutination inhibition activity (HAI). In general, the greater the carbohydrate content and HAI activity, the more resistant the strain to complement. However, some qualitative differences were observed in HAI activity of K antigen from complement sensitive and resistant strains. K antigen was also found to influence surface charge and decrease the binding capacity of both IgG and IgM antibody. Howard and Glynn (1971) found K rich, complment resistant strains were poorly phagocytosed and were virulent for mice on intracerebral injection. They suggest that K antigen protects the cell wall against the lytic activity of complement and impairs protein binding. K antibody was observed to be a poor activator of complement.

Glynn et al (1971) measured the HAI activity of strains from cases of UTI and normal faecal strains. The mean K content of urinary strains was significantly greater than faecal strains and this was attributed to the inclusion of patients with renal involvement. They conclude that strains reach the bladder in proportion to their frequency in the faecal flora, but K rich strains are able to overcome host

defences and invade the upper tract. In support of the concept that K rich strains are nephropathogenic Guze et al (1973) correlated the ability of strains to cause ascending pyelonephritis with high K content, resistance to phagocytosis and complement.

Kaijser (1973) detected both quantitative and qualitative differences in strains from UTI and those from faeces. Strains of O type 6 and K type 2a or 2c were more often associated with pyelonephritis, whilst O6:K13 strains were more often associated with cystitis. K1 strains were also more frequently isolated from urines than from faeces. In addition pyelonephritis strains were richer in K antigen than both cystitis or faecal strains. No difference in K content was detected between the latter two groups.

c) ANTIGENICITY

Smith and Bryant (1927) noted that capsular strains produced low-titre O antisera when injected into rabbits whereas acapsular mutants produced high titre sera which was inactive against the wild-type strains. These findings are indicative of both the poor antibody response evoked by K antigen and its blocking effect on the O antigen/antibody reaction.

Whilst studying the protective capacity of antibody in haematogenous pyelonephritis, Kaijser and Olling (1973) observed that not all the rabbits challenged showed a K antibody response. In those that did, titres were moderate compared to the O antibody response. Kaijser et al (1973) were able to induce tolerance to K containing cells by pre-immunising with purified K antigen.

Both quantity and quality of K antigen appear to be related to the ability of strains to resist phagocytosis and the bactericidal action of complement. K antigens also elicit poor antibody response and mask the strongly antigenic O componant. Evidence suggest that K rich strains are nephropathogenic, but strains isolated from the lower tract are similar to normal faecal isolates.

4) SERUM SENSITIVITY

The antibacterial properties of normal human serum were recognised as long ago as 1888; the early literature was reviewed by Mackie and Finkelstein (1932). Goldman *et al* (1969) demonstrated that complement componants C1, C2, C4 and C9 were essential for the bactericidal reaction, but considered that the whole complement sequence participated. Lysozyme was found not to be essential, but enhanced the action of complement. Strains of *E. coli* vary in their serum sensitivity.

a) RELATION TO VIRULENCE

Michael and Landy (1961) investigated the virulence of serum sensitive colonial variants derived from a serum resistant strain. The resistant parent strain was more toxic for mice than the sensitive variants when injected intraperitoneally, and dried extracts induced a greater degree of haemorrhagic necrosis in mouse sarcoma tumour. They considered the resistant strain to be a more potent source of endotoxin. Guze *et al* (1973) found that ability to produce retrograde pyelonephritis in mice undergoing diuresis was related to serum resistance.

b) OCCURRENCE OF RESISTANT STRAINS

Roantree and Rantz (1960) compared serum sensitivity of strains causing bacteraemia and UTI with faecal isolates. Nearly all the strains from cases of bacteraemia were resistant whilst only 21% of faecal and 25% of urinary isolates were resistant. Olling et al (1973) could not show any difference in the serum sensitivity of strains from children with cystitis or pyelonephritis and faecal strains from normal children. However, resistant strains belonging to the more common O groups were twice as prevalent in urine from those with symptomatic infection than in faeces. Children with asymptomatic infections had a higher frequency of sensitive strains belonging to the less common O groups. Kimball et al (1964) also found that urinary strains of high prevalence O groups (ie >7% of all typable strains) were more resistant than the low prevalence O groups (ie. <3% of all typable strains).

Whilst serum resistance has been shown to contribute to virulence in animal studies, resistant strains do not appear to predominate in UTI, although serum resistance does appear to be a feature of strains belonging to the more common O groups.

Howard and Glynn (1971) demonstrated that strains of E. coli with sufficient K antigen to resist killing by complement were also poorly phagocytosed. Thus, it is equally possible that increased virulence of serum resistant strains is due to their ability to resist phagocytosis.

5) TOXIN PRODUCTION

a) HAEMOLYSIN PRODUCTION

Colon bacteria capable of haemolysis were isolated as long ago as 1903 by Kayser (Vahlne 1945) and from early on pathogenic significance was attached to this property. However, haemolysins were not investigated in detail until recently.

A soluble haemolysin, demonstrable in the cell free supernatant of alkaline extract broth was reported by Lowell and Rees (1960). This was further investigated by Williams Smith (1963), who described the soluble haemolysin as alpha haemolysin, and distinguished it from beta haemolysin which was found to be an integral part of the cell. In a small number of strains alpha haemolysin (Hly) has been shown to be plasmid borne (Williams Smith and Lingood 1971). Williams Smith and Halls (1967) suggest that the integration of the Hly gene into the chromosome, loss of conjugation factors or repression account for the inability of strains to transfer the Hly gene.

(i) Cytotoxicity

Cooke and Ewins (1975) associated alpha haemolysin production with cytotoxicity to chicken or foetal colon fibroblasts in cell culture. Non-haemolytic strains were never shown to be cytotoxic. Chaturvedi *et al* (1969) showed alpha haemolysin to be closely linked, but not identical to a cytotoxic factor. Complete destruction of chick embryo cells occurred after 4 hours contact with alpha haemolysin, but monkey kidney and mouse embryo cells were not affected.

(ii) Animal toxicity

Williams Smith (1963) investigated the toxicity of alpha haemolysin for laboratory animals. Cell-free culture supernatant

from alpha, but not beta haemolysin producing strains was highly toxic for guinea pigs, rabbits and mice when injected intravenously. Death of some animals was associated with intravascular haemolysis. Although the toxic principal was identical to alpha haemolysin with regard to pH, temperature stability and neutralisation with specific antisera, qualitative differences in haemolysin from different strains were detected. Paradoxically, no difference could be found in mouse pathogenicity of haemolytic and non-haemolytic variants of the same strains. Rennie and Arbuthnott (1974) were unable to demonstrate toxicity of purified haemolysin in mice or rabbits. However, they observed that whilst haemolytic strains were not enteropathogenic for ligated intestinal loop, the organisms could be found circulating in the blood-stream. Non-haemolytic strains were confined within the gut wall.

(iii) Nephropathogenicity

During investigations into experimental pyelonephritis in rats, Fried and Wong (1970) observed that pyelonephritis occurred in the absence of renal obstruction when haemolytic strains were introduced into the animal intravenously. Non-haemolytic strains gave rise to infection only if the collecting tubules were obstructed by crystalline sludge. This finding was later confirmed using a non-haemolytic mutant derived from a haemolytic strain (Fried, Vermeulen, Ginsberg and Cone 1971). The mutant never produced pyelonephritis in rats and rarely in mice, whereas the wild-type produced infection in about 40% of animals when administered intravenously.

These findings were not confirmed by Kimball et al (1964). They could not produce infection in unligated rat kidneys by intravenous inoculation. Haemolytic strains did not produce infection more often in ligated kidneys than non-haemolytic strains.

(iv) Occurrence

Strains which are enteropathogenic for pigs are frequently haemolytic. However, there is no evidence to suggest that this relationship is anything but fortuitous (Williams Smith and Halls 1967 an 1968).

Vahlne (1945) isolated haemolytic strains more frequently from human pathological material than from faeces. Cooke and Ewins (1975) found haemolytic strains were commoner in UTI than in faeces from normal individuals. In contrast, McGeachie (1966) was unable to demonstrate any difference in the frequency of haemolytic strains in infected and uninfected urines.

Evidence suggests that the alpha haemolysin of E. coli possesses cytotoxic activity for certain cells, but its relationship to animal pathogenicity is not clear. The ability of haemolytic strains to cross the gut wall may be relevant to blood-borne disease. If haemolytic strains are truly nephropathogenic, this may be of great significance in the pathogenesis of upper tract disease. However, it is not known if haemolytic strains are more likely to cause disease when the route of infection is ascending. It has not been established whether haemolytic strains are more frequently associated with UTI than would be expected from the faecal carriage rate.

b) ENDOTOXIN

Endotoxin is a heat-stable, soluble cell extract particularly associated with gram negative bacilli. It is lipopolysaccharide in nature, incorporates the major somatic antigens and is concentrated at the cell surface. It can readily escape from intact cells

(Milner, Rudbach and Ribi 1971). Endotoxin is of pathogenic significance as it is believed to be responsible for some of the toxic manifestations of gram negative infections.

(i) Effect on phagocytosis

The more resistant an organism is to phagocytosis the more likely it is to multiply in the host. Brogan (1966) observed that low concentrations of endotoxin were sufficient to depress markedly serum-independent phagocytosis. This may be of considerable importance as phagocytosis in the bladder takes place in the absence of complement.

(ii) Effect on ureteral peristalsis

Grana et al (1968) infected canine ureters with various gram negative bacteria and noted that inflammatory changes and ureteral paralysis occurred with some species. Ureteral muscular activity was abolished during E. coli and Pseudomonas aeroginosa infection and did not return to normal until several months after treatment. Depression of peristalsis without inflammatory changes could be induced by treatment with purified endotoxin. Teague and Boyarsky (1968) attributed the rapid and reversable loss of ureteral peristalsis produced by E. coli and Citrobacter to endotoxin. Similarly, King and Cox (1972) were able to demonstrate ureteral paralysis in an in vitro model with many urinary tract pathogens, but did not consider endotoxin to be responsible. Cell-free culture filtrates of E. coli were found to be capable of abolishing ureteral activity but, unlike endotoxin, the active fraction was heat labile.

Depression of ureteral activity may be expected to interfere with hydrokinetic defence mechanisms of the upper tract. In particular,

raising of intravesical pressure during micturition is more likely to result in reflux. The antiperistaltic activity of endotoxin may be responsible for the finding that reflux is often resolved by treatment of infection (Rosenheim 1965).

(iii) Effect on renal function

Hinshaw et al (1961) found injection of E. coli endotoxin into the renal artery of dogs resulted in depression of renal function. This was attributed both to a drop in arterial blood pressure and a direct effect on tubular activity.

c) LIPOPROTEIN TOXIN

Mesrobeanu et al (1962) extracted a lipoprotein possessing cytotoxic activity, termed "neurotoxin", from E. coli, Proteus spp and Ps. aeruginosa. A contact time of one hour resulted in the detachment and necrosis of human embryo cells in tissue culture. They subsequently demonstrated its presence in soluble form in the urine of patients suffering from UTI caused by enterobacteria (Mesrobeanu, Mesrobeanu, Alamita, Draghici, Grigoresco and Georgesco 1964). It is not known whether this toxin provokes cell damage in vivo but it may be of significance in the aetiology of UTI.

Overall, evidence suggests that E. coli possesses a variety of toxins which are of value in overcoming host defences. Of particular interest is the ability of some gram negative bacteria to abolish ureteral muscular activity. This probably encourages reflux thus enabling organisms to ascend the ureters. Haemolysin production may contribute to the establishment of renal infection. Haemolytic strains have been shown to produce infection even in the absence of renal

obstruction. It is not known whether some strains are able to produce these toxins in greater quantity or potency than others, but this may be relevant to the pathogenicity of strains.

6) FIMBRIAE PRODUCTION

Filamentous appendages, other than flagella, were demonstrated in a number of gram negative bacilli by Anderson (1949) and Houwink (1949). These structures were further investigated in E. coli by Duguid et al (1955) who showed that they conferred haemagglutinating power by virtue of their adhesion to red cells. They named them fimbriae and electron microscope studies showed them to be usually between 0.3 and 1u in length, about o.o1u wide and to cover a large proportion of the cell wall.

a) FUNCTION

Fimbriae are considered to be organs of attachment and Hashimoto et al (1963) demonstrated adherence of fimbriate E. coli strains to red blood corpuscles, thrombocytes, yeasts, tumour cells and dyes. Adherence to different dyes appeared to be selective. However, Meadows (1971) found no difference in the ability of fimbriate and non-fimbriate strains to attach to solid surfaces. The plasmid borne K88 antigen, which is thought to be fimbrial, enables organisms to proliferate in the anterior small intestine by allowing them to adhere to the epithelium (Williams Smith and Lingood 1971) whilst strains lacking the K88 antigen are carried along the gut by normal peristaltic movement. In contrast, McNeish et al (1975) were unable to attribute to fimbriae adhesion of human enteropathogenic E. coli strains to the mucosa of foetal small intestine. Many strains which adhered to the mucosa were not fimbriate and vice versa.

Duguid (1968) postulated that fimbriae are involved in the uptake of nutrient molecules, or adherence to cell surfaces where nutrient molecules are readily available.

b) OCCURRENCE

In vitro evidence for the role of fimbriae in bacterial adhesion contrasts with epidemiological findings. Duguid (1968) considered fimbriae to be helpful but not essential for intestinal commensalism. Buck (1967) found that the proportion of fimbriate strains isolated from infected urines was similar to non-infected urines and faeces. Gupta et al (1958) found that more than half the strains from UTI cases were not fimbriate. Only in Salmonella typhimurium has fimbriae production been directly associated with pathogenicity. Darekar and Eyer (1973) found fimbriate strains were more pathogenic for mice infected by the oral or conjunctival routes, and more likely to spread to a susceptible host.

With the exception of the K88 antigen there is no evidence that fimbriae act as organs of attachment in vivo. Few strains possess the K88 antigen whereas most E. coli strains can be shown to be fimbriate. If fimbriae are capable of functioning as organs of attachment, fimbriate strains should be more frequently associated with UTI by virtue of their ability to resist urine flow.

7) SUGAR FERMENTATION

McCabe and Jackson (1960) noted that a high proportion of strains isolated from the urine of patients with initial episodes of pyelonephritis failed to ferment sucrose and salicin. Guze et al (1973) found that the ability of strains to cause ascending pyelonephritis in diuresing mice correlated with, amongst other things, ability to ferment dulcitol.

8) OTHER PROPERTIES

It seems likely that E. coli possesses many properties, as yet uninvestigated, which influence pathogenicity. Suppression of the immune response by bacterial products is an area which has received little attention although immunoglobulin secretion has been demonstrated in the urinary tract, and may be an important defence mechanism (Tourville, Bienenstock and Tomasi 1968; Spencer and Fairhead 1972).

The work of Kirpatovski and Stanislavski (1971) suggests that the cytoplasmic fraction of E. coli is immunosuppressive, and Friedman and Chakrabarty (1971) demonstrated the suppressive effect of L-asparaginase, derived from E. coli, on skin allograft rejection in mice.

SUMMARY

Escherichia coli is the single commonest pathogen in UTI. Invasion of the urinary tract usually takes place by the ascending route and depends on both the status of the host defence mechanisms and the pathogenicity of the invading strain.

The normal bladder is naturally resistant to colonisation by organisms residing in the urethra and introitus. Colonisation of the urethra may be minimised by the flushing action of voiding and possibly the presence of an antibacterial secretion. Hydrodynamic mechanisms and the antibacterial action of the bladder wall appear to be the main defence mechanisms of the lower tract. It has not been established whether the antibacterial action of the bladder mucosa is due to antibody, phagocytic processes or a bactericidal secretion.

Unobstructed urine flow and normal immunological mechanisms keep the upper tract free from infection even in the presence of bladder bacteriuria.

Certain serotypes of E.coli are more often associated with UTI than others. It seems likely that this reflects both increased virulence and epidemiologic prevalence. There is little evidence that certain O types are nephropathogenic. The relation of H serotype to UTI has not yet been established, but motility does not appear to be especially associated with strains causing UTI.

Possession of K antigen appears to be of paramount importance in the ability of strains to resist host defences. Type and quantity of K antigen have been found to influence antibody binding capacity, resistance to phagocytosis and resistance to the bactericidal action of

complement. K antigens also elicit poor antibody response and mask the strongly antigenic O antigen. There is some evidence that K rich strains are nephropathogenic.

Resistance to the bactericidal activity of normal serum appears to contribute to virulence in animal models and is a feature of strains belonging to the more common O groups. However, resistant strains are not especially associated with UTI.

There is some evidence that haemolytic strains are nephropathogenic. This may be related to the cytotoxic activity of alpha haemolysin.

Fimbriate strains have not been found to be more common in UTI than would be expected from the faecal carriage rate. There is ample evidence that fimbriae act as organs of attachment *in vitro*, but their *in vivo* role is less well established.

Endotoxin has been shown to markedly depress phagocytosis, ureteral peristalsis and renal function. In addition, a lipoprotein possessing cytotoxic activity has been demonstrated in *E. coli*. These toxins may be of significance in the pathogenesis of UTI, but it is not known whether different strains vary in their toxin producing ability.

Other products, such as L-asparaginase, may influence the immune response of the host.

PART II. EXPERIMENTAL WORK

SECTION A: ORIGIN, IDENTIFICATION AND MAINTENANCE OF E. COLI STRAINS

1) Origin

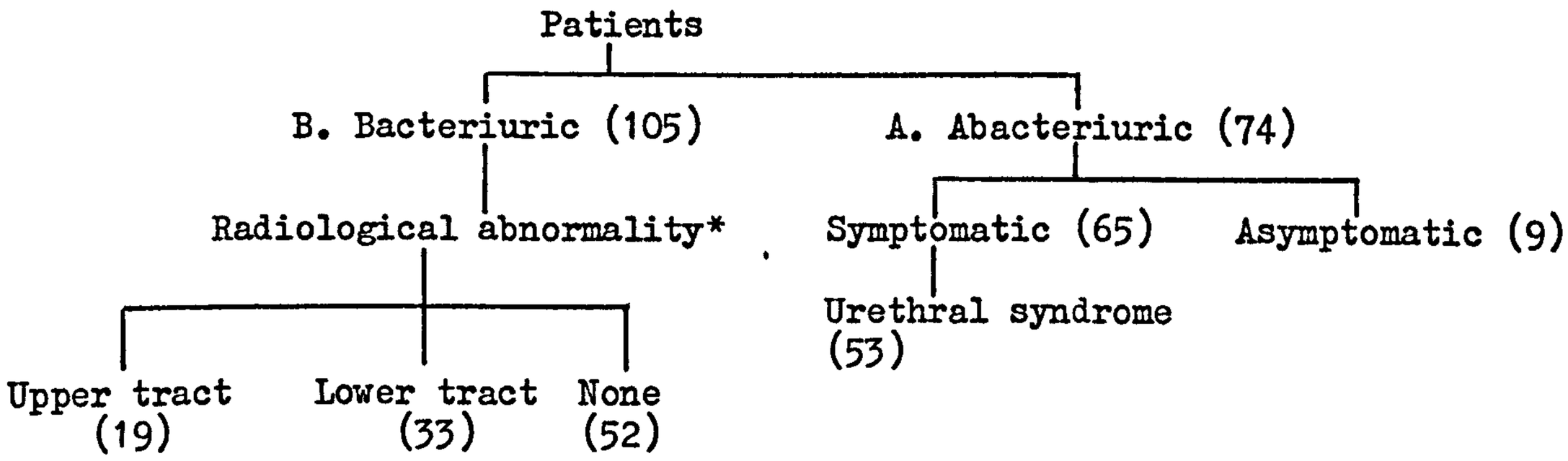
2) Identification and maintenance

1) ORIGIN

a) PATIENTS STUDIED

Patients were referred to the Nephrourological Clinic at St. Bartholomew's Hospital because of known or suspected urinary tract infection. Those studied had been followed for at least 3 months. Intravenous pyelograms were routinely performed on all patients attending the clinic by an independent consultant radiologist. Where necessary residual urine volumes were measured by the ^{131}I-hippuran method of Shand et al (1968).

Patients were categorised according to their symptoms and on the results of bacteriological and radiological tests as shown below:



Bacteriuric patients (B) had at least one documented episode of E.coli bacteriuria, and abacteriuric patients (A) were shown to remain free from infection with E. coli during the follow-up period, which was at least 3 months. The term 'urethral syndrome' was applied to those patients presenting with frequency alone or in combination with dysuria in the absence of bacteriuria. Radiological abnormalities recognised are given in appendix 1.

b) SAMPLING TECHNIQUES

i) Urines

Midstream urines were clean-catch specimens. Patients were

* Not available on one patient.

regarded as bacteriuric if two consecutive specimens yielded 10^5 or more organisms per ml on culture.

ii) Periurethral swabs

Patients were instructed verbally and presented with written instructions prior to sampling (appendix 2). Swabs were taken at home, using tampons, and cultured on MacConkey's medium (Oxoid) by the patient. The inner labia and urethral meatus were swabbed without prior vulval cleansing. Simultaneous urine cultures were obtained by passing a filter paper strip through the midstream and touching the end 1/4" onto a MacConkey plate. The inoculated plates were then returned to the laboratory and incubated overnight. Any enterobacteria isolated were identified and the number of colonies recorded. Filter paper strip cultures were interpreted according to the method of Leigh and Williams (1964). Cultures yielding 25 or more colonies of gram-negative bacilli, or 30 colonies or more of gram-positive cocci were regarded as potential infections, and those yielding between 5 and 25 colonies of enterobacteria were regarded as possible infections. For confirmation, these patients were recalled to the hospital and midstream, clean-catch samples collected and cultured in the usual way.

iii) Localisation tests

Localisation tests were performed on some patients using a modification of the method described by Stamey, Govan and Palmer (1965). All patients studied had a documented history of recurrent UTI and had significant bacteriuria (10^5 or more organisms per ml) at the time of study. A diagnosis of upper tract infection was based on the demonstration of a hundredfold difference in bacterial count in ureteral urine compared with washed bladder urine.

c) GROUPING OF STRAINS

Strains were categorised according to the following scheme: (also see fig. 2).

(i) Urinary strains

1) Bacteriuric group-BU

105 strains from 105 randomly selected female patients were isolated from midstream urines.

2) Upper tract group-UT

A total of 19 strains were isolated from the upper tracts of 18 patients. 15 strains were isolated from ureteral urine specimens of 5 male and 10 female patients undergoing localisation tests. Two strains were isolated from the renal tissue of an excised kidney from one patient with calculus disease, and 2 strains were isolated from the renal calculi of 2 patients.

3) Lower tract group-LT

Twenty nine strains were isolated from the lower tracts of 7 males and 22 females undergoing localisation studies. The ureteric urine of these patients was shown to be sterile. Strains were taken either from the catheter bladder or washed bladder specimens.

(ii) Periurethral strains

4) Bacteriuric patients between infections group-BP

A total of 43 strains were isolated from periurethral swabs of bacteriuric patients at a time when they were between infections, ie. they were abacteriuric for at least 6 weeks before and after the periurethral strain was isolated, and were not taking any antibiotics. Urinary strains from all patients in this group were included in the BU group.

The periurethral strains were subdivided as follows:

Non-infecting strains-BP/NP [@]

Thirty four strains could not be shown to give rise to infection in that the episode of bacteriuria immediately preceding, and any following the isolation of the periurethral strain, were either not due to E. coli or were caused by a different serotype. This does not exclude the possibility that these strains caused infection at some more remote time.

Infecting strains

Seven strains were considered to give rise to infection in that the episode of bacteriuria preceding or following isolation of the periurethral strain was due to an E. coli strain of the same serotype.

5) Abacteriuric group-AP

A total of 75 strains were isolated from periurethral swabs of 69 patients who were never shown to have E. coli bacteriuria:

a) 58 strains were isolated from 53 urethral syndrome patients, group AP/US.

b) 10 strains were isolated from 9 asymptomatic, abacteriuric patients.

c) 7 strains were isolated from 7 patients who had episodes of bacteriuria due to Proteus mirabilis (in 6 cases) and Klebsiella aerogenes (in 1 case), but never to E. coli. All 7 patients were followed for more than one year.

6) Control group -CP

Forty five periurethral strains were isolated from 28 normal,

[@]NP = "non-pathogenic".

female laboratory workers with no previous history of UTI. None had symptoms suggestive of UTI or were taking antibiotics.

Fig. 2 GROUPING OF STRAINS

- B. Bacteriuric patients (105)
  - At time of infection — 105 urinary strains: Group BU
  - Between infections — 43 periurethral strains: Group BP
    - 9 strains gave rise to infection
    - 34 strains did not give rise to infection: Group BP/NP
- Bacteriuric patients undergoing localisation studies (47)
  - Infection of the upper tract (18) — 19 strains: Group UT
  - Infection of the lower tract (29) — 29 strains: Group LT
- A. Abacteriuric patients (69) — 75 periurethral strains: Group AP
  - Urethral syndrome patients (53) — 58 strains: Group AP/US
  - Asymptomatic patients (9) — 10 strains
  - Bacteriuria — 7 strains never due to E. coli (7)
- C. Abacteriuric controls (28) — 45 periurethral strains: Group CP

2) IDENTIFICATION AND MAINTENANCE

a) IDENTIFICATION OF STRAINS

Strains giving the following reactions were identified as *E. coli* (Edwards and Ewing, 1972).

Indole production		+	+	-	+
Lactose fermentation		+	-	+	-
Glucose fermentation:	acid	+	+	+	+
	gas	+	+	+	-
Urea splitting		-	-	-	-
Citrate utilisation		-	-	-	-
M.R. test		+	+	+	+
V.P. test		-	-	-	-
		(296 strains)			
Adonitol fermentation			-	-	-
Inositol fermentation			-	-	-
Malonate			-	-	-
O.N.P.G.			+		+
			(17 strains)	(2 strains)	(1 strain)

All tests were incubated for 48 hours at 37°C.

b) MAINTENANCE OF CULTURES

Stock cultures were maintained on nutrient agar slopes or stabs without subculture at 4°C. "Working" subcultures were maintained at room-temperature and periodically renewed. A sweep of growth from the stock cultures was inoculated onto nutrient agar slopes and purity checked by plating onto MacConkey's medium.

SECTION B: STATISTICAL TESTS

STATISTICAL TESTS

Nonparametric techniques of hypothesis testing were considered particularly suitable for this type of date for two reasons:

a) they do not assume that the population under analysis is distributed in a certain way, thus avoiding the necessity for testing data for normal distribution.

b) they include "ranking tests" which are particularly suited to examination of ordinal data; for example where results are expressed as titres in the K antigen investigation or sensitivity grades as in the serum sensitivity investigation.

The chi-square test for two independent samples was used where data consisted of frequencies in discrete categories. The significance of differences between pairs of groups was determined by this method, the hypothesis being that the two groups differed with respect to the property being examined. The chi-square test was used to compare the frequencies of haemolysin and fimbriae production, certain serotypes, nutritionally fastidious strains, and fermentation of sucrose, salicin and dulcitol. The Mann-Whitney U test for two independent samples was used to compare sensitivity of the groups to serine, spermine and urea, K antigen content, mucinase production and serum sensitivity (Siegel 1956).

Urinary strains (group BU), periurethral strains from patients (groups BP and AP) and periurethral strains from controls (group CP) were compared with each other using the appropriate statistical tests. Upper tract strains (group UT) were compared with lower tract strains (LT). These groups contained much smaller numbers of strains and it was considered inappropriate to evaluate them against the larger groups BU, BP and AP.

SECTION C: PROPERTIES OF E. COLI

1) O and H serotyping

2) K antigen content

3) Serum sensitivity

4) Haemolysin production

5) Fimbriae production

6) Fermentation of sucrose, salicin and dulcitol

7) Sensitivity to serine, spermine and urea

8) Growth requirements

9) Mucinase production

1) O AND H SEROTYPE

a) INTRODUCTION

In view of the number of conflicting reports on the distribution of different O serotypes in UTI and the environment, O typing of strains was considered to be worthwhile. In addition, there are few publications concerned with H types in UTI.

Serotyping techniques were developed by Kauffmann and his co-workers (Kauffmann, 1947) during the 1940s, and are based on the study of various antigenic componants of the bacterial cell. In E. coli these are O antigens (cell-wall), H antigens (flagella), and K antigens (capsule or envelope). When injected into rabbits different bacterial strains evoke specific responses and the antibodies produced will react only with strains of similar antigenic type. A complex scheme has been evolved whereby E. coli species can be subdivided into a large number of serotypes, (Edwards and Ewing 1972).

O antigens are thermostable and specificity is due to the arrangement of sugar residues in the cell wall. Cross reactions between different O types have been related to the presence of the same sugar in strains which are otherwise antigenically dissimilar (Ørskov et al 1967). Autoagglutinating (rough) strains cannot be typed and are believed to have lost their sugar residues.

H antigen typing can only be accomplished in actively motile cultures which can be obtained by serial passage through soft agar in Craigie's tubes. The H typing scheme was not compiled until the development of this method by Vahlne (1945).

K antigens are responsible for the inability of many living strains to react with O antisera (Kauffmann 1947). Thus O agglutination tests are always carried out with heat treated cells which have lost their heat labile K antigens. A few strains have heat stable K antigens and are non-typable.

b) MATERIALS AND METHODS

Cell wall, O and flagella, H antigen types were determined by reacting suspensions of killed bacteria with antisera raised in rabbits. The method used was based on the work of Kauffmann (1943 and 1944). The antisera were prepared by Dr. K.A. Bettelheim and Mary Chandler who also supervised, and greatly assisted with, the typing.

(i) O typing

Preparation of antisera

Antiserum preparation was based on the methods of Edwards and Ewing (1955), Sojka (1965) and Kauffman (1944) as reviewed by Cooke (1974).

Steamed, autoclaved or formalinised six hour brain-heart infusion broths (Oxoid) of standard classical strains were inoculated into the marginal ear veins of New Zealand White rabbits. An increasing series of doses was given over a period of 6-8 weeks, and the rabbits were bled 4 times in all over a period of 2 weeks. Serum was separated by leaving the blood to clot overnight at 4°C and centrifuging to remove free red cells. The serum was stored at -20°C.

Typing

Strains were O typed according to the method of Bettelheim & Taylor (1969). Antigen suspensions were prepared by steaming overnight nutrient broth cultures (Oxoid) for one hour; 40% formalin was then

added to a final concentration of 0.5%. 1/8 dilutions of antisera were grouped into pools according to their cross-reactions and reacted with the antigen suspensions using an automatic machine, as described by Bettelheim et al (1975). Agglutination of the test suspensions was observed after overnight incubation at 50°C. The reactions of antigens with the constituent members of the pools were then determined. Strains were finally identified by titrating antigen against doubling dilutions of sera starting at 1/50, and comparing the resulting titre with that obtained with the standard classical strain. If a strain was agglutinated 'to titre' by more than one component, absorbed sera were used to erradicate cross-reactions.

(ii) H typing

Preparation of antisera

Antisera were prepared as described in the previous section, except that the rabbits were inoculated with formalised, fully motile suspensions.

Typing

Motility was induced by passaging strains up to six times in Craigie's tubes at 37°C and 30°C. On the third and sixth subcultures, nutrient broths were inoculated, incubated for 4-5 hours at 37°C or 30°C, (depending on the optimum temperature), and examined under the microscope by the hanging drop method. If 80% or more of the bacterial cells were motile, the strain was typed.

Strains were H typed according to the method of Chandler & Bettelheim (1974). Formalised, motile suspensions were reacted with H antisera as described in the previous section, except that the incubation period was only 2 hours.

c) RESULTS

(i) O Typing

The overall distribution of O types is shown in figure 3. Distribution of the fifteen commonest O types, that is types represented by a total of four or more strains, is shown in table III (percentages given are of the number of smooth, typable strains in each group).

With the exception of O75 and O18, differences in the incidence of individual O types between groups were small. Figure 4 shows the overall distribution of the fifteen commonest types. O types were arranged according to their frequency in the BU group; thus O73 occurred with the lowest, and O75 with the highest frequency. The percentage of smooth, typable strains belonging to each group was plotted in a cumulative fashion. The overall distribution between urinary and periurethral groups appears to be slightly different.

Strains of O type 75 were less often isolated from periurethral swabs of normal subjects (3%) than urines (17%); $X^2 = 4.0, 0.5 > p > 0.02$. Only 5% of periurethral strains which did not give rise to infection in bacteriuric patients were type O75. Over half (55%) the smooth, typable strains isolated from the upper tract were type O75. However, the total number in this group was small, and it was not significantly different from the lower tract group.

Six per cent of urinary strains were O18ab whilst this type was rarely isolated from periurethral swabs (only one strain in all). O18ac was commonly isolated from periurethral swabs but never from urines, although the number of strains was inusfficient for statistical analysis.

The most common urinary types were O2,O4,O6,O8, O18ab, and O75 which accounted for 54% of smooth, typable strains from midstream specimens (table IV). Periurethral carriage of these O types was

significantly less frequent in abacteriuric patients (X^2 = 6.8,0.01 > p > 0.001) and normal subjects (X^2 = 14.05,p = 0.001) but not in bacteriuric patients. Nearly all the upper tract strains were common 0 types (82%) whereas these types accounted for only 44% of lower tract strains. This difference was statistically significant (X^2 = 5.1,0.05 > p > 0.02). Strains from urethral syndrome patients (AP/US) were not different from the parent AP group. Periurethral strains which did not give rise to infection in bacteriuric patients resembled those from abacteriuric patients.

A total of 58 different 0 types were isolated including one new type 0153.

KEY

BU: Bacteriuric patients; urinary strains.

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

UT: Bacteriuric patients; strains isolated from the upper urinary tract.

LT: Bacteriuric patients; strains isolated from the lower urinary tract.

Fig.3 DISTRIBUTION OF O SEROTYPES

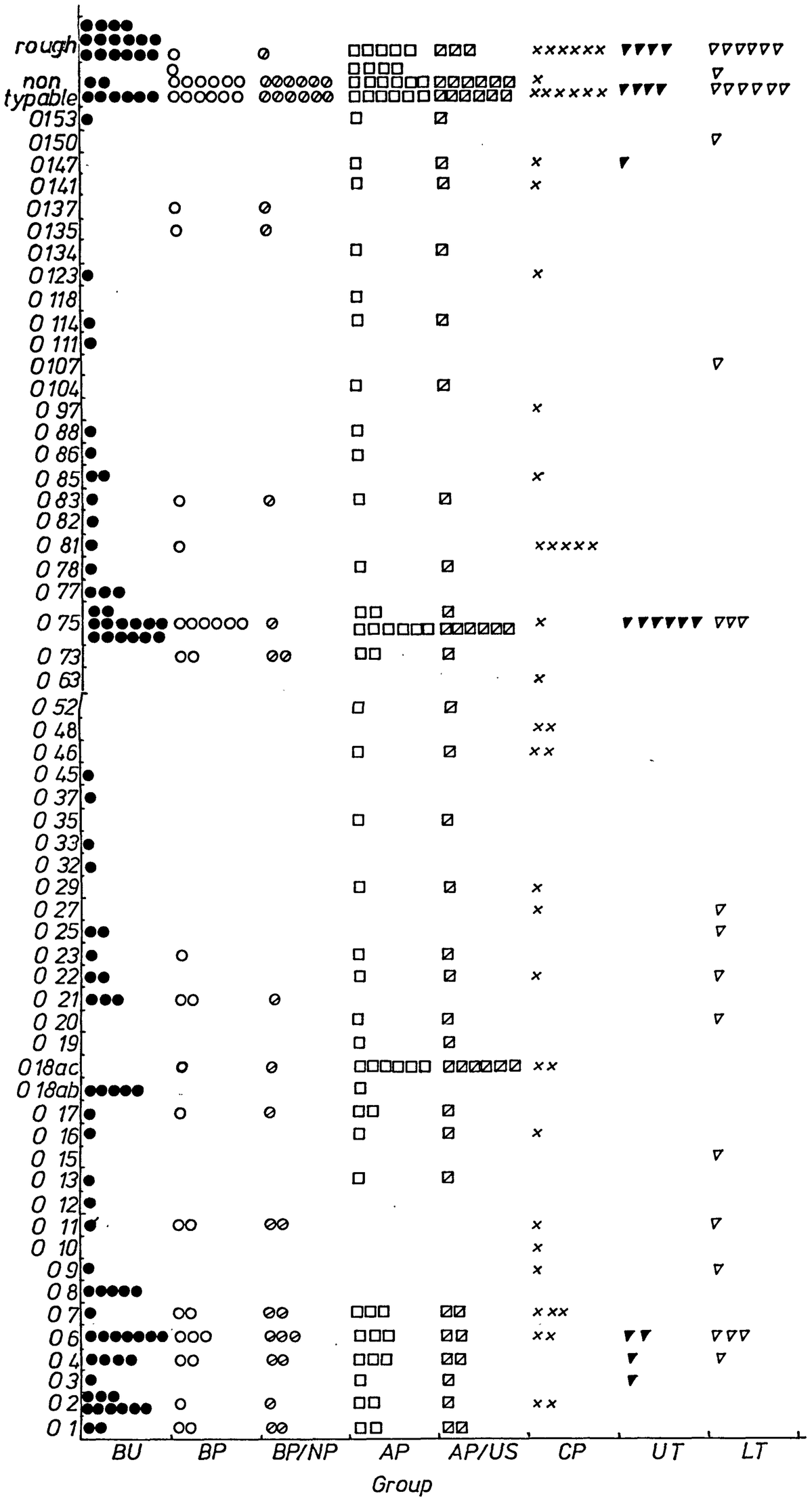


TABLE III: DISTRIBUTION OF 15 COMMONEST O TYPES

O TYPE	GROUP BU		BP		BP/NP		AP		AP/US		CP		UT		LT	
01	2	3%	2	7%	2	9%	2	4%	2	5%						
02	9	11%	1	3%	1	5%	2	4%	1	2%	2	6%				
04	4	5%	2	7%	2	9%	3	5%	2	5%			1	9%	1	6%
06	7	9%	3	10%	3	14%	3	5%	2	5%	2	6%	2	18%	3	19%
07	1	1%	2	7%	2	9%	3	5%	2	5%	3	10%				
08	5	6%														
011	1	1%	2	7%	2	9%					1	3%			1	6%
017	1	1%	1	3%	1	5%	2	4%	1	2%						
018ab	5	6%					1	2%								
018ac			1	3%	1	5%	6	11%	6	14%	2	6%				
021	3	4%	2	7%	1	5%										
022	2	3%					1	2%	1	2%	1	2%			1	6%
073			2	7%	2	9%	2	4%	1	2%						
075	14	17%	6	21%	1	5%	8	15%	7	16%	1	3%	6	55%	3	19%
081	1	1%	1	3%							5	16%				
Smooth typable strains	81		29		21		54		43		32		11		16	

Fig. 4 CUMULATIVE DISTRIBUTION OF THE FIFTEEN COMMONEST O SEROTYPES

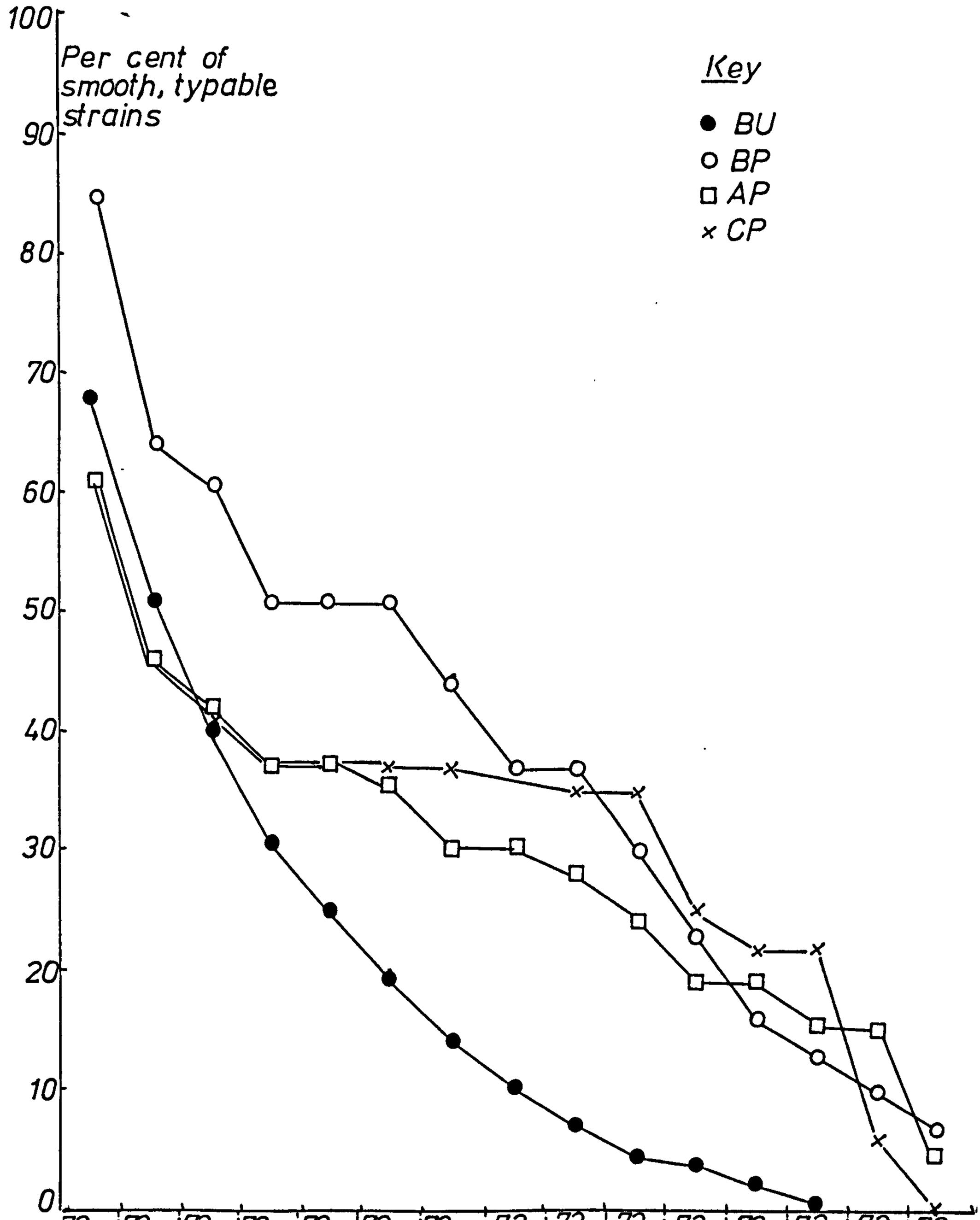


TABLE IV: DISTRIBUTION OF COMMON URINARY O TYPES

GROUP	NO. OF SMOOTH, TYPABLE STRAINS	NO. OF STRAINS TYPE 02, 04, 06 08, 018ab, 075	
BU	81	44	54%
BP	29	12	41%
BP/NP	21	7	33%
AP	54	17	32%
AP/US	43	12	28%
CP	32	5	16%
UT	11	9	82%
LT	16	7	44%

(ii) H typing

The overall distribution of H types is shown in figure 5. Distribution of the 11 commonest H types (types represented by a total of 4 or more strains) is shown in table V, (percentages given are of the number of motile, typable strains in each group).

With the possible exception of H5 particular H types were not associated with urinary infections. There was no difference in the overall distribution of the 11 commonest H types amongst the groups (fig. 6).

Type H5 strains were significantly more often isolated from urines (17%) than from periurethral swabs of normal subjects (0%), $X^2 = 4.5$, $0.05 > p > 0.02$., otherwise there were no significant differences between the groups. Twenty nine per cent of strains isolated from upper tracts were H5, and only 11% of strains from lower tracts were of this type. However, the number of strains was insufficient for statistical analysis.

The most common urinary types were H1,H4,H5, and H7 which accounted for between 39% and 60% of motile, typable strains (table VI). The small differences between periurethral and urinary isolates were not of statistical significance.

KEY

BU: Bacteriuric patients; urinary strains.

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral straihs.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

UT: Bacteriuric patients; strains isolated from the upper urinary tract.

LT: Bacteriuric patients; strains isolated from the lower urinary tract.

Fig.5 DISTRIBUTION OF H SEROTYPES

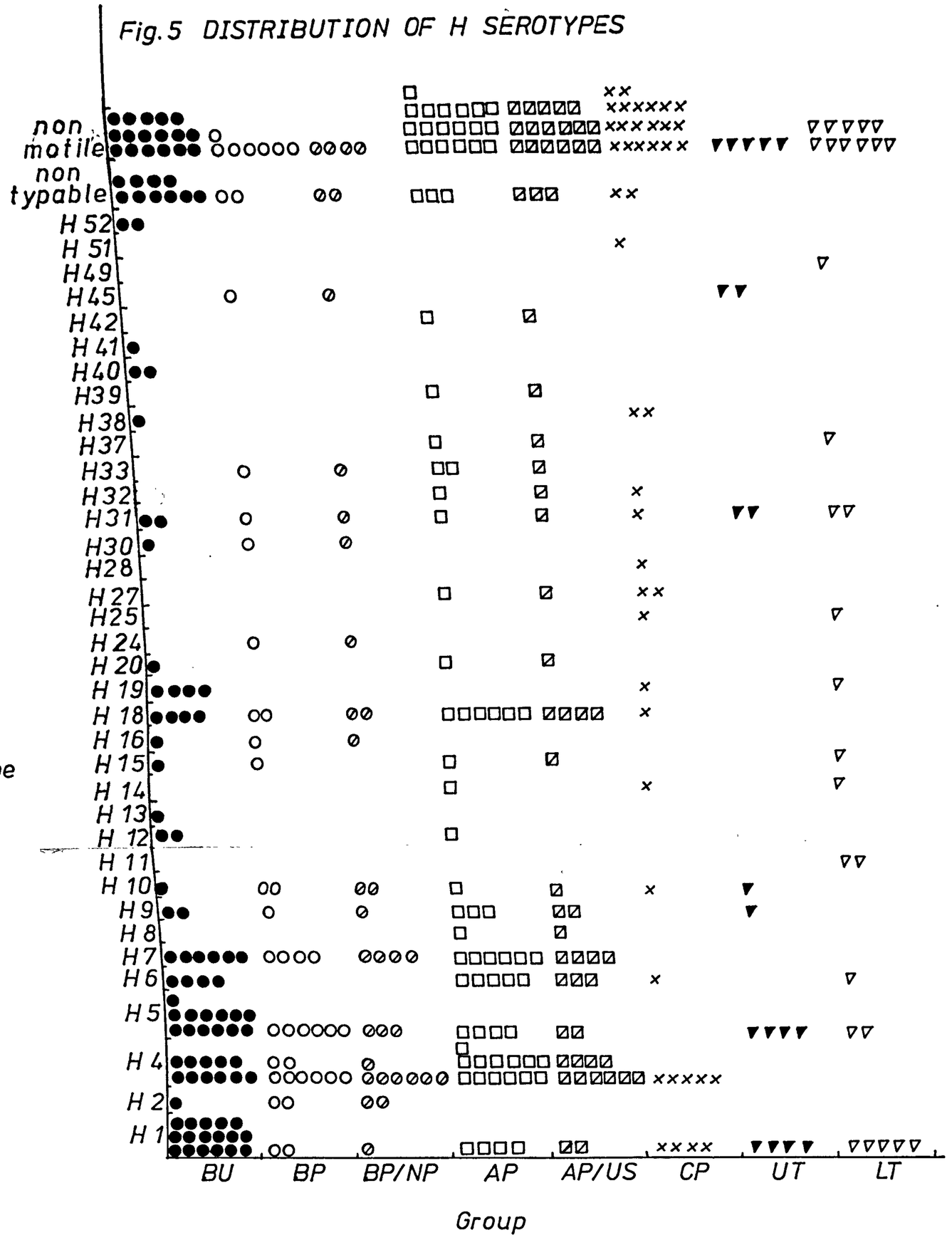


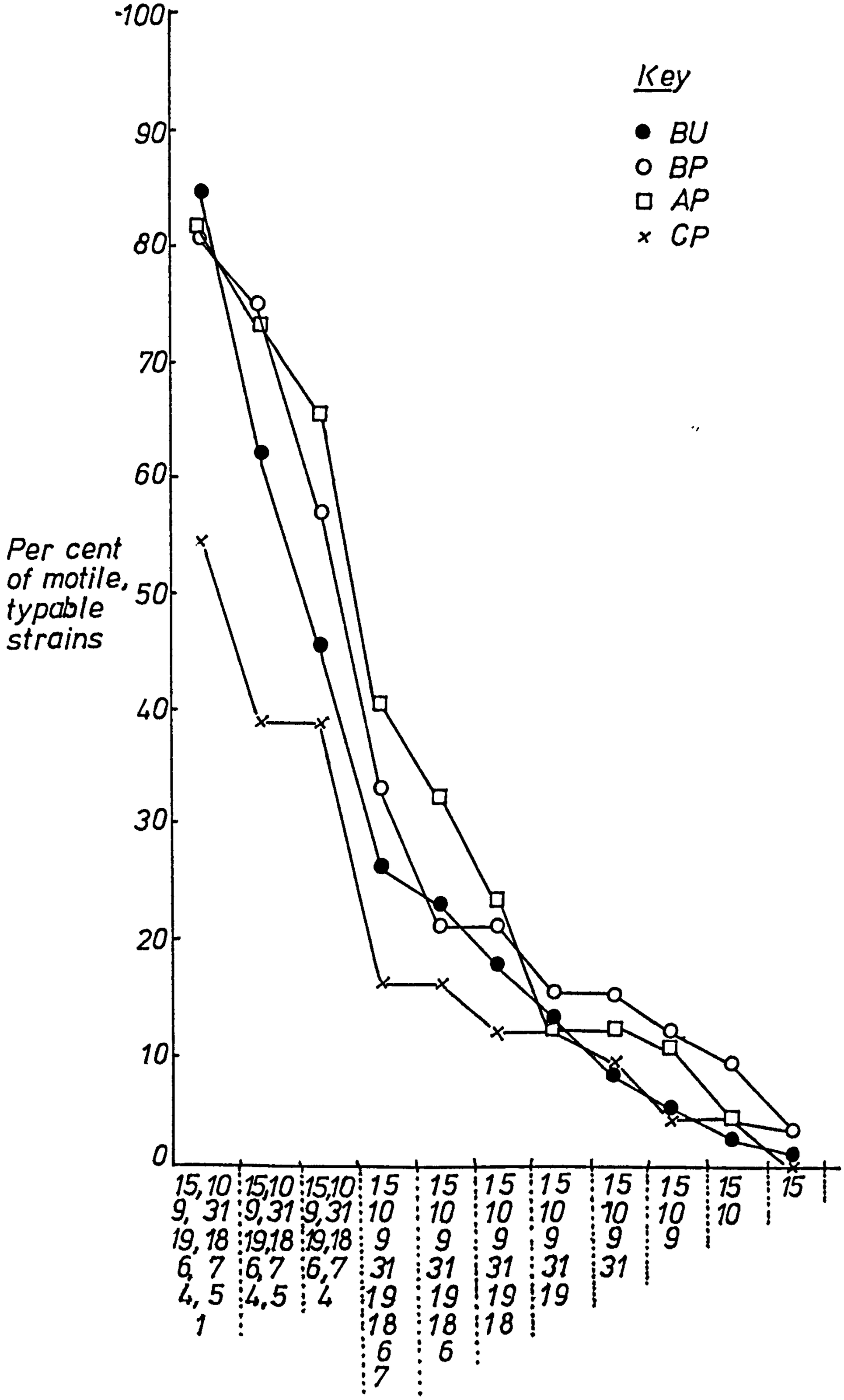
TABLE V: DISTRIBUTION OF THE 11 COMMONEST H TYPES

H TYPE	GROUP															
	BU		BP		BP/NP		AP		AP/US		CP		UT		LT	
H1	17	22%	2	6%	1	4%	4	8%	2	5%	4	17%	4	29%	5	28%
H4	11	14%	8	24%	8	24%	13	25%	10	26%	5	22%				
H5	13	17%	6	18%	3	11%	4	8%	2	5%			4	29%	2	11%
H6	4	5%					5	9%	3	8%	1	4%			1	6%
H7	6	8%	4	12%	4	14%	4	8%	4	11%						
H9	2	3%	1	3%	1	4%	3	6%	2	5%			1	7%		
H10	1	1%	2	6%	2	7%	1	2%	1	3%	1	4%	1	7%		
H15	1	1%	1	3%			1	2%	1	3%					1	6%
H18	4	5%	2	6%	2	7%	6	11%								
H19	4	5%									1	4%			1	6%
H31	2	3%	1	3%	1	4%	1	2%	1	3%	1	4%	2	14%	2	11%
Motile typable strains	78		34		28		53		38		23		14		18	

TABLE VI: DISTRIBUTION OF COMMON URINARY H TYPES

GROUP	NO. OF MOTILE, TYPABLE STRAINS	NO. OF STRAINS H1,H4,H5,H7	
BU	78	47	60%
BP	34	20	59%
BP/NP	28	16	47%
AP	53	25	47%
AP/US	38	18	47%
CP	23	9	39%
UT	14	8	57%
LT	18	7	39%

Fig. 6 CUMULATIVE DISTRIBUTION OF THE ELEVEN COMMONEST H SEROTYPES



(iii) Motility

Almost half (44%) the periurethral strains of normal subjects were non-motile (table VII). This was a significantly greater proportion than strains isolated from urines and periurethral swabs of both bacteriuric and abacteriuric patients ($X^2 = 13.5$, $p < 0.001$; $X^2 = 8.3$, $0.005 > p > 0.001$; $X^2 = 4.7$, $0.05 > p > 0.025$ respectively).

A surprisingly large proportion of upper and lower tract strains were non-motile (26% and 38% respectively), and were not significantly different from strains from normal subjects.

TABLE VII: MOTILITY

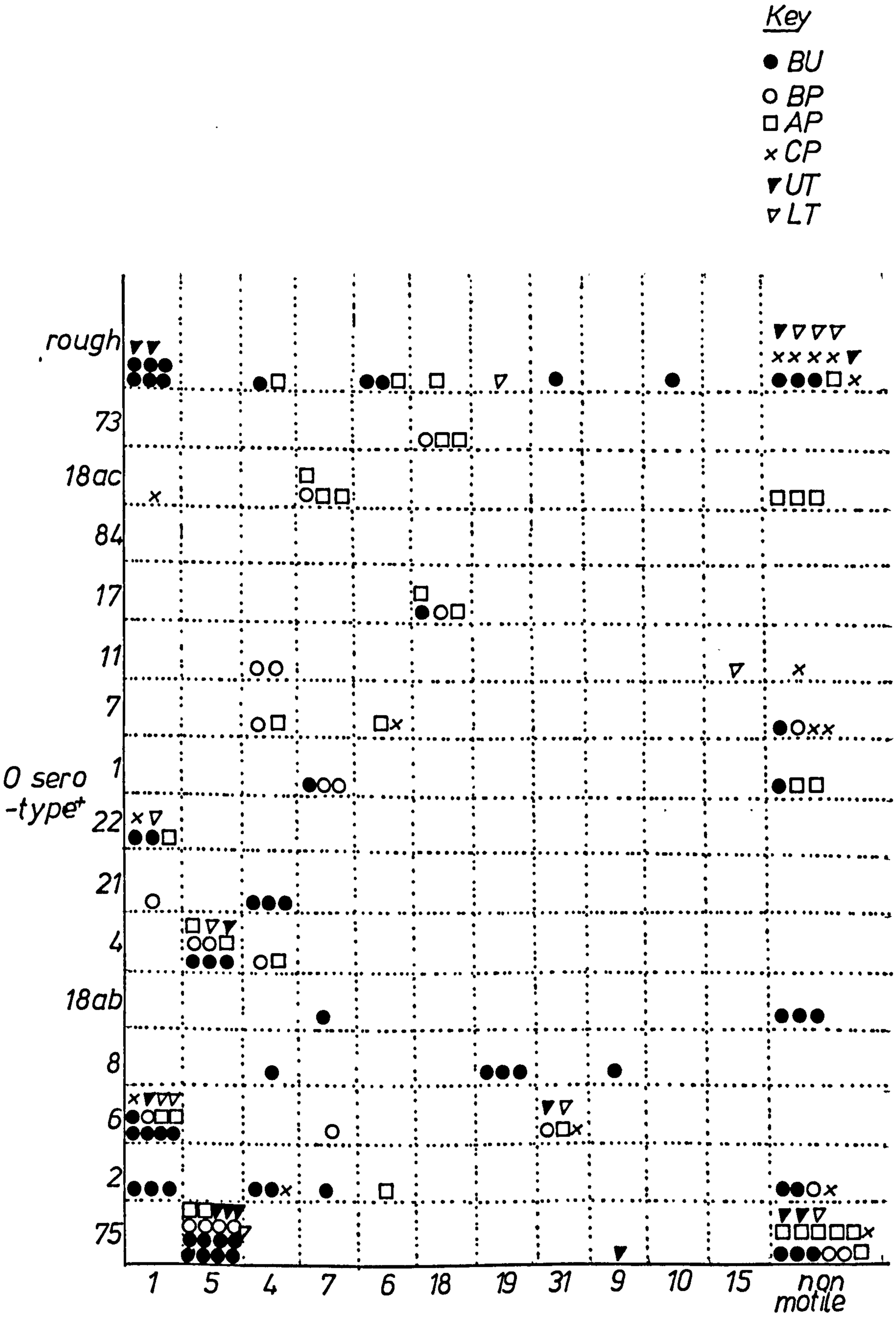
GROUP	TOTAL NO. OF STRAINS	NO. NON-MOTILE	
BU	105	17	16%
BP	43	7	16%
BP/NP	34	4	12%
AP	75	19	25%
AP/US	58	17	29%
CP	45	20	44%
UT	19	5	26%
LT	29	11	38%

(iv) O/H combinations

Combinations of common O and H types are shown in Fig. 7. O75 strains were almost exclusively H5 or non-motile. O6 was usually associated with H1 or H31, O4 with H5, and O22 with H1.

Particular combinations were not found exclusively in any group.

Fig.7 O/H SEROTYPE COMBINATIONS



(+=in order of frequency)

2) K ANTIGEN CONTENT

a) INTRODUCTION

K antigen appears to confer resistance to certain host defence mechanisms, thus it would be expected that strains rich in this antigen are commonly associated with disease. There are relatively few investigations into the K antigen content of strains from different sources, and measurement of K antigen content was considered a worthwhile investigation.

Kauffman (1947) defined K antigen as an envelope or surface antigen which renders living cells inagglutinable with O antiserum. There are 3 types of antigens, L,A and B, differentiated on the basis of their heat lability. A antigens include true capsules which can be seen microscopically. K antigens are usually acidic polysaccharides and can be considered to be an extension of the cell wall in some strains, but in others are not componants of the O antigen (Ørskov and Ørskov, 1971; Bettelheim, 1975). It is generally accepted that a few K antigens may not be acidic polysaccharides; K88(L) is a protein, probably a fimbrial antigen (Kauffmann, 1974). K antigens are serologically heterologous and can be typed by immunoelectrophoresis; about 90 serotypes have been described (Cooke, 1974).

Glynn and Howard (1970) utilised the knowledge that acidic polysaccharides inhibit agglutination in serologically unrelated systems in order to measure K antigen quantitatively.

b) MATERIALS AND METHODS

K antigen was estimated by the ability of saline extracts to inhibit the agglutination of sheep red cells by sheep red cell antibody raised in rabbits (Glynn and Howard 1970).

Preparation of K extract

Strains were cultured aerobically on 3 Blood agar Base plates (Oxoid). Blood agar Base was recommended as a suitable medium for K antigen production by Edwards and Ewing (1972). After overnight incubation at 37°C, cells were harvested in a minimum amount of saline and centrifuged for 20 mins. at 3,000 r.p.m. Deposits were suspended in acetone and refrigerated for 6 to 7 hours or overnight. After further centrifugation the deposits were dried overnight at room temperature.

The dried cells were ground to a fine powder and homogenised in saline at a concentration of 10mg/ml. Cell suspensions were cooled on ice prior to homogenisation, and homogenised on ice for a total of 3 mins. in 30 sec. bursts. Homogenates were centrifuged and the supernatants (K extracts) stored at -20°C until titration.

Dried cells were stored in air tight bottles in the dark up to 3 months without loss of titre. Frozen extracts were stored for up to 1 month without loss of titre; but repeated freezing and thawing resulted in false negative titres.

Preparation of antiserum

Haemagglutinating antiserum was prepared by the method of Darter (1953), except that the serum was heated to 56° for 40 mins. to destroy complement.

On alternate days a New Zealand White rabbit was inoculated, intracutaneously in the thigh, with a series of five different doses of whole sheep blood: 0.5, 1.0, 1.5, 2.0, and 2.5mls. On the twelfth and fifteenth days the rabbit was inoculated intravenously in the ear, with 0.1ml of a 20% suspension of red cells in normal saline containing 0.01% $MgSO_4$. A trial bleeding was taken from the rabbits ear on the

eighteenth day; as the titre was satisfactory (between 1/640 & 1/1280) blood was taken again on days 25 and 32.

Titration of K extracts

Doubling dilutions of K extract in phosphate buffered saline (PBS) were incubated with equal amount of a 2% or 3% suspension of washed sheep erythrocytes (Wellcome Laboratories) in a water bath at 37°C for 30 mins. 0.025 ml volumes of coated and control uncoated red cells were added to 0.025ml amounts of doubling dilutions of rabbit anti-sheep red cell serum in PBS starting at 1/160 to give a chequer board titration, such that antibody dilutions were read horizontally and K extract dilutions vertically.

Titration plates were incubated for one hour at 37°C, then refrigerated overnight to allow the red cells to settle. The titration end-point was the last dilution of K extract to inhibit the agglutination of sheep red cells by the antiserum by one doubling dilution, i.e. one well in the titration plate.

Controls

The red cell content of different sheep blood specimens was found to vary slightly. Thus, prior to every batch of K titrations sheep red cell antiserum was titrated against 1%, 2%, and 3% red cell suspensions. The red cell suspension chosen gave an end point with a 1/640 dilution of antiserum.

A strain with a titre of 1/256, and a strain with no titre were included in every batch of titrations. 10 strains were titrated on 3 separate occasions in order to check the reproduceability of the method.

c) RESULTS

On repeated testing K antigen titres were found to vary by at most + or - one doubling dilution.

K antigen titres for each group of strains are given in table VIII. The reciprocals of K antigen titres were plotted against percentage of strains in a cumulative manner (Fig. 8). No K antigen was detected in 26% to 54% of strains, about the same proportion of strains in each group had high K antigen titres, i.e. 1/1024. However, significantly more urinary strains had titres of between 1/32 and 1/512 than periurethral strains from normal subjects ($p = 0.008$). The incidence of K antigen titres of this order was greater in periurethral strains from both bacteriuric and abacteriuric patients than normal subjects (but this was just outside the 0.05 level of significance) and slightly less than in urinary strains. Upper tract strains more often had titres of between 1/32 and 1/512 than lower tract strains but this difference was not significant ($p = 0.06$). Lower tract strains were significantly richer in K antigen than those from normal subjects ($p < 0.001$). Periurethral strains from urethral syndrome patients (AP/US) were almost identical to the parent AP group and periurethral strains from bacteriuric patients which did not give rise to infection (BP/NP) were not different from group BP.

There was no correlation between the K antigen titre of urinary strains and site of radiological abnormality (table IX, fig. 9).

In contrast to the serum sensitivity @results, rough strains were not devoid of K antigen and thus were not excluded from the analysis of results (table X).

@See next section.

KEY

BU: Bacteriuric patients; urinary strains

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

UT: Bacteriuric patients; strains isolated from the upper tract.

LT: Bacteriuric patients; strains isolated from the lower tract.

TABLE VIII: K ANTIGEN TITRES

GROUP (No. of strains tested)	RECIPROCAL OF K ANTIGEN TITRE											
	0	1	2	4	8	16	32	64	128	256	512	1024
BU (105)	30 28%	2 2%	12 2%		1 1%	1 1%	1 1%	10 10%	9 9%	22 21%	15 14%	12 11%
BP (43)	16 37%			2 5%			1 3%	2 5%	7 16%	7 16%	4 9%	4 9%
BP/NP (34)	14 41%			1 3%				2 6%	5 15%	5 15%	4 11%	3 9%
AP (75)	29 39%	1 1%					1 1%	2 3%	12 16%	10 13%	14 19%	6 8%
AP/US (58)	21 36%	1 2%					1 2%	1 2%	11 19%	9 15%	9 15%	5 9%
CP (45)	24 54%				1 2%		2 4%	4 9%	2 4%	3 7%	4 9%	5 11%
UT (19)	5 25%			1 5%	1 5%		1 5%		2 11%	3 16%	2 11%	4 21%
LT (29)	14 48%				1 4%				5 17%	5 17%	3 10%	1 4%

TABLE IX: RELATION OF K ANTIGEN TITRE TO RADIOLOGICAL ABNORMALITY

SITE OF ABNORMALITY (No. of strains tested)	RECIPROCAL OF K ANTIGEN TITRE											
	0	1	2	4	8	16	32	64	128	256	512	1024
UPPER TRACT (19)	6 32%					1 5%	1 5%	1 5%	3 16%	2 11%	4 21%	1 5%
LOWER TRACT (33)	10 31%	1 3%						3 9%	4 12%	5 15%	6 18%	4 12%
NONE (52)	13 25%	1 2%	2 4%		1 2%			6 11%	3 6%	15 29%	5 10%	6 11%

TABLE X: K ANTIGEN TITRE OF ROUGH STRAINS

	RECIPROCAL OF K ANTIGEN TITRE											
	0	1	2	4	8	16	32	64	128	256	512	1024
ROUGH STRAINS (38)	20 53%							1 3%	2 5%	6 16%	7 18%	2 5%

Fig.8 K ANTIGEN CONTENT

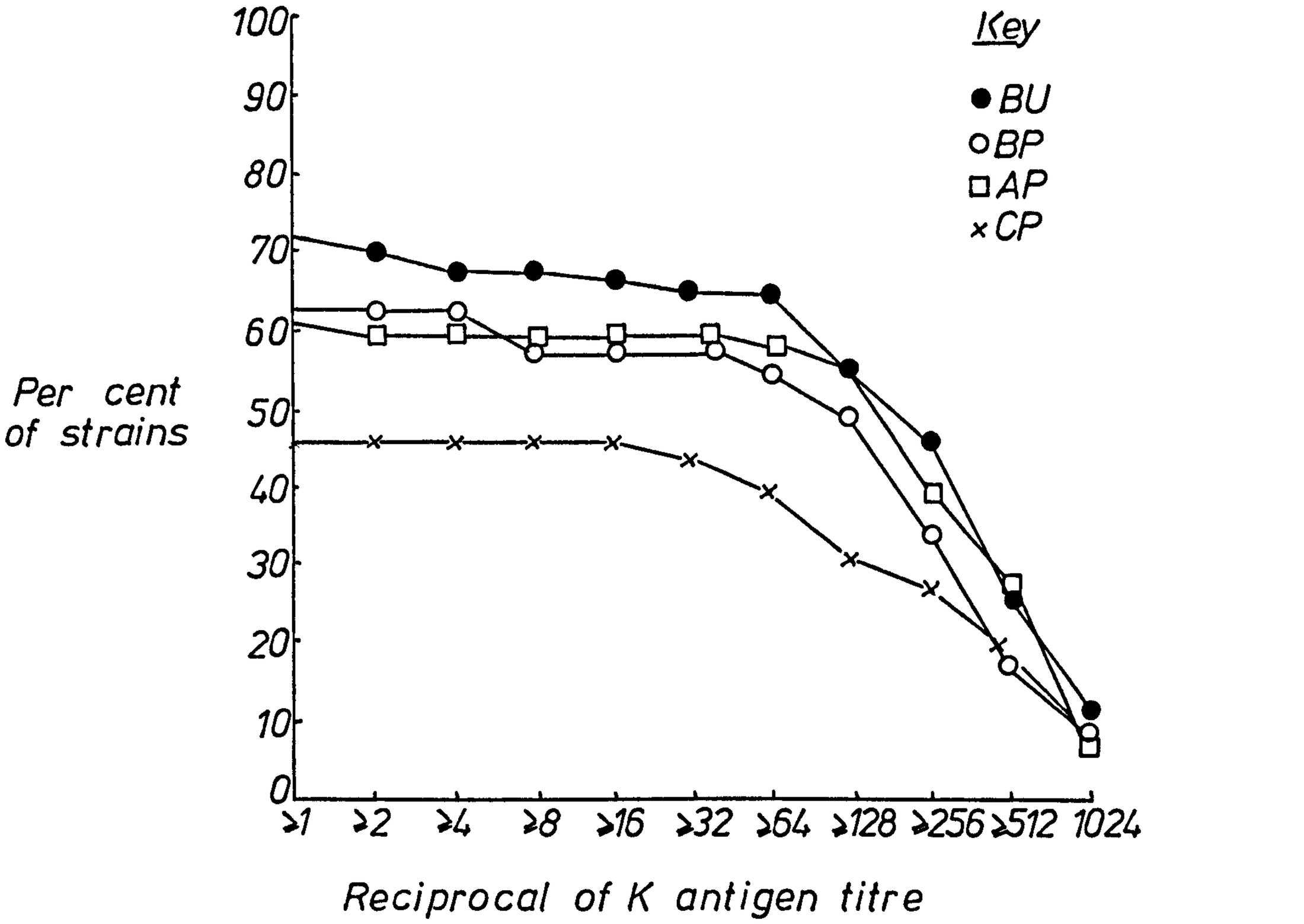
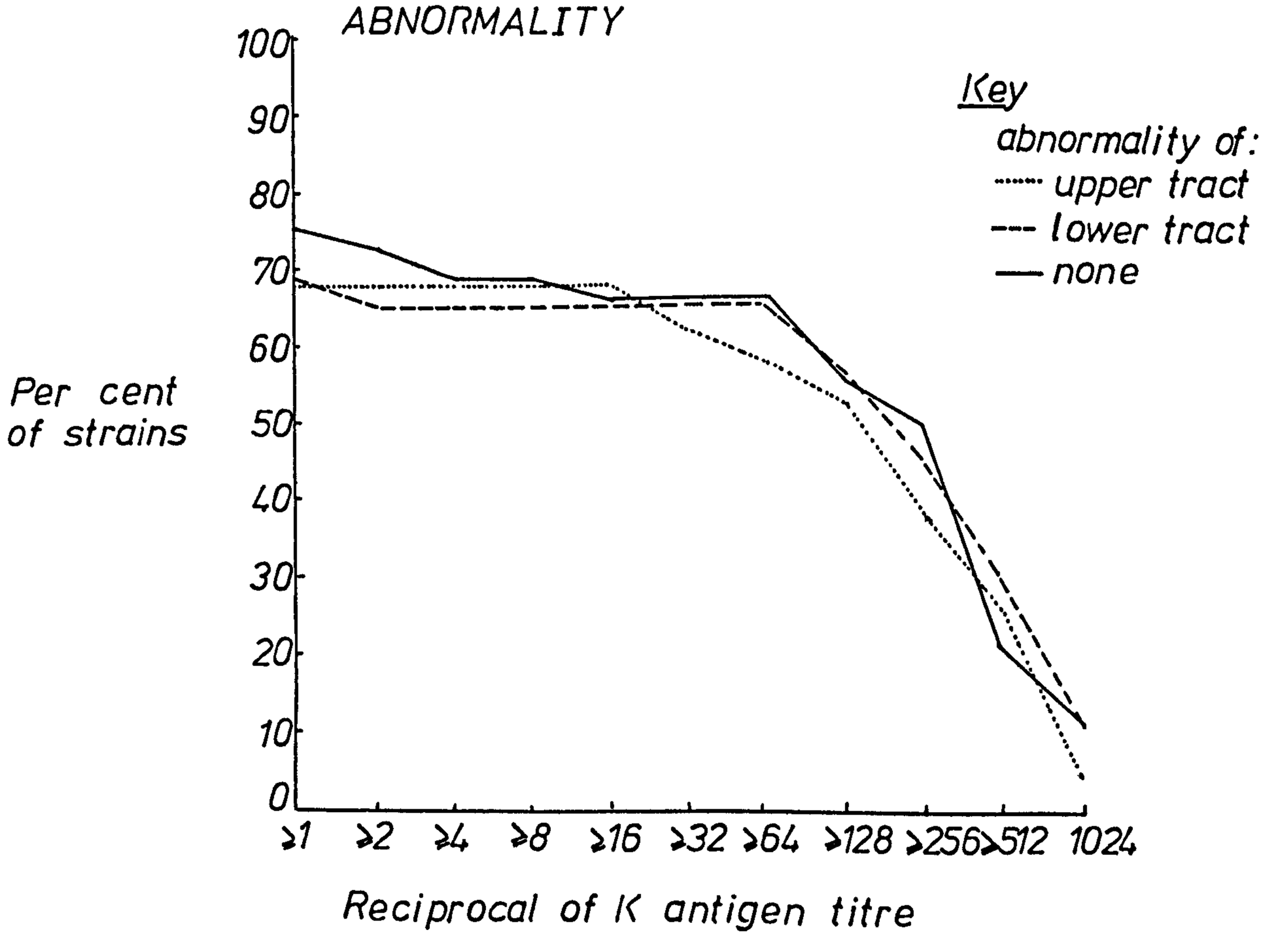


Fig.9 RELATION OF K ANTIGEN TO RADIOLOGICAL ABNORMALITY



3) SERUM SENSITIVITY

a) INTRODUCTION

It was postulated that strains resistant to the bactericidal action of serum would be more likely to survive and multiply in the host than sensitive ones. It was not expected that serum resistance would confer any advantage on strains confined to the lower tract which were not in intimate contact with serum bactericidal factors. However, strains which reach the upper tract might well benefit from such resistance.

Various techniques have been used to assess the sensitivity of bacteria to normal human serum (e.g. Kimball *et al* 1964; Kalmanson *et al*, 1965; Brumfitt and Heptinstall, 1960). Taylor *et al* (1972) investigated factors affecting the estimation of serum bactericidal activity *in vitro*. They found that this activity could be adversely affected by diluting or freezing the serum, and the use of water or minimal media as a suspending medium. Variation of pH of the suspending medium between 7 and 9 and small variation in the inoculum size did not affect the outcome of the test. In this study their recommendations, with minor adaptations, have been adhered to.

Rough enterobacteria have previously been shown to be serum sensitive, (Rowley, 1956; Michael and Landy, 1961). As loss of smoothness on storage can occasionally occur sensitivities of smooth and rough strains are reported separately.

b) MATERIALS AND METHODS

A modification of the method of Taylor, Roberts, and Gower (1972) was employed to determine the sensitivity of *E. coli* strains to normal, human serum.

Collection of serum

Approximately 25 mls of blood were taken once a week from four healthy volunteers with no previous history of urinary tract infection. The blood samples were left to clot at room temperature for 3 hours, refrigerated overnight, and the serum pooled and centrifuged at 2,000 r.p.m. for 10 mins to remove any free red cells. 0.75 ml aliquots were distributed into sterile tubes and stored at $4^{o}C$ for the duration of the experiment (3 days).

Preparation of cells

Tubes containing 9 mls of nutrient broth (Oxoid) were seeded with 1 ml of overnight broth cultures of the strains under test, and incubated in a shaking water bath for 90 mins at $37^{o}C$ (gentle agitation). Cultures were centrifuged, the cells washed once in Tris buffer (pH 8.4) and re-suspended to 10 mls in buffer (Cruickshank 1965). 1/1000 dilutions of the washed cell suspensions were prepared in buffer.

Determination of sensitivity

0.25 ml volumes of dilute cells were incubated with 0.75 ml of serum in a shaking water bath for 3 hours. Using a modification of the method of Miles and Misra (1938), 0.1 ml samples were taken at 0, 1, 2, and 3 hours and the number of colony forming units (CFU) per ml determined; four ten-fold dilutions were prepared in Tris buffer and 0.3 ml of each dilution spotted in triplicate onto nutrient agar plates (Oxoid), using an automatic pipette.

The plates were incubated overnight at $37^{o}C$, the dilution which yielded between 4 and 40 colonies per spot inoculum was noted and the exact number of colonies recorded. CFU per ml of serum was calculated as follows:

Average CFU count x 1/dilution x 100/3

Strains were graded according to their sensitivities as follows:

1. A progressive decrease in viable count at each hourly interval, the final count being less than 10% of the initial inoculum.

2. A progressive decrease in the viable count at each hourly inverval, the final count being between 10% and 50% of the initial inoculum.

3. No progressive overall decrease, but the viable count at 3 hours was less than 50% of the initial inoculum.

4. Viable counts both greater (at least double) and smaller (at least 50%) than the initial inoculum at 1, 2, and 3 hours.

5. Viable count at 3 hours was at least double the initial inoculum, but no progressive increase, or counts at 1, 2, and 3 hours were the same as the initial inoculum.

6. Progressive increase in the viable count at each hourly interval, the final count being at least double that of the initial inoculum.

Controls

The reproduceability of the method was predetermined using 8 random strains and a known sensitive strain (NCTC 9075).

a) Three aliquots of overnight cultures of three strains were tested for sensitivity using the same batch of serum.

b) All nine strains were tested using the same batch of serum on three separate occasions.

c) All nine strains were tested on four separate occasions with four different batches of serum.

Error inherent in the counting method was determined by preparing ten-fold dilutions in triplicate from aliquots of 3 strains and calculating the number of CFU per ml as previously described.

To ascertain whether diluting the hourly samples was sufficient to nullify the bactericidal effect of the serum 0.03 ml of each dilution was spread over the surface of a nutrient agar plate and the CFU count compared to that obtained using the method previously described.

Two strains, one serum resistant the other sensitive, were included in every batch of tests.

c) RESULTS

(i) Serum sensitivity

Results were reproduceable, + or - one serum sensitivity grade, whether the same or different batches of serum were used. The antibacterial activity of serum was not diminished by storage at $4^{o}C$ for 3 days. Error inherent in the counting method was small: + or - less than 40% of the inoculum. CFU counts of spot inocula were the same as those obtained when diluted serum was spread over the surface of the plate.

Serum sensitivities of smooth strains are given in table XI. Periurethral strains from abacteriuric patients were more sensitive than those from urines ($p = 0.02$), but otherwise there were no significant differences between the groups. (fig. 10). Lower tract strains were slightly more sensitive than upper tract strains, but this difference was not significant. Strains from urethral syndrome patients were similar to the AP group as a whole. Strains from bacteriuric patients which did not give rise to infection were similar to the BP group as a whole.

Resistant strains were marginally more common in patients

with radiological abnormalities of the upper tract, and marginally less common in patients with abnormalities of the lower tract compared to those with normal tracts (table XII fig. 11). However, these differences were not statistically significant.

Nearly all the rough strains (82%) were serum sensitive (grades 1-3); only 7 strains (18%) were resistant (table XIII).

KEY

BU: Bacteriuric patients; urinary strains.

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

UT: Bacteriuric patients; strains isolated from the upper tract.

LT: Bacteriuric patients; strains isolated from the lower tract.

TABLE XI: SERUM SENSITIVITIES

GROUP (No. of smooth strains tested)	SERUM SENSITIVITY GRADE 1	2	3	4	5	6
BU (89)	32 36%	6 7%	16 18%	8 9%	17 19%	10 11%
BP (42)	16 38%	2 5%	10 24%	4 9%	7 17%	3 7%
BP/NP (34)	14 41%		8 23%	4 12%	6 18%	2 6%
AP (70)	32 46%	6 9%	16 23%	8 11%	7 10%	1 1%
AP/US (55)	25 45%	5 9%	13 24%	6 11%	5 9%	1 2%
CP (39)	14 36%	1 3%	11 28%	2 5%	5 13%	6 15%
UT (15)	5 33%	1 7%	3 20%		4 27%	2 13%
LT (22)	11 50%		5 23%	1 5%	4 18%	1 4%

TABLE XII: RELATION OF SERUM SENSITIVITY TO RADIOLOGICAL ABNORMALITY

SITE OF ABNORMALITY (No. smooth strains tested)	SENSITIVITY GRADE 1	2	3	4	5	6
UPPER TRACT (15)	5 33%	1 7%	2 13%	1 7%	4 27%	2 13%
LOWER TRACT (27)	11 41%	3 11%	6 22%	2 7.5%	2 7.5%	3 11%
NONE (46)	17 37%	2 4.5%	7 15%	6 13%	8 17.5%	6 13%

TABLE XIII: SERUM SENSITIVITY OF ROUGH STRAINS

	SERUM SENSITIVITY GRADE 1	2	3	4	5	6
ROUGH STRAINS (38)	24 63.5%	1 2.5%	6 16%	1 2.5%	5 13%	1 2.5%

Fig. 10 SERUM SENSITIVITY

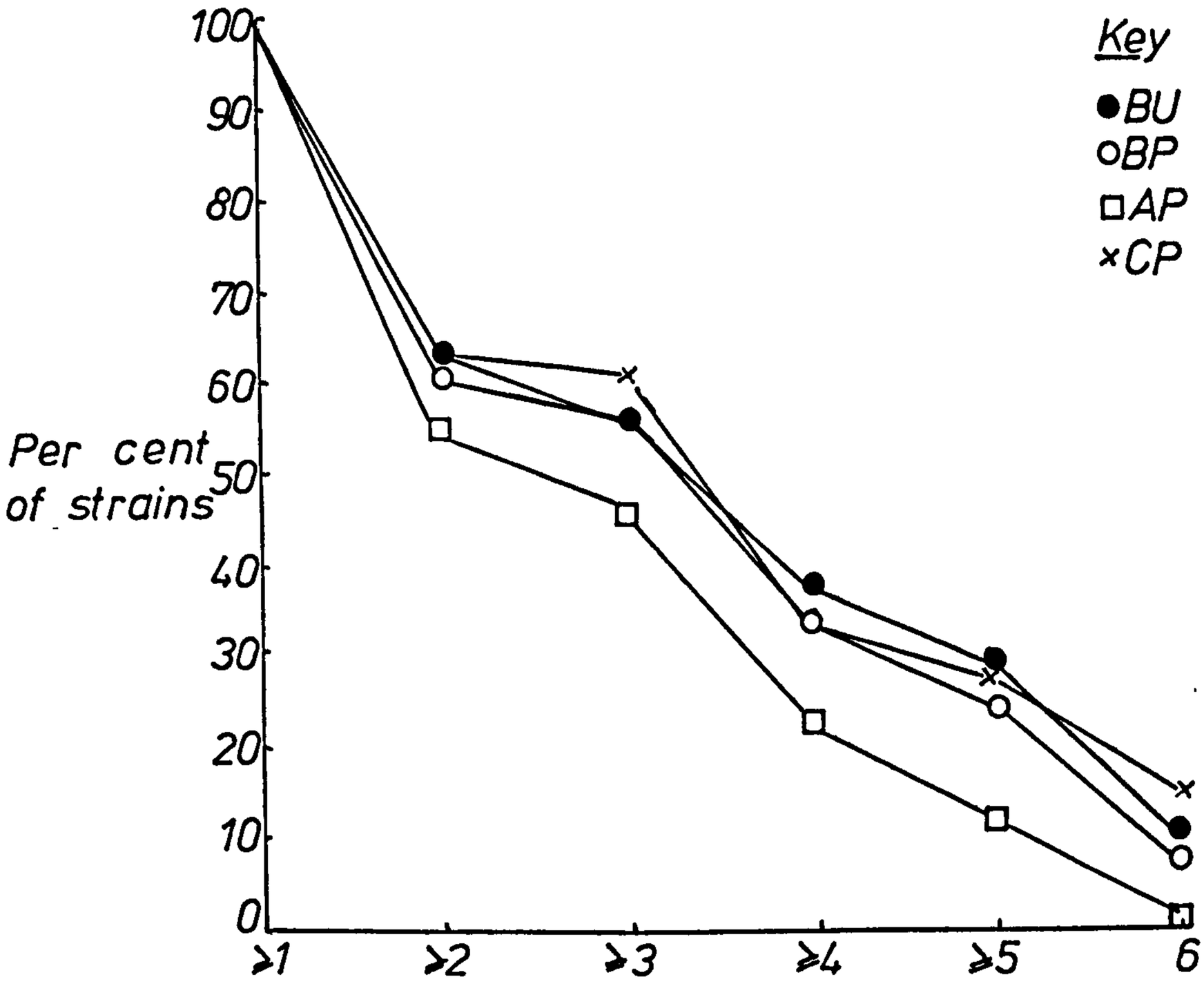
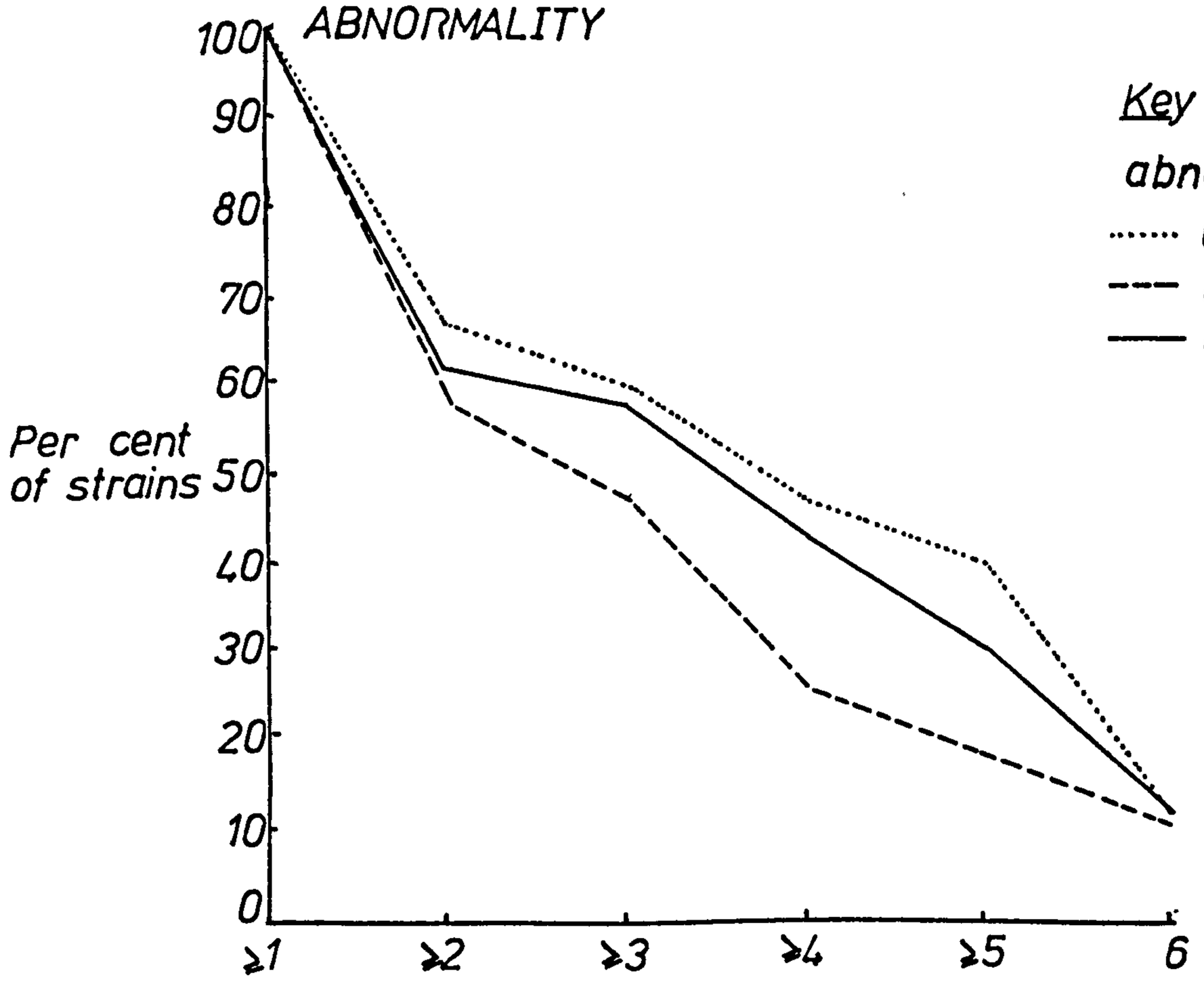


Fig. 11 RELATION OF SERUM SENSITIVITY TO RADIOLOGICAL ABNORMALITY



(ii) Relation of O type, K antigen and serum sensitivity

K antigen and serum sensitivity

The K antigen content of smooth strains was not directly related to serum sensitivity, but there were few (18) which had K antigen titres of less than 1/32 and were resistant to the bactericidal activity of serum (table XIVA). The 161 strains with titres of 1/32 or more were almost equally divided between serum sensitive and resistant categories (90 and 71 respectively).

Of the 38 rough strains, 7 were serum resistant and only 2 of these had K antigen titres of 1/32 or more. About half the rough strains were rich in K antigen; 18 had titres of 1/32 or more, 20 had titres of less than 1/32. (table XIVB).

O type, K antigen and serum sensitivity

O type could be related to K titre only in those serogroups containing a sufficient number of strains to make comparison worhtwhile. Results for the 6 serogroups containing at least 9 strains, rough and non-typable strains are given in table XV. Non-typable and rough strains were generally deficient in both K antigen and serum resistance compared to smooth typable strains (fig. 12). Both serum resistance and high K titre appear to be a feature of strains belonging to O types 2,6 and 7. 075 and 018ac strains usually had high K titres.

TABLE XIVA: K ANTIGEN AND SERUM SENSITIVITY

	SMOOTH STRAINS	
	K ANTIGEN TITRE	
SERUM SENSITIVITY GRADE	<1/32	>1/32
4-6	18	71
1-3	99	90

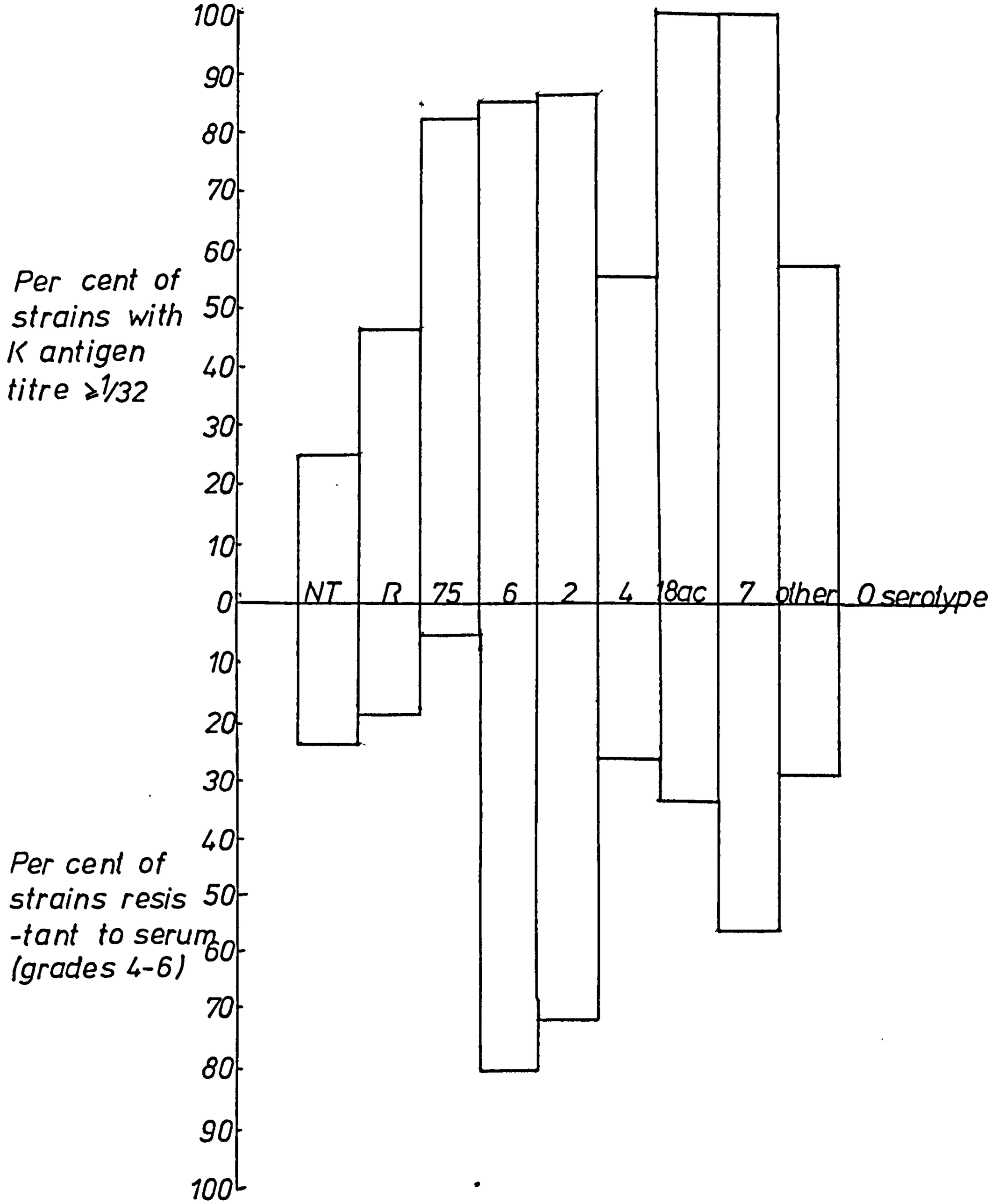
TABLE XIVB:

	ROUGH STRAINS	
	K ANTIGEN TITRE	
SERUM SENSITIVITY GRADE	<1/32	>1/32
4-6	2	5
1-3	18	13

TABLE XV: RELATION OF O TYPE TO K ANTIGEN AND SERUM SENSITIVITY

O TYPES IN ORDER OF FREQUENCY	K ANTIGEN TITRE				SERUM SENSITIVITY GRADE			
	<1/32		≥1/32		1-3		4-6	
NT	41	75%	14	25%	32	76%	13	24%
R	20	53%	18	47%	31	82%	7	18%
75	7	18%	31	82%	36	95%	2	5%
6	3	15%	17	85%	4	20%	16	80%
2	2	14%	12	86%	4	29%	10	71%
4	5	45%	6	55%	8	73%	3	27%
18ac	0		9	100%	6	67%	3	33%
7	0		9	100%	4	44%	5	56%
Other	53	43%	69	57%	86	71%	36	29%

Fig.12 RELATION OF O TYPE TO K ANTIGEN AND SERUM SENSITIVITY



NT = non typable
R = rough

4) HAEMOLYSIN PRODUCTION

a) INTRODUCTION

A variety of toxic activities have been attributed to haemolysins, particularly to alpha haemolysin, although the relation of alpha to beta haemolysin is not fully understood. Both haemolysins have the same spectrum of activity against erythrocytes of various species, but other differences gave rise to the view that they were separate entities. Beta haemolysin could not be obtained in cell-free preparation and Williams Smith (1963) found that alpha haemolysin was antigenic whilst beta haemolysin was not. Antiserum to alpha haemolysin would not neutralise beta haemolysin. In contrast, Rennie and Arbuthnott (1974) found that antibody to alpha haemolysin was capable of neutralising beta haemolysin in one strain. They suggest that alpha haemolysin is a released form of beta haemolysin, brought about by its interaction with large molecules in the medium. Alpha haemolysin production was enhanced by large molecular weight components of the medium and they suggest that quantitative differences in alpha haemolysin production may reflect its binding properties to the cell surface.

In view of the increased virulence of haemolytic strains for the rat kidney (Fried & Wong 1970; Fried *et al* 1971) and the conflicting reports of Cooke and Ewins (1975) and McGeachie (1966) on the incidence of haemolytic strains in UTI, investigation of haemolytic activity was considered worthwhile. No attempt was made to separate the two types of haemolysin in this study because of their uncertain nature. Thus the method of Cooke (1968) was favoured whereby production of haemolysin in solid and liquid media was tested.

b) MATERIALS AND METHODS

Production of solid and liquid haemolysins was examined as described by Cooke (1968).

Solid Haemolysin

Strains were examined for 'solid', ie. diffusable haemolysin production on blood agar layer plates. These plates consisted of a basal layer of Blood agar Base (Oxoid) and a surface layer of Blood agar Base containing 7% washed horse erythrocytes (Oxoid). Four strains were streaked onto each plate and after overnight incubation at 37°C haemolytic strains produced a clear zone of lysis.

Liquid haemolysin

Strains were inoculated into isotonic peptone water (peptone 10g/L, NaCl 8.5g/L) containing 0.1 ml of a 7% suspension of washed horse erythrocytes. Cultures were incubated overnight at 37°C, shaken well and refrigerated overnight to allow whole red cells to settle. The presence of free haemoglobin was indicative of lysis.

Two hundred strains were tested for lysis of sheep erythrocytes (Wellcome Laboratories) and human group O erythrocytes.

Controls

Two controls, a haemolytic and a non-haemolytic strain of E. coli were included in every batch of tests[@].

c) RESULTS

(i) Haemolysin production

All strains which lysed horse erythrocytes lysed sheep erythrocytes, but one strain did not lyse human group O erythrocytes.

The majority of strains produced both liquid and solid haemolysins and no strain produced solid but not liquid haemolysin. Five of the urinary

[@]Provided by S. O'Farrell, St. Bartholomew's Hospital.

strains and 1 of the periurethral strains from abacteriuric patients produced liquid but not solid haemolysin. Strains were termed haemolytic providing they produced liquid heamolysin.

Strains isolated from urinary infections were more frequently haemolytic (43%) than periurethral strains from abacteriuric patients (21%), and normal subjects (9%), table XVI, fig. 13). These differences were found to be highly significant ($X^2 = 9.0$, $0.01 > p > 0.001$ and $X^2 = 16.5$, $p < 0.001$ respectively).

The incidence of haemolytic periurethral strains was greater in abacteriuric patients (21%) than in normal subjects (9%), but this was not statistically significant ($X^2 = 3.1$, $0.1 > p > 0.05$). Periurethral strains from urethral syndrome patients (AP/US) were not different to the abacteriuric AP group as a whole.

Periurethral strains from bacteriuric patients, when they were between infections, were more frequently haemolytic (28%) than those from normal subjects (9%), and this difference was statistically significant ($X^2 = 5.4$, $0.05 > p > 0.02$). The incidence of haemolysis in periurethral strains which did not give rise to infection in bacteriuric patients (BP/NP) was not significantly different from that of normal subjects, and was slightly less (24%) than the BP group as a whole (28%).

The majority of upper tract strains were haemolytic (58%) but this group was not significantly different from the lower tract group, of which 27% were haemolytic ($X^2 = 3.5$, $0.1 > p > 0.05$). The small number of strains in the upper tract group may have been responsible for this finding. However, there was still a significantly higher incidence of haemolytic strains in the lower tract group compared to normal subjects ($X^2 = 6.0$, $0.02 > p > 0.01$).

Strains isolated from the urines of patients with radiological abnormalities of the upper tract were more often haemolytic than patients with lower tract or no abnormalities (table 4) although these differences were not of statistical significance.

KEY

BU: Bacteriuric patients; urinary strains.

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

UT: Bacteriuric patients; strains isolated from the upper tract.

LT: Bacteriuric patients; strains isolated from the lower tract.

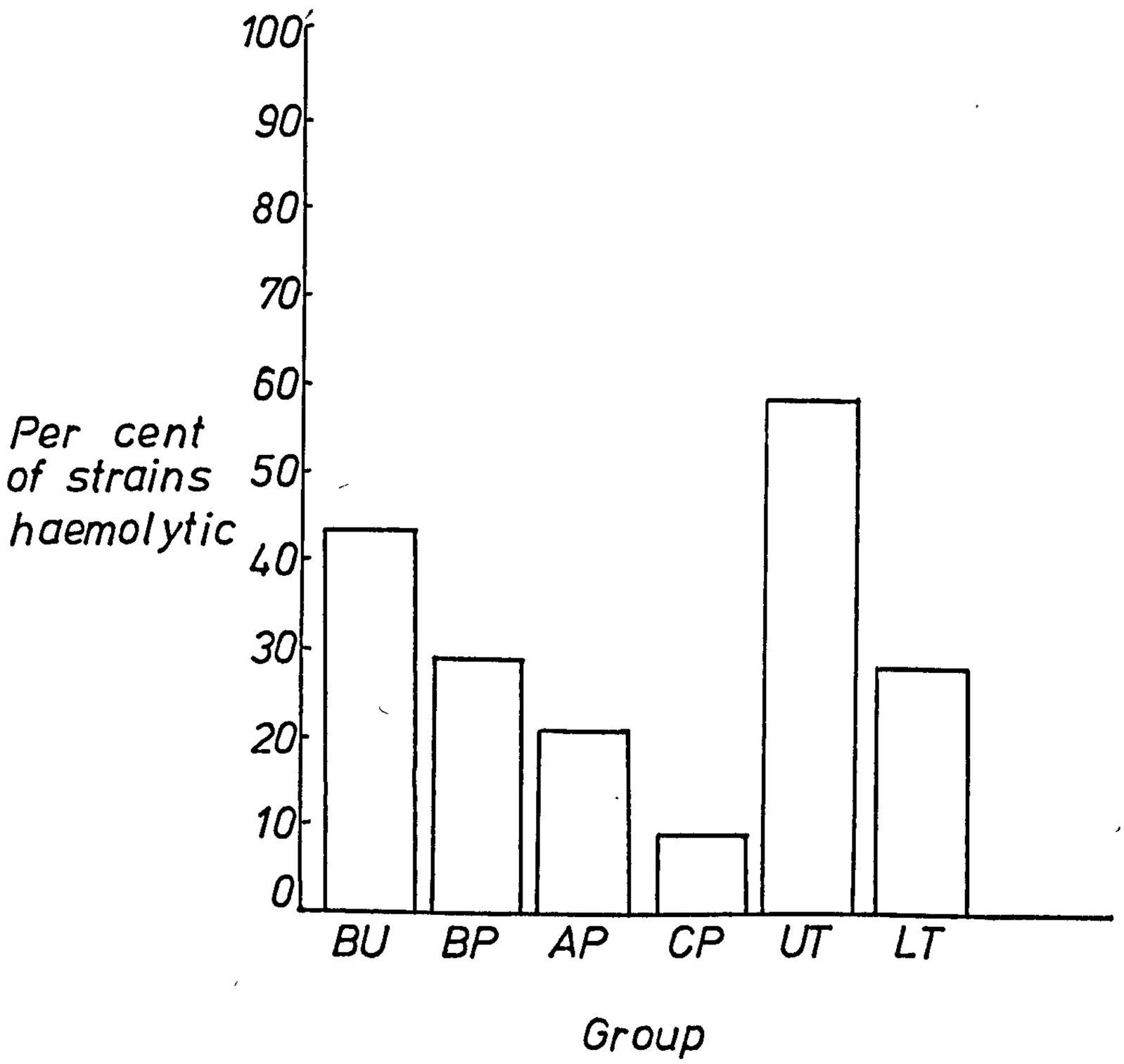
TABLE XVI: HAEMOLYSIN PRODUCTION

GROUP (No. of strains tested)	BU (105)	BP (43)	BP/NP (34)	AP (75)	AP/US (58)	CP (45)	UT (19)	LT (29)
No. haemolytic	45	12	8	16	12	4	11	9
	43%	28%	24%	21%	21%	9%	58%	27%

TABLE XVII: RELATION OF HAEMOLYSIN PRODUCTION TO RADIOLOGICAL ABNORMALITY

SITE OF ABNORMALITY (No. of strains tested)	UPPER TRACT (19)	LOWER TRACT (33)	NONE (52)
No. haemolytic	11	14	20
	58%	42%	39%

Fig.13 HAEMOLYSIN PRODUCTION



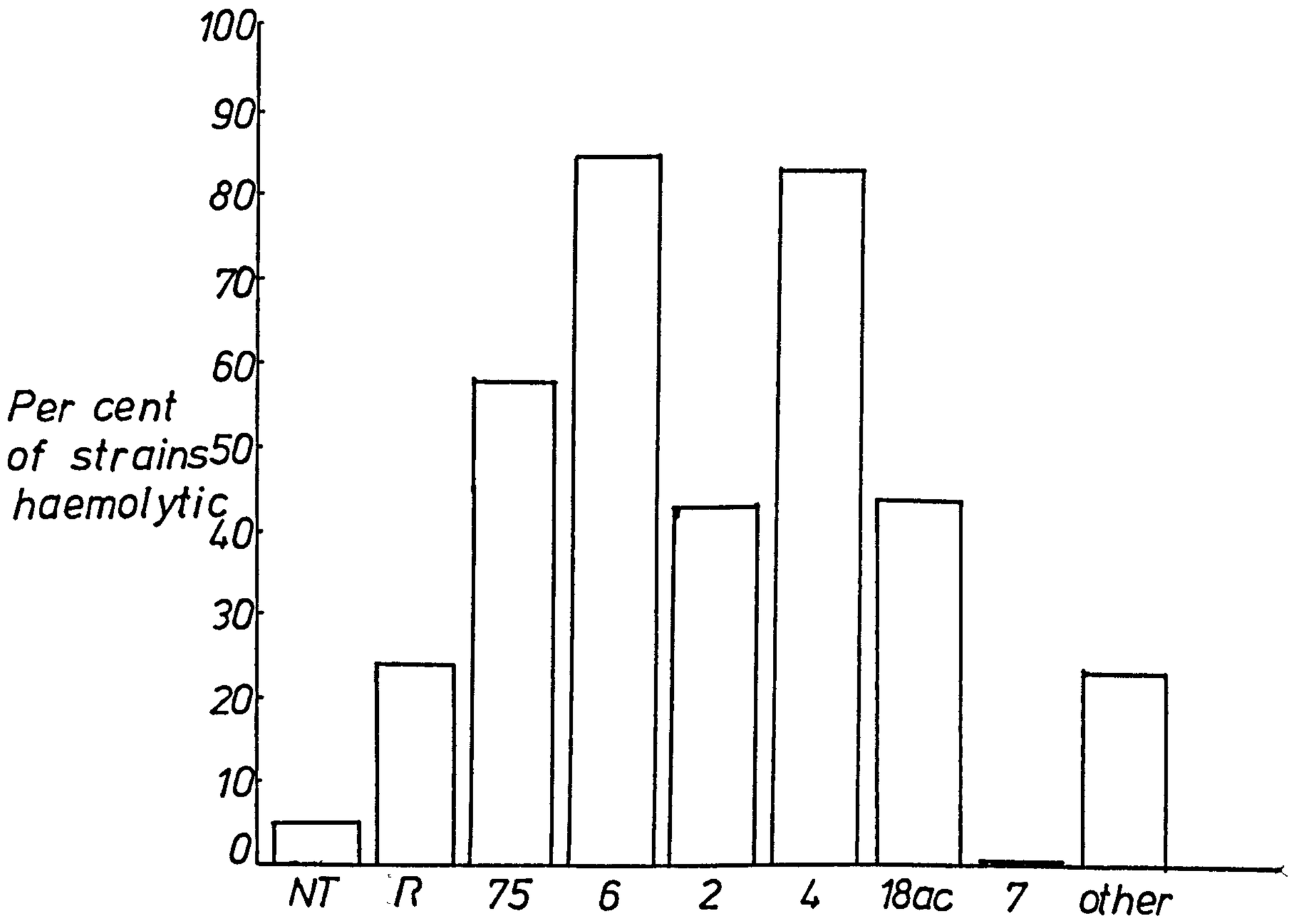
(ii) Relation of haemolysin production to O type

Haemolysin production was especially associated with O types 4 and 6; over 80% of strains belonging to these serotypes were haemolytic (table XVIII, fig. 14). Non-typable strains were rarely haemolytic. Only 3 non-typable strains (5%) produced haemolysin.

TABLE XVIII: RELATION OF HAEMOLYSIN PRODUCTION TO O TYPE

O-TYPE	HAEMOLYTIC		NON HAEMOLYTIC		TOTAL
Non-typable	3	5%	52	95%	55
Rough	9	24%	29	76%	38
075	22	58%	16	42%	38
06	17	85%	3	15%	20
02	6	43%	8	57%	14
04	9	82%	2	18%	11
018ac	4	44%	5	56%	9
07	0		9	100%	9
Other	28	23%	94	77%	122

Fig.14 RELATION OF HAEMOLYSIN PRODUCTION TO O SEROTYPE



NT=non typable
R=rough

5) FIMBRIAE PRODUCTION

a) INTRODUCTION

The adhesive powers of fimbriae, demonstrable *in vitro*, may confer resistance to hydrodynamic clearance mechanisms by enabling strains to adhere to the lining of the urinary tract. It was postulated that strains causing UTI would be more frequently fimbriate than commensal strains.

Duguid *et al* (1955) demonstrated biphasic strains which exhibited both fimbriate and non-fimbriate phases. Serial subculture in peptone water at 37°C was found to facilitate fimbriae production. Haemagglutination was found to be strongest at 3-5°C in most strains, and elution of red cells and bacteria took place at a higher temperature depending on the type of red cells used. Duguid (1968) found that haemagglutination in fimbriate strains could be neutralised by the addition of D-mannose and agglutination in the presence of this sugar was considered to be due to other factors.

b) MATERIALS AND METHODS

The method used for the detection of fimbriae was that described by Duguid *et al* (1955).

Strains were subcultured up to nine times in peptone water (Oxoid) and tested for fimbriae production on the third, sixth, and ninth subculture. Cultures were concentrated to approximately 10^{10} organisms per ml by centrifugation at 3,000 r.p.m. for 10 mins. Equal volumes (0.03ml) of 3% washed horse erythrocytes (Oxoid) and centrifugate were mixed thoroughly by gentle rocking on white tiles. Fimbriate strains agglutinated the erythrocytes within 15 mins. of mixing.

Haemagglutination in the presence of 0.5% D-mannose (B.D.H.)

was taken to indicate that agglutination was due to factors other than fimbriae.

All tests were carried out at 4°C.

Controls

@Two controls, a fimbriate and a non-fimbriate strain, were included in every batch of tests.

c) RESULTS

(i) Fimbria production

The majority of strains (between 60% and 83%) from all groups produced fimbriae on repeated subculture in a suitable medium, (table XIX, fig. 15). Fimbriae production was found to be a constant characteristic provided strains were subcultured up to nine times.

A significantly higher proportion of strains isolated from urines (79%) were fimbriate than those from periurethral swabs of normal subjects (60%), $X^2 = 5.84$, $0.02 > p > 0.01$. The incidence of fimbriate strains from periurethral swabs of both bacteriuric and abacteriuric patients was intermediate between these two groups and not significantly different from either. There was no difference in the incidence of fimbriation between strains isolated from the upper and lower urinary tract.

There was no correlation between the incidence of fimbriation in strains isolated from urines and the radiological abnormalities of the patients. (table XX).

@Provided by Professor Duguid, Dundee University.

KEY

BU: Bacteriuric patients; urinary strains.

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethralstrains.

UT: Bacteriuric patients; strains isolated from the upper tract.

LT: Bacteriuric patients; strains isolated from the lower tract.

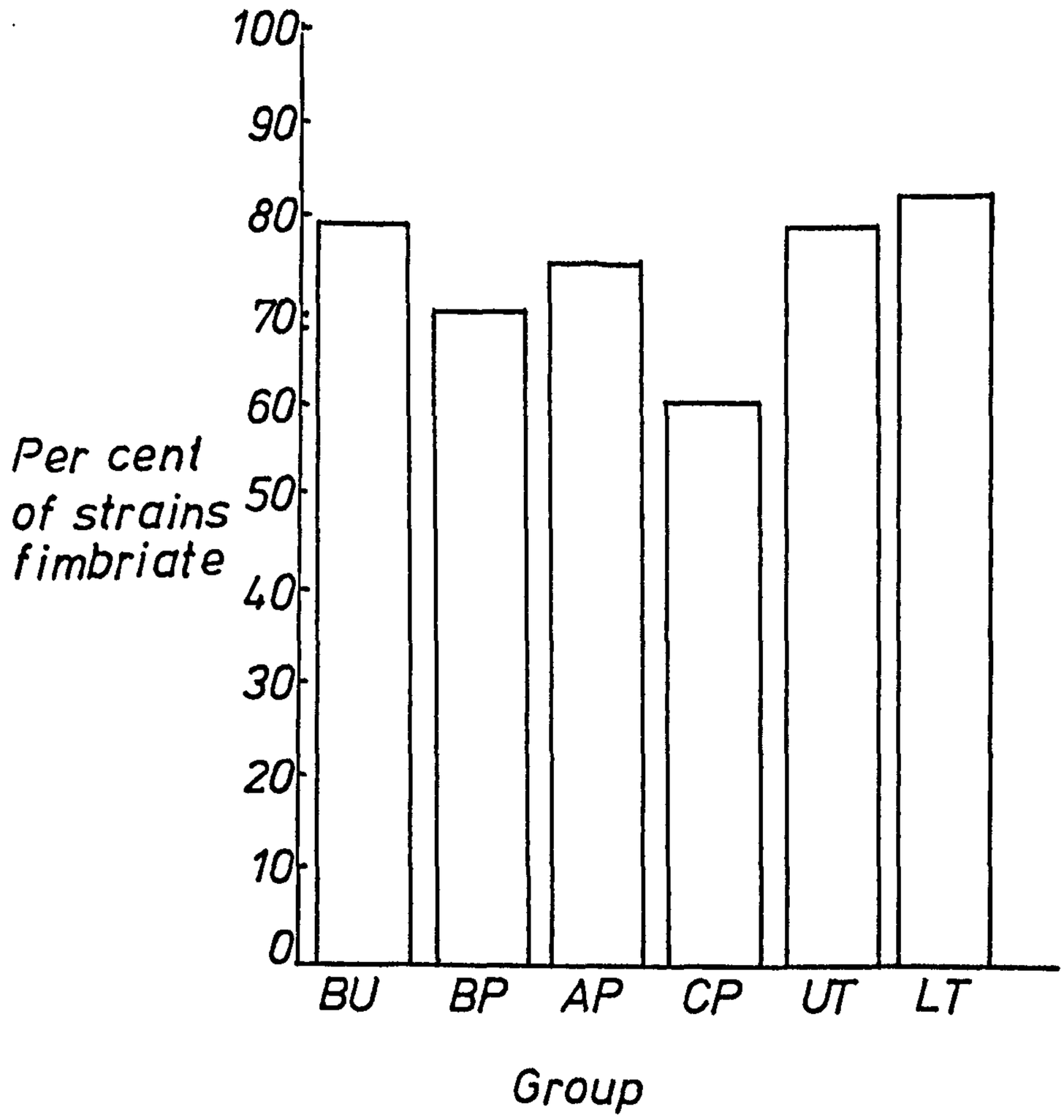
TABLE XIX: FIMBRIÆ PRODUCTION

GROUP (No of strains tested)	BU (105)	BP (43)	BP/NP (34)	AP (75)	AP/US (58)	CP (45)	UT (19)	LT (29)
No. fimbriate	83	30	22	56	43	27	15	24
% fimbriate	79%	70%	65%	75%	74%	60%	79%	83%

TABLE XX: RELATION OF FIMBRIAE PRODUCTION TO RADIOLOGICAL ABNORMALITY

SITE OF ABNORMALITY	UPPER TRACT	LOWER TRACT	NONE
(No. of strains tested)	(19)	(33)	(52)
No. fimbriate	14	24	44
% fimbriate	74%	73%	84%

Fig. 15 FIMBRIAE PRODUCTION



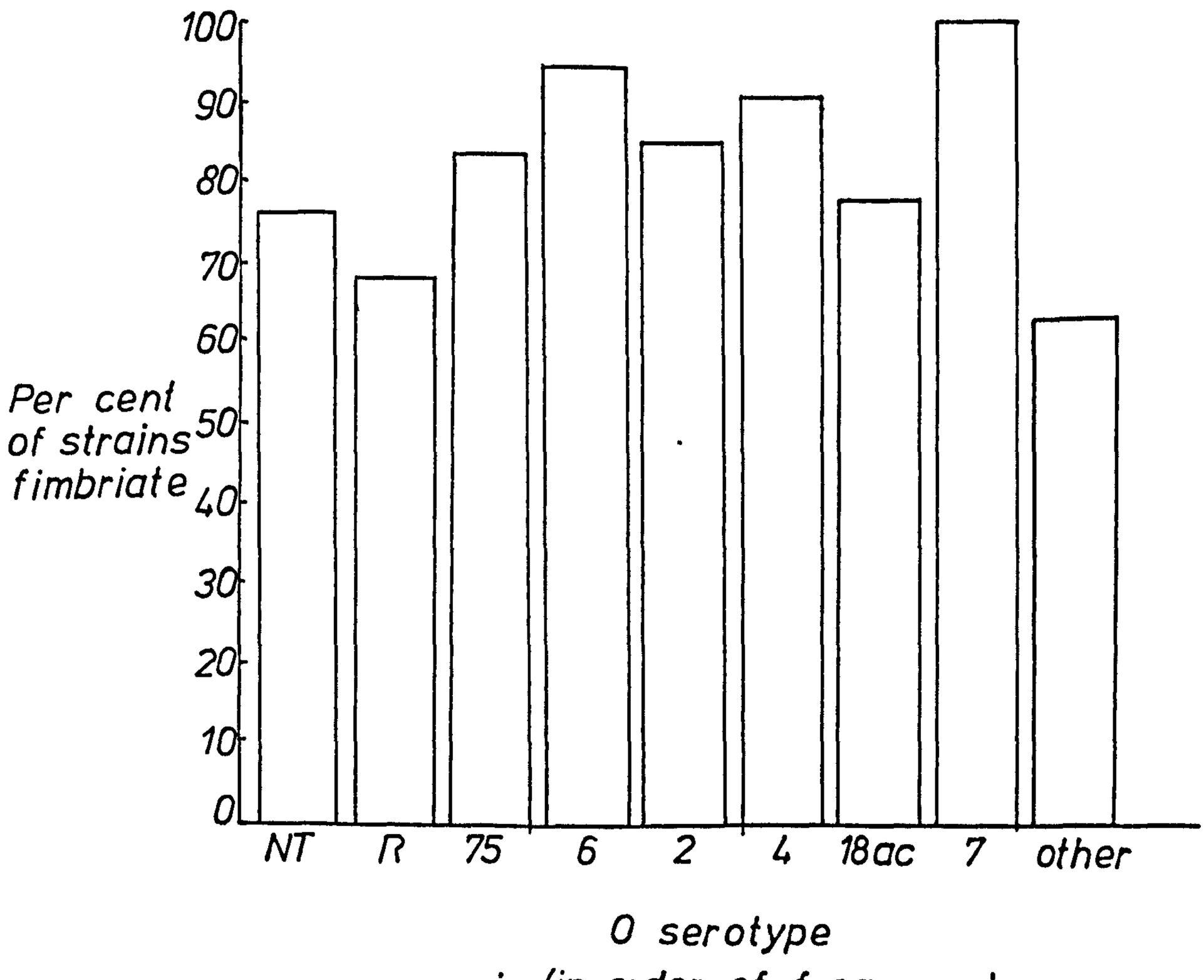
(ii) Relation of fimbriae production to O type

The majority of all types produced fimbriae (table XXI, fig. 16), and this property was not especially associated with any particular type.

TABLE XXI: RELATION OF FIMBRIAE PRODUCTION TO O TYPE

O-TYPE	FIMBRIATE		NON-FIMBRIATE		TOTAL
Non-typable	42	76%	13	24%	55
Rough	26	68%	12	32%	38
075	32	84%	6	16%	38
06	19	95%	1	5%	20
02	12	86%	2	14%	14
04	10	91%	1	9%	11
018ac	7	78%	2	22%	9
07	9	100%	0		9
Other	77	63%	45	37%	122

Fig16 RELATION OF FIMBRIAE PRODUCTION TO O SEROTYPE



NT=non typable
R = rough

6) FERMENTATION OF SUCROSE, SALICIN AND DULCITOL

a) MATERIALS AND METHODS

Fermentation of sucrose, salicin, and dulcitol was determined by the following method:

1) Peptone water sugars (Oxoid) were inoculated with the strains under test and incubated for one week at 37°C. Production of acid was recorded on days 1,2,3,4,5 and 7.

2) Fermentative properties were ascribed to each strain if acid was produced at any time during the observation period.

Two controls, a strain which fermented all three, and a strain which did not ferment any of the sugars, were included in every batch of tests.

b) RESULTS

Approximately half the strains in all groups fermented sucrose (tableXXII, fig. 17), and the majority of strains (70%-90%) fermented dulcitol. Significantly more urinary strains (80%) fermented salicin than periurethral strains from normal subjects (53%) and bacteriuric patients (54%), ($X^2 = 11.1$, $p < 0.001$ and $X^2 = 8.6$, $0.01 > p > 0.001$ respectively). The incidence of salicin fermentation in periurethral strains from abacteriuric patients was slightly greater than in normal subjects and slightly less than in urinary strains, but these differences were not statistically significant.

Combinations of fermentative properties are presented in table XXIII figure 18. Urinary strains more often fermented all three sugars (36%) than periurethral strains from normal subjects (16%) and abacteriuric

patients (23%), (X^2 = 6.4, $0.02 > p > 0.02$ and X^2 = 4.5, $0.05 > p > 0.02$ respectively). Periurethral strains from bacteriuric patients were not significantly different from any of these groups.

There was no difference in fermentative properties between the upper and lower tract groups. Periurethral strains from urethral syndrome patients (AP/US) were not different to the parent AP group. Similarly, periurethral strains from bacteriuric patients which did not give rise to infection (BP/NP) resembled the parent BP group in their fermentative characteristics.

KEY

BU: Bacteriuric patients; urinary strains

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

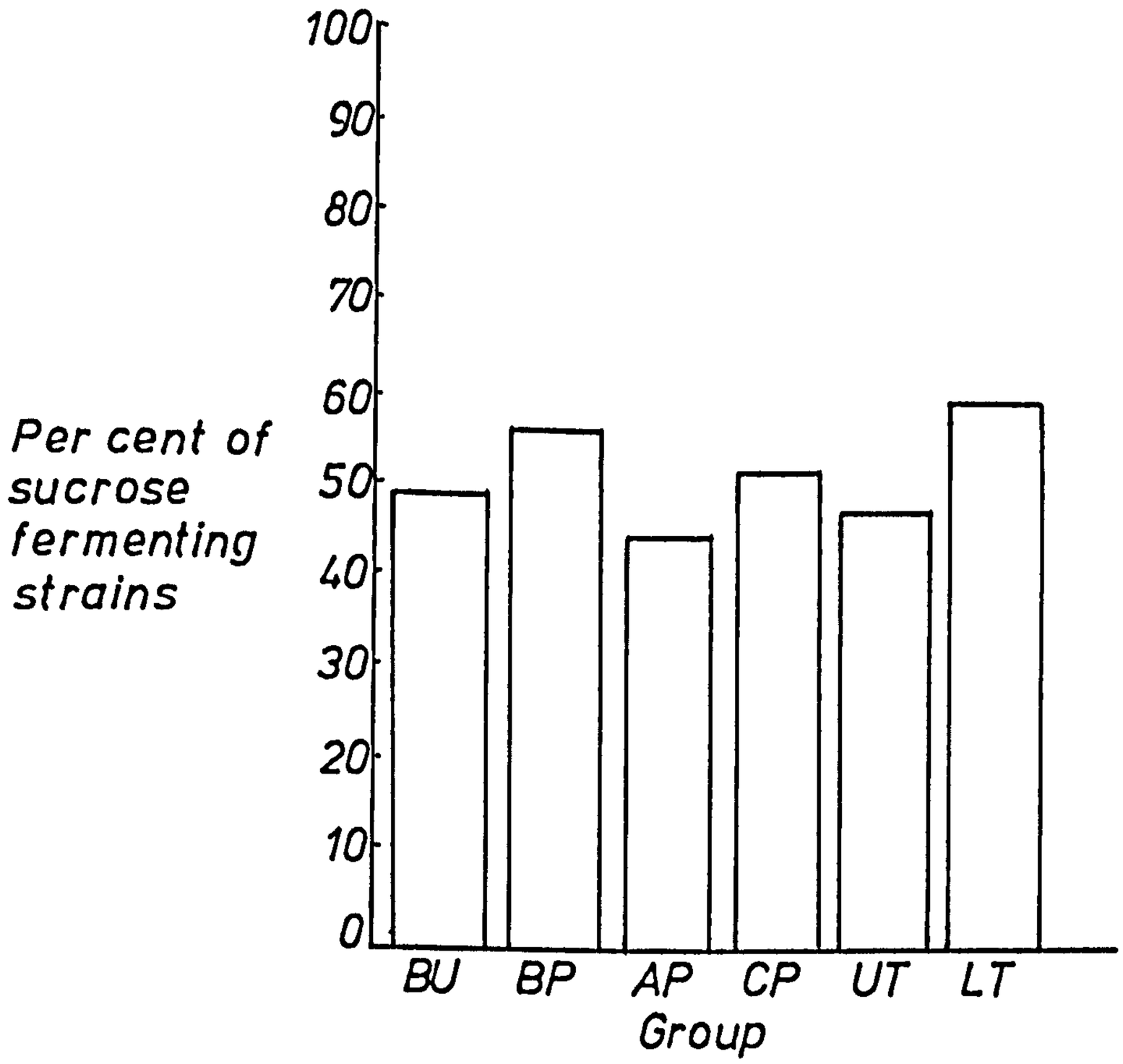
UT: Bacteriuric patients; strains isolated from the upper tract.

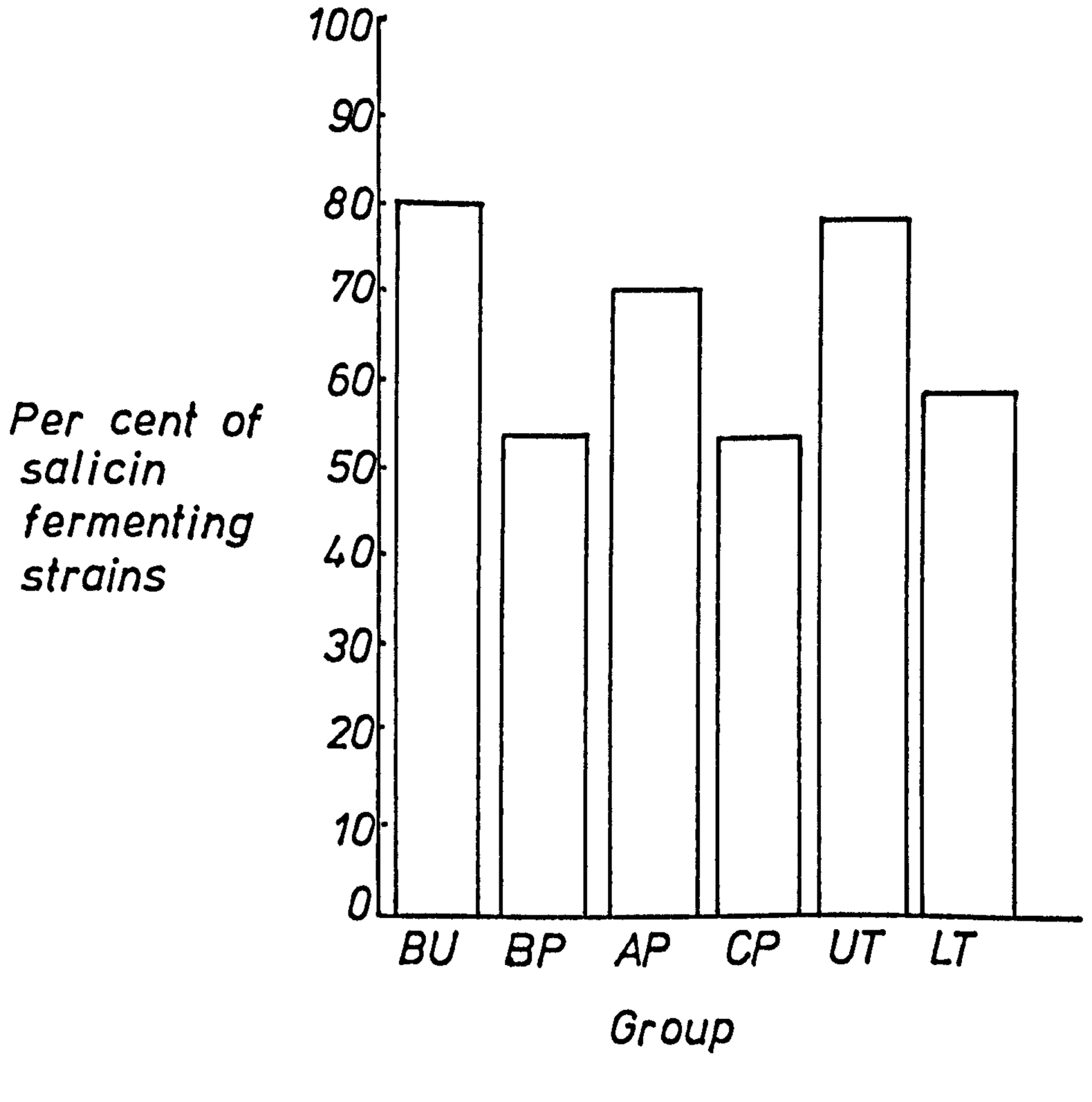
LT: Bacteriuric patients; strains isolated from the lower tract.

TABLE XXII: FERMENTATION OF SUGARS

GROUP (No. of strains tested)	SUCROSE		SALICIN		DULCITOL	
BU (105)	51	49%	84	80%	89	85%
BP (43)	24	56%	23	54%	38	88%
BP/NP (34)	17	50%	20	59%	29	85%
AP (75)	34	45%	53	71%	58	77%
AP/US (58)	26	45%	40	69%	46	79%
CP (45)	23	51%	24	53%	34	76%
UT (19)	9	47%	15	79%	17	90%
LT (29)	17	59%	17	59%	23	79%

Fig. 17 FERMENTATION OF SUCROSE, SALICIN & DULCITOL





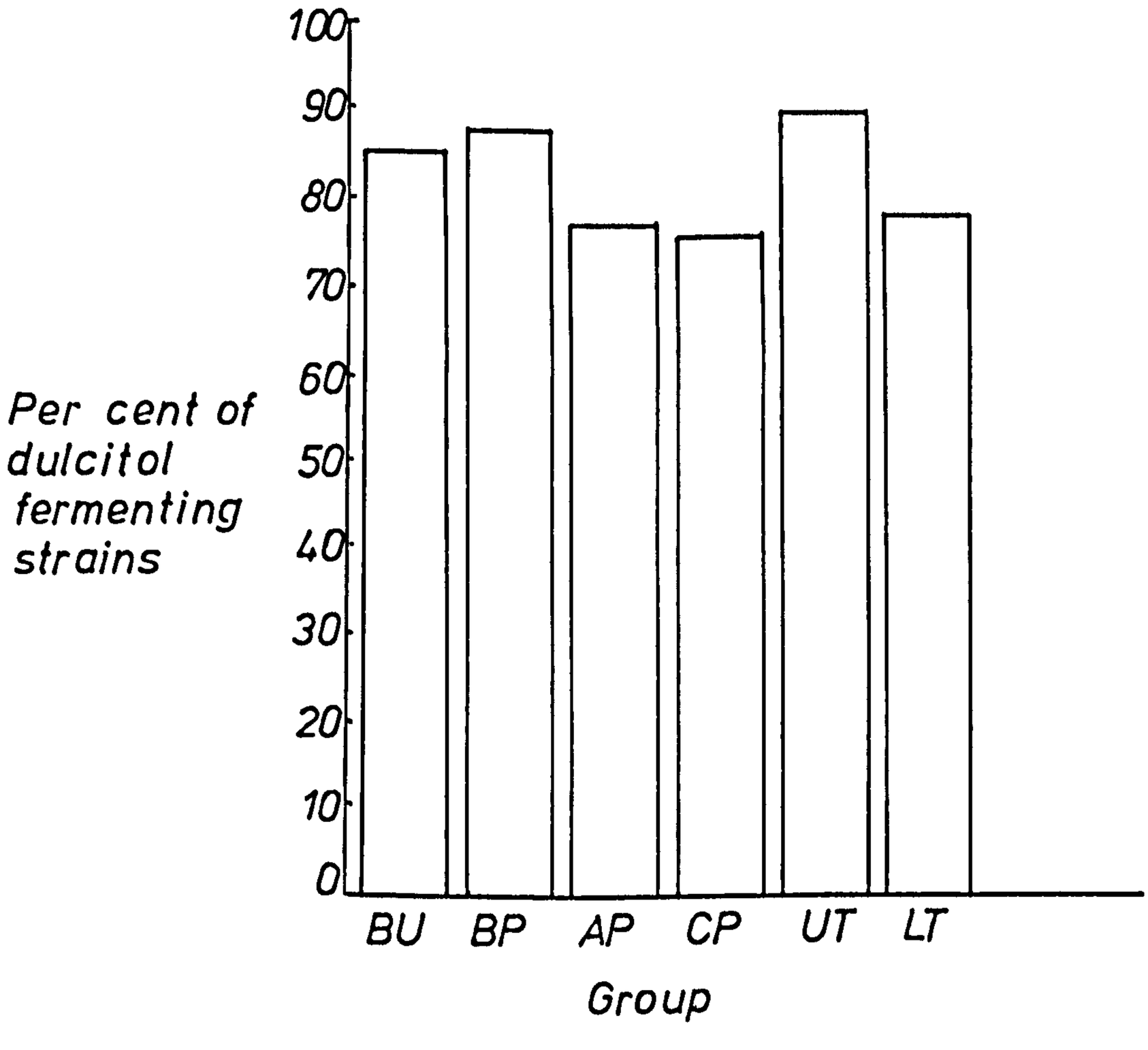
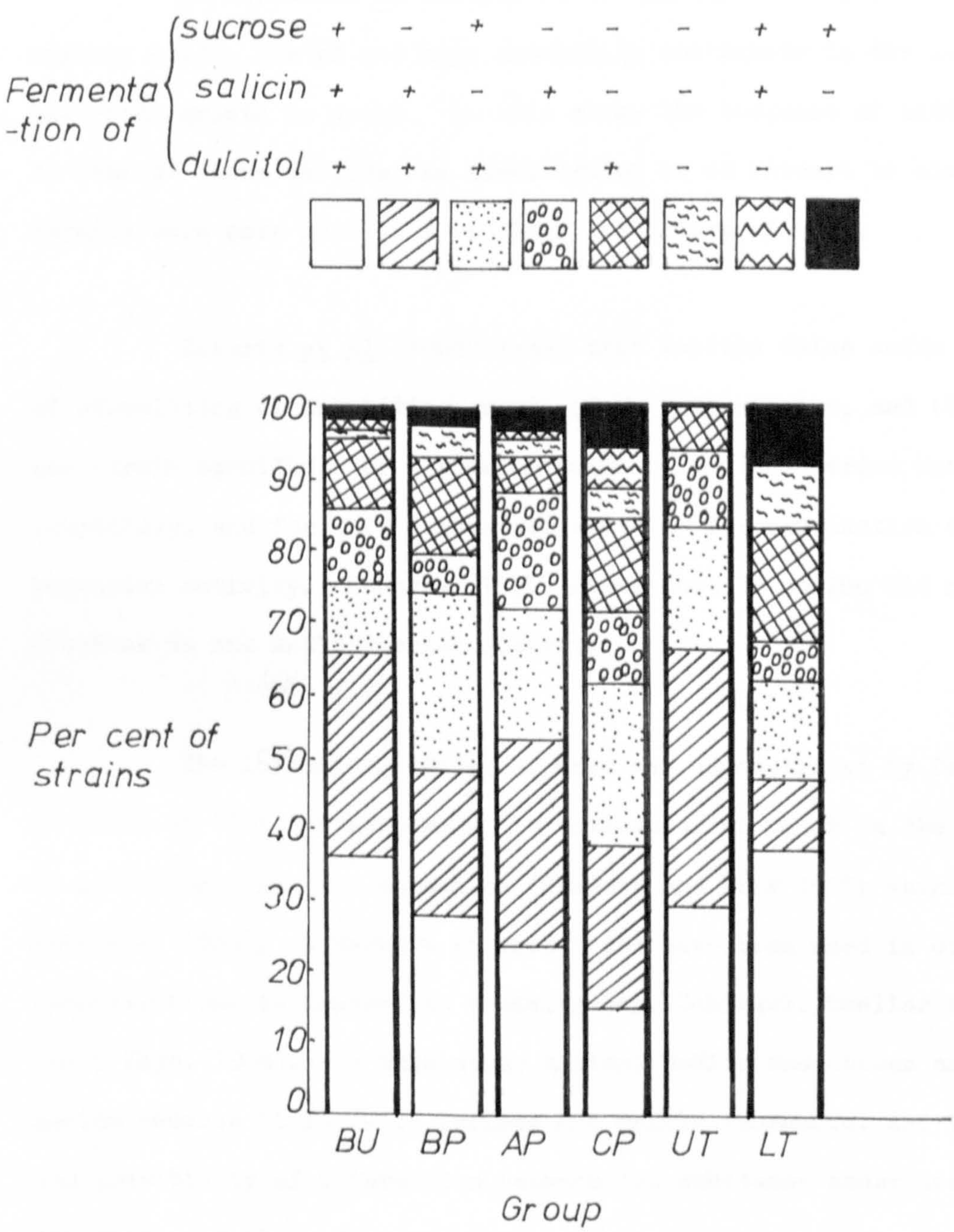

Per cent of dulcitol fermenting strains
100
90
80
70
60
50
40
30
20
10
0
BU
BP
AP
CP
UT
LT
Group

TABLE XXIII: FERMENTATION PATTERNS

GROUP (No of strains tested)	Suc+ Sal+ Dul+	Suc- Sal+ Dul+	Suc+ Sal- Dul+	Suc- Sal+ Dul-	Suc- Sal- Dul+	Suc- Sal- Dul-	Suc+ Sal+ Dul-	Suc+ Sal- Dul-
BU (105)	38 36%	32 30%	9 9%	12 11%	10 10%	1 1%	2 2%	1 1%
BP (43)	12 28%	9 21%	11 25%	2 5%	6 14%	2 5%		1 2%
BP/NP (34)	9 26%	9 26%	7 21%	2 6%	4 12%	2 6%		1 3%
AP (75)	17 23%	23 30%	14 19%	12 16%	4 5%	2 3%	1 1%	2 3%
AP/US (58)	13 22%	18 31%	11 19%	8 14%	4 7%	2 3%	1 2%	1 2%
CP (45)	7 16%	10 22%	11 24%	4 9%	6 13%	2 4.5%	3 7%	2 4.5%
UT (19)	6 30%	7 37%	3 16%	2 11%	1 5%			
LT (29)	11 38%	3 10%	4 14%	1 4%	5 17%	3 10%	2 7%	

Fig.18 FERMENTATION PATTERNS



7) SENSITIVITY TO DL SERINE, SPERMINE AND UREA

a) INTRODUCTION

As discussed in section 2c of the introduction, urea concentration, organic acids, low pH and high osmolality contribute to the inhibition of bacterial growth in urine. In this study the response of individual strains to some of these factors was investigated in an attempt to discover if some strains were more resistant to their inhibitory effects.

Roberts *et al* (1968) found that various amino acids were capable of stimulating or inhibiting growth of *E. coli* strains, and that this effect was strain specific. Of the amino acids he tested, serine was the most inhibitory, and for this reason was selected for examination of its antibacterial activity. Roberts (1973) found that DL serine did not differ from L serine in its antibacterial activity.

The inhibitory effect of urea was demonstrated by Peju and Rajat as early as 1906 and its use as an antibacterial agent in the treatment of infections was even advocated (Symmers and Kirk 1915; Weinstein and McDonald, 1945). A variety of techniques have been used in other investigations to assess its activity (eg. Schlegel, Cuellar and O'Dell, 1961; Kaye, 1968). In this study minimal medium was chosen as the culture medium because it is fully defined and mainly inorganic, and thus avoids the possibility of interaction between the substance under test and unknown components of the medium.

Although spermine has been previously shown to exhibit marked antibacterial activity (Razin and Rozansky, 1959) the response of individual strains has not been investigated. Spermine is excreted in the urine of healthy females in low concentration but in pregnancy urine levels are

considerably elevated (Russell, Levy, Schimpff and Hawk, 1971). The antibacterial activity of prostatic fluid and semen is attributed, at least in part, to the presence of spermine (Bachrach, 1973).

The low incidence of urinary tract infection in males, as compared to females, is considered to be partially due to the antibacterial action of prostatic fluid, although spermine was not considered to be the active agent by Stamey et al (1968).

In their study of the capacity of urine to sustain bacterial growth Asscher et al (1966) noted the pH to vary from 4.6 to 7.25. In this study the effect of pH on the activity of serine, spermine and urea was assessed.

b) MATERIALS AND METHODS

Sensitivity of strains to various concentration of D-L serine, spermine, and urea in minimal medium at pH 7.2 was assessed by measuring growth inhibition. The effect of pH on the sensitivity of a random sample of strains to these compounds was examined. In addition all strains were tested for urea sensitivity at pH 5.5.

Preparation of plates

The minimal medium of Davis and Mingioli (1950) was solidified with 1.0% Agarose (Miles Serevac). The formula at different pH values is given below:

10% dextrose (sterile, added after autoclaving)		20 mls
Sodium citrate, $Na_3C_6H_5O_7.2H_2O$		0.5g
Magnesium sulphate, $MgSO_4.7H_2O$		0.1g
Ammonium sulphate, $(NH_4)_2SO_4$		1.0g
Disodium hydrogen phosphate, Na_2HPO_4	pH7.2	8.3g
	pH6.0	1.2g
	pH5.5	0.6g
Potassium dihydrogen phosphate, KH_2PO_4	pH7.2	2.7g
	pH6.0	8.2g
	pH7.2	11g

Minimal medium and Agarose were prepared at double strength and autoclaved separately to avoid 'browning'.

Freshly prepared two-fold dilutions of D-L serine (Koch Light Laboratories) and spermine (Aldrich Chemical Co.) were added to minimal medium broths to give final concentrations in the plates ranging from 1 mcg/ml to 1024 mcg/ml and 32 mcg/ml to 512 mcg/ml respectively. Urea was added to give final concentrations of 0.5% to 6% in 0.5% steps.

Equal volumes of cooled, melted Agarose were mixed with the supplemented broths and plates poured. Plates were stored up to 3 days with no loss of activity.

The use of chemically clean glass-ware was found to be essential in the preparation of these plates.

Growth inhibition

A multi-pronged inoculator (Denley Instruments Ltd.) capable of depositing 20 spot inocula per plate was used; each prong delivered 0.001ml of an overnight nutrient broth culture diluted 1/1000 in deionised water (10^2-10^3 organisms). Plates were incubated for 20 hours at 37°C and growth compared to a control plate not supplemented with inhibitory agents. The minimum inhibitory concentration was the last dilution to suppress growth completely, allow growth of less than 5 colonies, or a barely discernable haze of growth.

Controls

Two controls with different sensitivities were included in every batch of twenty strains.

mcg/ml = micrograms per ml.

c) RESULTS

Sixty seven per cent of all strains (214) were able to grow on minimal medium. Sensitivity was found to be a constant characteristic within the limits + or - one doubling dilution of serine and spermine, + or - 0.5% urea.

Minimum inhibitory concentration (MIC) ranges and medians are given in table XXIV. Sensitivity to serine was strain specific; some strains were inhibited by as little as 1 mcg/ml whilst others were resistant to more than 1024 mcg/ml. Variation in urea and spermine sensitivity was less marked.

MICs of strains in each group to serine, spermine and urea at pH 7.2 are given in tables XXV a,b and c. The distribution of strains according to their sensitivity was almost identical in each group (figs. 19a, b and c). Fewer periurethral strains from normal subjects were resistant to serine compared with the other groups, but this difference was not statistically significant. MIC values for urea at pH 5.5 are given in table XXV d. On the whole these were lower than at pH 7.2 and the small differences observed between groups were not statistically significant (fig. 19d).

Sensitivities of between 36 and 40 randomly selected strains to serine, spermine, and urea at pHs 7.2, 6.0 and 5.5 are shown in table XXVI a-c. Strains became progressively moreresistant to serine as the pH was lowered (fig. 20a). At pH6 the inhibitory effect of spermine was almost abolished; no further decrease in inhibition was observed at pH 5.5 (fig. 20b). Overall, strains became more sensitive to urea as the pH was lowered (fig. 20c), although a few strains were not affected by pH change. It must be pointed out that the growth of strains was not diminished by lowering the pH alone.

TABLE XXIV: MINIMUM INHIBITORY CONCENTRATIONS OF SERINE, SPERMINE AND UREA

	MEDIAN	RANGE
D-L SERINE pH 7.2	8 16 mcg/ml	1 - 1024 mcg/ml
SPERMINE pH 7.2	128 256 mcg/ml	32 - 512 mcg/ml
UREA pH 7.2	4.0 4.5%	2 - 6%
UREA pH 5.5	3.5 4.0%	1 - 5.5%

KEY

BU: Bacteriuric patients; urinary strains

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

UT: Bacteriuric patients; strains isolated from the upper tract.

LT: Bacteriuric patients; strains isolated from the lower tract.

TABLE XXVa: SERINE SENSITIVITY: pH 7.2

GROUP (No. of strains tested)	MIC in mcg/ml											
	1	2	4	8	16	32	64	128	256	512	1024	1024
BU (69)		5 7%	8 12%	16 23%	13 19%	4 6%	3 5%	1 1%	2 3%	1 1%	1 1%	15 22%
BP (32)	2 6%		6 19%	4 13%	8 25%	2 6%	1 3%			1 3%	1 3%	7 22%
BP/NP (28)	1 4%		5 18%	4 14%	7 25%	1 3.5%	1 3.5%			1 3.5%	1 3.5%	7 25%
AP (55)	2 4%	1 2%	8 14%	14 25%	8 14%	6 11%	2 4%			1 2%		13 24%
AP/US (44)		1 2%	6 14%	10 23%	7 16%	6 14%	1 2%			1 2%		12 27%
CP (35)	2 6%	1 3%	8 23%	6 17%	3 9%	5 14%	4 11%					6 17%
UT (6)				2	2	2						
LT (17)	3		4	4	5		1					

TABLE XXVb: SPERMINE SENSITIVITY : pH 7.2

GROUP (No. of strains tested)	MIC in mcg/ml					
	32	64	128	256	512	512
BU (70)	1 1%	4 6%	11 16%	35 50%	19 27%	
BP (21)			4 19%	9 43%	7 33%	1 5%
BP/NP (17)			3 18%	7 41%	6 35%	1 6%
AP (55)		3 5%	12 22%	26 47%	13 24%	1 2%
AP/US (44)		2 4%	11 25%	20 46%	10 23%	1 2%
CP (35)		3 9%	6 17%	13 37%	12 34%	1 3%
UT (5)			1	3	1	
LT (17)	1		3	6	7	

Fig.19a SERINE SENSITIVITY (at pH 7.2)

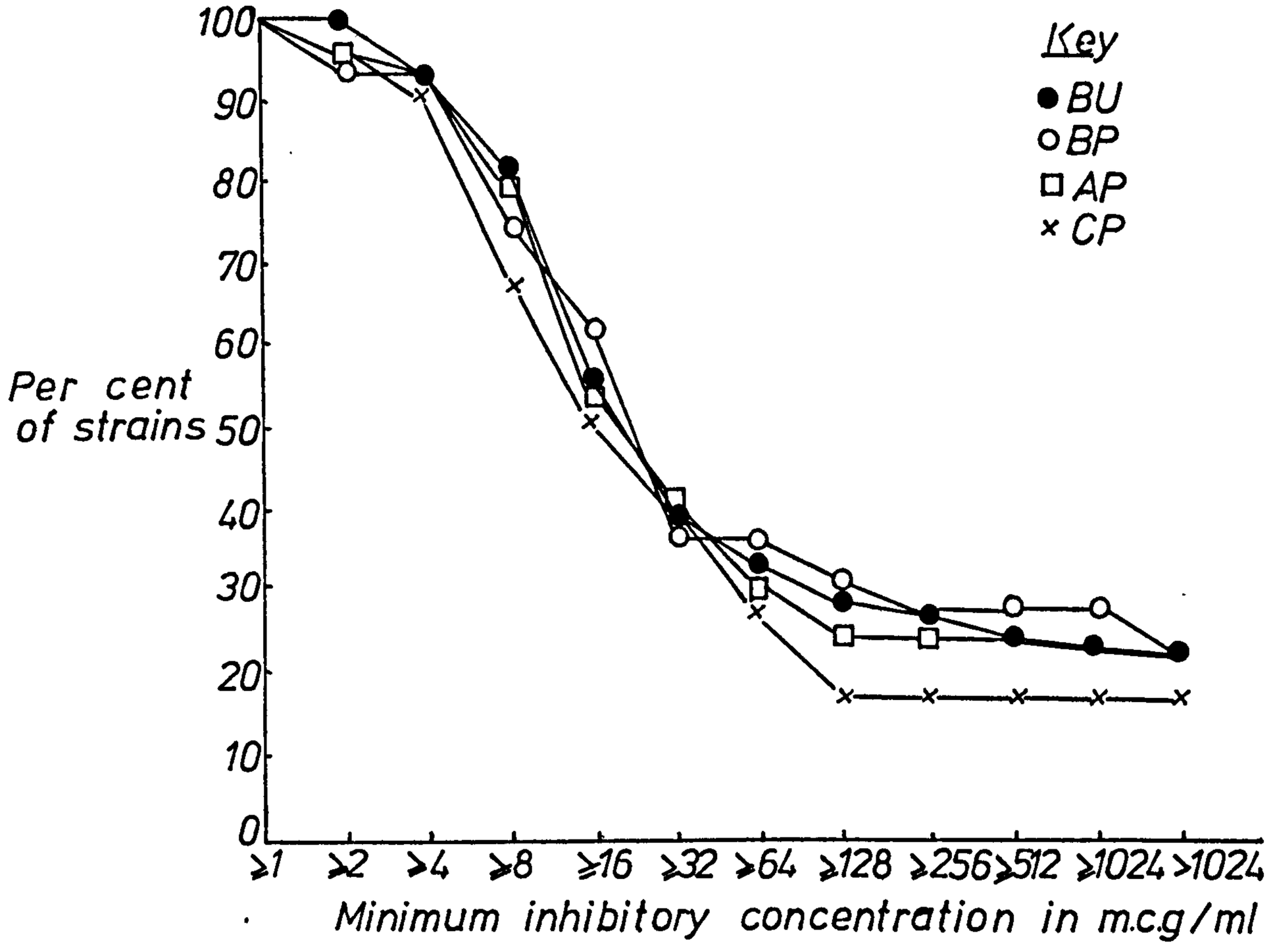


Fig.19b SPERMINE SENSITIVITY (at pH 7.2)

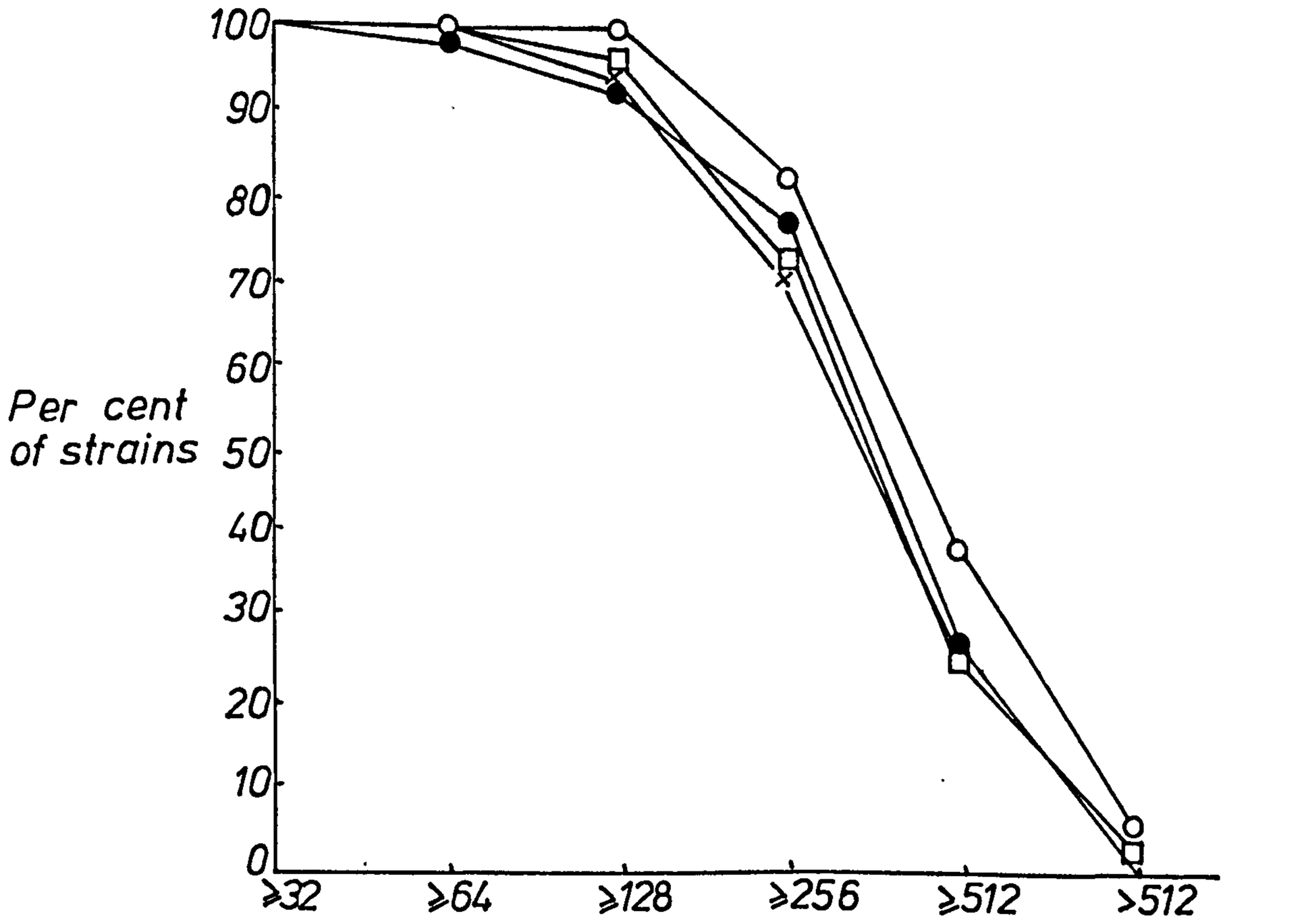


TABLE XXVc: UREA SENSITIVITY: pH 7.2

GROUP (No of strains tested)	MIC: % UREA								
	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
BU (70)		2 3%	4 6%	10 14%	15 21.5%	15 21.5%	17 24%		7 10%
BP (32)	1 3%	2 6%	1 3%	3 9.5%	8 25%	3 9.5%	13 41%		1 3%
BP/NP (28)	1 3.5%	1 3.5%		3 11	8 28.5%	2 7%	12 43%		1 3.5%
AP (55)		5 9%	1 2%	4 7%	7 13%	19 35%	9 16%	4 7%	6 11%
AP/US (44)		5 11%		4 9%	6 14%	13 29%	7 16%	3 7%	6 14%
CP (35)	2 6%	2 6%	2 6%		6 17%	12 34%	2 6%	5 14%	4 11%
UT (6)	1	2		1	2				
LT (17)		3	3	3	7	1			

TABLE XXVd: UREA SENSITIVITY: pH 5.5

GROUP (No of strains tested)	MIC: % UREA									
	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
BU (70)		2 3%	8 11.5%	5 7%	12 17%	2 3%	8 11.5%	16 23%	17 24%	
BP (32)	1 3%	2 6%	2 6%	2 6%		4 13%	3 9%	5 16%	8 25%	5 16%
BP/NP (28)	1 4%	2 7%	1 4%	1 4%		4 14%	3 11%	4 14%	8 28%	4 14%
AP (55)	1 2%	5 9%	8 15%	7 13%	2 4%	3 5%	3 5%	16 29%	5 9%	5 9%
AP/US (44)	1 2%	3 7%	8 18%	6 14%	1 2%	2 5%	2 5%	12 27%	5 11%	4 9%
CP (35)		5 14%	1 3%	2 6%	4 11%	3 8%	9 26%	8 23%	2 6%	1 3%
UT (6)				3		1	1	1		
LT (17)			2	3	2		1	6	1	2

Fig.19c UREA SENSITIVITY (at pH 7.2)

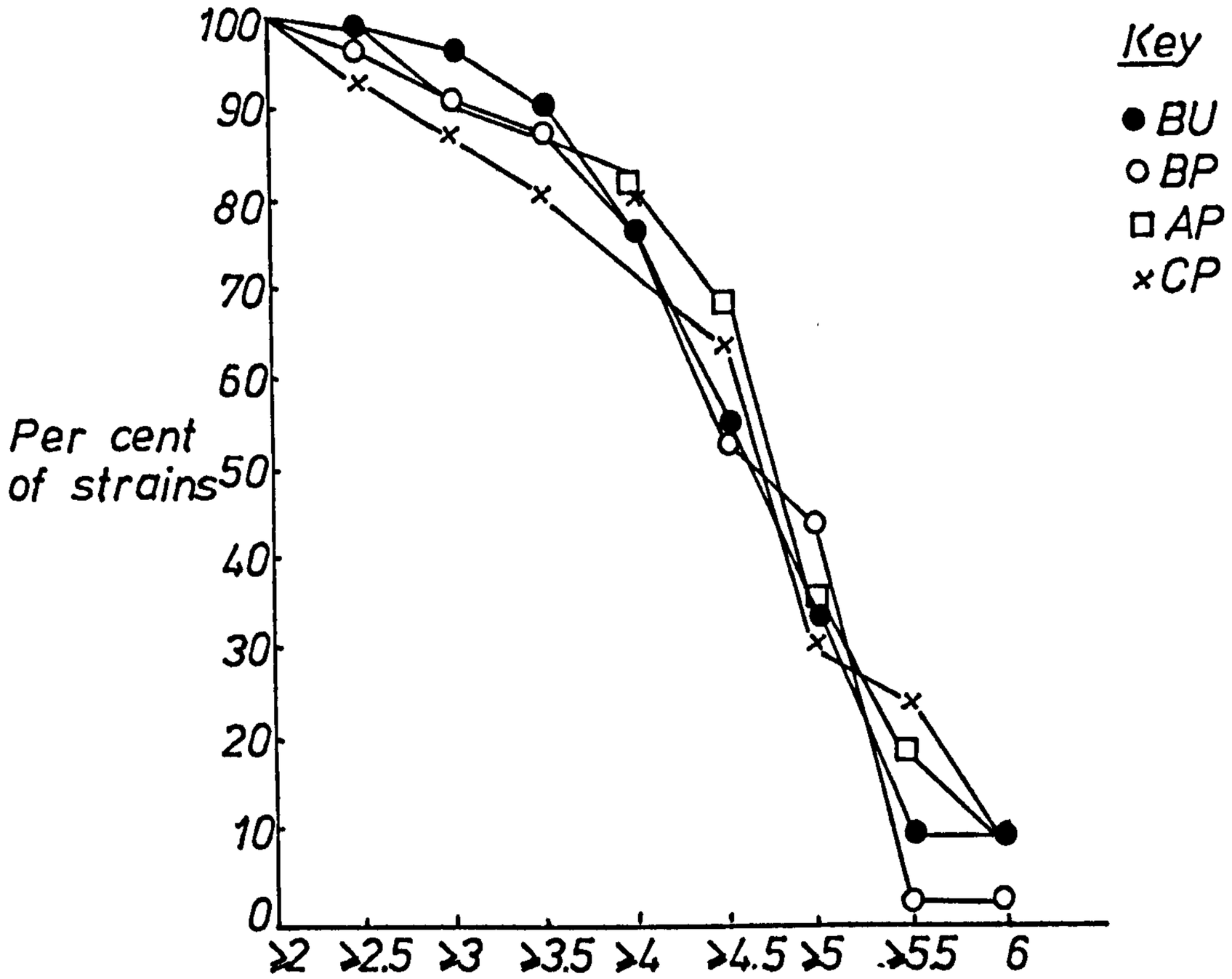
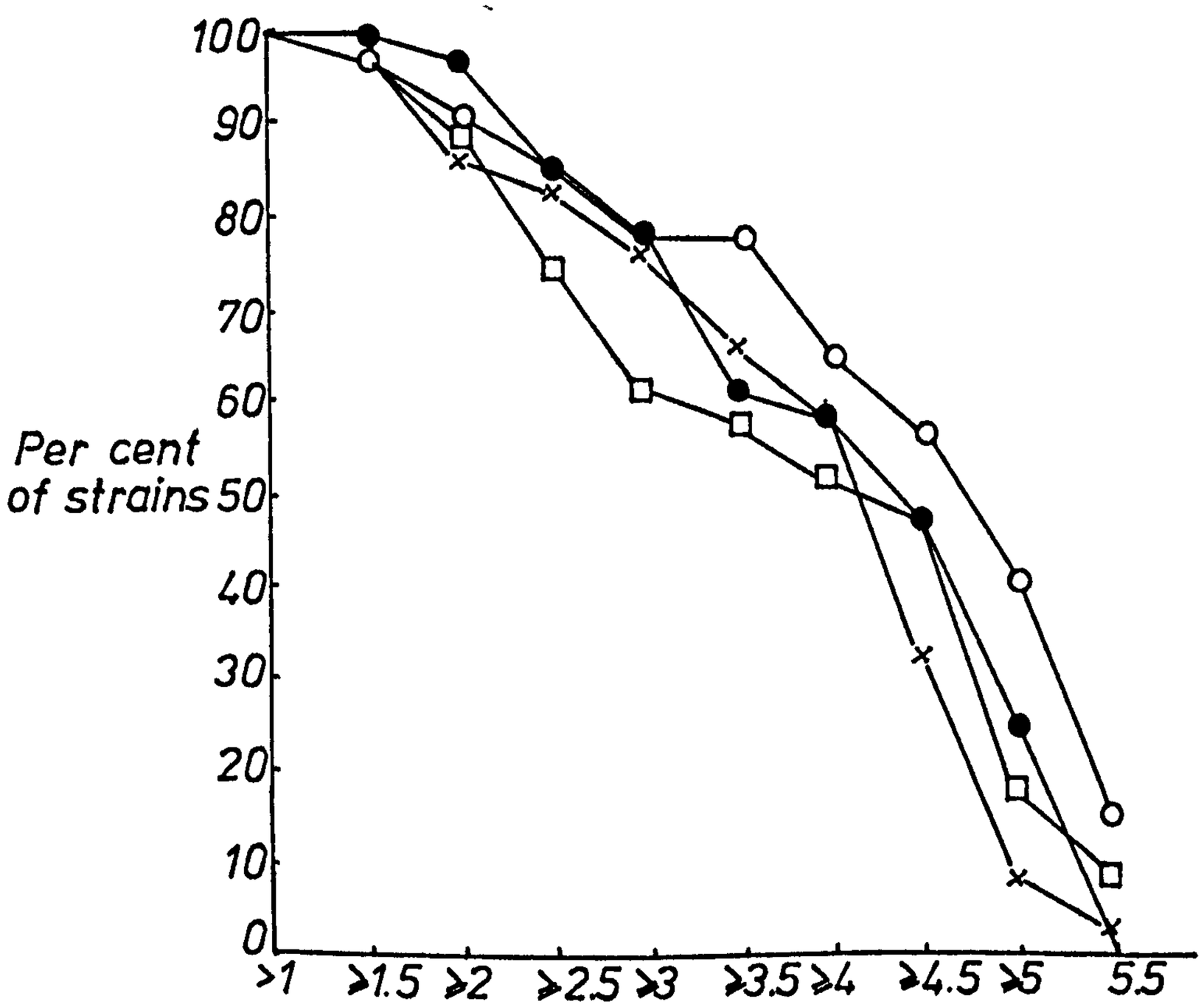


Fig.19d UREA SENSITIVITY (at pH 5.5)



EFFECT OF pH ON SERINE, SPERMINE & UREA SENSITIVITY

TABLE XXVIa

D-L Serine (40 strains)

MIC mcg/ml	pH 7.2		pH 6.0		pH 5.5	
0.5						
1						
2	3	8%				
4	2	5%	1	2.5%		
8	8	20%	2	5%		
16	5	12%	6	15%		
32	3	8%	2	5%		
64	1	2%	2	5%	2	5%
128			1	2.5%	1	2.5%
256	1	2%	1	2.5%	1	2.5%
512	2	5%	1	2.5%	3	8%
1024	3	8%			2	5%
1024	12	30%	24	60%	31	77%

TABLE XXVIb

Spermine (36 strains)

MIC mcg/ml	pH 7.2		pH 6.0		pH 5.5	
32						
64						
128	13	36%				
256	12	33%				
512	9	25%	6	17%	7	19%
512	2	6%	30	83%	29	81%

TABLE XXVIc

Urea (37 strains)

MIC (%)	pH 7.2		pH 6.0		pH 5.5	
1.0					1	3%
1.5					2	5%
2.0			1	3%	8	22%
2.5			2	5%		
3.0	1	3%	8	22%	1	3%
3.5	2	5%	2	5%	1	3%
4.0	1	3%	1	3%	2	5%
4.5	6	16%	8	22%	19	51%
5.0	17	46%	14	37%	3	8%
5.5	6	16%	1	3%		
6.0	4	11%				

Fig.20a EFFECT OF pH ON SERINE SENSITIVITY

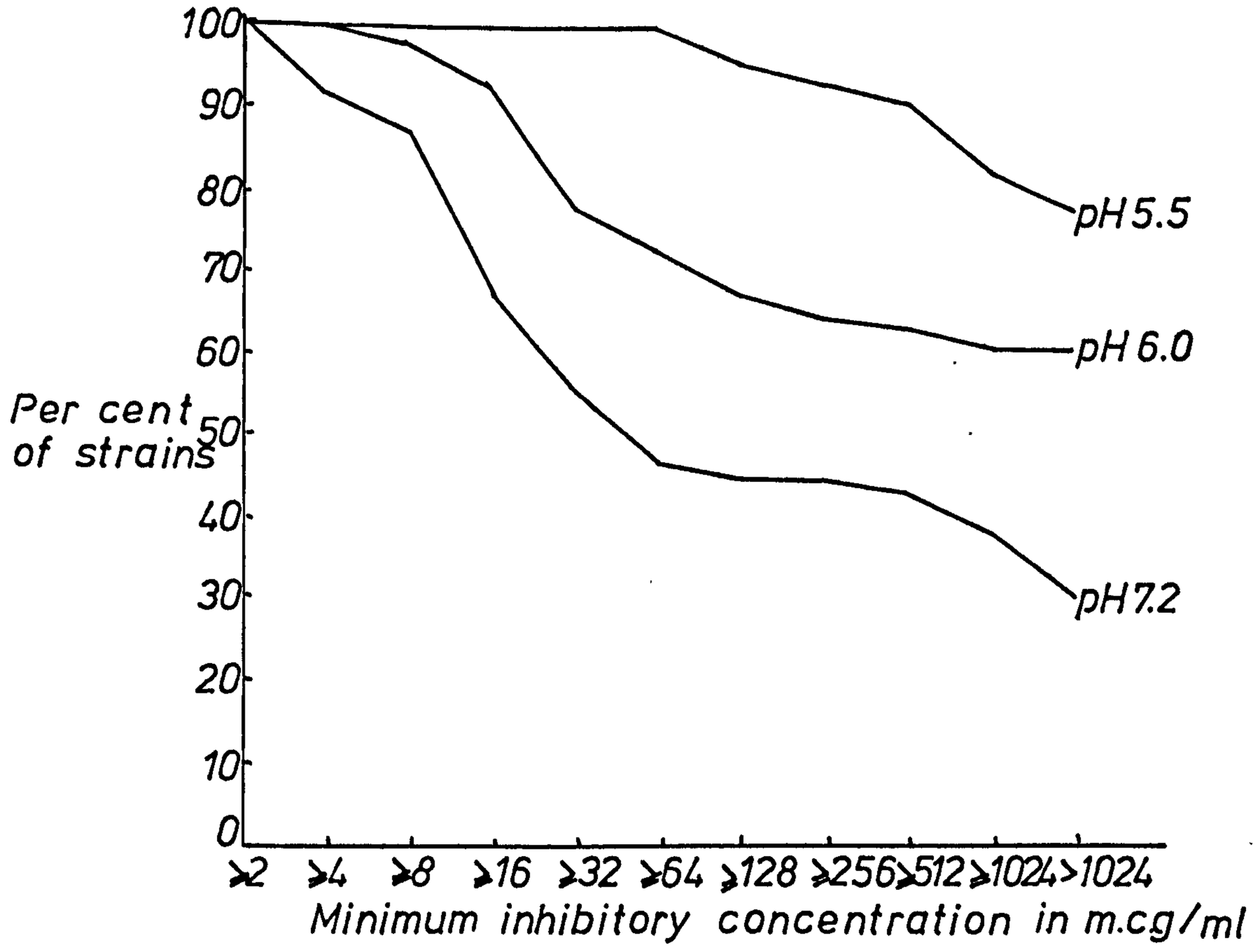


Fig.20b EFFECT OF pH ON SPERMINE SENSITIVITY

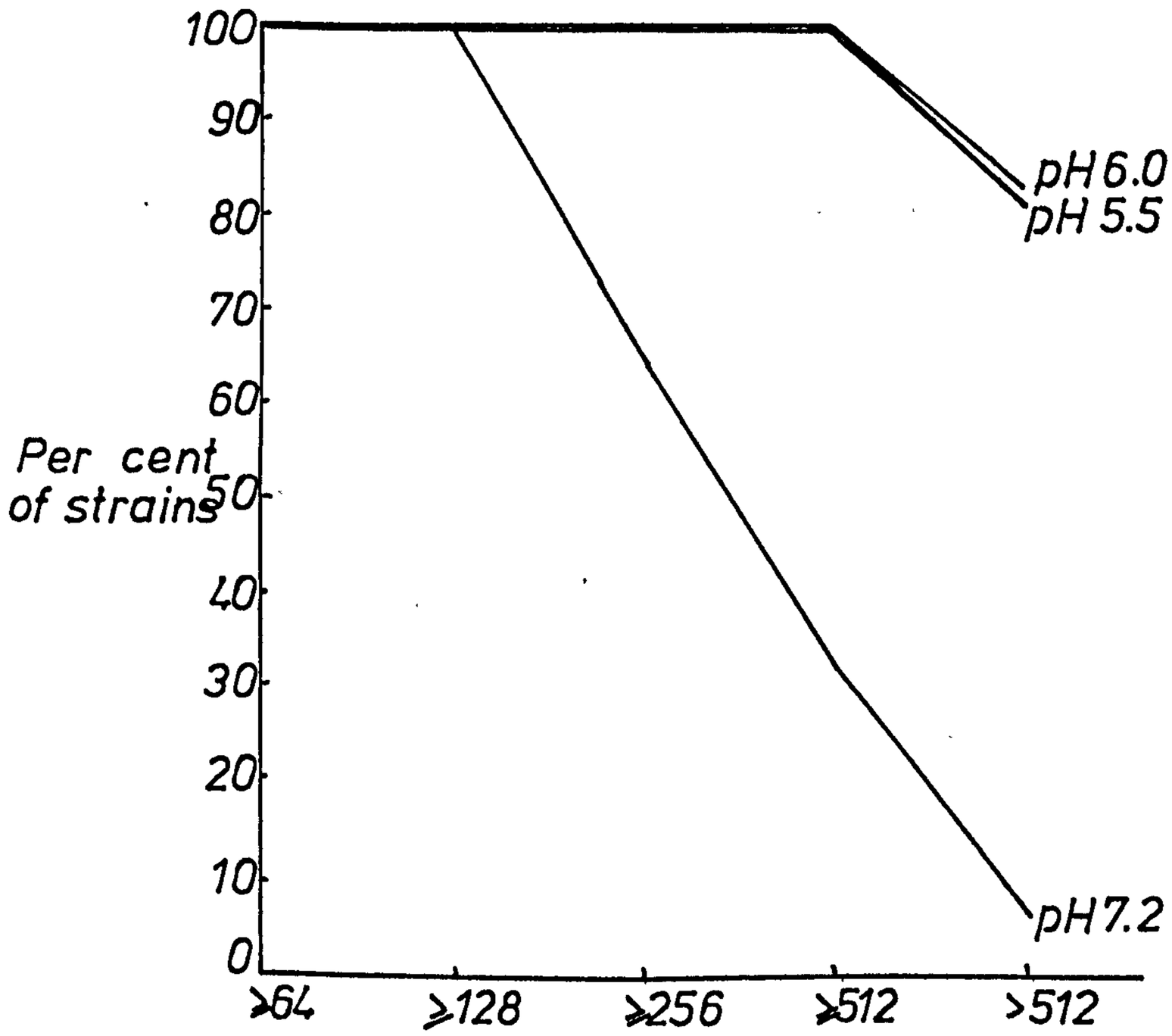
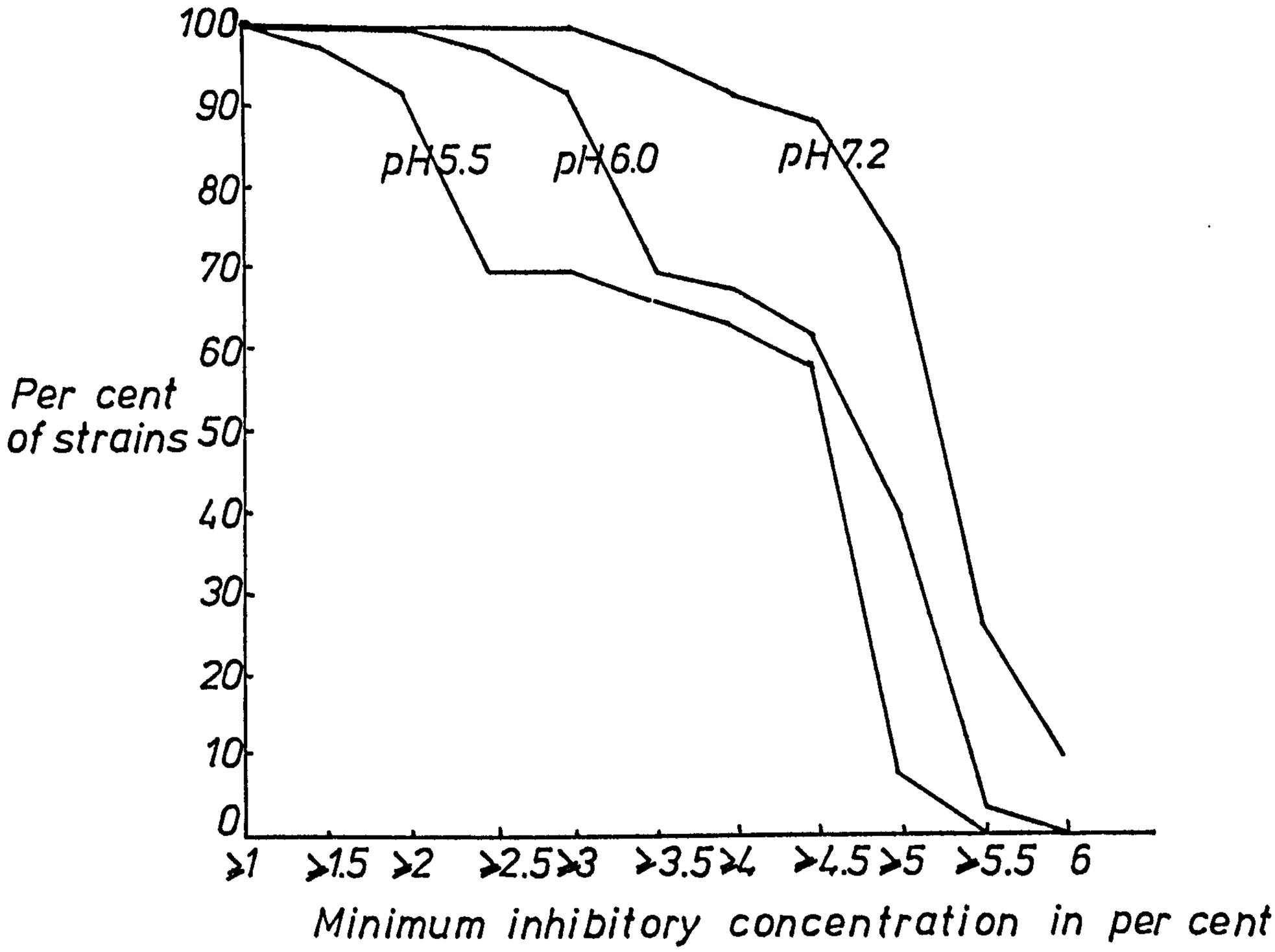


Fig.20c EFFECT OF pH ON UREA SENSITIVITY



8) GROWTH REQUIREMENTS

a) MATERIALS AND METHODS

Strains were tested for their ability to grow on the minimal medium of Davis and Mingioli (1950). The growth requirements of strains that failed to grow on minimal medium were assessed by the method of Holliday (1956).

Preparation of plates

Minimal medium was solidified with 1% Agarose and supplemented with amino acids and vitamins as shown on page 156.

Dilute NaOH was required to dissolve tyrosine, glutamic acid, and aspartic acid; tryptophan was dissolved in dilute HCl, (Meynell & Meynell 1970).

Inoculation of plates

Using an automatic multi-point inoculator delivering 20 spot inocula of 0.001ml, plates A-K were inoculated with the strains under test. The inoculating wells contained overnight broth cultures diluted 1/1000 in sterile deionised water so that 10^2 to 10^3 organisms were deposited per spot inoculum.

Interpretation of results

Plates were incubated for 18 hours at 37°C and growth compared with two control plates, one containing unsupplemented minimal media the other nutrient agar. Plates supporting growth comparable to that obtained on nutrient agar were considered to contain the required nutrients. Strains were graded according to the number of plates which sustained growth:

1. No growth on any plate; growth requirements of strains in this group were not met by any of the supplements.

2. Growth on three or less plates; a single amino acid or vitamin, or specific combination was essential for growth.

3a. Growth on 4 - 7 plates: } Nutrient requirements satisfied

3b. Growth on 8 -11 plates: } by any of a number of supplements.

Controls

Two strains able to grow on minimal medium were included in every batch of tests in order to ensure that none of the supplement combinations were inhibitory.

b) RESULTS

The nutritional requirements of strains are given in table XXVII, fig. 21. Only 6 of the 19 upper tract strains (32%) grew on unsupplemented minimal medium compared to 20 of the 29 lower tract strains (69%). This difference was statistically significant ($X^2 = 4.9$, $0.05 > p > 0.02$). Between 67% and 82% of strains from all other groups grew on minimal medium; there were no other significant differences between groups.

Strains unable to grow on minimal medium varied in their nutrient requirements; a few strains did not grow on any of the supplemented media whilst others grew well in the presence of any of a number of supplements. Distribution of these strains according to their fastidiousness was similar in all groups.

	A	B	C	D	E
F	Biotin	DL B-Phenylalanine	DL Alanine	L Arginine hydrochloride	L Leucine
	0.005 mcg/ml	10 mcg/ml	10 mcg/ml	10 mcg/ml	10 mcg/ml
G	Folic acid	DL Serine	L Cysteine	L Ornithine hydrochloride	Glycine
	0.05 mcg/ml	2 mcg/ml	10 mcg/ml	10 mcg/ml	10 mcg/ml
H	Pyridoxal-5-phosphate	L Tryptophan	L Threonine	L Aspartic acid	L isoleucine
	1 mcg/ml	10 mcg/ml	10 mcg/ml	10 mcg/ml	10 mcg/ml
I	Thiamin	L Tyrosine	DL Methionine	L Proline	L Histidine
	1 mcg/ml	10 mcg/ml	10 mcg/ml	10 mcg/ml	10 mcg/ml
J.	d-Pantothenic acid	p-Amino benzoic acid		DL Glutamic acid	L Lysine hydrochloride
	1 mcg/ml	0.05 mcg/ml		10 mcg/ml	10 mcg/ml
K	Riboflavin	Nicotinic acid			DL Valine
	1 mcg/ml	1 mcg/ml			10 mcg/ml

KEY

BU: Bacteriuric patients; urinary strains.

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

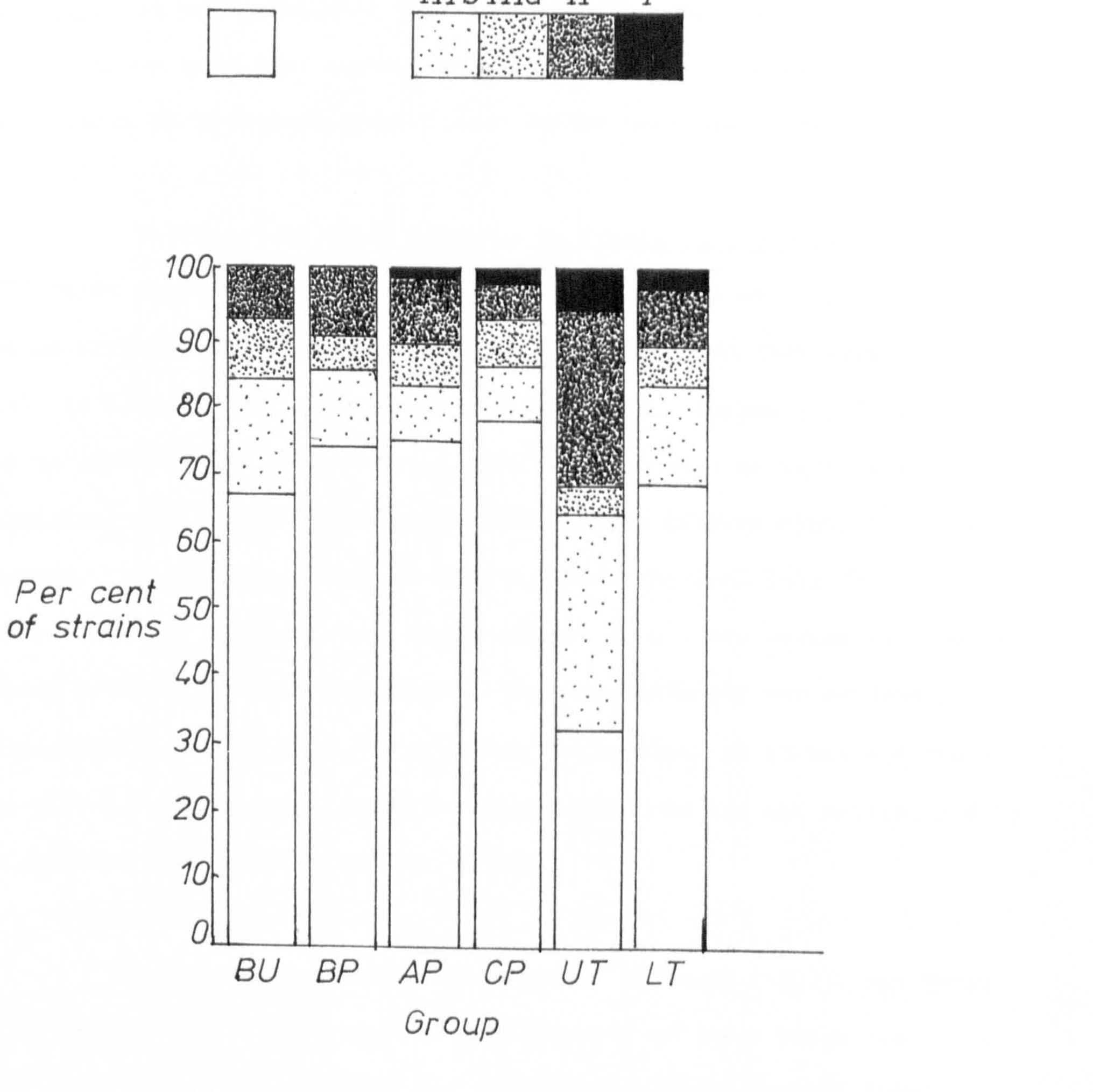
UT: Bacteriuric patients; strains isolated from the upper tract.

LT: Bacteriuric patients; strains isolated from the lower tract.

TABLE XXVII: GROWTH REQUIREMENTS

GROUP (No. of strains tested)	Growth on minimal medium	No growth on minimal medium: Grade I	II	IIIa	IIIb
BU (105)	70 67%		6 6%	11 10%	18 17%
BP (43)	32 74%		4 9%	2 5%	5 12%
BP/NP (34)	28 82%		4 12%	1 3%	1 3%
AP (75)	56 75%	1 1%	7 9%	5 7%	6 8%
AP/US (58)	45 77%		5 9%	4 7%	4 7%
CP (45)	35 78%	1 2%	2 4%	3 7%	4 9%
UT (19)	6 32%	1 5%	5 26%	1 5%	6 32%
LT (29)	20 69%	1 3%	2 7%	2 7%	4 14%

Fig. 21 GROWTH REQUIREMENTS



9) MUCINASE PRODUCTION

a) INTRODUCTION

Ross (1959) observed mucinase production in E. coli and Streptococcus faecalis. Titres were generally low except in enteropathogenic E. coli. It was postulated that mucinase production might enable strains to penetrate introital mucous, thus facilitating colonisation which has been shown to be a permissive factor in the development of UTI.

Mucinase was first detected in Vibrio cholerae culture filtrates which were found to liberate in vitro squamous epithelial cells from ileum and colon guinea pig segments, when they were normally held in place by mucin (Burnet and Stone 1947). Burnet (1947) went on to demonstrate the denaturing effect of mucinase on various mucins including those of guinea pig intestine, human ovarian cyst, and uterine cervix. Mucinase was found to have no action against hyaluronic acid and synovial fluid mucin whereas hyaluronidase from other bacterial species could split both these substrates. Mucinase activity was evidently independent of O antigen and endotoxin production, as it was not present in washed suspensions or autolysates of cells and was not neutralised by O antisera (Singer, Wei and Hoa 1948).

Ovomucin, a mucoprotein described by Young (1937), was found to be a useful substrate for the detection of mucinase which renders it non-precipitable by cetrimide and protamine sulphate (Burnet 1949).

b) MATERIALS AND METHODS

Mucinase was detected by its ability to render ovomucin non-precipitable with cetrimide. 1% cetrimide was found to produce an easily observable clot, although various substances have been used in

previous studies. Ross recommended barium sulphate as a flocculating agent, but because of its insoluble nature clot formation was not easily seen.

Preparation of ovomucin

Ovomucin was prepared according to the method of Freter (1955). The whites of six eggs were freed of chalazae and mixed with 1 litre of deionised water chilled to 4°C. The white precipitate formed after 30 minutes of stirring was left to settle and as much of the supernatant as possible was poured off. The precipitate was further separated by centrifugation, washed once in chilled deionised water and dissolved in an equal volume of 5% NaCl

Any precipitate remaining undissolved was removed by centrifugation, and the supernatant diluted with 0.085% NaCl until the addition of 1% cetrimide produced a precipitate at a dilution of 1/2 but not 1/4.

Preparation of mucinase

Mucinase extracts were prepared as described by Ross (1959). Nutrient agar slopes in Universal bottles were inoculated with 0.001ml of overnight broth cultures (10^5-10^6 organisms) and incubated for 18-24 hours at 37°C. The cultures were then frozen at -20°C overnight and thawed at room temperature. The expressed fluid was centrifuged at 3,000 r.p.m for 30 mins., and the supernatant tested for the presence of mucinase.

Mucinase titration

Doubling dilutions of mucinase extracts, upto 1/1024 were prepared in borate buffered saline at pH8 containing 0.1% $CaCl_2$ (Burnet 1949).

A control tube containing no mucinase extract was included. An equal volume of ovomucin was added to each tube, thoroughly mixed and incubated at 37°C for one hour. A drop of 1% cetrimide was added to each tube. The titration end-point was the highest dilution of mucinase required to prevent the formation of a fibrous clot.

Controls

A strain of Vibrio cholerae (NCTC 4Z8367) was used as a positive control; mucinase extracts gave a titre of 1/512. For reasons of safety the extract was prepared as previously described except that a cell-free preparation was obtained by filtration.

Two strains of E. coli which did not produce mucinase were included in every batch of tests. Six mucinase producing strains were titrated on five occasions in order to ascertain the reliability of the method.

c) RESULTS

Results were found to be reproduceable within the limits + or - one doubling dilution.

Mucinase production was not detected in the majority of strains from all groups. Titres obtained with mucinase producing strains were generally low; only 2 strains gave titres of more than 1/64 (table XXVIII).

There was no correlation between mucinase titre and periurethral colony count (fig. 22).

No significant differences were detected in the distribution of mucinase producing strains between the different groups, (fig. 23).

KEY

BU: Bacteriuric patients; urinary strains.

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections, periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains

UT: Bacteriuric patients; strains isolated from the upper tract.

LT: Bacteriuric patients; strains isolated from the lower tract.

TABLE XXVIII: MUCINASE TITRE

GROUP (No of strains tested)	0	1	2	4	8	16	32	64	128	256
BU (105)	89 84%		2 2%	4 4%	1 1%	2 2%	3 3%	2 2%	1 1%	1 1%
BP (43)	33 76%	1 2%	2 5%	1 2%	2 5%	2 5%		2 5%		
BP/NP (34)	26 76%	1 3%		1 3%	2 6%	2 6%		2 6%		
AP (75)	62 82%		5 6%	2 3%	2 3%	2 3%	2 3%			
AP/US (58)	46 79%		5 9%	2 3%	2 3%	1 3%	2 3%			
CP (45)	38 84%	2 5%	1 2%	3 7%		1 2%				
UT (19)	16 84%		2 11%	1 5%						
LT (29)	27 93%					1 3.5%	1 3.5%			

Fig.22 RELATION OF MUCINASE PRODUCTION TO PERIURETHRAL COLONY COUNT

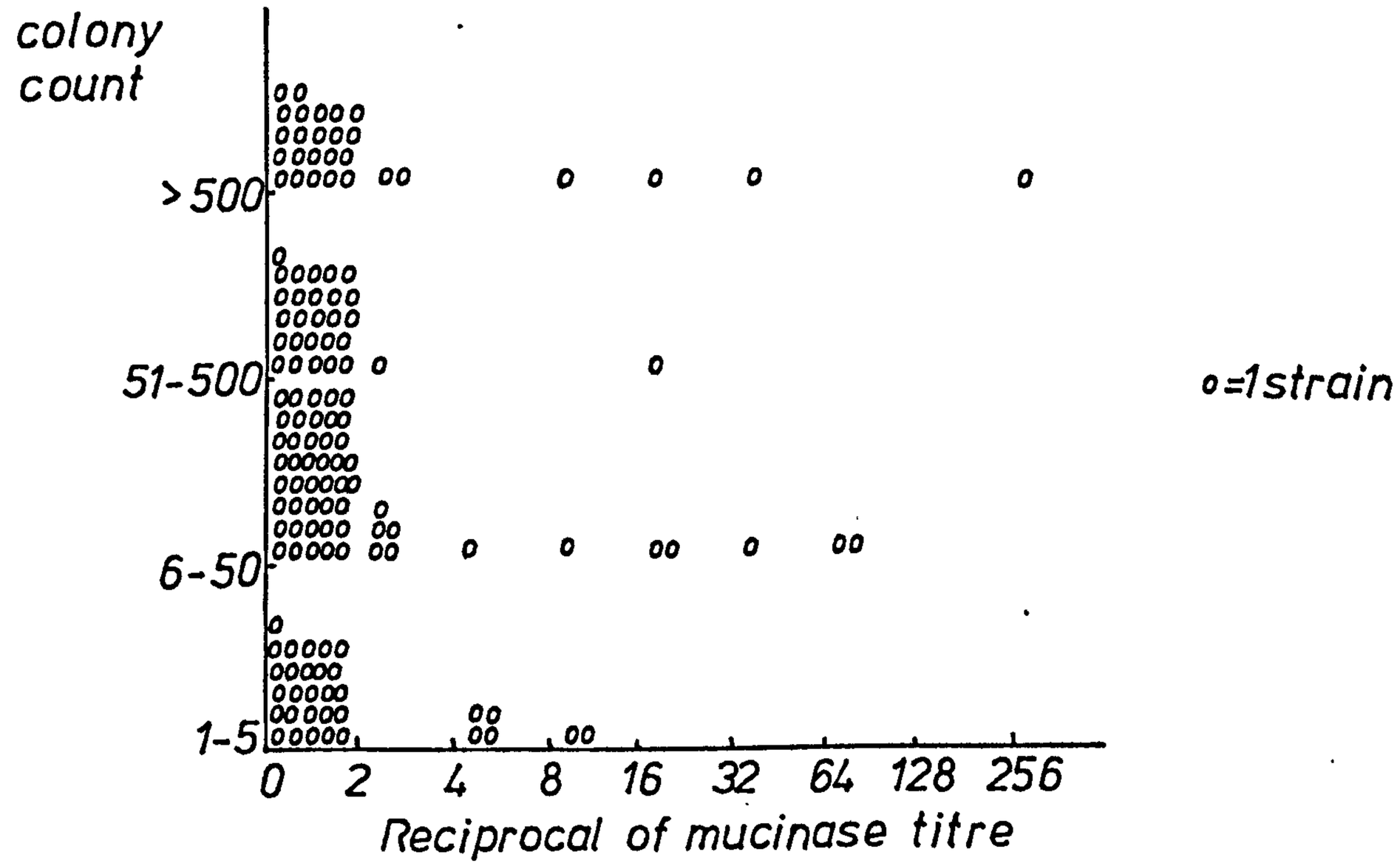
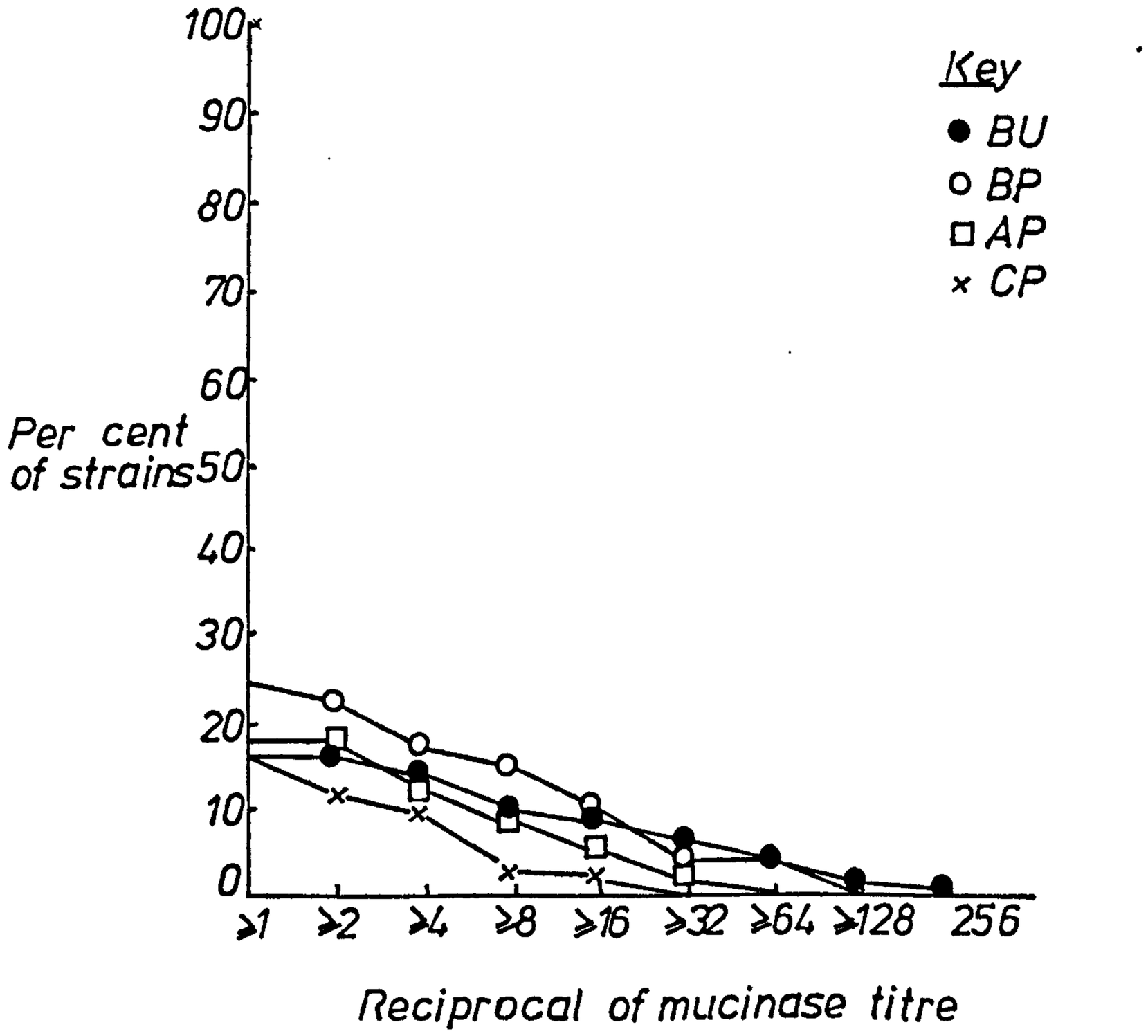


Fig.23 MUCINASE PRODUCTION



SECTION D: COMBINED PROPERTIES OF E. COLI

a) INTRODUCTION AND METHODS

It is possible that combinations of properties rather than individual properties determine pathogenicity. An examination of the frequency with which all the different combinations of properties found in individual strains occurred within the different patient groups was not possible by manual means but is currently being attempted by computer-based cluster analysis. There were, however, five properties that appeared to be particularly associated with pathogenicity and their combined occurrence in the different groups was assessed by reducing them to simple scores and treating the resulting numbers by conventional statistics.

The chosen properties were fimbriae production, haemolysin production, salicin fermentation, K antigen titre of 1/32 or more and O type 2,4,6,8,18ab or 75 all of which were especially associated with, although not exclusive to, strains from urines. Periurethral strains from normal subjects less often possessed these five properties, the differences in frequency with which the properties were found in urinary or periurethral strains being in each case statistically significant ($p < 0.05$). The other properties tested, H serotype, serum sensitivity, sensitivity to serine, spermine and urea, ability to grow on minimal medium and mucinase production were not found significantly more frequently in urinary or periurethral strains from normal subjects.

Since the five 'pathogenic properties' were individually found more frequently in urinary strains but nevertheless also occurred in strains from other sites, it was postulated that combinations of the five properties would be even more strongly associated with urinary strains and still less commonly encountered in non-invasive strains. Each of the five properties was scored '0' or '1' according

to whether the strain did or did not (1) possess fimbriae (2) produce haemolysin (3) ferment salicin (4) possess K antigen in a titre of 1/32 or more (5) belong to O serogroup 2,4,6,8,18ab or 75. Each strain was then given a score of 0-5 according to the number of properties exhibited and the frequency of scores in the different patient groups compared by standard statistical tests.

b) RESULTS

Results are given in table XXIX, and figures 24 and 25. The groups were compared using the Mann-Whitney U test for two independent samples. Strains from urines had significantly higher scores than periurethral strains from bacteriuric patients between infections, abacteriuric patients or normal individuals ($p=0.0018$, $p=0.0015$, $p<0.001$ respectively). Periurethral strains from abacteriuric patients (AP) and bacteriuric patients (BP) between infections were not significantly different from each other ($p=0.19$), but both groups had significantly higher scores than those from normal individuals ($p<0.001$ in both cases). The exclusion of periurethral strains which gave rise to infection did not significantly alter group BP. Strains from urethral syndrome patients were not different from the AP group as a whole.

Strains isolated from the upper urinary tract had significantly higher scores than those isolated from the lower tract ($p=0.03$), and lower tract strains had significantly higher scores than periurethral strains from normal individuals ($p=0.006$). There was no correlation between possession of the five properties by urinary strains and site of radiological abnormality in bacteriuric patients (table XXX, fig. 26).

KEY

BU: Bacteriuric patients; urinary strains.

BP: Bacteriuric patients between infections; periurethral strains.

BP/NP: Bacteriuric patients between infections; periurethral strains which did not give rise to infection.

AP: Abacteriuric patients; periurethral strains.

AP/US: Urethral syndrome patients; periurethral strains.

CP: Normal subjects; periurethral strains.

UT: Bacteriuric patients; strains isolated from the upper tract.

LT: Bacteriuric patients; strains isolated from the lower tract.

TABLE XXIX COMBINED PROPERTIES

GROUP (No. of strains tested)	SCORE OUT OF 5					
	0	1	2	3	4	5
BU (105)	1 (1%)	14 (13%)	22 (21%)	23 (22%)	28 (27%)	17 (16%)
BP (43)	1 (2%)	12 (28%)	15 (34%)	5 (12%)	5 (12%)	5 (12%)
BP/NP (34)	1 (3%)	10 (29%)	13 (38%)	3 (9%)	4 (12%)	3 (9%)
AP (75)	3 (4%)	11 (15%)	28 (37%)	17 (23%)	11 (15%)	5 (6%)
AP/US (58)	3 (5%)	8 (14%)	22 (38%)	13 (22%)	8 (14%)	4 (7%)
CP (45)	5 (11%)	16 (36%)	14 (31%)	5 (11%)	4 (9%)	1 (2%)
UT (19)	0	3 (16%)	3 (16%)	4 (21%)	5 (26%)	4 (21%)
LT (29)	2 (7%)	3 (10%)	10 (34.5%)	10 (34.5%)	2 (7%)	2 (7%)

Fig. 24 COMBINED PROPERTIES

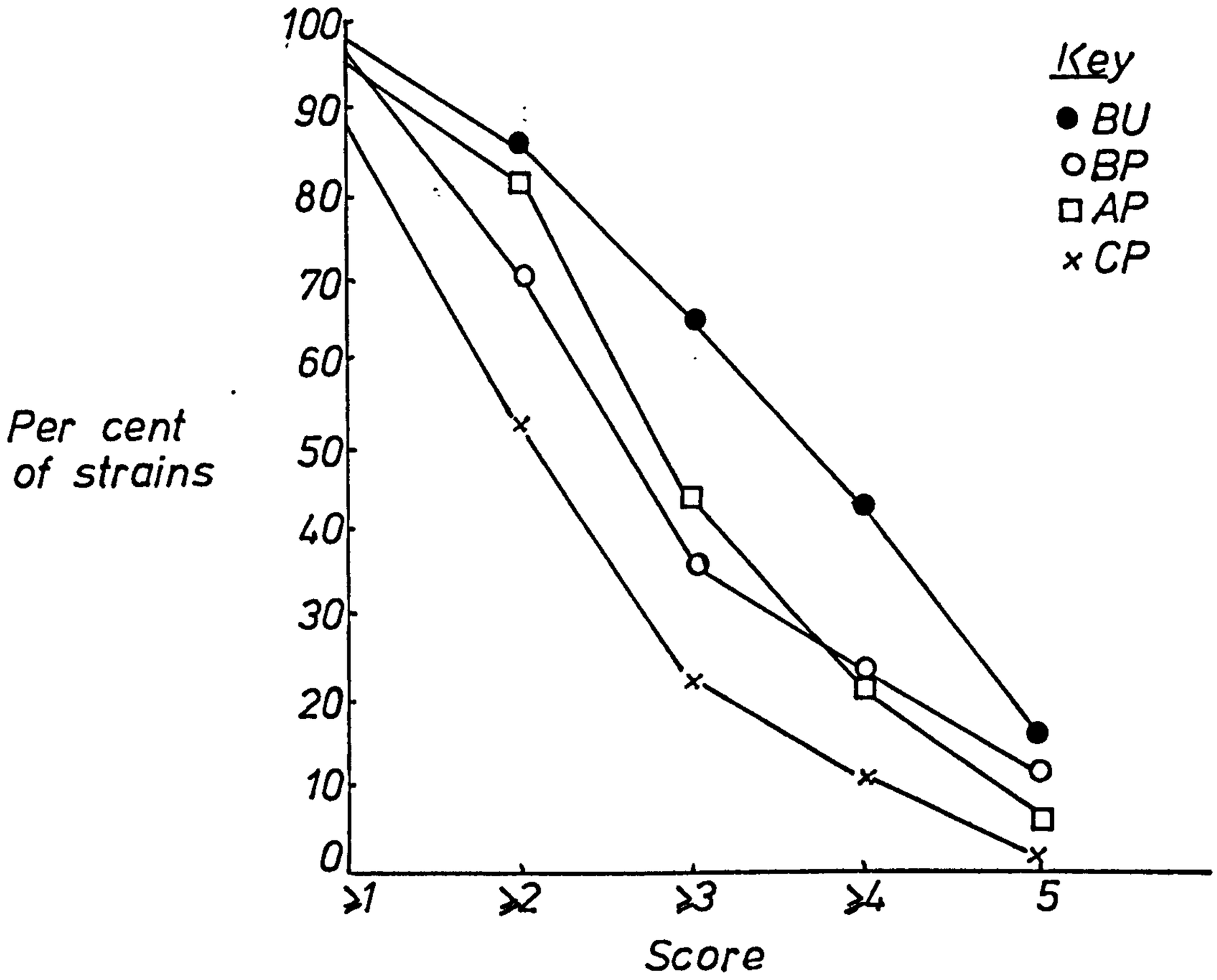


Fig. 25

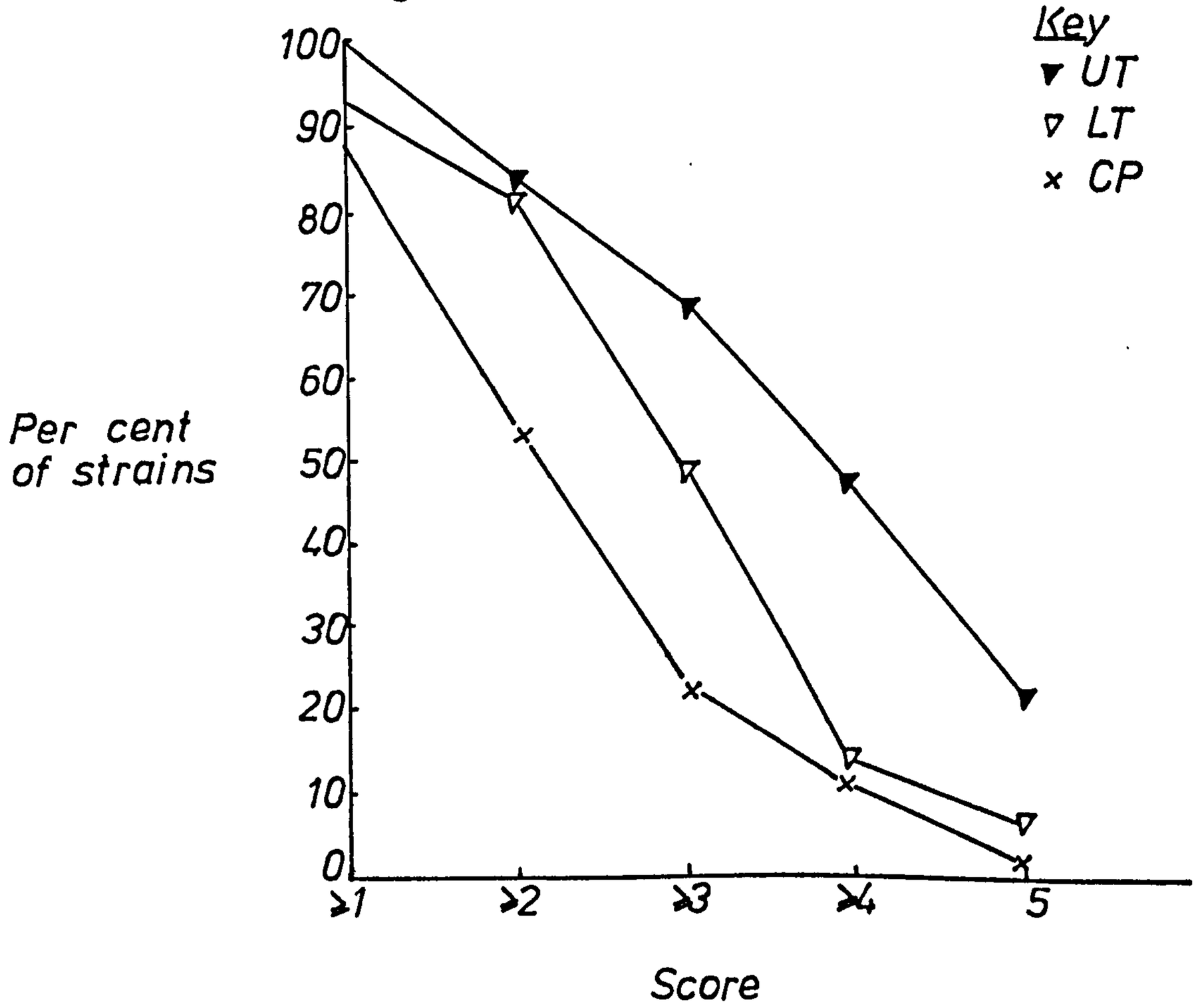
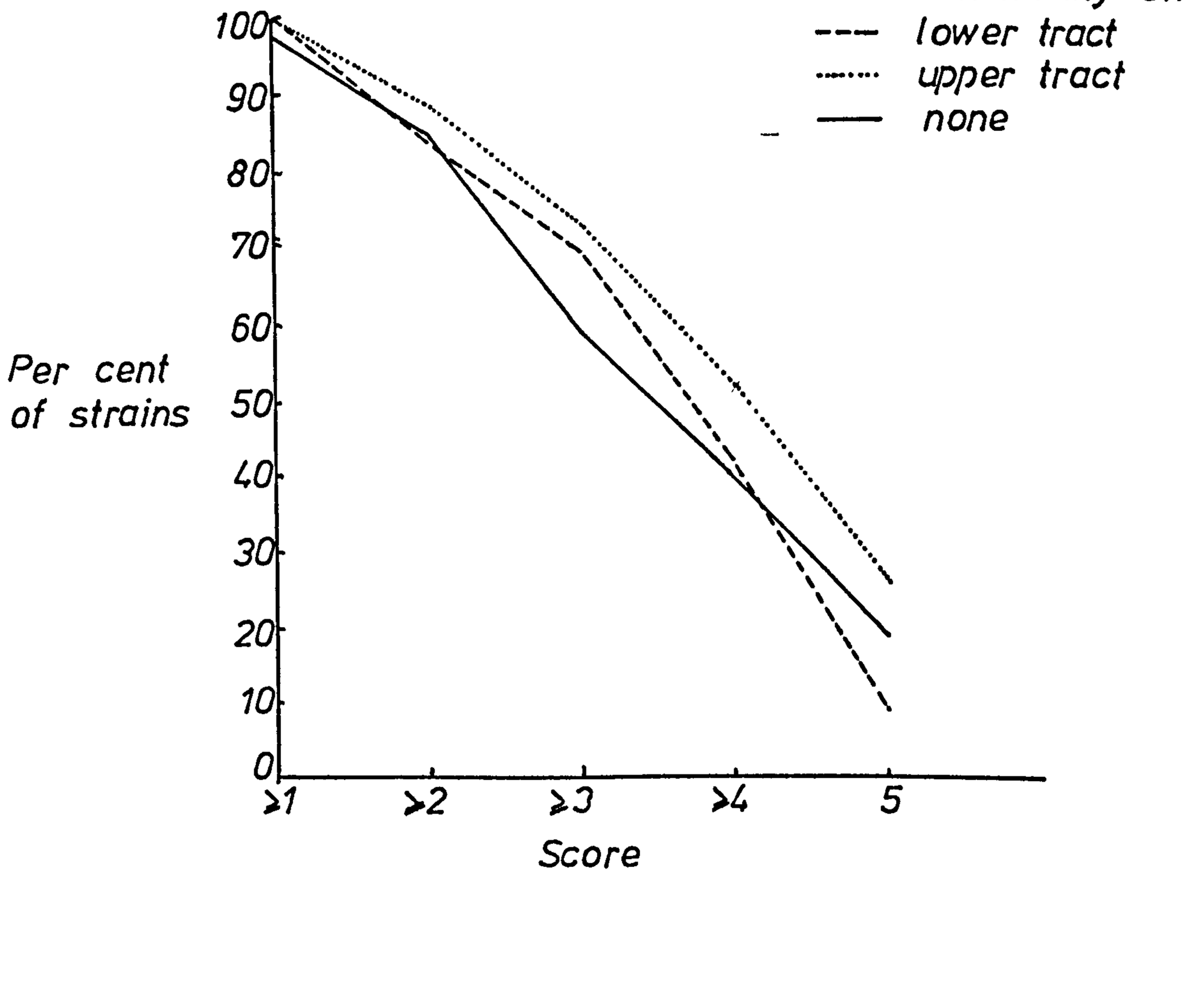


TABLE XXX RELATION OF COMBINED PROPERTIES TO RADIOLOGICAL ABNORMALITY

Site of radiological abnormality (No. of strains tested)	SCORE OUT OF 5					
	0	1	2	3	4	5
Upper Tract (19)	0		2(11%)	3(16%)	4(21%)	5(26%)	5(26%)
Lower Tract (33)	0		5(15%)	5(15%)	9(27.5%)	11(33.5%)	3(9%)
None (52)	1(2%)		6(12%)	14(27%)	9(17%)	12(23%)	10(19%)

Fig.26 RELATION OF COMBINED PROPERTIES TO RADIOLOGICAL ABNORMALITY



PART III: DISCUSSION

SECTION A: PROPERTIES OF E. COLI

1) O and H serotype
2) K antigen content
3) Serum sensitivity
4) Haemolysin production
5) Fimbriae production
6) Sugar fermentation
7) Sensitivity to serine, spermine and urea
8) Growth requirements
9) Mucinase production

1) O AND H SEROTYPE

a) O SEROTYPE

In agreement with other authors, a small number of strains were found to be responsible for the majority of infections. More than half the urinary isolates were of types O2, O4, O6, O8, O18ab, and O75. Other studies have shown types O1, O2, O4, O6, O7 and O75 to be most often associated with infection. Only three types O2, O6, and O75 were sufficiently common to each represent at least 7% of smooth typable strains. O2, O4, O6 and O75 were found to be the dominant types in other domicilliary investigations.

The common urinary types accounted for a significantly smaller proportion of periurethral isolates from normal subjects and abacteriuric patients indicating that these types were disproportionately associated with UTI. Although differences in the distribution of individual types was less striking, a small but significant excess of types O75 was observed in UTI compared to periurethral commensals from normal subjects. O18ab was rarely isolated from periurethral swabs, but the numbers of strains were insufficient for statistical tests to be performed. Grüneberg *et al* (1968) found O6 was more often associated with UTI than would be expected from the faecal carriage rate in normal individuals, and Roberts *et al* (1975) also noted a high prevalence of O6 strains in UTI. O18 has not been especially associated with infection in other studies, but this may be because the two sub-groups, ab and ac were not distinguished.

The results of this study lend support to the concept of uropathogenic O types, the common O types accounting for more cases of infection than would be expected from their prevalence in the

periurethral area. The reverse situation appeared to occur with O18ac. This type was responsible for less cases of infection than would be expected from the periurethral carriage rate, but the significance of this finding is doubtful because of the small number of strains involved. However, it must be emphasised that a considerable number of infections (46%) were due to types other than the common ones and it seems likely that any difference in virulence of common and rare O types is small. In addition, the immune status of the host must also influence selection of the infecting strain.

Only a small number of strains were isolated during localisation studies but O6, O4 and O75 accounted for a surprisingly large proportion (82%) of upper tract strains. Rantz (1962) observed that 72% of infections due to O4 and O6 were accompanied by the clinical signs and symptoms of pyelonephritis. Nimmich et al (1975) reported that types O6, O9, and O75 were associated with chronic pyelonephritis, although other authors failed to demonstrate any such correlation. There is no evidence from animal studies that certain O types are nephropathogenic, but further research on this topic may be worthwhile.

In addition to being a method for labelling strains, O typing is a qualitative measure of the lipopolysaccharide componant of the cell wall and may be directly related to virulence. The terminal O antigen sugar, which is responsible for specificity, has been shown to influence virulence in Shigella flexneri (Gemski, Sheahan, Washington, and Formal 1972). O antigen type may be related to resistance to the bactericidal activity of serum (see discussion, Section A, 3b), and there is evidence to suggest that certain O types induce immunological tolerance. Drach et al (1971) found many urinary tract pathogens had antigens in common with human blood group substances. They postulated

that such bacterial mimicry results in failure of the host to respond to antigenic stimuli. Marget et al (1975) found the O groups most commonly associated with UTI in children, O4 and O6, cross-reacted with blood-group substances especially A and B, and Cruz-Coke et al (1965) found a correlation between the incidence of E. coli UTI and blood-group. The incidence in group B subjects was unusually high, but group A subjects were no different to those belonging to groups AB or O. However, in view of the multitude of factors involved in host susceptibility, it would be surprising if such a simple relationship could be convincingly demonstrated.

There are many difficulties in testing the theory of special pathogenicity, especially where serotyping is used as a marker. A substantial proportion of strains are rough (15-20% in this series) and cannot be typed. It is possible that many of these strains belong to a single serotype. It is also necessary to use a full set of antisera; if limited sera are used the majority of UTI strains can be typed but strains which occur frequently in the environment and not in disease processes will not be detected. If hospitalised patients are studied the acquisition of nosocomial flora may invalidate comparisons between faecal strains and those from cases of infection. This is well illustrated by Winterbauer et al (1965) who found the carrier rate of types O4, O6, and O75 to be 60% in hospitalised patients and only 15% in non-hospitalised patients. Furthermore, types O4, O6 and O75 were responsible for the majority of hospital acquired UTI. If certain serotypes are more virulent than others their presence in the hospital environment would not be entirely unexpected. It is notable that those authors who were unable to demonstrate any difference in the distribution of serotypes in UTI and the environment all included hospitalised patients in their studies (Jackson, Kozij, Jao 1965; Turck and Petersdorf 1962; McGeachie 1965).

b) H SEROTYPE

In agreement with other workers H1, H4, H5 and H7 were found to be the most common urinary types (Maiztegui and Kass 1965; Pryles and Glagovsky 1965; Kunin, Deutscher and Paquin 1964). H5 was isolated more frequently from urines than would be expected from the periurethral carriage rate in normal subjects, but otherwise the groups were similar. In contrast to O type, the common H types were not especially associated with UTI and were found in approximately the same proportions whatever the site of isolation. The excess of H5 may reflect the increased frequency of O75 strains in UTI. Motile strains of this type were usually of H type 5.

Periurethral strains from normal subjects were less often motile than those from patients or urinary strains. However, the proportion of non-motile strains isolated from patients undergoing localisation studies was unexpectedly high (26% of upper and 38% of lower tract strains). These strains were isolated prior to those in the other groups and consequently had been stored for a longer period. Six passages through Craigies tubes may have been insufficient to induce motility in some of them. Previous work provides no evidence that motility is a feature of strains causing UTI, but immobilising flagellar antibodies have been demonstrated in the serum and urine of experimentally infected animals (Pazin and Braude 1969). Guze et al (1973) was unable to correlate motility or speed of motility with ability to produce pyelonephritis in mice, and McCabe and Jackson (1960) were unable to demonstrate superiority of a motile over a non-motile strain in ability to produce retrograde pyelonephritis in rats. Motility might be expected to confer some advantage by enabling strains to transverse the urinary tract but it is likely that mechanical factors, for example retrograde ureteric flow, are much more important.

c) O AND H TYPE COMBINATIONS

Particular O/H combinations were not peculiar to any group, but the numbers of strains were probably not sufficiently large for this type of investigation to be meaningful. Mabeck et al (1971) found no correlation between O, H or K types alone and localisation of infection, but certain combinations of types (O2:K1:H4, O4:K12:H5, and O6:K2ac:H1) were frequently associated with acute pyelonephritis.

2) K ANTIGEN CONTENT

Strains from urines commonly had high K antigen titres and they were significantly different from the periurethral strains of normal subjects. This difference was mainly due to an excess of urinary strains with K antigen titres of 1/32 or more, but there were also more strains without detectable K antigen in the control group. Periurethral strains from both bacteriuric and abacteriuric patients were richer in K antigen than those from normal subjects and the significance of this in relation to other differences is discussed on page 192. The result obtained in this study are comparable to those of Glynn et al (1971) who examined the K titres of strains from UTI and from faeces of uninfected individuals. In fact, if the haemagglutination inhibition test is taken to be one doubling dilution more sensitive in the present study the results of the two investigations are almost identical.

Glynn et al found that patients with renal involvment, as determined by raised serum O antibody titre, were usually infected with K rich strains. In patients in whom the O antibody titre was normal and infection was therefore considered to be restricted to the lower tract, the urinary strains were similar to faecal isolates. Thus, Glynn et al attributed the high frequency of K rich strains

in infected urines to the presence of patients with renal involvment. In the present study evidence for renal sequestration of K rich strains was not so clear cut. Strains isolated from the upper tract during localisation studies were somewhat richer in K antigen than those from the lower tract, but this difference was not statistically significant. There was no correlation between K antigen titre and site of radiological abnormality in bacteriuric patients, although Cattell et al (1972) demonstrated that patients with radiological abnormalities of the upper tract frequently have upper tract infection. In addition, lower tract strains were significantly richer in K antigen than periurethral strains from normal subjects. Glynn et al observed that the difference in K antigen content of upper and lower tract strains was not so striking if localisation was based on the results of the neomycin bladder wash-out test. They considered that this was because the presence of organisms in ureteric urine does not necessarily indicate renal involvment. The possibility that the association of high O antibody titre with infection by a K rich strain could be explained by the ability of such strains to stimulate a better O antibody response than strains lacking K antigen seems unlikely since K antigen is believed to mask O antigen.

Kalmanson et al (1975) were unable to correlate site of infection with K antigen. Strains isolated from the upper tract of patients during ureteric catheterisation did not possess K antigen more often than those isolated only from the lower tract. Brumfitt and Heptinstall (1960) compared the efficacy of a small number of E. coli strains to produce infection in rat kidney and concluded that K antigen was not of fundamental importance. Kalmanson et al (1975) were able to confirm the findings of Guze et al (1973) that

strains possessing K antigen were more virulent for the mouse kidney than those without K. Strains isolated from the upper tracts of patients were not more virulent for mouse kidney than those from the bladder. There are several possible explanations for these apparently paradoxical results:-

a) Mouse kidney may be an inappropriate experimental model as strains virulent in this animal may not be virulent in man.

b) As Glynn and Nicholson (1975) point out, ureteric bacteriuria may not always be accompanied by renal involvment. Thus strains isolated from ureteric urine cannot necessarily be considered nephropathogenic.

c) Perhaps most pertinent, Kalmanson et al did not correlate the quantity of K antigen with site of isolation but recorded only its presence or absence. In the present study, differences between groups would not have been so striking if only, the presence or absence of K antigen had been considered.

The occurrence and importance of K rich strains in UTI requires further clarification, but overall the results of the present study and previous publications suggest that selection of K rich strains takes place at two levels; firstly, during invasion of the lower tract and subsequently during invasion of the upper tract.

K antigen appears to confer resistance to the bactericidal action of complement, phagocytosis and antibody binding in porportion to the amount and type present (Howard and Glynn 1971). Evidence exists that suggests phagocytosis and local antibody production are

of importance in defence of the urinary tract (Vivaldi, Muñoz, Cotran and Kass 1965; Cobbs and Kaye 1966 and 1967; Burdon 1972). Thus K antigen could be of prime importance in overcoming host defence mechanisms. In particular, resistance of K rich strains to complement and antibody binding may facilitate multiplication in the kidney. Presumably K rich strains will also be more difficult to eliminate once disease processes have been initiated.

The relation of K quantity to serotype has not been fully investigated but recent evidence suggests that K serotype is an important pathogenicity determinant. Robbins et al (1974) found that 84% of neonatal meningitis cases due to E. coli were caused by K type 1 strains, whilst this type was present in only about 13% of stool cultures. It would be of interest to examine the K serotype of strains causing UTI and relate this to the amount of K present.

3) SERUM SENSITIVITY

Periurethral strains from both bacteriuric patients and normal individuals were similar to urinary isolates. This is in agreement with various other reports which were unable to show that serum resistance is a special feature of strains causing UTI (Roantree and Rantz 1960; Olling, Hanson, Holmgren, Jodal, Lincoln and Lindberg 1973). As complement is absent in urine it is not surprising that serum resistance seems to confer no advantage in invasion of the lower tract. However, periurethral strains from abacteriuric patients were significantly more sensitive to the bactericidal action of serum than urinary strains. The former group was similar to periurethral strains from bacteriuric patients for all other properties tested and as there is no adequate explanation for this anomaly, it seems likely that this result is merely fortuitous.

Serum resistant strains were slightly more common in patients with upper tract abnormalities and in those patients shown to have upper tract infection but these differences were not statistically significant. The numbers of strains in the upper and lower tract groups were small, and further work is required to clarify the relationship of serum sensitivity to localisation of infection. Guze et al (1973) demonstrated that serum resistance was related to ability to produce retrograde pyelonephritis in mice, although Olling et al (1973) did not find that serum resistant strains were especially associated with pyelonephritis in children. An important factor in the pathogenesis of renal infection may be the ability of the patients serum to kill the infecting strain. Gower et al (1972) and Waisbren and Brown (1966) reported an inhibitory factor in the serum of patients with chronic pyelonephritis which prevented bactericidal activity against the invading strain, regardless of its sensitivity to normal human serum.

a) RELATION OF O AND K ANTIGEN TO SERUM SENSITIVITY

In contrast to other studies, K antigen content was not found to be directly related to serum sensitivity, although resistant strains poor in K antigen were uncommon. Glynn and Howard (1970) demonstrated K rich strains were resistant to serum killing and Feingold (1969) found conversion of a serum resistant strain to serum sensitivity was accompanied by inhibition of surface polysaccharide synthesis.

Rowley (1971) postulates that sensitivity to complement depends on the thickness of the cell wall and only if complement is

activated near to the cell membrane will the organism be killed. He suggests that sensitive strains have insufficient lipopolysaccharide to cover the rough core and cell wall proteins and in the present study the majority of rough strains were serum sensitive. However, 7 rough strains were serum resistant and this apparently paradoxical finding is better explained if the model proposed by Taylor (1975) is accepted. He considered roughness and serum resistance to be due to specific factors in the cell wall which cannot be retained if the lipopolysaccharide layer is degraded. It is possible that the 7 rough, resistant strains had lost the "smooth" factor but retained the "serum resistance" factor.

Presumably K antigen causes complement to be activated at a distance from the cell membrane, but K rich strains were commonly found to be serum sensitive. It is conceivable that the K antigen layer is not evenly distributed and activation of complement close to the cell membrane occurs at some points on the cell surface.

b) <u>RELATION OF O TYPE TO K ANTIGEN AND SERUM SENSITIVITY</u>

Both serum resistance and high K titre were associated with serotypes O2, O6 and O7. In addition, strains of O type 18ac and 75 commonly had high K titres. The significance of these findings is not clear. O18ac and O7 were not amongst the common urinary types, although O7 was found to be a common urinary pathogen in other studies (eg. Pryles and Glagovsky 1965; Ewing and Davies 1961; Kunin and Halmagyi 1962; Grüneberg, Leigh and Brumfitt 1968). The validity of such correlations would be better confirmed by the examination of a larger number of strains. Such comparisons were severely limited by the small number of serogroups containing sufficient numbers of strains for analysis.

4) HAEMOLYSIN PRODUCTION

Haemolytic strains were responsible for more cases of UTI than would be expected from the periurethral carriage rate in normal subjects and abacteriuric patients. Haemolytic strains were relatively rare in normal subjects, occurring with a frequency of only 9%. Can haemolytic strains be considered uropathogenic? Cooke and Ewins (1975) found strains from cases of UTI more commonly produced haemolysin than those from normal faeces, but McGeachie (1966) was unable to demonstrate any such difference. This may have been due, at least partially, to his selection of strains for study. He compared strains isolated from urines in significant numbers (ie. $\geq 10^5$/ml) with those considered to be contaminants in an undefined group of patients. In the present study, comparison of urinary strains with periurethral contaminants in the same group of patients, ie. bacteriuric group, would have revealed little difference in the occurrence of haemolytic strains. In addition, he does not state whether patients were hospitalised and, as discussed in connection with serotyping studies, acquisition of nosocomial flora may obscure the relationship between prevalence and virulence. Fried and Wong (1970) found only 11% of strains from urines received in a diagnostic laboratory were haemolytic. A proportion of these infections can be assumed to be secondary to debilitating disease, instrumentation and immunosuppression, and it is accepted that under such circumstances patients can acquire infections with organisms of normally low pathogenicity. However, this seems unlikely to wholely account for such low frequency of haemolytic strains compared to the present study.

Haemolytic strains appear to have a special predilection for the upper tract. More than half the strains isolated from the upper tracts of patients who underwent localisation tests were

haemolytic and patients with radiological abnormalities of the upper tract were more often infected with haemolytic strains than those with lower tract or no abnormality. Supportive evidence is provided by the work of Fried et al (1971) and Fried and Wong (1970). Their studies indicate that haemolytic strains have a superior ability to produce renal infection than non-haemolytic strains. De Pauw et al (1971) demonstrated that haemolytic E.coli were capable of renal lysosome disruption, and this may be relevant to the mechanism of renal infection. In contrast, Kimball et al (1964) were unable to demonstrate nephropathogenicity of haemolytic strains. Both Fried et al and Kimball et al used similar experimental models, the only difference being that Kimball used femab Sprague-Dawley rats and Fried used males.

The excess of haemolytic strains in UTI does not appear to be due solely to the presence of patients with upper tract infection. Significantly more haemolytic strains were isolated from patients shown to have lower tract disease only, than from normal subjects. It seems likely that haemolytic strains are selected to some extent during invasion of the lower tract and to a greater degree in upper tract infection.

Alpha haemolysin has been shown to have a variety of toxic effects (Cooke and Ewins 1975; Williams Smith 1963; Chaturvedi, Mathura, Khan and Mehrotra 1969). Cooke and Ewins also observed that alpha haemolysin was closely associated with liquid, but not solid, haemolysin production. In contrast to their work all strains in the present study which produced solid haemolysin produced liquid haemolysin also. The relation between these types of haemolytic activity requires further clarification.

a) RELATION OF HAEMOLYSIN PRODUCTION TO O TYPE

The majority of strains belonging to O types 4 and 6 were haemolytic, and these serotypes have been associated with haemolysin production by several other authors. Vahlne (1945) found strains of types O2, O4 and O6 were usually haemolytic, Sjöstedt (1946) strains of types O2, O3, O4, O6 and O22 and Cooke and Ewins (1975) strains of types O2, O4, O6 and O75. It is possible that the prevalence of these O types in UTI is at least partially related to the toxic activity of haemolysin.

5) FIMBRIAE PRODUCTION

Fimbriate strains more often caused infection than would be expected from the periurethral carriage rate in normal subjects suggesting that some selection had taken place. However, non-fimbriate strains were still capable of causing infection. The periurethral carriage of fimbriate strains in both bacteriuric and abacteriuric patients were greater than in normal subjects, although not significantly so. The work of Buck (1967) and Gupta *et al* (1958) does not support the concept that fimbriate strains are more frequent in UTI. They found that only about half their urinary strains were fimbriate. In contrast, Duguid (1968) reported fimbriae production in 83% of urinary strains, and in this study 79% of urinary strains were capable of producing fimbriae. There is no apparent reason for this discrepancy; Buck certainly employed similar techniques to those used by Duguid and in the present study.

Many strains exhibited phase variation, requiring repeated subculture in order to select out sufficient fimbriate organisms to give the haemagglutination reaction. The phase state of organisms

in vivo has not been investigated and in this study only the potential of strains to produce fimbriae was assessed. This may not necessarily reflect the in vivo situation.

The adhesive powers of fimbriae, readily demonstrable in vitro, might be expected to confer resistance to removal by urine flow. The scanning electron microscope studies of Skoluda et al (1974) suggest that organisms adhere to the surface of the bladder mucosa during the course of infection and hydrodynamic clearance is important in keeping the urinary tract sterile (Cattell, Kelsey Fry, Spiro, Sardeson, Sutcliffe, O'Grady 1970; Shand, Mackenzie, Cattell and Cato 1968; Hinman 1968). The failure of some patients to eliminate infection by drinking large volumes of water and frequent micturition was attributed in a theoretical study by Mackintosh et al (1975) to the presence of organisms attached to the bladder wall. Patients with small bladder residues, but otherwise normal tracts, in whom infection is confined to the bladder should be able to eliminate 10^8 organisms per ml of urine from the bladder in 4-9 hours by a process of dilution and discharge at micturition. However, patients commonly exhibited a biphasic response consisting of an initial rapid fall in concentration followed by a steady output of about 5×10^4 organisms per ml. Mackintosh et al demonstrated mathematically that 10^6 wall-bound organisms per cm^2 could result in the biphasic response seen in patients. Adherence may also be an important factor in urethral colonisation as voiding exerts a flushing action (see p.26), and organisms firmly attached to the mucosa should resist removal. If the hypothesis that fimbriae assist in bacterial adherence is accepted, it is perhaps surprising that fimbriae could not be demonstrated in 21% of urinary isolates. It is of course likely that there are other factors involved in adherence.

6) SUGAR FERMENTATION

Fermentation of all three sugars tested and salicin alone were more frequently properties of urinary strains than commensal strains from normal subjects. In contrast, McCabe and Jackson (1960) observed that strains isolated from patients with pyelonephritis more often failed to ferment sucrose and salicin, whilst Guze *et al* (1973) correlated virulence for mouse kidney with dulcitol fermentation, but not sucrose or salicin fermentation. Kalmanson *et al* (1975) confirmed that dulcitol fermenting strains were significantly more virulent for mouse kidney than non-fermenters, but found strains isolated from the ureteric urine of patients were no more likely to ferment dulcitol than those isolated only from the bladder. In the present study no differences were detected between upper and lower tract strains.

It is possible that the association between fermentation of certain sugars and pathogenicity in man is fortuitous, and reflects the relatively small number of strains implicated in UTI as compared to the much larger number present in the environment. Guze *et al* were unable to offer an adequate explanation for the increased virulence shown by dulcitol fermenting strains.

7) SERINE, SPERMINE AND UREA SENSITIVITY

a) SERINE SENSITIVITY

A marked variation in the sensitivity of strains to serine was observed over a wide range of concentrations. It was somewhat surprising that as little as 1mcg/ml was inhibitory for some strains. Serine is a common constituent of proteins in *E. coli* and is a precursor of a large number of compounds made from single-carbon units (Mandelstam and McQuillen 1973). Rowley (1953) demonstrated

that inhibition was by bacteriostasis and could be reversed by the addition of other amino acids. Glycine was found to totally neutralise the antibacterial activity of serine whilst methionine or alanine gave partial inhibition. However, Roberts et al (1968) were unable to abolish inhibitory activity completely by the addition of other amino acids.

Approximately 5-50 mcg/ml of urine is considered to be the normal range of free serine levels in adult females (Diem 1962). Many strains were sensitive to less than 50 mcg/ml at pH 7.2 or 6.0 but at pH 5.5 only 2 of the 40 strains tested were inhibited by 64 mcg/ml. It is possible that serine contributes to the antibacterial activity of urine at higher pHs but this effect is probably minimised by the antagonistic activity of other amino acids normally present. Examination of the sensitivity of strains to different concentrations of serine in urine, rather than in conventional media, may elucidate its in vivo antibacterial activity more fully. It is of interest that serine became less inhibitory as the isoelectric point (pH 5.68) was approached. Antibacterial activity may be a function of ionic charge.

b) SPERMINE SENSITIVITY

Less strain variation was observed in spermine sensitivity and there was no overall difference between the groups. All strains were able to tolerate 32 mcg/ml and this is well above the average urine levels of 1.7 mcg/ml in normal females and 5.25 mcg/ml in pregnant women (Russell, Levy, Schimpff and Hawk 1971). Its inhibitory action was virtually abolished at pH 6.0 or less, thus this substance probably does not contribute to the antibacterial activity of urine.

c) UREA SENSITIVITY

Some strains were more sensitive to inhibition by urea than others, but there was little difference between the groups. The normal physiological range of urea is 0.1-3% (Asscher, Sussman and Weiser 1968). At pH 7.2 90% of strains had MICs of 3.0% or more; at this pH urea probably has little inhibitory effect in urine. However, as the pH was lowered strains became more sensitive, and at pH 5.5, which is well within normal physiological limits, approximately 30% of the strains tested were inhibited by 3% urea. Thus, the conditions of low pH and high urea content which prevail at night could be implicated in the prevention of bacterial growth. The decrease in sensitivity to urea withincrease in pH may also at least partially explain the finding that urine loses much of its inhibitory activity as pH is increased (Asscher, Sussman, Waters, Davis and Chick 1966; Yeaw 1940).

The inhibitory effect of urea was not considered to be osmotic in origin by Weinstein and McDonald (1945). They found that concentrations which inhibited bacterial growth had no effect on human erythrocytes over 24 hours. Kay (1968) observed that supplementation of urine with NaCl or NH_4Cl_2 had little inhibitory effect compared to supplementation to an equal osmolality with urea. In addition, removal of urea from urine by means of urease depleted inhibitory activity.

There was no evidence that strains which were resistant to the urinary constituents tested more often caused UTI. Investigations into the sensitivity of strains to normal urine may prove more fruitful.

8) GROWTH REQUIREMENTS

Most of the lower tract strains were able to grow on minimal medium but the majority of upper tract strains grew only in the presence of amino acids or vitamins. Although the numbers of strains in these two groups were small this difference was of statistical significance. In contrast Guze et al (1973) correlated virulence of E. coli strains for the mouse kidney with ability to multiply in minimal medium. The reason for the nutritional fastidiousness of upper tract strains is not apparent but it is possible that strains adapted to life in the upper tract eventually lose some of their chemosynthetic abilities if a rich supply of organic nutrients is available.

Some strains required a combination of growth supplements whilst others utilised any one of a number, and strains were graded according to their requirements (p154). Although the number of strains unable to grow on unsupplemented minimal medium varied between the groups, the percentages belonging to the different grades remained approximately the same in each group.

9) MUCINASE PRODUCTION

Mucinase activity was detected in only a few strains and was generally of low titre. It was postulated that penetration of the mucous layer would aid colonisation of the periurethral area by allowing contact of the organisms with the cell surface, enabling them to resist the normal movement of mucus. However, there was no correlation between mucinase production and periurethral colony count. It must be emphasised that a crude bacterial extract was used for mucinase detection and this method may not be very sensitive. Ross

(1959) found mucinase production was a feature of enteropathogenic E. coli strains and reported mucinase titres of 1/32 or more in only 7% of commensal strains. The proportion of commensal strains producing low titres was greater than in the present study although the reason for this is not apparent. There was only one difference in technique between the two studies, the use of CTAB instead of barium sulphate as a flocculating agent.

The presence of E. coli on the periurethral area is considered to be a permissive factor in the development of UTI, but there is no evidence to suggest that the number of organisms is important. Marsh et al (1972) found that the number of colonies isolated from introital swabs did not influence the development of UTI; those patients with high counts being no more likely to develop bacteriuria than those with low counts. This suggests that other overriding factors influence the development of UTI.

SECTION B: COMBINED PROPERTIES OF E. COLI

1) Strains isolated from urines

2) Periurethral strains from bacteriuric patients

3) Periurethral strains from abacteriuric patients

4) Periurethral strains from normal subjects

5) Upper and lower tract strains

1) STRAINS ISOLATED FROM URINES (BU)

Strains isolated from urines were richer in five properties (fimbriæ production, haemolysin production, high K content, and O type 2, 4, 6, 8, 18ab or 75) than strains isolated from periurethral swabs of normal subjects and this difference was highly significant ($p < 0.001$). Only 11% of periurethral strains from normal subjects possessed four or more of these properties compared to 43% of strains from urines. At the other end of the scale, 47% of periurethral strains from normal subjects possessed none or one property compared to 14% of urinary strains. This suggests that strains rich in these five properties (abbreviated to FP) are superior in ability to invade the urinary tract, and supports the "special pathogenicity" theory that the frequency with which different strains occur in UTI is dependent not only on their prevalence in the environment, but also on their pathogenicity.

The difference between these two groups of strains was more striking when all five properties were considered together rather than singly. It is, of course, likely that there are other characteristics which influence pathogenicity and it is possible that if more properties had been investigated the difference between the two groups would have been even greater.

2) PERIURETHRAL STRAINS FROM BACTERIURIC PATIENTS (BP)

Periurethral strains from bacteriuric patients when they were between infections were significantly richer in FP than those from normal subjects ($p < 0.001$). Exclusion of the nine strains shown to give rise to infection did not decrease the percentage of such strains in this group. Thus, the periurethral area of bacteriuric patients was more frequently colonised by FP rich strains than the

periurethral area of normal subjects, although they did not always give rise to infection. Such colonisation may predispose to infection simply by acting as a source of potential pathogens ready to invade the urinary tract at a time when host defence mechanisms are impaired. The reason for the difference in the periurethral E. coli flora of normal subjects and bacteriuric patients between infections is not altogether clear. There are two likely explanations: firstly, FP rich strains were seeded onto the periurethral area during previous episodes of infection and remained there. Secondly, colonisation of the gut occurred during previous episodes of UTI with the infecting FP rich strain and the periurethral flora was derived from this source.

Support for the "special pathogenicity" theory was also obtained when strains from urines were compared with periurethral isolates in the same, ie. bacteriuric, group of patients. Urinary strains were significantly richer in FP than periurethral strains isolated when the patients were between episodes of infection ($p = 0.0018$), indicating that some selection of strains occurred.

3) PERIURETHRAL STRAINS FROM ABACTERIURIC PATIENTS (AP)

Periurethral strains from abacteriuric patients were almost identical to periurethral strains from bacteriuric patients. They were significantly less often FP rich than urinary strains ($p = 0.0015$) but significantly richer in FP than those from normal subjects ($p < 0.001$). When strains from patients displaying the classical frequency/dysuria symptoms of the urethral syndrome were considered in isolation (AP/US) they were not different from the AP group as a whole. This somewhat unexpected finding cannot be easily explained.

It has been shown that patients presenting with symptomatic abacteriuria will, if followed for a sufficient length of time, fall into two categories; those who are between episodes of infection and those whose symptoms are unrelated to urinary infection (O'Grady, Charlton, Kelsey Fry, McSherry and Cattell 1972). In the present study patients in the first category were, where possible, excluded from the urethral syndrome group. All patients were followed for at least 3 months during which time several routine urine cultures, and if necessary supra-pubic aspirations, were performed in an attempt to define those with transient bacteriuria of persistantly low, ie. $<10^5$/ml, bacterial counts. Patients also had access to an "on demand" urine culture service in the casualty department of St. Bartholomew's Hospital at times when the usual laboratory and clinic facilities were not available. It is still possible that some urethral syndrome patients had episodes of transient bacteriuria which were missed, and it would be expected that their periurethral strains would resemble those from bacteriuric patients. However it seems unlikely that there was a sufficient number to account for the close similarity of the two groups of strains. Cattell et al (1975) demonstrated transient bacteriuria in 13 of 98 symptomatic abacteriuric patients by obtaining urine cultures at the very onset of the symptomatic episode. Seven subsequently became sterile whilst the remainder had reduced bacterial counts. However, bacteriuria could not be demonstrated in the remaining 85 patients.

Both Fair (1971) and Moore and Hira (1965) consider that some urethral syndrome cases are due to urethral inflammation of infectious origin. It is tempting to postulate that in some urethral syndrome patients UTI occurs which is confined to the urethra. If

it is further postulated that infection is caused by FP rich strains then the high frequency of such strains in this group of patients would not be unexpected. However, Charlton et al (1972) and O'Grady et al (1972) were unable to demonstrate any inflammatory response in symptomatic abacteriuric patients suggesting that the aetiology of this disease does not involve an infectious agent. Hormonal disturbances were considered to play a significant role in the symptomatology of some patients, but in the majority the cause was not known. Whatever the cause of this disease,or group of diseases, if it has a physical origin then it is possible that the periurethral environment is changed and the presence of FP rich strains may be the result of this rather than the cause of the symptoms.

4) PERIURETHRAL STRAINS FROM NORMAL SUBJECTS (CP)

FP rich strains appear to be rarely carried by normal subjects. Only 5 strains (11%) exhibited four or more of the five properties. It would be of interest to examine strains from other environmental sites, such as hospitals, for these properties.

5) UPPER AND LOWER TRACT STRAINS

The results of this study indicate that possession of FP not only influences the ability of strains to invade the urinary tract, but also localisation of infection. In spite of the small number of strains in these two groups, those isolated from the upper tract of patients undergoing localisation studies were significantly richer in FP than those isolated only from the lower tract. This may reflect either the superior ability of these strains to reach the upper tract or better ability to combat upper tract defence mechanisms, or indeed combination of both these factors. Lower tract strains were still significantly different from periurethral strains

of normal individuals indicating that the excess of FP rich strains in group BU was not due merely to the presence of patients with upper tract infection. Thus, selection of strains appears to take place firstly during invasion of the lower tract, and secondly during ascent to the upper tract.

It was expected that a similar relationship would be detected between sites of radiological abnormality and the FP score of the infecting strain. Apart from haemolysin production, which was frequently associated with strains from patients with upper tract abnormalities, no such correlation was observed. However, it must be emphasised that the site of radiological abnormality provides only a crude indication of localisation (Cattell, Charlton, McSherry, Kelsey Fry and O'Grady 1972).

CONCLUSION

The aims of this study were two-fold; firstly to determine whether some strains of E. coli were disporportionately associated with UTI and secondly to investigate properties which might be of value in overcoming host defence mechanisms. Strains isolated from cases of UTI were compared with periurethral commensal strains in the same group of patients, ie. those suffering from recurrent UTI, from persistantly abacteriuric patients and from normal individuals. The properties investigated were O and H serotype, K antigen content, sensitivity to the bactericidal activity of serum, haemolysin production, fimbriae production, fermentation of sucrose, salicin and dulcitol, sensitivity to serine, spermine and urea and mucinase production.

Five properties (FP) were significantly more often exhibited by strains from urines than by commensal strains from normal individuals

and these were O type 2, 4, 6, 8, 18ab or 75, high K antigen titre, haemolysin production, fimbriae production and fermentation of salicin. Some of these properties can be directly related to inhibition of host defences: K antigen has been shown to confer resistance to phagocytosis, complement and antibody binding. Although not conclusively demonstrated, fimbriae are believed to anchor organisms to surfaces thus enabling them to resist removal by urine flow. Haemolysins have been implicated in a variety of toxic activities and of particular interest is the ability to disrupt renal lysosomes. Certain O antigenic types may be immunologically tolerated and this may be particularly relevant where there is renal involvement and organisms are in more intimate contact with circulating antibody. High K antigen content and and haemolytic ability appear to be unduly associated with certain O types and the increased frequency in UTI of strains belonging to these O groups may be partially explained on this basis. The reason for the superior fermentative ability of urinary strains is not yet understood.

Strains were awarded scores according to the number of these properties exhibited and the different groups were compared statistically. Strains isolated from urines had significantly higher scores than those isolated from periurethral swabs of both patients and normal individuals. Thus, different E. coli strains are not found in the urinary tract solely in proportion to their availability on the periurethral area. This supports the concept of "special pathogenicity" in that some strains (in this study those which were FP rich) are more pathogenic for the urinary tract than others.

The importance of the status of host defence mechanisms in determining onset of infection was well illustrated by a study of

periurethral strains from bacteriuric patients isolated at a time when they were between infections. Although all these patients were susceptible to infection, in that they had a previous history of recurrent UTI, FP rich strains were often present on the periurethral area but either could not be shown to give rise to infection at all or were responsible for episodes of infection weeks after they were demonstrated on the periurethral area. This suggests that, in common with many other microbial infections, pathogens may be present but only give rise to infection when host defence mechanisms temporarily become ineffective.

FP rich strains were disproportionately associated with upper tract infection suggesting that these properties endow a superior ability to overcome defence mechanisms in the upper tract. Strains isolated from the lower tract only were still significantly different from periurethral strains of normal individuals. Thus, the excess of FP rich strains in UTI was not considered to be solely due to the inclusion of patients with upper tract disease, rather selection of FP rich strains was considered to take place during invasion of both the lower and upper tracts. Inability to grow on minimal medium was also a feature of upper tract strains, although the reason for this is not apparent.

Of particular interest was the finding that periurethral strains from urethral syndrome patients were similar to those from bacteriuric patients isolated at a time when they were between infections. They were significantly less often FP rich than strains from urines, but were still significantly different from those from normal individuals. Although there is at present little evidence to suggest that the urethral syndrome is infectious in origin these

results indicate that these patients are in some way bacteriologically different from normal individuals.

Other properties investigated in this study, H serotype, mucinase production, resistance to the bactericidal activity of serum and sensitivity to serine, spermine and urea were not found to be useful parameters of pathogenicity but some points of interest emerged from these investigations. The inhibitory activity of urea was found to be pH dependent. Inhibitory activity was increased as the pH was lowered and this may partially explain the antibacterial activity of acid, concentrated urine noted by so many workers. In contrast to other studies serum sensitivity was not found to be directly related to K or O antigen content as measured by haemagglutination inhibition activity and smooth/roughness respectively. This supports the concepts proposed by Taylor (1975) that roughness and serum sensitivity are due to specific cell-wall factors and not merely a function of its thickness.

It seems reasonable to suppose that there are many other factors which influence the pathogenicity and virulence of bacterial strains. Some possibilities are differences in quantity and quality of toxins and production of immunosuppressive substances. The finding that some strains of E. coli possess a superior ability to overcome the defence mechanisms of the urinary tract promotes the possibility that some also possess a superior ability to overcome defence mechanisms in other parts of the body and it would be of interest to investigate further properties of strains isolated from other types of infection.

APPENDICES

Appendix 1 Radiological abnormalities of bacteriuric patients

Appendix 2 Instructions for: a) Use of filter strips

b) Introital (periurethral) swab technique

Appendix 3 Table headings and figure legends

APPENDIX 1: RADIOLOGICAL ABNORMALITIES

Upper tract abnormalities

Calculus disease

Congenital calyceal cysts

Polycystic kidney

Papillary necrosis

Duplex system

Medullary sponge kidney

Reflux

Pelvi-ureteric obstruction

Renal tuberculosis

Non-functioning kidney

Hypoplastic kidney

Pseudotumour

Renal arterial stenosis

Uretecoele

Pyelonephritis

Lower tract abnormalities

Bladder residue (1ml or more)

Bladder diverticuli

Trabeculation of the bladder

Outflow obstruction

Bladder pouch

Patients with dilatation of the right ureter associated with pregnancy were considered normal.

APPENDIX 2

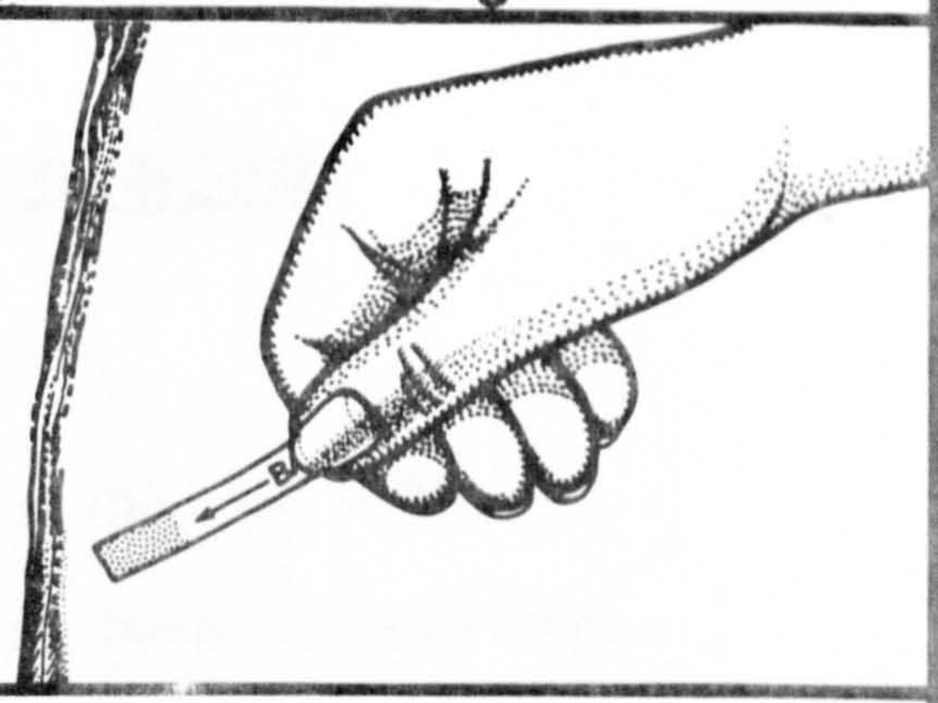
Instructions for: a) Use of filter strips

b) Introital (periurethral) swab technique

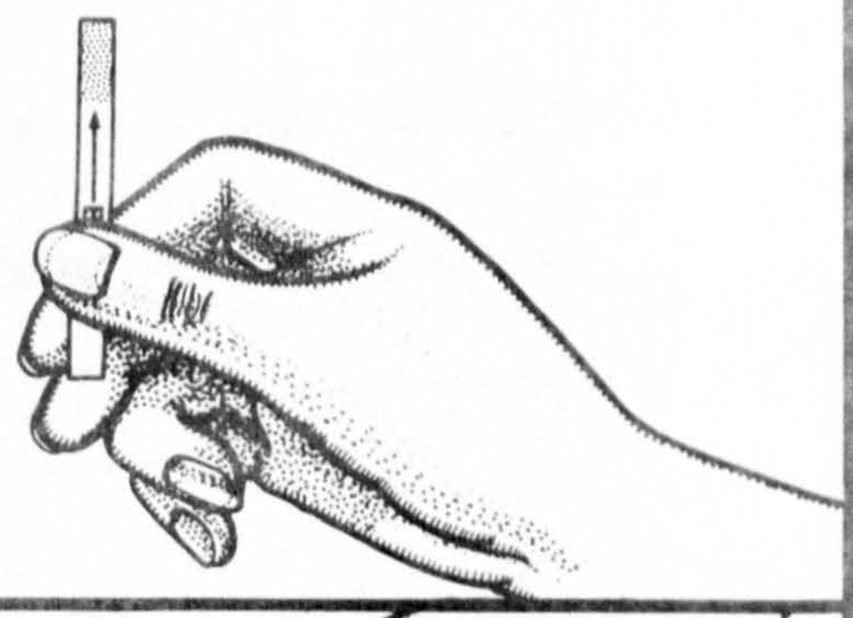
INSTRUCTIONS FOR THE USE OF FILTER STRIPS

Test to be done first thing in the morning

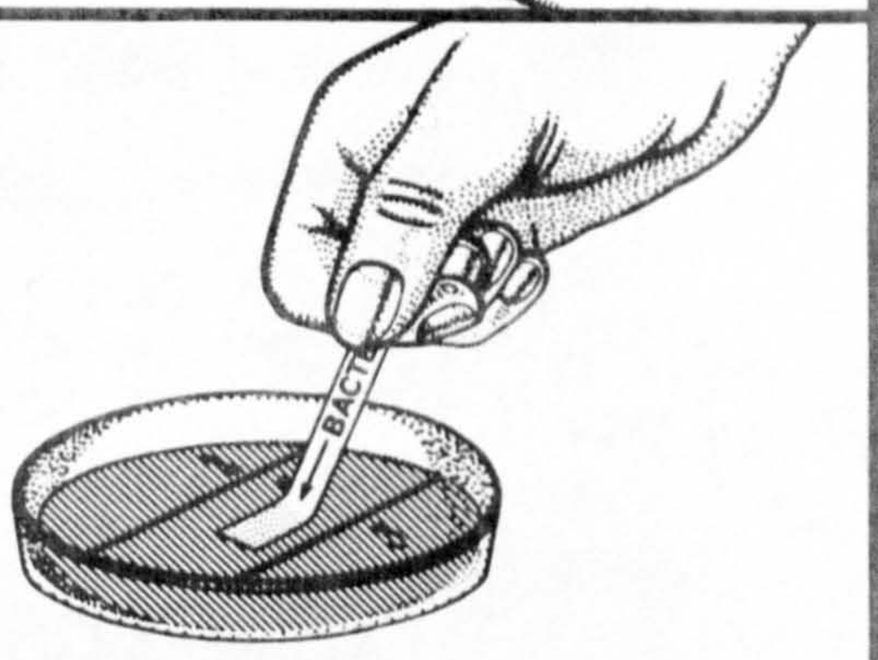
1. Pass the paper strips through the stream of urine halfway through bladder emptying so that about an inch of the strip is wetted.



2. Hold the strip up vertically until all the urine has completely soaked in



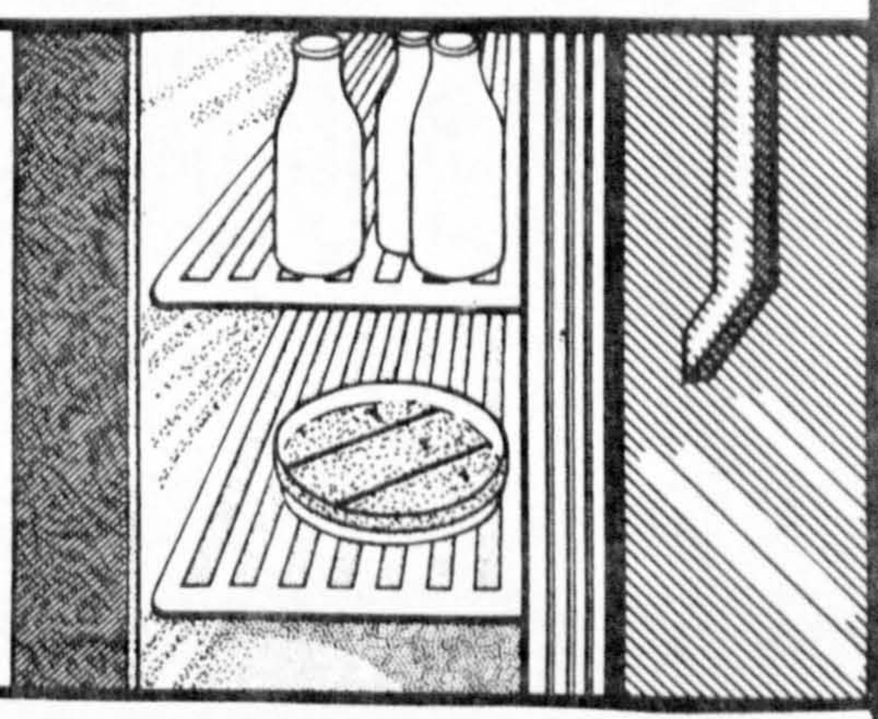
3. Place the wet end of the strip down flat on the surface of the plate so that all the wet part comes into contact with the jelly.



4. Pull strip off vertically so that impression is not smudged.

5. Discard the strip in the lavatory.

6. Replace the lid of the plate and keep it in a fridge or a cool place with the gel uppermost



7. When completed:— place the plate in the addressed padded bag and mail.

Don't forget to complete symptom sheet and enclose with plate.

INTROITAL SWAB TECHNIQUE

1. Remove paper wrapping.
   Push the tampon halfway out of tube.
2. With the fingers of one hand spread the labia (lips) apart.
3. With the other hand, take the tampon and swab over the urinary opening and inside the inner labia (lips). Do not push the tampon into the vagina.
4. Rub the used end of the tampon gently over the jelly plate, covering as much of the surface as possible.
5. Discard the tampon.
6. Place the plate in the addressed padded bag (with the strip plate) and mail.

APPENDIX 3: TABLE HEADINGS AND FIGURE LEGENDS

	Table	Page No.
Urethral and introital carriage of E. coli and enterobacteria	I	24-25
Predominant O types in UTI	II	45
Distribution of 15 commonest O types	III	83
Distribution of common urinary O types	IV	85
Distribution of the 11 commonest H types	V	89
Distribution of common urinary H types	VI	90
Motility	VII	93
K antigen titres	VIII	101
Relation of K antigen titre to radiological abnormality	IX	102
K antigen titre of rough strains	X	102
Serum sensitivities	XI	109
Relation of serum sensitivity to radiological abnormality	XII	110
Serum sensitivity of rough strains	XIII	110
K antigen titre and serum sensitivity of:		
smooth strains	XIVA	113
rough strains	XIVB	113
Relation of O type to K antigen and serum sensitivity	XV	114
Haemolysin production	XVI	120
Relation of haemolysin production to radiological abnormality	XVII	120
Relation of haemolysin production to O type	XVIII	123
Fimbriae production	XIX	127

	Table	Page No.
Relation of fimbriae production to radiological abnormality	XX	127
Relation of fimbriae production to O type	XXI	130
Fermentation of sugars	XXII	134
Fermentation patterns	XXIII	137
Minimum inhibitory concentrations of serine, spermine and urea	XXIV	143
Serine sensitivity : pH7.2	XXVA	144
Spermine sensitivity: pH7.2	XXVB	145
Urea sensitivity : pH7.2	XXVC	147
Urea sensitivity : pH5.5	XXVD	148
Effect of pH on serine, spermine and urea sensitivity:		
D-L serine	XXVIA	150
spermine	XXVIB	150
urea	XXVIC	151
Growth requirements	XXVII	157
Mucinase titres	XXVIII	162
Combined properties	XXIX	167
Relation of combined properties to radiological abnormality	XXX	169

	Figure	Page No.
Urethral and introital carriage of E. coli and enterobacteria	1	23
Grouping of strains	2	70
Distribution of O serotypes	3	82
Cumulative distribution of the 15 commonest O serotypes	4	84

	Figure	Page No.
Distribution of H serotypes	5	88
Cumulative distribution of the 11 commonest H serotypes	6	91
O/H serotype combinations	7	95
K antigen content	8	103
Relation of K antigen to radiological abnormality	9	103
Serum sensitivity	10	111
Relation of serum sensitivity to radiological abnormality	11	111
Relation of O type to K antigen and serum sensitivity	12	115
Haemolysin production	13	121
Relation of haemolysin production to O type	14	124
Fimbriae production	15	128
Relation of fimbriae production to O serotype	16	131
Fermentation of sucrose, salicin and dulcitol	17	135-136
Fermentation patterns	18	138
Serine sensitivity (at pH 7.2)	19a	146
Spermine sensitivity (at pH 7.2)	19b	146
Urea sensitivity (at pH 7.2)	19c	149
Urea sensitivity (at pH 5.5)	19d	149
Effect of pH on serine sensitivity	20a	152
Effect of pH on spermine sensitivity	20b	152
Effect of pH on urea sensitivity	20c	153
Growth requirements	21	158
Relation of mucinase titre to periurethral colony count	22	163
Mucinase production	23	163
Combined properties	24 & 25	168
Relation of combined properties to radiological abnormality	26	170

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