THE CLINICAL SIGNIFICANCE

OF

KLEBSIELLA INFECTIONS IN THE TROPICS

a Thesis

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"One of the most significant changes in the pattern of bacterial disease since the introduction of antimicrobials has been the increasing prevalence of serious infections with certain organisms generally considered as constituents of the normal bowel flora."

Fred A. Gill and Edward W. Hook
"Changing Patterns of Bacterial Resistance to Antimicrobial Drugs"
Amer. J. Med. (1965) 39. 780.

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1. SUMMARY OF FINDINGS

This thesis is a bacteriological and clinical analysis of cases of klebsiella infection occurring in patients in the University Hospital of the West Indies from April, 1966, to March, 1967. Matched control specimens are taken from infected and normal sites.

The literature on the various clinical infective syndromes associated with this organism is reviewed, but few of these papers coordinated clinical findings with full bacteriological investigations.

Two hundred and ninety isolates of Klebsiella aerogenes from 248 patients are considered. The incidence of isolation from normal and infected throats is similar; in sputum, klebsiella isolation is related to a variety of clinical infective syndromes.

Urinary tract infection is related to urethral stricture. In the female genital tract klebsiella is probably not a primary invader.

Cases of klebsiella abscess formation and wound infection occur in this hospital. Diabetes mellitus does not predispose to klebsiella infection.

Approximately one third of all klebsiella isolates are hospital acquired, but cross infection does not play an important role since a great variety of serotype and biotype

combinations are encountered. Multiple infection with different serotypes and biotypes of klebsiella is a feature of several cases, and the importance of determining the serotype and biotype in any studies of this type is emphasised.

Eighty-nine out of 248 patients were on antibiotics at the time of first isolation of klebsiella, and this compares closely to the incidence of antibiotic prescription in a carefully matched control group of hospital patients showing no evidence of klebsiella infection. When the antibiotic history is further analysed, there is seen to be a predisposition to develop klebsiella infection after seven or more days multiple chemotherapy.

Strains of K. aerogenes isolated from specimens of urine are more biochemically heterogeneous than isolates from other sources.

Part Two of this study demonstrates that the various klebsiella serotypes produce quite different cross reactions in two species of laboratory animals, rabbit and mouse. It is thought that these differences are probably genetically related. Further work is indicated on the immunological specificity of the capsular polysaccharides of klebsiella, and on the modifying effect of adjuvants, if any, on the antigenic determinant site.

Similarities are apparent between infection due to type III pneumococcous and Klebsiella aerogenes.

2. INTRODUCTION AND PURPOSES OF INVESTIGATION

Isolation of klebsiella species from specimens submitted for bacterial culture from a variety of clinical infective syndromes is of frequent occurrence in Jamaica. The increase in infections due to members of the gram negative group of organisms since the advent of antibiotics and chemotherapeutic agents is well known and has been extensively documented. The primary purpose of this investigation is to analyse the nature and severity of infections due to the various serotypes of Klebsiella aerogenes, as isolated from patients studied in the University Hospital, Jamaica.

The questions asked in this survey are:

1. What is the etiological significance of isolation of a particular serotype of Klebsiella aerogenes from clinical material?
2. What is the significance of previous chemotherapy in establishing infection by this group of gram negative bacteria?
3. Is any particular serotype more frequently related to infection at any one site, or to hospital acquired klebsiella infections?

During the course of the preparation of antisera in the rabbit to the various serotypes of klebsiella species, for purposes of typing the above strains, opportunity was

also taken to assess the ability of the Swiss colony strain of white mouse to produce a hyperimmune response to klebsiella stock cultures. Comparison was made of the antibody titres and cross reactions to the same antigen in the two host species, rabbit and mouse.

The questions asked in this section of the work are:

1. Does the mouse produce antibody titres comparable to the rabbit?
2. How do differences in host species affect the cross reactions?

Since this work is mainly an investigation into the clinical signs found in association with isolation of specific serotypes in humans, detailed animal investigations had to be of necessity limited.

If the final section succeeds in asking even more questions than it answers, this is partially due to the fact that our knowledge of the Immunochemistry of the klebsiella antigens is still incomplete, and that further work is needed on the effects of adjuvants on antigenic specificity.

PART I

3A. HISTORICAL REVIEW OF THE LITERATURE

(1) Fourteen years ago, Grant (1958a) commented on the frequent culture of klebsiella species from specimens submitted from patients in Jamaica - most commonly from urines, and then in decreasing order of frequency from high vaginal swabs, sputa, ulcer swabs and throat swabs. During the 1955-1957 period he stated that 31 deaths were due primarily or secondarily to klebsiella infection, and suggested that cross-infection may be an important factor. The same year, Grant (1958b) surveyed the bacterial aetiology of urinary tract infections in the University Hospital, Jamaica, and found that during the 1954-1956 period, of 1,542 bacterial infections of the urinary tract, 17.4% were due to pure cultures of Klebsiella aerogenes.

Sandison et al (1967), in a survey of the Intensive Care Unit of the University Hospital, Jamaica, noted quite a degree of bacterial contamination of the air (of 0.67 orgs/ cm ^{2}/30 mins.), but the organism most commonly found was staphylococcus. All three patients on the intensive care side of the unit at the time of the survey had a high population of klebsiella organisms on their bedclothing and the floor by the bedside, and klebsiella was isolated from suction tubes and ventilators in use. Two of these three patients had had klebsiella, in mixed culture, isolated from tracheostomes. James et al (1967), in a more extensive bacterial survey of

the hospital noted a high level of bacterial contamination of the air, by the settle-plate technique, but the commonest organism isolated was the staphylococcus, and klebsiella isolation was relatively rare, and in all cases plate counts were low.

In none of these surveys was an attempt made to classify the isolated strains of klebsiella serologically, or to assess the incidence of klebsiella in association with active clinical infection and normal control groups. Since the advent of antibiotic therapy there have been many reports on the increasing number of infections associated with the more resistant gram negative organisms. Wilson and Miles (1964) point out the difficulty of assessing the significance of the numerically higher capsular serotypes of klebsiella in respiratory tract infections, particularly in patients on antibiotic treatment in hospital.

Helmholtz and Sung (1944) found a high degree of resistance to penicillin in strains of K. aerogenes isolated from urine, and Herrell and Nichols (1945) stressed the value of streptomycin in urinary and tracheo-bronchial suppurations caused by klebsiella. By 1946 it was becoming clear that prolonged penicillin therapy could result in striking changes in the bacterial flora, and this was well demonstrated by Lipman et al (1946), who reported the emergence of gram negative organisms in the throat flora of patients on penicillin

therapy for rheumatoid arthritis, replacing the penicillin-sensitive gram positive cocci isolated before onset of therapy.

Streptomycin was not to remain of unquestioned value in klebsiella eradication for long. In 1946, Finland, Murray and Harris showed the development of streptomycin resistance to a variety of organisms including klebsiella, during therapy, in cases of chronic pyelonephritis and these observations were supported by the work of Herrell and Nichols (1945) at the Mayo Clinic, and by Buggs and others (1946).

The emergence of resistant strains of the same species, and spontaneous occurrence of new infection during antibiotic therapy aroused much interest in the 1947-1952 period. Weinstein (1947) observed a failure in clinical cure of patients suffering from pneumococcal pneumonia after penicillin therapy, concommitant with the appearance of K. aerogenes in the sputum. He pointed out the danger of spontaneously-occurring, new infections during the course of penicillin or streptomycin therapy, due to disturbance by antibiotics of the normal state of bacterial antagonism. Super-infections with gram negative bacilli were also reported by Appelbaum and Leff (1948), by Yow (1952), McCurdy and Neter (1952), Smith (1952), Spink (1953) and Weinstein et al (1954). Smith (1952) emphasised the complex balance which normally exists among the normal microbial flora of the body, and when this is disturbed by prolonged antibiotic therapy, may result in

the development of secondary vitamin deficiencies or new infectious disease syndromes. McCurdy and Neter (1952) compared the conversion of the throat flora after the use of penicillin in combination with a broad spectrum antibiotic with the changes following treatment with broad spectrum antibiotic therapy alone. The combination of drugs was more likely to produce a predominantly gram negative bacillary flora than the use of one broad spectrum antibiotic alone, and these results were statistically significant. Of great interest were the observations of Weinstein at al (1954) that the clinical disease appearing when superinfection occurred involved the same organ system that had been involved first. However, in superinfection, involvement of other tissues was also noted, and the respiratory tract was usually affected. Most of the superinfections occurring during chemotherapy were noted between the third and sixth days of treatment of the primary disease, and 44% became evident on the fourth or fifth day of antibiotic therapy. The authors pointed out that the appearance of superinfection during the administration of an antibiotic may convert a benign, self limiting disease into a serious, prolonged, or even fatal one.

Yow (1952) presented clinical details of Pseudomonas and Proteus infection occurring during chemotherapy. In a subsequent paper, Yow (1955) pointed out that infections due to resistant strains of gram negative bacilli are especially likely to appear in patients with decreased resistance such as

malnutrition, alcoholics, blood dyscrasias and other debilitating conditions. In the same article, Yow drew attention to a report from the Philadelphia General Hospital by Weiss and others (1954) indicating a rapid rise in the frequency of pulmonary infection due to klebsiella over the decade 1942-1952, with over 50% of these patients in the 40-59 age group.

In 1956, Steiner and Putnoky described 94 instances of isolation of klebsiella from premature and newborn babies and infants and claimed a greater frequency of this organism becoming pathogenic. No statistical evidence, however, was given to substantiate whether the increase was a real or apparent one. They found type A the most common and type C the most rare.

Eisenberg and other (1958) noted that klebsiella organisms often appear in respiratory secretions in the absence of specific disease, and serotypes 1-4 were more often responsible for infection than higher types. They studied 14 patients with penicillin-treated pulmonary infections and failed to demonstrate a casual relationship between penicillin therapy and higher serotype klebsiella. They pointed out, however, that the use of antibiotics plays a role in the selection of organisms in a hospital environment and the consequent establishment of a nosocomial reservoir. Antibiotics have thus shifted the clinical importance from the pneumococcus to the staphylococcus, klebsiella and other gram negative enteric bacteria.

In 1959, Finland, Jones and Barnes reported that K. aerogenes as a cause of gram negative bacteraemia had been rare in 1935, and had steadily risen in incidence, along with a rise in Pseudomonas and Proteus infections. In 1964, Kislak, Eickhoff and Finland reported an interesting survey of 115 hospital infections in the Boston City Hospital. Klebsiella and Aerobacter groups represented 8.7% of cases in which this organism occurred in pure culture, and was found in 18.3% of all isolates. It was more frequent than E. coli (7%), Proteus (2.6%) and Pseudomonas (3.5%) as a cause of hospital infection. Collectively, the gram negative bacilli equalled the Staphylococci (34%) in the aetiology of hospital infection, and as the former were frequently found in mixed cultures an appraisal of their specific aetiologic role was difficult.

In 1959, Rogers observed that in the New York Hospital the enteric bacteria were the most common cause of death from infection in medical patients from 1957-1958, and the majority of infections developed while the patient was in hospital.

Gill and Hook, in 1965, most convincingly drew attention to changes in antibiotic resistance of several important pathogens. In considering the increased prevalence of serious infections caused by enteric bacteria, they drew attention to the natural resistance of these strains to antimicrobial drugs, and also to the possible emergence of resistant mutants from originally sensitive strains, under the influence of antibiotics.

Klebsiella aerogenes is ubiquitous in nature, and was isolated from river water by Jordan (1903). It has been shown to exist in soil, air, dust and canal water (Ford, 1927). Gray (1932) demonstrated the isolation of klebsiella from the soil even when faecal contamination by man or animals was unlikely; the same organisms have also been isolated from drinking water, and milk, and have been shown to be transmitted by flies. Clemensha (1912) found B. aerogenes to be more prevalent in rivers and lakes during the wet season, and attributed this to the rapid multiplication of this organism in water.

Escherich, in 1885; first demonstrated the presence of B. lactis aerogenes in the intestinal flora of neonates. MacConkey (1905) studied no less than 241 lactose fermenting strains isolated from 23 samples of human faeces, but only about 1% were identified as B. lactis aerogenes, and he could find no differences between strains of human and animal origin. Dudgeon (1926) found klebsiella in only 5.5% of faeces examined and this confirmed a previous report by Levine (1921) who summarised the results of several workers and found that only 6% of 4,000 strains isolated from human and animal faeces were K. aerogenes type. However, Mollari, Randall and Reedy (1939), working in Washington, found that, by using a preliminary enrichment media, they had a 40% isolation rate from the faeces of adult, healthy males. Similar figures were obtained in Danish infants, when Ørskov (1955c) demonstrated a 32.8%

isolation rate of klebsiella from faecal material. Among 74 capsulate isolates she demonstrated 44 different capsular serotypes, establishing that there was no special type frequently isolated from the faeces of normal infants.

Once outside its normal habitat in the gastro-intestinal tract, Klebsiella aerogenes may be associated with a diversity of clinical lesions, and the literature on this will now be summarised.

(II) Respiratory tract infections related to klebsiella

The literature on the isolation and clinical features associated with klebsiella in the respiratory tract is extensive. In 1882, Von Frische isolated a capsulated bacillus from cases of rhinoscleroma, and this is the first recorded description of klebsiella infection.

In 1882, Friedlander described the presence of a "micrococcus" in effusions of fibrin from the bronchi and from alcohol fixed sections of lung at autopsy, in eight patients ranging in age from ten to 70 years. The following year he characterised this organism on serum culture in "Die Mikrokokken der Pneumonie" (1883) and noted that a live culture produced murine pneumonia. Two other authors, Etienne in 1895 and Conba in 1896, each reported a fatal case of Friedlander's pneumonia in children. However, Talaman (1883), Fraenkel (1886) and Sternberg (1885) showed conclusively that the common cause of lobar pneumonia was pneumococcus, and

expressed the view that Friedlander's organism was a secondary invader. In 1886, Weichselbaum implicated Diplococcous pneumonia in the majority of cases of lobar pneumonia and Friedlander's bacillus was relegated to a less important role.

In 1912, Lamar and Meltzer showed that a lobar pneumonia could be induced in dogs by intrabronchial insufflation of either pneumococcous or Friedlander's bacillus. Three years later, Sisson and Thompson (1915) reported four fatal cases of Friedlander's pneumonia, and in reviewing 33 cases from the literature noted that the organism may be a primary or secondary invader and characteristically produced a universally fatal pneumonia, sudden in onset with severe toxaemia and characteristic slimy sputum. They described an initial lobular distribution with mottled, marble-like appearance of the lungs, progressing to a lobar distribution with abscess formation and necrosis. In 1919, Zander described an epidemic of Friedlander's pneumonia in Germany comprising 411 cases in four months, with 144 deaths. The highest mortality was in the 40-50 age group, with a predominantly lower lobar distribution. Bloomfield, in a series of articles (1920, 1921a and b), reported the results of his studies on chronic respiratory carriage of Klebsiella. He was unable to induce the carrier state, or produce disease in healthy individuals, but studied two chronic carriers with no history of preceding infection. He found a 5.8% incidence of Klebsiella isolation from the upper respiratory tract of 85 unselected hospital cases.

Subsequently the carrier state has received scant attention in the literature, but there have been numerous reports of pulmonary infection due to Klebsiella. Belk (1926) studied 18 cases and noted abscesses as early as the fourth day of illness. He was the first to demonstrate their characteristic, thin-walled appearance. Westermark (1926) confirmed the presence of multiple thin-walled abscesses in a case of Friedlander's pneumonia, and the aetiology of this organism in chronic nasal catarrh in children was demonstrated by Mackay in 1927. A wider variety of pulmonary lesions was well described by Collins and Kornblum (1929), who described a bronchopneumonic type of lesion in two cases and a lobar consolidation in one. They pointed out that chronic infection could radiologically mimic pulmonary tuberculosis, due to the extensive fibrosis produced.

Ferguson and Tower (1933), in describing the occurrence of Friedlander's pneumonia in infant twins, one of whom died, demonstrated that the X-ray signs characteristic in adults may not occur in children. The same year, 1933, Olcott reported five cases of Friedlander's pneumonia with positive blood culture all in males aged 38-55 years. Four of these cases had a leucopenia and although sections of lungs showed many bacilli, the author felt that the ability of these organisms to exist commensally in the respiratory tract made its role in pneumonia doubtful. This view was not upheld by Hyde and Hyde (1943), who demonsrated that the incidence in which

the organism can be recovered from the normal respiratory tract was less than one percent. They demonstrated that in a series of 51 patients with Friedlander's pneumonia, 88% were over 40 years, with a male:female ratio of 5:1. The case fatality rate was much higher in those with positive blood culture, and reached 82%.

Solomon (1937) suggested that alcoholism, malnutrition and other debilitating factors were common in Friedlander's pneumonia. He noted that most of these patients had foci of infection in the upper respiratory tract - such as dental caries, tonsillitis, sinusitis, but as these lesions were not cultured, the significance of these observations cannot be assessed. Bhatngar and Singh (1935), working in India, noted an incidence of 13% of klebsiella isolation in acute lobar pneumonia, and an association with a group 4 pneumococcus was noted, the latter being of low animal virulence. Unfortunately, the klebsiella isolates were not typed, but again these patients were all in older age groups. Baehr and others (1937), working at Mount Sinai Hospital, claimed that Friedlander's infection was more commonly associated with abdominal infections and urinary tract infections. They found respiratory tract infections due to klebsiella to represnet less than 13% of all klebsiella infections and recommended a change in nomenclature from K. pneumoniae to K. Friedlanderi. In only two cases did clinical and bacteriological studies show a primary Friedlander pneumonia, and in

seven other cases of lobar or bronchopneumonia mixed cultures were obtained.

Bullowa, Chess and Friedman (1937) demonstrated a clinical similarity between Friedlander's and pneumococcal lobar pneumonia, and showed that the highest mortality rate was among Group A (type 1) infections.

Feder (1938) studied cases of Friedlander's pneumonia occurring in the Pennsylvania Hospital over a decade, and showed an overall incidence of 8% Friedlander's pneumonia of all pneumonias, and an 80% mortality rate. An extensive review of the literature from 1882-1938 was made by Hartmann (1940) who found a case/fatality rate of 94%. An overall mortality of 82% was quoted in the series by Julianelle (1941), with a high mortality in Group A infections, Group C being less virulent (7%). Frisch (1940a, 1940b), however, claimed that the prognosis in both pneumococcal type 3 and Friedlander's pneumonia was dependent primarily on the amount of capsular polysaccharide produced.

A chronic type of Friedlander's pneumonia characterised by remissions has been described. This variety followed an episode of acute pneumonia in the case described separately by Berglund (1925) and Westermark (1926) and persisted for nine years in the case reported by Brulé and others (1927). These authors characterised the chronic form as being frequently mistaken for pulmonary tuberculosis, and typically

showing pulmonary cavitation, fibrosis and bronchiectasis. Muschenheim (1940) described a case in the New York Hospital who had harboured the organism for at least four years, and had repeated attacks of pneumonia.

Soloman (1940), Perlman and Bullowa (1941) experienced a higher incidence of Friedlander's pneumonia in older age groups, predominantly male, and one of the earliest cases to be successfully treated with sulphonamides also occurred in a middle-aged male and was reported by Sinclair in 1941.

Wylie and Kirschner (1950), in describing 16 cases of Group A infection in middle-aged males, noted that half of the cases were alcoholic, and with the more frequent survival of patients the problem of residual suppurative pneumonia requiring lobectomy or pneumonectomy would assume greater importance. Weiss, Eisenberg and others (1954) noted a high incidence of middle-aged males admitted to the Philadelphia General Hospital in 1952-1953 with klebsiella pneumonia, and the duration of symptoms prior to admission averaged 31 days. Serological typing was done, and of 21 cases three were type 1, two were type 4, and one each of types 2 and 3. Higher serotypes accounted for 14 cases, and there was a tendency to leucopenia. In contrast, Weiss and others (1956) showed type 2 to be the most common respiratory strain, and the authors noted that destructive lung disease was more than three times as common with lower type infection than with higher. Klebsiella of lower types was isolated usually prior to antibacterial therapy, while higher types tended

to emerge during penicillin or other resistant drug therapy. They postulated that since klebsiella may be carried in the respiratory tract as a commensal, decreased host resistance may lead to infection. It may be noted that their patterns of antibiotic resistance showed 93% of strains to be sensitive to chloramphenicol, streptomycin or both.

Limson, Romansky and Shea (1956) showed most of their respiratory klebsiella isolates to be sensitive to tetracycline, chloramphenicol and streptomycin, and demonstrated a decline inmortality rate in chronic cases to 1 in 9, but nine out of 13 acute infections were fatal. Erasmus (1956) reported 17 cases of klebsiella pneumonia at Pretoria General Hospital, in which he claimed that 14 cases were due to primary infection; the rest occurred as mixed cultures in cases such as asthma and neoplastic disease, but most cases resembled a suppurative pneumonia.

In contrast to previous reports (Olcott, 1933; Weiss et al, 1954), the Pretoria cases showed a moderate polymorpholeucocytosis; all cases recovered. Morris and Yates (1956) stressed the importance of predisposing diseases sich as diabetes, in the aetiology of Friedlander's pneumonia, confirmed the observations of earlier workers (Solomon, 1937; Yow, 1952) and its resemblance to pulmonary tuberculosis, and stressed that isolation did not prove a causal relationship. Kahler (1951) described a patient with bronchial carcinoma

who developed pneumonia and proctitis due to an organism of the klebsiella group.

In the late 1940s reported of Friedlander's pneumonia in infancy became more frequent. Miller, Orris and Taus (1947) reported for the first time cystic changes in the lung in a three-week old infant with klebsiella pneumonia, which responded well to streptomycin; the organisms was also isolated from the nose and throat. Faucett and Miller (1948) described six infants with mouth lesions identical to thrush from which K. aerogenes was isolated, but there was no systemic reaction to the organism, and its significance here is doubtful since in three cases Monilia albicans was also isolated. Grotts (1949) reported seven cases of Friedlander's pneumonia in infants but unlike Miller's cases (1947), did not encounter pulmonary abscess or cavity formation, although positive chest signs persisted for weeks. He could only demonstrate klebsiella in patients with pulmonary infection. All children had gastrointestinal symptoms, and in this series stool cultures would have been of interest, but were not done. Levine (1950), in commenting on infection control in premature nurseries, reported klebsiella in the lungs at autopsy of premature infants. Obrinsky et al (1950) brought evidence to show that the widespread use of penicillin by 1950 may be producing a real increase in klebsiella infections. They described ten cases of klebsiella infections in infants occurring over a 12-month period - three

causing pneumonia, five of sepsis, one meningitis and one urinary tract infection, but the absolute incidence of isolation of this organism in this series was not clear.

Thaler (1962) reviewed 36 cases of klebsiella infection in infants, and noted that while serotypes 1, 2 and 4 were usually implicated in severe respiratory diseases in adults, any type may prove pathogenic in infants. A type 16 klebsiella pneumonia in an infant was described. Steiner and Putnoky (1956) described an outbreak of klebsiella infection in Budapest where previous antibiotic therapy did not appear to play any part. In a very clear dissertation, they pointed out that the higher incidence of diseases due to Klebsiella pneumoniae has not yet been adequately explained, and in the state of present knowledge, the causal role of Klebsiella pneumoniae could only be proven:

(i) in positive blood or pleural effusion cultures;

(ii) in C.S.F. which shows inflammatory symptoms;

(iii) in urine collected aseptically and associated with pyuria;

(iv) in faeces during a dysentery epidemic, and when the carriers have been removed the epidemic ends.

Considerable interest arose in the late 1940s in the mechanisms involved in the development of Friedlander's pneumonia. Sale and Wood (1947) produced pulmonary lesions in white rats by intrabronchial inoculation, and showed the progressive development of hyperaemia, consolidation and abscess

formation. An important finding was that advanced pulmonary lesions were associated with heavy deposits of fibrin in the alveoli, and the main differences from pneumococcal pneumonia were (1) many more bacteria in alveoli in Friedlander's pneumonia; (2) abscess formation was common; (3) organisation of the alveolar exudate was much more prominent in Friedlander's pneumonia. In a further paper (1947), Smith and Wood showed that destruction of the alveolar wall by necrosis prevented phagocytosis from occurring, as the latter was essentially a surface phenomenon. They also demonstrated reduction in phagocytosis in the low oxygen concentration prevailing in the centre of the abscesses, and failure to phagocytose bacteria. Frisch (1943) stained sputum smears from cases of pneumococcal and Friedlander's pneumonia, and described two separate appearances - a non-reticulated type of sputum, and one showing reticulation of extensibe fribrin network in which organisms were interspersed. The latter had a high case fatality rate of 83%. He claimed that the outcome in Friedlander's pneumonia depended on the degree of reticulation and the amount of capsular material produced. He claimed that bacteriostasis induced by chemotherapy reduced the amount of polysaccharide produced.

Øskov (1955a and b), in a series of papers on serological investigations in the klebsiella group reported that 102 out of 350 sputa samples from hospital patients grew klebsiella, and these belonged to a minimum of 61 different antigenic types.

She found types 1-6, which other authors had found especially often in the respiratory tract, in only 16.6% of her cases. Other serotypes found in sputum were 16, 26, 29, 41, 47 and 68 - four cases each. Epstein and Friedmann (1958) studied 50 strains isolated from patients at the Institute of Laryngology and Otology in London. Only 2% of patients harboured pure klebsiella in the ear and upper respiratory tract, and 21 of 26 typable strains were serotype 1; they considered these strains to be primarily pathogens. The authors also isolated acapsulate strains in pure cultures from infection, thus confirming the statement in Wilson and Miles (1964) that capsule formation is often associated with, but not essential for virulence. In contrast to the findings of Epstein and Friedmann (1958) on the pathogenicity of type 1 strains, Dubay (1960) detected type 1 in 0.4% of 1,000 normal subjects, in the upper respiratory tract, and the same incidence of type 4 klebsiella was found. However, in 30 patients with chronic pulmonary disease, types 1, 3 and 4 were also isolated.

Foster and Bragg (1962) classified 50 klebsiella isolates from sputum, in terms of severity of clinical signs, and found that both K. aerogenes and K. edwardsii v edwardsii occurred in the lung at necropsy associated with abscess formation, but while K. aerogenes could be associated with trivial infection, K. edwardsii v edwardsii was invariably highly virulent. Their incidence of isolation of klebsiella at Dudley Road Hospital, Birmingham, in 1962, was 50 out of 900 sputum samples.

In Helsinki, Mäkitalo and Widholm (1962) attempted to determine the extent to which hospital personnel were nasal and pharyngeal carriers of intestinal organisms. They studied 450 individuals, of which 142 (32%) carried an organism normally constituent in the bowel flora - 24 of these 450 individuals were nasal or pharyngeal carriers of Klebsiella. Kossowski and others (1964) examined the nasopharyngeal secretions of 81 patients, and of these Klebsiella was isolated in 56 patients. They appeared, however, to be working predominantly with ozena patients since a chronic fetid atrophic nasopharyngitis associated with Klebsiella serotype 4 infection was demonstrated in the patient group, and this serotype was not found in healthy controls. Darrell and Hurdle (1964), in a biochemical survey of Klebsiella isolated from chest infections, found K. aerogenes was the biochemical type most frequently isolated, but expressed the opinion that this species was of doubtful pathogenicity in sputum, and frequently appeared following antibiotic therapy for other organisms. They stressed that it should be clearly distinguished from other Klebsiella species occurring as primary pathogens in sputum, viz. Klebsiella edwardsii-edwardsii, Klebsiella pneumoniae and Klebsiella atlanta, and for these latter, more virulent species, they suggested the collective term "Friedlander's bacillus".

In July, 1965, the U.S. Department of Health Education

and Welfare reported an outbreak of 18 cases of klebsiella pneumonia in a psychiatric hospital in Washington, D.C. In six of the fatal cases, K. pneumoniae was isolated from purulent material from the lungs at autopsy. No information of the serotype isolated in these cases was given, so no conclusions as to cross-infection can be drawn.

Nagesha and Laxman Pai (1966) investigated 363 cases of broncho-pulmonary infection and isolated klebsiella from 61 of these; their investigations, however, were largely biochemical.

Davis and Woolhouse (1966), working at the University of Lagos, found that the majority of klebsiella isolates from clinical material were K. aerogenes species, and pointed out the need for further information on the role of these bacteria and other enterobacteriaceae in human disease. In 1968, Burns demonstrated the presence of precipitins to klebsiella and other enterobacteria in the serum of patients with chronic respiratory disorders.

An excellent and comprehensive survey of klebsiella-enterobacter-serratia infection occurring in the Boston City Hospital in 1964 was reported by Steinhauser and others (1966). Of 205 isolates of klebsiella, types 1, 3, 4 and 5 were more frequently associated with infections of the respiratory tract, but only two of the 14 patients from whom these four capsular types were isolated had lobar infiltration

characteristic of Friedlander's pneumonia. In most patients these types were associated with either bronchitis or bronchopneumonia. Twelve of the 16 strains of type 2 klebsiella isolates in this survey were associated with urinary type infections, and type 24, which was the commonest type isolated, was almost exlusively associated with urinary tract infections. The authors noted a predominance of males over females in their series. Klebsiella type 2 tended to be of major significance in illness more often that the other strains. About half the organisms of respiratory tract origin were contributing but not major factors in the clinical illness; but this assessment was, of course, subjective.

(iii) Klebsiella infection in the Urinary Tract

Denys (1892) reported B. aerogenes in urine and attempted to correlate its presence with similar organisms in the faeces of children. In 1894, Nicolaier reported renal tract infection due to an encapsulated bacillus. The probable intestinal origin of these infections was emphasised by Goldberg (1895) and the role played by retention was first emphasised by Guyon in 1889. Trumpp (1896) described 29 cases of cystitis in children due to B. aerogenes, and Montt-Saavedro (1896) also reported cases of cystitis due to this organism. Differential bacterial cultures of urine were reported by Rostoski (1898), Brown (1902), Lenhartz (1907) and Raskai (1905), but these articles are mainly now of historical interest. A comprehensive review of the literature was made by Luetscher

(1911) but he claimed that the incidence of B. aerogenes in urinary tract infections was overestimated. This was not confirmed by Meyer and Hinman (1920), Lacy and Murdoch (1922), nor by Hill and his co-workers (1929). The latter, working at John Hopkins Hospital, Baltimore, studied 200 cases of urinary tract infections due to gram negative coliforms, and found Klebsiella aerogenes to be the second most frequent isolate (E. coli = 100 cases, K. aerogenes = 79 cases). Their observations were substantiated by adequate biochemical classification, and they stressed the impertance of demonstrating capsulation, which could be shown in 90% of their cultures of K. aerogenes.

A lower incidence of Klebsiella in the aetiology of urinary tract infections was reported by Gendolf and Stringer (1929) who found only a 22% incidence in their study of 50 cases.

A wider approach, from the Public Health standpoint, was made by Burke-Gaffney in 1933, who found that of 1,000 urinary strains of coliform bacilli, 52% were aerogenes-type on a biochemical basis, and of 500 faecal cultures, 8% were aerogenes, thus establishing that in bacterial examination of water, the presence of Aerobacter aerogenes could not be regarded as non-excretal in origin.

In the late 1940s several articles dealt with the frequency of Klebsiella aerogenes infection in cases of

urinary retention - which had first been demonstrated by Guyon (1889). Petroff and Lucas (1946) studied 87 patients with neurogenic bladders and A. aerogenes was by far the commonest pathogen (39%). Warner (1948) found that 64% of infections of the urinary tract in paraplegics were due to K. aerogenes, compared to only 24% due to E. coli, and pointed out the increased resistance of K. aerogenes to 200 units of penicillin compared to E. coli. A comparative incidence of the two organisms in two different groups of patients was well demonstrated by Coleman and Taylor (1949); they studied 100 consecutive cases of pyuria, and in 60 cases where no gross lesion of the urinary tract was present, E. coli represented the commonest pathogen - 49/60 cases, compared to 1/60 due to klebsiella. Where infection was shown to be secondary to a surgical or medical condition affecting the urinary tract, the incidence of klebsiella was 18/40 compared to E. coli 7/40. The authors suggested that in the absence of a gross lesion, urine flows too freely to allow bacterial multiplication, but where there was urinary stasis, saprophytes could flourish. This makes no attempt to suggest why "pathogens" should not equally flourish in static urine. However, the increased incidence of K. aerogenes over E. coli has been certainly confirmed - Owen and Finch (1949) demonstrated a 62.4% incidence of K. aerogenes in urinary infections in paraplegia; Milner (1963) showed K. aerogenes to be the commonest lactose fermenter

isolated from the urine of male paraplegics, and Levy-Bruhl and Viala (1935) found the urinary tract to be the most frequent site of disease due to Friedlander's bacillus following blood stream invasion, and noted that localisation was facilitated by any anatomical abnormality. A case of perirenal abscess due to Klebsiella aerogenes was reported by Kindall (1941), and in 1949 Wilhelm and Orkin reported a striking increase in the incidence of urinary tract infections caused by klebsiella in the Beth Israel Hospital. In 1940-41 this organism represented 7.4% of the urinary tract infections, whereas by 1945-48 it had risen to 49% - in the latter period 126 cases were studied, four had positive blood culture and the authors postulated an increase in virulence in the organism because its isolation was becoming associated with more clinical signs and symptoms. This may well have been due to the fact that only 12 cases were available for study in the 1940-41 group. In a later paper from the same hospital, Wilhelm and others (1949) showed that 85% of strains of K. aerogenes from the urinary tract were streptomycin resistant, and in this group they reported 24 cases of bacteriaemia with seven deaths.

In 1951, Worfel and Ferguson isolated a new Klebsiella serotype 15 from faeces of infants in an epidemic of gastro-enteritis in Michigan, and isolated a similar antigenic strain from a catheterised bladder specimen of urine. Ørskov,

in a series of articles from Denmark (1952, 1954b and 1954c) also examined urinary isolates of Klebsiella serologically. She studied 322 strains of Klebsiella from various hospitals in Copenhagen, and clearly demonstrated nosocomial infection in a ward where type 8 was the predominant strain. Fifty per cent of 67 patients treated with indwelling catheters were infected with type 8 within two weeks following catheterisation. Ørskov (1954b) demonstrated type 8 in the faeces of one of these patients as well as from cleaned urinals, but ten sterilised catheters gave no growth. Eleven of twenty-one patients infected with Klebsiella, who were treated by general practitioners appeared to have been infected in a clinic with the same endemic type. Ørskov pointed out that adequate precautions are necessary to prevent urinary cross infections in hospital patients.

It was demonstrated by Dutton and Ralston (1957) that organisms could be transferred from the catheter of one patient to the catheter of another by the hands of attendants. It is possible that asymptomatic bacteriuria follows catheterisation with great frequency, since Kass (1956) showed that significant numbers of bacteria could be found in the urine of patients who had no urinary symptoms. Kass also showed (1955) (1956) that the use of an indwelling catheter led to bacilluria within four days in 95% of cases and prophylactic antibiotic therapy was ineffective in its

prevention. Hospital acquired urinary tract infections were shown to be more resistant to antibiotics than infections acquired elsewhere - Kirby, Carpron and Tanner (1956), and Lattimer, Seneca and Zinsser (1959) demonstrated an increased increase in chronic urinary infections since the advent of antibiotic therapy, and noted a rising mortality due to klebsiella septicaemia. Lattimer and his coworkers (1959) clearly demonstrated induction of tetracycline resistance in K. aerogenes and when grown in subinhibitory concentrations of tetracycline, four strains achieved a resistance of 850 mcg/ml - far beyond the reach of therapeutic blood levels. They concluded that this was the probable explanation of the increase in number of chronic clinical infections.

Hojo (1965), working in Japan, studied 27 patients with chronic urinary tract infections and could type only 11% of these strains using antisera types 1 - 72, so no conclusions of type incidence could be drawn.

Steinhauser, Eichoff and others, in 1966, demonstrated that of 142 urinary tract isolates of klebsiella, 124 were typable, and the type incidence was as follows :

Types 1, 3, 4, 5	- 3 cases
Type 2	- 12 cases
Type 9	- 8 cases
Type 19	- 9 cases
Type 20	- 7 cases

Type 21	- 6 cases
Type 24	- 35 cases
Type 27	- 5 cases
Others	- 39 cases
Non-typable	- 18 cases

Thus, apart from a predominance of type 24, a wide variety of serotypes of klebsiella could be incriminated in urinary tract infections. Their report covered klebsiella isolated from various clinical sources, and of 16 strains of type 2 klebsiella, 12 came from urinary tract infections. The serotypes 1-4 had hitherto been generally associated with respiratory tract origin. Over 50% of their patients were 70 years or over, but only 43% of patients with infections due to klebsiella types 1, 3, 4 and 5 were over 60 years, whereas 78% of those who had type 24 urinary tract infections were over 60 years. The lowest proportion of patients with associated major illness (44%) was among those with klebsiella type 2 infection, and the highest (88%) among those with klebsiella types 1, 3, 4 and 5, but as the authors pointed out, the total number of observations was small (14 in types 1, 3, 4 and 5 groups and 16 in type 2 group). However, this paper is of great interest as it presents recent data on incidence of various klebsiella serotypes isolated from different sites of infection. They also established that few of the low klebsiella types were hospital acquired in contrast to type 24, 88% of which were definitely hospital acquired infections.

(IV) Klebsiella in the Genital Tract

In contrast to the extensive literature on urinary tract infections due to klebsiella, this organism has not been extensively reported in infections of the genital tract. An incidence of 3.5% was reported by Baehr, Schwartzman and Greenspan (1937), and other case reports where *K. aerogenes* has been incriminated in infections of the female genitalia have been made by Scheyer (1926) (1933), and Reichart (1921).

Hepp (1936) described a type A klebsiella where the primary focus of infection was the lungs, spreading to cause postoperative wound abscess, salpingitis and peritonitis after abortion. Another case of acute salpingitis due to *K. pneumoniae* was reported by Botsford and Kinney (1946), the patient being an 80 year old female.

Gordon, Hughes and Barr (1966) made an interesting survey of the varied nature of the vaginal bacterial flora in the presence of *Trichomonas vaginalis* infection, and in severe inflammation states. They noted a more frequent rate of isolation of the enterobacteriaceae, as well as anaerobic streptococci, mycoplasma and *H. vaginalis* in cases of severe inflammation, as well as *T. vaginalis* infection, and these are presumably due to alterations in vaginal pH. No especial mention was made of klebsiella isolates in this series.

Klebsiella associated with abscess formation

One of the cases included in this survey was a fatal case of klebsiella liver abscess (Talerman, Finnie and Wontumi (1968)).

Since 1940, klebsiella has been incriminated in 19 cases of liver abscess - Boettiger, Weinstein and Wern (1940); Rocher and Dubarry (1941); Kinney and Ginsberg (1943); Norman and Binford (1945); Sheridan (1945); Sweden and Liljestrand (1945); Davidson (1948); Bordes (1949); DiFiglia and Cramer (1951); Boardman and O'Brien (1961); Sharma and Singh (1966).

As pointed out elsewhere (Talerman, Finnie and Wontumi, 1968), the great majority of these cases occurred in adults and were often associated with other debilitating conditions such as agranulocytosis, diabetes or biliary tract disease. Serotyping of the klebsiella organism isolated was only done in three cases - Kinney and Ginsberg (1943) and Boardman and O'Brien (1961).

Occasional cases of prostatic abscess due to klebsiella have been reported. The first publication of this type of infection was by Tennenbaum and Ravid (1936), who described a 45 year old diabetic male with a urethral discharge, urinary infection, blood culture and CSF all showing Friedlander's bacillus. At autopsy there were multiple prostatic abscesses and pyaemic abscess in the lungs and kidneys with incipient

hydronephrosis. Pfeiffer (1937) described a case of klebsiella prostatic abscess with blood, faecal and urine cultures also positive for K. pneumoniae. No typing or biochemical results were given in this report, so we cannot conclude that the same strain was isolated from all sources.

In 1947, Solomon reported three fatal cases of wound infection with Friedlander's bacillus, followed by meningitis. The mode of spread of the organism to the meninges in these three cases is of interest - in one it was secondary to bacteraemia, in another there was direct extension through a torn duramater, and the third followed rupture of a brain abscess. The author was of the opinion that these wound infections were hospital acquired. Swartz and Rohde (1946), in their report "Klebsiella infections in an Army Hospital", drew attention for the first time, to the relatively high incidence of klebsiella in osteomyelitis resulting from war wounds. They reported on 189 patients who had gunshot wounds in the extremities - when the medullary cavity was cultured at the time of sequestrectomy, 13 cases (6.8% of all cultures) grew Friedlander's bacillus group A. The authors claimed that the klebsiella bacillus was one of the most important organisms in osteomyelitis due to war wounds, and stressed the need for awareness on the part of laboratory workers in the identification

of Klebsiella from sites other than the respiratory tract.

Lemke and Gates (1951) reported on the distribution and antibiotic sensitivity of 55 strains of K. pneumoniae. 54.5% were isolated from the respiratory tract and the remaining 45.5% from other sources such as osteomyelitis, abscesses and wound infections. Thirty-two of the 55 isolates were resistant to 100 µgm/ml of penicillin, and only one strain was inhibited by 1 µgm/ml. Chloramphenicol was reported as being the most effective drug in treatment. The importance of the gram negative rods in postoperative wound infection in Cardiff was reported by Quick and Brogan (1968); in 117 postoperative wounds, 11% developed infection and more than half of these were due to gram negative rods. The authors classified the degree of wound infection, and in 13 cases showing frank infection, one was due to K. aerogenes and was thought to have contributed to a fatal termination. E. coli predominated as the pathogen in this series, but the diverse biochemical and serological reactions obtained suggested that many strains were involved. No gram negative rods were isolated from any of the pre-operative skin swabs. The authors pointed out that previously little work had been done to produce experimental evidence that gram negative rods can cause soft tissue infections in their own right, and they demonstrated that all the gram negative rods in their series were capable of

causing lesions in the gluteal muscle of mice - but could demonstrate no correlation between the extent of the lesion and the number of organisms injected.

(v) Meningitis associated with K. aerogenes

An excellent review of the literature to 1931 on this aspect of klebsiella infection was made by Rothschild (1931). It is interesting to note that cases were being described at the end of the 19th Century before the practice of lumbar puncture was known - Weichselbaum (1888) and Mills (1892) obtained the organism from the meninges at autopsy, and a very early case of otitis media and mastoiditis was described by Brunner in 1896. Malis (1930) described a fatal case of klebsiella meningitis secondary to otitis media. Rothschild (1931) described the first case of klebsiella meningitis in which recovery occurred. The infection was in a 34 year old adult, where subdural abscess occurred secondary to otitis media and mastoiditis. Cultures from the subdural abscess and CSF were biochemically typical Friedlander's bacillus, and the viscous nature of the CSF was noted.

Neal (1931) reviewed 2,000 cases of meningitis which had come under the surveillance of the New York Department of Health, and only two of these were due to Friedlander's bacillus. Two years later, in 1933, Fothergill and Sweet reported only one case of klebsiella meningitis in a series

of 705 cases (0.14%), and this was in a four-week infant. Another case in an infant was reported by Mori (1943), the patient recovering after sulphonamide therapy. In 1946, a fatal case of klebsiella meningitis was reported by Tartakoff, where the CSF became markedly less viscid after streptomycin therapy, but pulmonary embolism led to a fatal result. At autopsy there was evidence of a "nearly healed" meningitis.

Jacob and Top (1948) reported seven cases of Friedlander's meningitis, five of which were fatal, three of these five had klebsiella septicaemia, two of the patients were diabetic and in five out of the seven the primary focus of infection was the middle ear. The authors postulated that bacteraemia in the presence of meningitis indicated a graver prognosis than in pneumonia. Their two patients who survived never had positive blood cultures. Their work was done in the Herman Kiefer Hospital, Detroit and Henry Ford Hospital, and some indication of the rarity of klebsiella meningitis in these hospitals is illustrated by the fact that over a 19 year period, only seven out of 3,377 (0.02%) cases of meningitis were due to klebsiella. The age range of these seven patients was 39 - 56 years, and five were males. Three of the seven cultures were reported as type A - no information was given on the serotype of the other cases.

Ramsmeier and Major (1943) described a fatal case of Klebsiella septicaemia and meningitis in a 73 year old male. The organism was serotype B (type 2) and in this patient at autopsy miliary abscesses of the myocardium, liver, kidney and cultures from the blood, meninges and bronchi yielded an organism identical to the isolates from the blood, urine and CSF before death. The authors, in reviewing a further 29 cases from the literature, noted that Klebsiella meningitis occurs chiefly in infants, and in adults after the fourth decade, where it is frequently associated with debilitating lesions. In over half the cases reviewed in adults, infection of the middle ear, mastoid and sinuses were regarded as predisposing causes, and in the infant group the primary lesion was unknown. Pneumonia was considered a precursor in only five cases and pharyngitis in one.

In the same year, 1943, Jaffe noted that most emphasis had been placed on the pulmonary lesions produced by Friedlander's bacillus and described two interesting cases to demonstrate that infections may arise from foci other than the meninges, respiratory and renal tracts. Jaffe's first case was a diabetic, with severe localised abscesses of lateral and medial malleoli, and over the dorsum of the ankle - all associated with a pure growth of K. pneumonia Group A (type 1). Mid-thigh

amputation was required after accumulation of pus in the fascial spaces and muscle tissue of the entire leg, and this pus again yielded pure culture of group A klebsiella. The second case was of gangrene of the gall bladder and subphrenic abscess, which yielded pure culture of K. pneumoniae. Both cases were cured by sulphonamide therapy and surgery.

(VI) Klebsiella septicaemia

The literature on the actual incidence of Klebsiella aerogenes in cases of septicaemia is very sparse. In 1934, Kolmer noted that klebsiella septicaemia was rare and Meyer and Amtman (1939) reviewed the literature to that time and noted that 109 cases had been reported. Knorr et al (1953) reported a case of septicaemia secondary to hepatic cirrhosis, which at autopsy revealed diffuse purulent peritonitis, pyaemic abscesses and meningoencephalitis. Since 1956 there appears to have been an increase in bacteraemia due tc the gram negative coliforms, including klebsiella; Koch (1956) described 50 cases, all males, with gram negative bacteraemia in 60% of which some urological manipulative procedure had preceeded the bacteraemia. Maiztegui, Biegeleissen, Cherry and Kass (1965) studied 100 patients with gram negative septicaemia. Klebsiella was the second commonest organism isolated - 30% of cases (E. coli = 35%). This is the only study where detailed serotyping was done - 15 capsular serotypes of klebsiella were isolated, the commonest being types 2, 21, 24, 25 and 54.

Unterman and others (1957) noted that Aerobacter aerogenes was recovered from the blood immediately after sigmoidoscopy in one in 50 patients. Weil and Spink (1958) reported on 400 patients at the University of Minnesota Hospitals in the five year interval 1950-1955, all of which had had gram negative organisms recovered from blood culture. In 278 of these cases the bacteriological findings correlated with the clinical course, indicating that bacteraemia existed, i.e. the results were not due to contamination. There were 165 deaths in this group, klebsiella being incriminated in 35 of these, and a less frequent cause of death than pseudomonas (44 cases), proteus (41 cases) and E. coli (37 cases). However, it is interesting to note that when mortality rates are compared with isolation 35 out of 59 isolates of klebsiella were fatal, i.e. 59% compared to only 35% of pseudomonas, 42% of proteus and 20% of E. coli.

An eight year survey of gram negative bacteraemia was carried out by McCabe and Jackson (1962a and 1962b). Of 173 episodes of bacteraemia in this eight year survey, the commonest organisms incriminated were E. coli and Aerobacter aerogenes - each 28%, pseudomonas 18% and proteus 5%. The authors found a progressive increase in fatalities and Aerobacter aerogenes bacteraemia occurred most frequently in males after genito-urinary operations. Seventy percent of the patients acquired the bacteraemia in hospital, and

this group had a higher mortality. Thirty-eight percent of all cases had had antibiotics prior to infection.

Rogers, Koenig and Holmes (1965) noted a five to eight fold increase in grap negative sepsis over the previous 20 years. Factors which they considered to be contributory were advancing patient age, frequent catheterisation, and wider use of antibiotics which had altered the intrinsic protective bacterial flora in many patients. They also pointed out that gram negative bacteraemia was predominantly hospital acquired.

(VII) Antibiotic resistance studies on Klebsiella species

In view of the postulated aetiology of antibiotics in the increasing infections caused by the gram negative bacilli, it is relevant to consider the more important papers on this subject. Reimann, Price and Elias (1945) noted that orally administered streptomycin was almost entirely excreted in the faeces and could suppress a growth of streptomycin resistant coliforms, but these reappeared within a few days of cessation of therapy. Heilman (1945) studied nine strains of Klebsiella, and demonstrated protection of mice by streptomycin, to 1,000 - 10,000 times the lethal dose of Klebsiella.

Schoenbach (1949), in comparing in vivo minimal inhibitory concentrations of aureomycin, chloromycetin and

polymyxin against klebsiella, showed that polymyxin was most effective, with a M.I.C. of 0.312 - 1.25 micrograms/ml compared to aureomycin and chloramphenicol, which had a M.I.C. range of 1.25 - 5 micrograms/ml.

Coffey, Schwab and Ehrlich (1950) demonstrated that klebsiella could acquire resistance to chloramphenicol in vitro after successive exposure to greater concentration of antibiotic.

Eisenberg, O'Louglin and Flippin (1954) studied 305 klebsiella isolates from various sources, and from all sources the highest incidence of sensitivity was to chloramphenicol. Types 1-4 were most sensitive and type 7 least sensitive to antibiotics. Epstein (1959a), in a biochemical analysis of 82 klebsiella strains described an anaerogenic type 3 strain which, unlike all other klebsiella strains, was sensitive to erythromycin, oleandomycin and novobiocin. Coppo (1957) confirmed the relative resistance of aerogenes strains to novobiocin.

Resistance transfer to streptomycin was demonstrated by Hughes (1961) using streptomycin when a population with increased resistance over the parent strain emerged. When these two strains were grown together and retested, a uniform population was found to be present, with streptomycin resistance intermediate between the initial two.

Koch and Rose (1966) compared the resistance of klebsiella, aerobacter, cloaca and serratia to 30 ug cephalothin and 10 ugm ampicillin. None of 25 strains of klebsiella were resistant to cephalothin in contrast to 92% resistance in aerobacter and 100% resistance in the cloaca group. Ampicillin, however, failed to inhibit the growth of 72% of the klebsiella strains, 72% of the cloaca strains, but was more effective for serratia which only had 36% resistance. In 1963, Fleming demonstrated that the enzyme cephalosporinase, which is highly active against cephalosporin C could not be demonstrated in klebsiella, but could be produced by aerobacter and serratia.

Benner, Micklewait and others (1965), working at Seattle, studied 161 klebsiella-aerobacter strains and found that all non-motile klebsiella were susceptible to cephalothin and cephaloridine, in contrast to the motile aerobacter, which were highly resistant. One other point of interest in this paper was that one third of the klebsiella strains were resistant to tetracycline, chloramphenicol, streptomycin, kannamycin, furadantin and sulphonamide.

Eickhoff, Steinhauser amd Finland (1966) studied in vitro susceptibility of 256 strains of klebsiella to 16 antibiotics. Gentamycin was the most active, with 90% of

strains inhibited by 3.1 µg/ml. Kanamycin, neomycin and paromomycin were next most active, but about 16% of Klebsiella strains were resistant to 100 ug/ml of kanamycin. About half the strains were resistant to streptomycin, chloramphenicol and tetracycline. Types 1 to 5 were more sensitive to tetracycline, chloramphenicol and streptomycin than were the other types. Type 24 was much more resistant than the other types. The authors also demonstrated that respiratory tract strains were more sensitive to cephalothin, chloramphenicol, tetracycline, streptomycin and, to a lesser extent, polymyxin B, than strains from other sources, whereas there was no difference in sensitivity to kanamycin and neomycin when the source was considered.

The value of kanamycin in treating Klebsiella infection was stressed by Bulger (1967), who studied 25 clinical isolates and found only two kanamycin resistant strains. Bulger demonstrated synergism for 13 of the 25 strains with kanamycin and cephalothin, and no antagonism was observed. However, with one strain, rapid in vitro resistance developed with both kanamycin and cephalothin, and this was delayed when the combination was used. The author recommended that, despite its toxicity, kanamycin should be considered when selecting a chemotherapeutic agent to treat a severe Klebsiella infection.

PART I

4A MATERIALS AND METHODS

Primary cultures of Klebsiella aerogenes were collected from MacConkey plates, and stored on Worfel Ferguson slopes at 4°C, in sealed Bijou bottles.

Worfel Ferguson media (Difco)

Sodium chloride	2.0 g
Potassium sulphate	1.0 g
Magnesium sulphate	0.25g
Saccharose	20.0 g
Yeast extract	2.0 g
Agar	15.0 g

40.3 g. medium was rehydrated in 1 litre of cold distilled water, and heated to boiling to dissolve. Media was sterilised for 15 minutes at 121°C.

The source of Klebsiella isolation was noted, and the following biochemical tests were done on all specimens :

1. Acid and gas from glucose and lactose.
2. Methyl red and Voges Proskauer reactions.
3. Ability to utilise citrate.
4. Urease production.
5. Gluconate and malonate tests.
6. Lysine, arginine and orthinine decarboxylase production.

7. Production of acid from dulcitol.
8. Ability to grow on KCN.
9. Motility.
10. Acid and gas production from adonitol, starch and glycerol.
11. Indole production.
12. Gelatin liquefaction.

The nomenclature proposed by Cowan and Steel (1960) is used throughout this work. Only non-motile strains were included in this series, and only strains showing typical K. aerogenes biochemistry, or deviating from this in not more than three tests were studied.

Sugar Fermentation

Acid and gas production from glucose, lactose, adonitol, starch and glycerol, and acid production from dulcitol were tested.

All media for sugar reactions contained 5 g. sugar per litre of base. Bacto purple broth base was reconstituted to 16 g. per litre of distilled water. The indicator was Brom-cresol purple.

All sugars after inoculation, were incubated at 37°C and read at 24 and 48 hours. Negative sugars were reincubated for up to 14 days, and read daily. Starch fermentation was read initially after four days and reincubated for up to 14 days.

Methyl Red and Voges-Proskauer Tests

Bacto methyl red Voges-Proskauer medium rehydrated to 17 g. per litre distilled water. Distributed in 10 ml volumes in screw top bottles and pH adjusted to 6.9. Inoculated cultures were incubated at 37°C for 48 hours.

Methyl red test performed as in Cowan and Steel (1965).

Positive control - Escherichia coli

Negative control - Enterobacter cloacae

Voges-Proskauer test

Voges-Proskauer test performed by Barritt's method (1936) as described in Cowan and Steel (1965).

Positive control - Enterobacter cloacae

Negative control - Escherichia coli

Citrate Utilisation Test

Bacto-Simmons Citrate Agar - 24.2 g. per litre distilled water; pH adjusted to 6.8. Test performed as in Cowan and Steel (1965) modified from Simmons (1926).

Urease Production

Bacto urea agar base 29 g. dissolved in 100 ml distilled water and this concentrated base filter sterilised. 15 g. Bacto agar dissolved in 900 ml distilled water by boiling, and sterilised at 15 lbs. pressure for

15 minutes (121°C). The media was cooled to 50°C and 100 ml of filter-sterilised concentrated Bacto-urea agar added aseptically. Final reaction pH 6.8. Test performed as in Cowan and Steel (1965).

Positive control - Proteus vulgaris

Negative control - Salmonella typhi

Gluconate Test

Gluconate broth was prepared as follows :

Peptone	1.5 g
Yeast extract	1.0 g
K_2HPO_4	1.0 g
Potassium gluconate	40.0 g
Distilled water	1,000 ml

Solids dissolved in water by heating; pH adjusted to 7. Mixture was filtered, and sterilised at 115°C for 20 minutes, and dispensed in 2 ml volumes in cotton-top tubes. Inoculated broth was incubated at 37°C for 48 hours. 1 Clintest tablet was added (Carpenter (1961)) and media was observed for formation of brown, orange or tan precipitate, indicating a positive reaction.

Positive Control - Klebsiella aerogenes NCTC 9142

Negative Control - E. coli

Malonate Utilisation

Malonate test was performed as in Cowan and Steel

(1965) by method of Shaw and Clarke (1955).

Positive control - Klebsiella aerogenes NCTC 9142

Negative control - Proteus vulgaris

Decarboxylase Tests

Decarboxylase medium base - Difco. The rehydrated medium was distributed in four equal volumes, sterilised at 115°C for 20 minutes and the following additions made :

1. L arginine HCL 1%
2. L lysine HCL 1%
3. L ornithine HCL 1%
4. No addition.

pH adjusted to 6. The four media were distributed in 1.5 ml volumes in 3 x 3/8" tubes containing liquid paraffin to a height of 5 mm.

Tests were performed by Møller's method (1955) using the following controls :

	Arginine	Lysine	Ornithine
Proteus vulgaris	-	-	-
Proteus morganii	-	-	+
Klebsiella aerogenes	-	+	-
Salmonella typhi	+	+	-
Salmonella typhimurium	+	+	+

KCN Test

KCN broth prepared as follows :

Peptone	3 g
NaCl	5 g

KH_2PO_4	0.225 g
$Na_2HPO_4 2H_2O$	5.64 g
Distilled water	1,000 ml

The solids were dissolved in the water, filtered and distributed in 100 ml volumes in screw capped containers. Sterilised at 115°C for 20 minutes. Before use, 1.5 ml of freshly prepared 0.5% KCN solution was added to 100 ml base and the mixture distributed in 1 ml amounts into sterile Bijou bottles. Method of Møller (1954) and Rogers and Taylor (1961) used. Cultures read after 24 and 48 hours.

Positive control - Klebsiella aerogenes NCTC 9142

Negative control - E. coli

Motility

Motility was tested by the use of semi-solid motility media by the method of Edwards and Bruner (1942).

Positive control - Proteus vulgaris

Negative control - Klebsiella aerogenes NCTC 9142

Gelatin Liquefaction

Nutrient Gelatin

Beef extract	3 g
Peptone	5 g
Gelatin	120 g
Water	1,000 ml

Gelatin was added to the water and mixture stood for 15 minutes to dissolve gelatin. The other constituents were added and pH adjusted to 7. Sterilised at 115°C for 20 minutes.

Method: Nutrient gelatin inoculated with a straight wire and incubated at 37°C for 14 days. Every three days medium was cooled to 4°C for two hours and examined for liquefaction.

Positive control - Serratia marcescens

Negative control - Uninoculated tube incubated for same time as test culture.

Indole Test

1% Bacto-tryptone medium. 0.2 ml Kovac's reagent added to 5 ml of 24 hour culture.

Positive control - E. coli

Negative control - S. typhi

Antibiotic Sensitivity Tests

Sensitivity tests were performed on Mueller and Hinton agar, using bacterial broth suspensions prepared as described by Bauer, Kirby et al (1966).

Klebsiella cultures isolated from urines were tested with Oxoid multidiscs 1186E, containing the following concentrations of antibiotics :

Penicillin	5 mcg
Streptomycin	25 mcg

	Tetracycline	50 mcg
	Chloramphenicol	50 mcg
	Sulphonamide	300 mcg
	Furadantin	100 mcg
	Polymyxin	250 mcg

and in addition, the following single-discs were used :

Mast Lab.	Ampicillin		25 mcg
Upjohn	Albamycin T	Novobiocin	15 mcg
		Tetracycline HCL	15 mcg
	Ceporin		25 mcg

Klebsiella cultures from all other sites except urine were tested with Oxoid multidiscs 846E containing :

Penicillin	1.5 mcg
Streptomycin	10 mcg
Tetracycline	10 mcg
Chloramphenicol	10 mcg
Sulphonamide	50 mcg
Polymyxin	100 mcg

and also the following single discs :

Mast Labs.	Ampicillin		25 mcg
Upjohn	Albamycin T	Novobiocin	15 mcg
		Tetracycline HCL	15 mcg
	Ceporin		5 mcg
	Framycetin		30 mcg

Inhibition was read after 18 hours incubation at 37°C.

Demonstration of Capsules

All cultures of Klebsiella isolated were tested for capsule production by the method described in Harris and Coleman (1963). One colony from Warfel Ferguson agar was suspended in physiological saline solution and 1 loop of this suspension placed on a slide. Adjacent to it was placed one drop of Pelican India ink, the two drops joined with a loop and covered with a glass cover slip, and examined for capsules microscopically.

Preparation of antigens used in Antisera Production

Stock cultures of Klebsiella species serotypes 1 to 72 were obtained from the National Collection of Type Cultures, Colindale, London. The list of stock strains used for antisera preparation is given in Table 25 of the appendix.

Storage of Cultures

Stock cultures were stored at 4°C on Warfel Ferguson slopes in sealed Bijou bottles.

Formalisation of Antigens

Fresh subcultures of the stock antigens were made onto Warfel Ferguson plates. Mucoid colonies

not producing excess of slime were inoculated into 50 ml of 0.2% glucose broth, and incubated for four hours at 37°C.

40% Formalin was added to the broth cultures to a final concentration of 0.5%. Formalised cultures were incubated at 37°C for 18 hours, and tested for sterility by 48 hours aerobic and anaerobic incubation on Blood agar plates. The formalised cultures were tested for capsule formation by adding one drop of Pelican India ink to one loop of suspension and examining wet preparations under a coverslip microscopically. Only cultures showing good capsulation were used as antigens.

Preparation of Antisera

Antisera to capsular serotypes 1 - 40 were prepared in two groups of animals, rabbit and mouse. Only the rabbit antisera were used in typing, and the immunisation schedule will now be described.

Rabbits: Strain - Pink-eyed Belgium rabbits of either sex, weighing 4 - 6 Kgms.

Before inoculation, 10 ml of blood was removed from the lateral vein of the ear and serum tested neat and at ½ dilution for naturally occurring antibodies to each of the 72 capsular serotypes. Two drops of suspension of antigen in physiological saline were made on a slide and one drop of serum added to each. To one of the antigen/

serum suspensions one drop of India ink was added, and the two preparations compared for the Quellung reaction. Circulating antibodies to the 72 serotypes of Klebsiella could not be detected in any of the animals used.

Inoculation schedule

The schedule recommended by Harris and Coleman (1963) was followed. Each rabbit was inoculated with the formalised culture of capsular specific antigen at four day intervals, intravenously via the ear vein. The volumes of antigen given were 0.5, 1, 2, 3, 3 and 3 ml. Four days after the last injection, at day 25 of the inoculation schedule, a small amount of serum was tested for precipitating antibodies by the capsular swelling test. Sera giving a positive Quellung reaction with homologous antigen to a dilution of $^{1}/16$ or more were acceptable. Where the titre was $^{1}/8$ or less, animals were re-inoculated with 3 ml at four day intervals. However, in some cases it was impossible to read a titre greater than $^{1}/8$ (see results section, part 2). Animals were exsanguinated by intra cardiac puncture, blood collected in sterile tubes, allowed to separate overnight at 4°C, and serum collected after centrifugation at 5,000 rpm for 30 minutes. The volume of serum collected from each animal ranged from 15 - 25 ml, and this was stored at -20°C in 5 ml aliquots to which one

drop of methiolate was added.

Testing of Antisera

a) For homologous titre

Serial doubling dilutions of antisera were made in physiological saline from neat to $^{1}/_{64}$. Fresh cultures of homologous antigen were suspended in formol-saline, and these tested with each dilution of antisera by the Quellung reaction. The end point was taken as the highest dilution to give clear cut, positive Quellung reaction.

b) For cross reactions

Each antiserum prepared was tested at a one quarter dilution with fresh formol-saline suspensions of Klebsiella serotypes 1 - 72 for cross reactions. Where cross reactions occurred Quellung reactions were performed with serial dilutions of antiserum.

Absorption Tests

In order to obtain pure homologous antisera, the method described by Edwards and Ewing (1962) was followed. A fresh suspension of the cross reacting serotype was suspended in 1 ml saline and this added dropwise to 1 ml of the serum to be adsorbed, with continuous shaking, until the supernatant fluid remained cloudy. The serum was then centrifuged at 5,000 rpm for 15 minutes and the supernatant again

titred by the Quellung reaction with homologous culture, and with the absorbing culture to ensure that all cross reactions had been removed. Where it was necessary to adsorb an antisera with more than one heterologous serotype, the adsorptions were done successively.

Typing of unknown cultures

For this purpose, antisera prepared in the rabbit were used.

Eight pools of antisera were prepared, each containing 1 ml of $\frac{5}{\wedge}$ different undiluted antisera. Fresh cultures of the organism to be typed were grown on Wörfel Ferguson slopes and a suspension made in physiological saline. One drop of suspension was added to each drop of each of the eight pools of antisera on a glass plate, and tested for agglutination. Pools of antisera giving naked eye agglutination were then tested for specific capsular swelling with each of the component antisera in turn, at optimal dilutions. Cultures which could not be typed by antisera 1 - 40 were re-examined for capsule formation by India ink staining, and reported as either capsulate not 1 - 40, or non-capsulate.

PART I

5A RESULTS

(1) From April, 1966, to March, 1967, 290 isolates of Klebsiella aerogenes were collected for study.

All these cultures came from clinical material submitted for bacteriological culture from patients of the University Hospital of the West Indies.

Source of 290 cultures was as follows:

Respiratory Tract			
	Throat swabs	46	
	Tracheotomy aspirates	7	(38%)
	Sputum	58	
Urinary Tract			
	Urines	79	(27.2%)
Female Genital Tract			
	High Vaginal swabs	24	(8.3%)
Other Pyogenic Lesions			
e.g.	Wound infections, Abscesses, Infected umbilical cords	55	(19%)
Circulatory System			
	Blood cultures	6	(2%)
Central Nervous System			
	Cerebrospinal fluid	1	(0.35%)

Miscellaneous		
Ear swabs	2	(1.6%)
Eye swabs	2	
Autopsy Material	10	(3.5%)

A total of 248 patients were involved in this survey, 16 of these showed multiple Klebsiella infection at different sites. There were three deaths in this series, directly attributable to infection by Klebsiella aerogenes, and from these three patients 10 autopsy specimens were collected.

Where the same serotype and biotype of Klebsiella was isolated from the same source in any one particular patient on two or more occasions, only one of these strains was included in the survey, to avoid misleading repetition. Where a patient showed a change in either serotype or biotype in specimens submitted from the same source, these were classified as separate strains, for purpose of analysis of antibiotic sensitivity.

The 248 patients in this survey had an age range of two days to 85 years.

Age Distribution		
Less than 1 year	34 cases	(13.7%)
1 - 9 years	24 cases	(9.7%)
10-19 years	14 cases	(5.7%)

20-29 years	28 cases	(11.2%)
30-39 years	31 cases	(12.5%)
40-49 years	27 cases	(11.0%)
50-59 years	37 cases	(14.9%)
60-69 years	35 cases	(14.1%)
70+ years	18 cases	(7.2%)

Sex Distribution (248 patients)

119 males	(44%)
129 females	(56%)

In order to assess the clinical significance of Klebsiella aerogenes isolation, the majority of cases in the survey were restricted to cases where Klebsiella aerogenes occurred in pure culture. In all cases Klebsiella was the predominant growth, and specific mention will be made in the text where it occurred concommittently with any other organism.

The following details were taken from all patients

1. Name. Hospital number.
2. Provisional admission diagnosis.
3. Ward. 4. Age. 5. Sex.
6. Full clinical examination of the system where Klebsiella infection occurred. Details were obtained from the patients' case notes of any other illnesses, which may have predisposed to infection.

7. History of antibiotics given immediately prior to isolation of Klebsiella; the nature of the antibiotics given, the daily dose, and the duration of therapy.
8. 8:00 am oral temperature on day of submitting positive specimen.
9. White blood count where available.
10. An assessment of whether the infection was hospital acquired - the latter included :
    (i) any neonatal infection occurring in hospital after a hospital delivery;
    (ii) any case where previous negative culture had been obtained from the same site since the current admission to hospital;
    (iii) where symptoms and signs of infection clearly developed after admission.

Some cases were clearly not hospital acquired, e.g. specimens taken on admission, or early thereafter. Where more than a three-day interval had elapsed between admission to hospital and submission of the first specimen for culture, the environmental source of infection was classified as unknown, with the exceptions of groups (i) to (iii) above. Where any doubt existed, patients were classified in this unknown group, which may contain a proportion of hospital acquired infections; this evidence could only have been confirmed by daily swabbing

of all patients. Nevertheless, in spite of the rigid criteria for defining hospital acquired infection, 94/248 patients (37%) were shown to have acquired Klebsiella infection from a hospital source.

Serological typing was attempted on all specimens, using adsorbed antisera to types 1-40, prepared in rabbits as described in Part I of Materials and Methods.

In the presentation of the results the following points are considered :

1. The serotypes isolated from the various sources of infection and their association with disease. The incidence of isolation of commensal and pathogenic strains will be considered.
2. The strains of respiratory origin will then be compared with strains from other sources for biochemical heterogeneity and antibiotic resistance.
3. The signs and symptoms of urinary tract infection due to Klebsiella will be compared with an age and sex matched group having E. coli urinary tract infection.
4. Patients with multiple Klebsiella infection will be considered.
5. The role of antibiotics in predisposing to Klebsiella infection will be assessed by comparing

the antibiotics given to a control group free from Klebsiella infection, and matched for age, sex, duration of hospital admission, and provisional admission diagnosis.

6. Finally the serotype, biotype and ward distribution of hospital acquired strains of Klebsiella will be analysed, to investigate the extent to which cross-infection is occurring in the hospital.

(ii) Analysis of Respiratory Strains of Klebsiella Aerogenes

Incidence of throat swabs

Two hundred throat swabs were examined from patients with clinical signs of infection in the throat, e.g. inflamed throat, tonsillitis - with or without cervical lymphadenopathy, and laryngitis.

The age distribution of this sample was as follows:

0 - 1 years	30 cases	(15.0%)
1 -10 years	110 cases	(55.0%)
11-20 years	37 cases	(18.5%)
21-30 years	12 cases	(6.0%)
31-40 years	6 cases	(3.0%)
41-50 years	5 cases	(2.5%)
51 and over	0 cases	

Sex Distribution of sample:

95 males

105 females

Twenty seven of this group had Klebsiella aerogenes in predominant culture in the throat, and in all 27 cases there was heavy growth of Klebsiella and no isolation of either Streptococcus pyogenes or Corynebacterium diphtheriae. Therefore the incidence of K. aerogenes in cases of throat infection = 27/200 (13.5%).

The age distribution of this group of 27 patients showing K. aerogenes in the throat was much the same as the distribution of the total sample, with the exception of a higher proportion of Klebsiella isolates in the first year of life, and a lower number in the age group 1 - 20 years.

Age Distribution (throat swabs)	Total No. Cases (200)	% Total	27 showing K. aerogenes	% Total
0 - 1 year	30	(15.0%)	11	42.0%
1 -10 years	110	(55.0%)	9	33.3%
11-20 years	37	(18.5%)	2	7.0%
21-30 years	12	(6.0%)	2	7.0%
31-40 years	6	(3.0%)	1	3.7%
41-50 years	5	(2.5%)	2	7.0%
51 and over	0		0	

The sex distribution of the Klebsiella group was 15 males : 12 females.

It is seen that 70% of all throat swabs examined came from children under 10 years, and 75% of all klebsiella isolates in this group fell into this under-10 age distribution, with the highest incidence (42%) occurring in the first years of life.

In the total sample of 200 cases of throat infection Streptococcus progenes was isolated from 35/200 patients (17.5%, and C. diphtheria mitis from one case (0.5%). In no instance was klebsiella culture associated with the presence of these organisms.

Normal Controls

Two hundred patients showing no evidence of throat infection, or other respiratory signs, were matched with the above group for age, sex, duration of hospitalisation and history of antibiotic therapy. In this group of normal cases, klebsiella was isolated in 23/200 cases, i.e. an incidence of 11.5%.

The age distribution of these patients showing Klebsiella aerogenes was as follows:

Age Distribution (throat swabs from normal persons)	Total No. Cases	% Total	Klebsiella (23)	% of Total Klebsiella Isolates
0- 1 year	30	(15.0%)	9	39.0%
1-10 years	110	(55.0%)	8	34.8%
11-20 years	37	(18.5%)	3	13.0%

Age Distribution (throat swabs from normal persons)	Total No. Cases	% Total	Klebsiella (23)	% of Total Klebsiella Isolates
21-30 years	12	(6.0%)	1	4.4%
31-40 years	6	(3.0%)	1	4.4%
41-50 years	5	(2.5%)	1	4.4%
51 and over	0			

In this control group, one case of Streptococcus pyogenes isolation occurred, and there were no C. diphtheriae isolates. The remaining cases showed normal upper respiratory tract flora.

Therefore, incidence of Klebsiella aerogenes in throat infections = 27/200 (13.5%). Incidence of Klebsiella aerogenes in normal throats = 23/200 (11.5%).

t test on difference in proportion curves

t = 0.60, giving p = 0.55 which is not significant.

Strains of Klebsiella aerogenes isolated from throat swabs.

Of the 23 patients showing Klebsiella aerogenes in normal throats, 16 patients were available for this survey. All 27 of the cases where Klebsiella was associated with upper respiratory tract infection were available for study, giving a total of 42 patients, from which 46 different isolates were made (Table 1 - Appendix).

Klebsiella from normal throat swabs

Sixteen patients, 12 of whom were under ten years of age, and five were under one year, are considered. Two patients showed a change of strain of klebsiella in the throat, giving 18 separate isolates.

In the group showing evidence of throat infection, 28 different strains of klebsiella were obtained from 27 patients. Eleven out of these 27 patients were under one year, and a further nine were under ten years.

A comparison will now be made of the serotypes isolated from the normal and infected throats, the biochemical findings and the proportion of hospital acquired cases.

Table 1 shows that serotypes 3, 9, 11, 15, 29 and 35 were isolated from both normal and infected throats, so the isolation of these serotypes is probably of no significance.

Serotypes 1, 2, 8, 13, 16 and 24 were isolated in association with local signs in the throat, but as the total sample size was small (46) it would not be justifiable to ascribe a pathogenic role to these serotypes. Table 1 does illustrate the diversity of serotypes of Klebsiella aerogenes which can be isolated from throat swabs.

Table 1

Serotypes of Klebsiella aerogenes Isolated from Normal and Infected Throats

Serotype	1	2	3	4	8	9	11	12	13	15	16	23	24	29	35	Not 1-40	Acapsulate	Total
Normal throats (figures indicate no. of isolates)	-	-	3	2	-	1	2	1	-	1	-	1	-	2	3	1	1	18
Infected throats	3	5	2	-	1	1	2	-	1	1	1	-	1	2	4	4	-	28

In the group showing normal throats a total of 18 different isolates were made from 16 patients, and six out of 16 patients were on antibiotics at the time of first isolation of klebsiella.

In the infected group, 28 isolates were made from 27 patients, and nine out of those 27 patients were on antibiotics. Thus, 28/43 patients acquired a klebsiella invasion of the throat in the absence of antibiotic therapy.

Biochemically typical Klebsiella aerogenes is defined by the following reactions :

Gas produced from glucose
Acid from lactose
MR +, V-P negative
Citrate +
Urea +
Gluconate +
Malonate +
Lysine decarboxylase +
Acid dulcitol variable
KCN +
Motility negative
Acid and gas from adonitol, starch and glycerol
Indole negative
Gelatin not liquefied

Any deviations from the above pattern will be listed in the text.

In the Klebsiella isolates from normal throat swabs, 12/18 were biochemically typical, and from infected throats 20/28 were biochemically typical (Table 1 - Appendix).

Table 2 in the Appendix analyses the different combinations of serotypes and biotypes found in 46 isolates of Klebsiella from the throat. If each strain is given the numerical prefix T (throat swab) (Table 2 - Appendix), at least 27 different strains are present. T1, T4, T13, T12, T24 are each represented by three isolates. If strains 41 - 45 could have been serotyped they may have represented more than the three strains demonstrated biochemically. It is thus apparent that one is dealing with a very heterogeneous population of Klebsiella aerogenes, even when considering specimens obtained from one site alone.

Clinical features associated with Klebsiella isolation from the throat

Of the 18 cases carrying Klebsiella commensally in the throat, eight were hospital acquired infections, i.e. the admission throat swabs were negative.

In this group of 18 patients only three could be regarded as completely healthy carriers, and these were routine throat swabs from nurses, carrying types 12, 29 and 35. The remaining cases included a diversity of

signs and symptoms, such as haemophilus meningitis, malnutrition, acute glomerulonephritis, patent ductus arteriosus, haemophilia, a severely ill case of acute leukaemia, and E. coli 055 B5 gastroenteritis, chronic pyelonephritis, and two neonates - one with pyloric stenosis, and the other a premature infant of 3 lbs., who developed a hospital acquired klebsiella in a healthy throat on the 18th day after delivery.

27 Cases of Klebsiella Isolation Associated with Local Signs in the Throat

Table 2 illustrates the local signs associated with K. aerogenes isolated from throat swabs. Of these 27 cases, 12 were hospital acquired strains and nine patients were on antibiotics at the time of first isolation of klebsiella from the throat.

Consideration of Table 3 shows that in series numbers 3, 4, 5, 6, 7, 8 and 38 throat lesions were occurring in healthy adults not on antibiotics, and klebsiella was apparently the primary invader - these were all serotypes 1 and 2, traditionally associated with respiratory infection, except for case 38, when a type 35 apparently caused the tonsillitis. Cases 37 and 31 are obvious examples of super-infection after antibiotic therapy; the throat signs persisted until klebsiella had been eradicated.

In series numbers 1, 9, 10, 11, 19 and 39, (Table 3), sputum samples were not submitted so a klebsiella aetiology cannot be attributed to the signs in the lower respiratory tract. In all cases, however, there was clinical improvement after eradication of klebsiella from the throat, but this may have been fortuitous.

In series numbers 20, 27 and 2, the presence of Candida albicans infection in the mouth makes the role of klebsiella impossible to assess, but it is probably of secondary importance. Similarly, in case 29, where Staphylococcus pyogenes was isolated twice from a pleural effusion, the throat klebsiella is probably of little importance.

Three cases in this series showed change in klebsiella type in the throat. Case number 44, a child of five months with Pierre Robin syndrome, acquired an untypable, biochemically typical Klebsiella aerogenes after ten days in hospital, but with no antibiotic therapy. Five days later this strain was replaced by a second untypable strain (No. 45 - see Table 1 - Appendix) which differed from the first in :

(1) failure to produce a lysine decarboxylase;

(2) ability to ferment dulcitol;

(3) increased resistance to antibiotics.

Table 2

LOCAL SIGNS IN THE THROAT IN ASSOCIATION WITH PURE CULTURE OF KLEBSIELLA

	Local Signs	Number of Cases	Serotypes Isolated
I	Throat inflamed (without exudate)	9 (Series Nos. 2,4,6,29,30, 31,37,40,44.)	1,2,2,24,29, 29,35,35, 1 strain not 1-40.
II	Acute follicular tonsillitis.	6 (Series Nos. 3,7,8,17,26, 38.)	1,2,2,9,15, 35.
III	Acute follicular tonsillitis with laryngitis or sinusitis.	2 (Series Nos. 5,42.)	2, 1 strain not 1-40.
IV	Inflamed throat and bronchitis or acute bronchitis or broncho-pneumonia.	10 (Series Nos. 1,9,10,16,19 20,24,27,39, 43.)	1,3,3,8,11, 11,13,16,35, 1 strain not 1-40.

Table 3

CLINICAL DETAILS OF 27 CASES OF KLEBSIELLA ASSOCIATED WITH UPPER RESPIRATORY INFECTION

Series No.	Local signs in throat	Other diseases	Sero-type	On anti-biotics
1	Inflamed throat.	Acute bronchitis. Prematurity.	1	No
2	Inflamed throat with mucoid sputum.	Oral *candida albicans*. Hydrocephalus. Lumbar meningo-myelocoele.	1	No
3	Acute follicular tonsillitis.	Healthy adult.	1	No
4	Inflamed throat. Laryngitis.	Healthy adult.	2	No
5	Acute follicular tonsillitis. Sinusitis.	Healthy adult.	2	No
6	Inflamed throat.	Healthy child	2	No
7	Acute follicular tonsillitis and cervical adenitis.	Healthy adult	2	No
8	Acute follicular tonsillitis.	Healthy adult	2	No
9	Inflamed throat.	Acute bronchitis.	3	No
10	Inflamed throat.	Tracheo-bronchitis.	3	No

(Continued)

Table 3 (Continued)

CLINICAL DETAILS OF 27 CASES OF KLEBSIELLA ASSOCIATED WITH UPPER RESPIRATORY INFECTION

Series No.	Local signs in throat	Other diseases	Sero-type	On anti-biotics
16	Inflamed throat.	Right middle lobar pneumonia.	8	No
17	Acute follicular tonsillitis.	Gastroenteritis. (no pathogens isolated)	9	No
19	Inflamed throat.	Bronchiolitis.	11	No
20	Inflamed throat.	Acute bronchitis. Oral *candida albicans*. Prematurity.	11	Yes
24	Inflamed throat.	Bronchopneumonia. Enteropathic *E. coli* infection.	13	Yes
27	Inflamed throat.	Oral *candida albicans*. Bronchopneumonia. Gastroenteritis. (no enteric pathogens isolated)	16	No
26	Acute follicular tonsillitis.	Gastroenteritis. (no pathogens isolated)	15	Yes
29	Inflamed throat. (Staphylococcus also isolated)	Staphylococcoal empyema.	24	No
30	Inflamed throat.	Nephrotic syndrome.	29	Yes

Table 3 (Continued)

CLINICAL DETAILS OF 27 CASES OF KLEBSIELLA ASSOCIATED WITH UPPER RESPIRATORY INFECTION

Series No.	Local signs in throat	Other diseases	Sero-type	On antibiotics
31	Inflamed throat. Klebsiella appeared after 6 days penicillin therapy for B-haemolytic streptococcal infection. Throat remained inflamed until Klebsiella eradicated.	Haemophilia.	29	Yes
37	Inflamed throat - after 10 days penicillin therapy for BH/S throat infection.	Von Gierkes disease.	35	Yes
38	Acute follicular tonsillitis.	Healthy adult	35	No
39	Inflamed throat.	Bronchitis. Atelectasis. Congenital deformities. Prematurity.	35	Yes
40	Inflamed throat.	Piperazine neurotoxicity, in coma.	35	Yes
42	Acute tonsillitis and laryngitis.	Healthy female.	not 1-40	Yes

Table 3 (Continued)

CLINICAL DETAILS OF 27 CASES OF KLEBSIELLA ASSOCIATED WITH UPPER RESPIRATORY INFECTION

Series No.	Local signs in throat	Other diseases	Sero-type	On anti-biotics
43	Inflamed throat.	Bronchiolitis. Klebsiella otitis media.	not 1-40	No
44	Inflamed throat.	Pierre Robin syndrome Malnutrition.	not 1-40	No

Case number 11, a child of 21 months, acquired a type 3 klebsiella culture in the throat after five days penicillin and streptomycin therapy, which was sensitive to four antibiotics (albamycin, framycetin, ceporin and polymyxin). Five days later this was replaced by a more resistant type 3 variant, sensitive only to ceporin and polymyxin. The throat remained normal throughout (see strains Nos. 11 and 12 in Table 1 - Appendix).

Finally, case number 14 was a six-year-old child with acute stem cell leukaemia. A serotype 4 was isolated from her normal throat on two occasions. Of these, strain 15 was dulcitol negative and sensitive to all antibiotics except penicillin. Four days later

strain 14 (see Table 1 - Appendix) was isolated in pure culture; this strain was dulcitol positive, and sensitive only to chloramphenicol, framycetin and polymyxin.

Cases 14 and 44 illustrate that colonisation of the throat in hospital with more resistant organisms does not require administration of antibiotics, and all three cases illustrate the constantly changing nature of the upper respiratory flora.

Tracheotomy Specimens

Seven different isolates of K. aerogenes were obtained from tracheotomy aspirates of six patients during the course of this study. The incidence of klebsiella isolation was 6/10 patients (Table 4).

In these observations, which are too small for statistical analysis, two out of six patients showed no signs of infection but grew klebsiella, compared to four out of six patients who showed infected tracheotomies. Case number 50 is of interest (Table 4) in that no signs of infection were associated with a heavy growth of klebsiella type 11 and when this was replaced by a klebsiella type 1 the same patient remained asymptomatic. Klebsiella type 9 was commensal in isolate number 52.

Table 3 - Appendix shows the strain designation

Table 4

ORGANISMS ISOLATED FROM 10 PATIENTS WITH TRACHEOSTOMES

Strain Number	Age Distribution of Patients with Tracheotomy	Sex	Organisms grown	Signs of Infection
	3 years	F	No growth	None
47	5 years	M	Klebsiella type 24 + Pseudomonas	Yes
48	11 years	M	Klebsiella type 21	Yes
	12 years	M	No growth	None
	18 years	F	No growth	None
49	28 years	F	Klebsiella type 3	Yes
50 (same patient)	29 years	F	Klebsiella type 11	None
51 (same patient)			Klebsiella type 1	None
	36 years	M	Staphylococcus pyogenes	Yes
52	52 years	M	Klebsiella type 9	None
53	61 years	M	Klebsiella type 8 + Pseudomonas	Yes

Table 5

COMPARISON OF KLEBSIELLA STRAINS ISOLATED FROM NORMAL AND INFECTED TRACHEOTOMIES

	Normal Sites (2 patients - 3 strains)	Infected Sites (4 patients)
Serotypes Isolated	1, 9, 11	3, 9, 21, 24
Hospital Acquired	3/3	4/4
Biochemical Pattern	All strains typical *K. aerogenes*	All strains typical *K. aerogenes*
Previous history of Antibiotic Therapy	2/2	3/4

of the tracheotomy isolates. Five of these seven strains had already been encountered in the throat swab series and two additional strains, T29 and T30 were encountered in tracheotomy aspirates.

In this small series (Table 6) type 1, normally regarded as a respiratory pathogen, occurred in a tracheotomy apparently as a commensal, as also did types 9 and 11. Type 21 and type 3 (Table 6) are clearly related to infection, being isolated in pure culture repeatedly during the course of infection. The roles of types 8 and 24 are difficult to assess because the cultures were repeatedly mixed with pseudomonas, the latter probably contributing to infection.

Table 6

SIGNS ASSOCIATED WITH ISOLATION OF KLEBSIELLA FROM TRACHEOSTOME

Series Number	Respiratory Signs	Other Diseases	Serotype	On Antibiotics
47	Left lower lobar pneumonia. (Temperature 99.9°F) WBC 16.9	Cerebellar ataxia	24 (+ Pseudomonas)	No
48	Right upper lobar pneumonia. (Temperature 99°F) WBC 13.3	Tetanus	21	Yes
49	Bronchopneumonia. (Temperature 100°F)	Tetanus S.L.E.	3	Yes
same patient 50	None. (Temperature 98.6°F)	Tetanus	11	Yes
same patient 51	None (Temperature 98°F)		1	Yes
52	None (Temperature 99°F)	# skull Motor aphasia	9	Yes
53	Hypostatic pneumonia. Pulmonary collapse. (Temperature 101°F)	Myaesthenia gravis.	8 (+ Pseudomonas grown also)	Yes

An analysis of klebsiella isolated from the lower respiratory tract

In the following section, a minimum of two sputum samples was obtained from each patient, and only where each grew klebsiella was the patient included in the survey. In all cases, klebsiella occurred in a heavy growth, and all cultures mixed with other lower respiratory pathogens were excluded, in order to facilitate assessment.

Two hundred cases showing clinical evidence of respiratory infection ranging from acute bronchitis to lobar pneumonia were sampled.

A control group matched for age, sex, ward and duration of antibiotic therapy were selected. In order to get a reasonable degree of antibiotic matching, the age matching of the controls had to be modified to ± 5 years, except in the case of children under ten years where a matching of ± one year was achieved.

The sex distribution in both groups was 52% males and 48% females - 38/200 patients in the group showing respiratory infection grew a heavy culture of klebsiella on two or more occasions = 19%.

The control group had no positive chest signs, no fever and a normal WBC. In order to sample sputum

effectively, and not saliva, this group was mainly composed of cases of asthma, congestive cardiac failure and bronchiectasis, but in all cases the sputum was non-purulent. The incidence of Klebsiella in this group was 20/200 = 10%.
t test on differences in proportion curves t = 2.76, giving $p < 0.01$ which is highly significant.

There was no special type incidence seen in either infected or normal sputum.

Six out of 20 (30%) of the samples of normal sputum and 16/38 (38%) of samples of purulent sputum came from patients on antibiotics. Forty percent (eight out of 20) of the Klebsiella isolated from normal sputum and 25% (nine out of 36) from infected sputum were hospital acquired infections.

The biotypes and serotypes of Klebsiella isolated from normal sputum specimens are shown in Table 4 - Appendix.

In these 20 isolates, the only two identical serotypes and biotypes are represented by isolates number 60 and 61 (Table 4 - Appendix).

On the basis of biotype only, isolates 71 and 72 are the same (Table 4 - Appendix), so from 20 isolates from normal sputum there are a minimum of 18 different

Table 7

COMPARATIVE AGE DISTRIBUTIONS IN SPUTUM GROUPS

	Age Distribution of Total Samples (200 cases)	Age Distribution of *Klebsiella* associated with Respiratory Infection (38 cases)	Age Distribution of Commensal *Klebsiella* Isolates (20 cases)
0-10 years	14	4	0
11-20 years	20	4	0
21-30 years	26	6	2
31-40 years	30	5	3
41-50 years	34	5	3
51-60 years	30	3	8
61-70 years	30	5	3
70+ years	16	6	1
Sex Males	104	26	13
Females	96	12	7

Table 8

SEROTYPES OF KLEBSIELLA AEROGENES ISOLATED FROM NORMAL AND PURULENT SPUTUM

Serotype	1	2	3	5	7	8	9	10	11	13	15	16	19	20	22	29	31	35	not 1-40
Normal sputum (figures indicate Number of isolates)	1	1	1	-	2	-	3	-	1	2	-	1	-	-	1	3	-	1	3
Purulent sputum	-	5	-	1	-	1	7	1	2	-	2	2	1	1	-	-	2	1	12

strains represented, and possibly 19, if isolates 71 and 72 are different serotypes.

Where a particular serotype and biotype combination has already been designated in the section on throat swabs and tracheotomy aspirates, the same T classification was used to avoid repetition of strain designation. Where a particular strain had not previously been encountered, the designation S (sputum) followed by a numeral was used in the strain designation (Table 4 - Appendix); nine additional strains were isolated from sputum samples. Table 5 - Appendix shows the type of cases from which Klebsiella was isolated. Table 6 - Appendix shows the biotypes and serotypes of 38 isolates of Klebsiella from sputum in association with respiratory infection. In these 38 cases the following isolates are the same biotype and serotype combination, viz :

75, 76, 77, 78	- Type 2, dulcitol negative
82, 84, 86	- Type 9, dulcitol negative
85, 87	- Type 9, dulcitol positive
93, 94	- Type 16, dulcitol positive

The rest are all different, giving a minimum of 21 strains associated with lower respiratory tract infection (see Table 6 - Appendix).

Klebsiella isolated in association with lower respiratory tract infection (Table 9) was seen in 13 cases of acute bronchitis, nine cases of broncho-pneumonia, 13 cases of lobar pneumonia and three cases of lung abscess. Six of the 13 cases of acute bronchitis were superimposed on other pulmonary disorders, such as Sarcoidosis, Bronchiectasis, Asthma and chronic bronchitis and emphysema.

Similarly, five out of nine cases of broncho-pneumonia had an underlying pulmonary disorder. In the cases of lobar pneumonia, however, ten out of 13 of these cases occurred in the absence of other lung disease and only two of these ten were secondary infections. All the cases of lung abscess were in normal persons and five out of 13 cases of lobar pneumonia were caused by a klebsiella type 9. Leuco-cytosis was only a feature of 13/38 cases of respiratory infection due to klebsiella, but 21/38 cases were pyrexial (see Table 7 - Appendix).

In the group of 13 cases showing klebsiella in association with acute bronchitis, 11/13 cases showed klebsiella in pure culture from the admission specimen. Only one of these (isolate number 95), a case of asthma with bronchiectasis, had been on antibiotics at the time of first isolation of klebsiella (Table 9). Therefore we may assume klebsiella to be the primary invader in at least ten out of 11 of these cases.

Table 9

SEROTYPES ASSOCIATED WITH LOWER RESPIRATORY INFECTION

Type of Infection	Isolate Number	Sero-type	Hospital Acquired	Other Clinical Factors
Acute Bronchitis (13 cases)	74	2	No	Hodgkin's disease.
	75	2	No	None
	76	2	No	Sarcoidosis.
	81	9	No	Obstructive jaundice with uraemia.
	91	15	No	Congestic cardiac failure. Hyper-tension.
	93	16	No	Mitral stenosis; cardiac failure.
	94	16	No	Prostatic hyper-trophy.
	95	19	No	Bronchiectasis. Asthma.
	100	Not 1-40	No	Chronic bronchitis and emphysema.
	101	Not 1-40	Yes	Mitral stenosis. Asthma.
	102	Not 1-40	Yes	Bronchiectasis. Kwashiokor.
	103	Not 1-40	No	None
	104	Not 1-40	No	Ischaemic heart disease.
Broncho-pneumonia (9 cases)	77	2	Yes	C.V.A. with right hemiplegia.
	79	5	No	Bronchiectasis.

Continued.....

Table 9 (Continued)

SEROTYPES ASSOCIATED WITH LOWER RESPIRATORY INFECTION

Type of Infection	Isolate Number	Sero-type	Hospital Acquired	Other Clinical Factors
Broncho-pneumonia (9 cases) (cont.)	82	9	No	Well controlled diabetic.
	89	11	No	Carcinoma of larynx.
	92	15	Yes	None.
	96	20	No	Bronchial asthma.
	105	not 1-40	No	Bronchogenic carcinoma.
	106	not 1-40	Yes	None
	107	not 1-40	Yes	Bronchiectasis.
Lobar pneumonia (13 cases)	80	8	No	None. Healthy adult.
	83	9	Yes (2° to BHS)	Well controlled diabetic.
	84	9	No	Carcinoma right upper lobe.
	85	9	No	None.
	86	9	No	Pulmonary fibrosis.
	87	9	Yes (2° to E.coli)	Pulmonary embolism and infarction.
	90	11	No	None. Healthy adult.
	98	31	No	Benign prostatic hypertrophy.
	99	35	No	None. Healthy adult.

Continued.....

Table 9 (Continued)

SEROTYPES ASSOCIATED WITH LOWER RESPIRATORY INFECTION

Type of Infection	Isolate Number	Sero-type	Hospital Acquired	Other Clinical Factors
Lobar pneumonia (13 cases) (cont.)	108	not 1-40	No	Pregnancy.
	109	not 1-40	Yes	None (2° to pneumococcus)
	110	not 1-40	No	Pyelitis of pregnancy.
	111	not 1-40	Unknown	Myaesthemia gravis. Benign prostatic hypertrophy.
Lobar pneumonia and lung Abscess	78	2	No	None.
	88	10	No	None.
	97	31	No	None.

In these 11 cases, cases 75, 81, 94, 103 had no lesion especially predisposing to respiratory tract infection, but only cases 75 and 103 were otherwise completely normal adults (Table 9).

The two hospital acquired klebsiella isolates associated with acute bronchitis were isolates number 101 and 102, cases of mitral stenosis and bronchiectasis respectively (Table 9). Both had been on prolonged chemotherapy and previous bacteriology was negative in both cases on the first sputum examination. However, since the onset of clinical signs in the chest coincided with the appearance of K. aerogenes in the sputum, this, together with previous absence of respiratory pathogens would seem to suggest a klebsiella aetiology.

In the nine cases of klebsiella bronchopneumonia (Table 9) five patients grew klebsiella within two days of admission (numbers 79, 82, 89, 96 and 105), but all of these 5 had other factors predisposing to infection and, with the exception of number 82 who was diabetic, all of these cases had some abnormality of the respiratory tract.

Of the four hospital acquired klebsiella isolates - numbers 77, 92, 106 and 107 - the first three all had negative sputum culture on admission, and acquired klebsiella in the sputum after hospitalisation. Case 107 was a klebsiella secondary to a primary Beta haemolytic

streptococcal infection in a case of bronchiectasis.

In the 13 cases of lobar pneumonia a primary klebsiella aetiology can be attributed to ten out of the thirteen cases, in that this organism was isolated on admission. Case number 84 (Table 9) had had five days penicillin and two days tetracycline therapy by this time, so this may have eradicated another pathogen. Case 85 had had one day penicillin therapy, so this is unlikely to have contributed greatly to a change in flora, and case number 110 had had two days chloramphenicol therapy for pyelitis. The appearance of klebsiella in the sputum, however, coincided with the onset of respiratory signs.

Isolates 83, 87 and 109 (Table 9) were hospital acquired, and all secondary to antibiotic therapy, because the following organisms primarily occurred in sputum:

Case 83	- Beta haemolytic streptococcus	- 10 days penicillin and streptomycin therapy
Case 87	- E. coli	- Eight days ampicillin
Case 109	- Pneumococcus	- 10 days chloramphenicol. Two days penicillin and sulphonamide.

Thus, in 12/13 of the cases of acute bronchitis; eight out of nine of the cases of bronchopneumonia; and nine out of 13 of the cases of lobar pneumonia, klebsiella

may be regarded as being of primary aetiological significance.

In the three cases with lobar pneumonia and lung abscess (Table 9), isolates 78, 88 and 97 all grew klebsiella in pure culture on admission. Isolates 88 and 97 had had no previous chemotherapy, so it is justifiable to attribute primary klebsiella aetiology to at least two out of three of these cases. This gives a total of 31/38 cases of lower respiratory infection associated with klebsiella where klebsiella species could be regarded as the primary infective agent. However, when considering the type of patients involved, only numbers 75, 103, 92, 106, 80, 85, 90, 99, 108, 78, 88 and 97, i.e. 12/31 cases, can be regarded as completely healthy subjects with no other factors predisposing to infection. It would seem to be indicated that in these cases klebsiella has shown the ability to attack a previously normal, healthy respiratory tract.

In the 13 cases of acute bronchitis a leucocytosis (Table 7 - Appendix) was only shown in cases number 100 and 101, and five out of 13 cases showed pyrexia. In the bronchopneumonia group pyrexia was a feature in only five out of nine cases and only two showed a leucocytosis.

In the lobar pneumonia and lung abscess groups (16 cases), nine out of 16 cases showed leucocytosis and 12/16 cases showed pyrexia exceeding 99°F (table 7 - Appendix).

It is of interest that only one out of three of the cases of lung abscess showed leucocytosis (Table 7 - Appendix). Radiological appearances are presented in the Appendix of three cases of acute bronchitis, two cases of bronchopneumonia, four cases of lobar pneumonia and two cases of lung abscess.

(III) Klebsiella Isolates from the Urinary Tract

A total of 79 isolates of Klebsiella aerogenes was made from urine specimens submitted by 74 patients. Of all urines showing microscopic pyuria and an organism count of 100,000/ml or more measured by the quantitative loop method, the commonest urinary pathogen over the period of study was E. coli, which represented 33% of all significant cultures. Klebsiella aerogenes was the second most frequent pathogen, occurring in 28% of all significant urinary tract infections, in comparison to proteus species, 24%, and pseudomonas, 6%.

An investigation was made of the prevalent serotypes in urinary tract infections in Jamaica. The age and sex distribution of the 74 patients showing klebsiella

urinary tract infection was as follows:

Females 42 Males 32

Infection was predominantly in the over-50 age group.

Age Distribution of Klebsiella Urinary Tract Infection

Age	Number of Cases
1- 9 years	5 cases
10-19 years	3 cases
20-29 years	6 cases
30-39 years	8 cases
40-49 years	8 cases
50-59 years	21 cases
60+ years	24 cases

Seventy-two of these 74 were cases of primary klebsiella of the renal tract, i.e. no other organism had been isolated in the preceeding 12 months. Two cases were secondary to a previous *E. coli* infection.

Five patients changed either their biotype or serotype of klebsiella during the course of infection.

Table 8 - Appendix gives the incidence of the various biotypes and serotypes found in 79 isolates of klebsiella from 74 patients. Where a strain had already been designated in the previous section on respiratory tract, the same designation was used. New strains

were given numerical classification prefixed by U (urine). Twenty-two additional urinary strains were designated (Table 8 - Appendix).

The following isolates were from the same patients and showed a change in biotype or seretype :

156 and 157

162 and 163

136 and 183

113 and 185

151 and 190

Antibiotic Resistance Pattern of Urinary Klebsiella

The resistance patterns of all urinary Klebsiella were compared to (a) the biochemically typical, (b) the biochemically atypical strains, to see if there was any significant difference in resistance patterns associated with biochemically heterogenicity (see Table 10). (Page 98).

Table 11 compares the number of antibiotics to which the various urinary isolates of Klebsiella are resistant. (Page 99).

Pyrexia in Association with Klebsiella Urinary Infection

Table 9 - Appendix shows the temperature range of 30/74 patients with fever on day of submitting a positive urine culture. In seven of these 30 cases

there were other sources of infection to which the pyrexia may have been wholly or partially attributable, viz:

Case Number	Diagnosis	WBC
115	prostate abscess	24.5
128	carcinoma cervix	5.4
132	carcinoma vulva	7.4
144	gastroenteritis	9.7
150	acute epididymo-orchitis	8.6
152	carcinoma vulva	6.5
167	30% burns	15.2

There were 23/74 patients where pyrexia was clearly associated with Klebsiella urinary infection. In the above cases, numbers 115 and 167 showed peripheral leucocytosis, possibly in association with other causes.

In this series only three out of 70 cases of Klebsiella urinary infection showed an elevated white count above 11,000.

In order to determine whether the association between Klebsiella urinary tract infection and urine retention, postulated by other authors, applied here, a control group of patients suffering from significant E. coli infection were matched with the Klebsiella

Table 10

	Total Urines	Biochemically typical Klebsiella	Biochemically atypical Klebsiella	t	p
Total Number of Observations	79	51	28		
Resistant to Penicillin	79	51	28		
Resistant to Streptomycin	43	27	16	0.36	0.72
Resistant to Tetracycline	41	23	18	1.68	0.09
Resistant to Chloramphenicol	25	18	7	0.97	0.33
Resistant to Sulphonamide	55	34	21	0.79	0.43
Resistant to Albamycin T	27	15	12	1.19	0.23
Resistant to Ampicillin	37	24	13	0.05	0.96
Resistant to Furadantin	10	4	6	1.58	0.12
Resistant to Cesorin	24	13	11	1.25	0.21
Resistant to Polymyxin B	10	6	4	0.31	0.75

t test shows no difference between individual antibiotics in the two groups, and none of the values are significant.

Table 11

COMPARISON OF THE NUMBER OF ANTIBIOTICS TO WHICH THE VARIOUS URINARY ISOLATES OF KLEBSIELLA ARE RESISTANT

	Total Urines	Biochemically Typical	Biochemically Atypical
Total Number of Observations	79	51	28
No. of strains resistant to :			
1 antibiotic	19	13	6
2 antibiotics	11	9	2
3 antibiotics	5	4	1
4 antibiotics	4	1	3
5 antibiotics	7	4	3
6 antibiotics	12	7	5
7 antibiotics	9	6	3
8 antibiotics	5	3	2
9 antibiotics	5	3	2
10 antibiotics	2	1	1

($x^2_q = 5.47$)

x^2 overall (Table II) shows no difference between the biochemically typical and atypical groups.

group for age, sex, ward distribution, and compared for such symptoms and signs as frequency, dysuria, retention, catheterisation and calculus formation.

Table 12

COMPARISON OF KLEBSIELLA (74 CASES) AND E. COLI (74 CASES) URINARY TRACT INFECTIONS

	Klebsiella	E. coli	p value
Frequency of micturition	48%	68%	* 0.01
Dysuria	62%	58%	0.62
Retention	40%	30%	0.20
Calculus formation	7%	8%	0.82
History of catheterisation	27%	28%	0.89
Urethral stricture (usually gonococcal)	27%	8%	* 0.002
Prostatic hypertrophy	6.4%	4%	0.51

There was no significant difference in the two groups as far as calculus formation, history of catheterisation and urinary retention were concerned. Dysuria, which admittedly being subjective, was difficult to assess, but a significantly lower proportion of the klebsiella group complained of frequency of micturition (Table 12).

In considering the causes of retention, the incidence of prostatic hypertrophy in the two groups was similar, as perhaps could be expected from a series which was age and sex matched, but the Klebsiella group contained a much higher proportion of cases of urethral stricture, which was usually gonococcal. It can be concluded that in this environment, although Klebsiella infection is not significantly related to retention more so than E. coli infection, urethral stricture is definitely a significant predisposing cause of infection.

(IV) Klebsiella in the Female Genital Tract

Twenty-four cases of isolation of Klebsiella aerogenes from high vaginal swabs were considered, in an attempt to assess the significance of isolation of this organism.

The age distribution of these patients was 19 to 60 years.

19-29 years	11 cases
30-39 years	7 cases
40-49 years	3 cases
50+ years	3 cases

The patients were sub-divided into the following groups:

		No. of Cases
1.	Non-pregnant females of child-bearing age.	9
2.	Pregnant females.	1
3.	Puerperium.	13
4.	Post-menopausal	1

Fifty non-pregnant females of child bearing age complaining of vaginal discharge were investigated. The incidence of Klebsiella aerogenes isolation was six in 50 or 12%.

High vaginal swabs taken routinely for investigative purposes in females with no evidence of vaginal infection were used as controls, and age matched to within five years. The incidence was three in 50 or 6%.

One hundred high vaginal swabs were taken from patients during the puerperal stage. Fifty of these patients had a normal lochia and no pyrexia or leucocytosis. Klebsiella aerogenes was found in seven out of 50 or 14% of these cases.

Fifty age matched patients with an offensive lochia showed a Klebsiella incidence of six in 50 or 12%.

Two patients, one pregnant and one post-menopausal, both showing signs of vaginal infection associated with klebsiella, are also included in this survey, but the material available from these latter groups was too inadequate to permit any analysis of incidence.

The biotypes and serotypes found in 24 isolates of klebsiella from the female genital tract are given in Table 10 - Appendix. Table 11 - Appendix shows the clinical signs present in these patients. Eighteen out of 24 strains obtained from the female genital tract were untypable 1 - 40 (Table 11 - Appendix).

Two out of six cases of discharge where klebsiella was isolated in the non-pregnant group were associated with Trichomonas vaginalis infection - these were type 3 and one untypable strain. Types 3, 7, 15 as well as higher serotypes were found in association with perfectly normal lochia (Table 11 - Appendix).

In six cases of offensive lochia, only one strain could be typed, and this was strain 194, serotype 9. All cases of offensive lochia were

associated with pyrexia of 100°F or more, but only three out of these six cases had a leucocytosis in excess of 11,000 (Table 11 - Appendix).

Puerperal cases showing a normal lochia, klebsiella isolation and fever, usually had some other reason for pyrexia (see Table 11 - Appendix) with the exception of isolates number 198 and 207.

(v) Klebsiella Isolated from the Central Nervous System and from Ear and Eye Swabs

During the period of study, one case of klebsiella meningitis occurred, and two isolates of klebsiella were made from eye swabs and from ear swabs.

The total incidence of klebsiella meningitis was one in 52 positive cerebral spinal fluid cultures, or less than 2%. It occurred in a male of 62 years with klebsiella prostatic abscess and septicaemia. A type 7 Klebsiella aerogenes was recovered twice from the cerebro spinal fluid during life, and from the meningeal swabs at autopsy. This cases will be more fully described in the section discussing multiple klebsiella infection.

The two cases where klebsiella was recovered from eye swabs were both hospital acquired infections (isolates 216 and 217), associated with sticky eye in children delivered in hospital (Table 12 - Appendix). Both occurred in full term infants after normal deliveries, and the birth weights were normal (216 = 8 lbs., 217 = 5 lbs., 13 ozs.). In both cases repeated examination for gonococcus was negative, by both cultural and Fluorescent Antibody methods, nor was there a maternal history of gonorrhoea. Klebsiella occurred in pure culture, and produced a mild degree of sticky eye which cleared spontaneously after two and four days respectively.

Over the one year period of investigation, klebsiella was obtained in <u>pure</u> culture from only two ear swabs (Table 12 - Appendix). Isolate 218 was a routine ear swab taken from a case of fractured temporal bone, in a six-year-old child with no history of antibiotic therapy. The ear swab was positive on admission, and there were no signs of infection in the ear, which remained normal. The second isolate, 219, was more difficult to assess, as it occurred in a one-year-old child with bronchopneumonia - klebsiella being obtained from both the ear and throat swabs. There was a mild degree of otitis media and the child

had previously had no antibiotics.

~~Pyrexia and leucocytosis~~ TEMPERATURE RANGES associated with klebsiella isolated from the central nervous system, and from ear and eye swabs are shown in Table 13 - Appendix.

White counts were normal in cases 216, 217 (sticky eye), 218 (normal ear). Isolates 219 and 215 both showed leucocytosis and 219 had a WBC of 12,000, but the patient also had a bronchopneumonia. Case 215 had a WBC of 25,000 in association with klebsiella meningitis.

(VI) Klebsiella from Blood Cultures

Six blodd cultures were positive for Klebsiella aerogenes (see Table 14 - Appendix). Five patients showed klebsiella septicaemia, one showing a change in seretype during the course of infection, giving six different isolates in all. Each isolate was recovered on two or more occasions from the same patient, so are not likely to be contaminants, and all patients showed clinical signs of septicaemia.

The five patients showing klebsiella septicaemia were three males and two females. Cases 220 and 222 were fatal (Table 14 - Appendix). The age range was 41 to 62 years. Isolates 224 and 225 came from a case of subphrenic abscess, with an interval of five days

penicillin and streptomycin therapy between the two different isolates. Case 220 had had seven days furadantin therapy.

All of the other isolates of klebsiella from blood cultures were made prior to onset of antibiotic therapy. WBC and temperature on the day of taking each positive specimen are shown in Table 15 - Appendix. Over the year of study the incidence of klebsiella septicaemia was five out of 48 cases of significant septicaemia (= 10.2%).

(VII) Klebsiella from Wound Infections, Abscesses, Umbilical Stumps and from Ascitic Fluid

Fifty-five isolates of klebsiella from these miscellaneous sources were available for study. These came from :

Ascitic fluid	3 cases
Umbilical stumps	8 cases
Draining abscesses	7 cases
Wound swabs	37 cases

Klebsiella isolated from Ascitic Fluid

Klebsiella was cultured from ascitic fluid aspirates in two males and one female. All cultures were pure Klebsiella aerogenes.

Table 13

KLEBSIELLA ISOLATED FROM THREE ASCITIC FLUIDS

Clinical details and diagnosis	Age	Seretype	Series Number
Peritonitis with perforated ilium.	60	2	226
Acute glomerular nephritis; uraemia; hypertension.	26	25	227
Hepatic cirrhosis.	36	not 1-40	228

The significance of Klebsiella isolated from the Umbilical Stump

Klebsiella aerogenes was isolated from eight swabs taken from the umbilical stump. All were children born in hospital so were clearly hospital acquired infections. None of these babies were on antibiotics at the time of isolation of klebsiella from the umbilicus. There is difficulty in assessing the clinical significance of Klebsiella aerogenes isolated from the umbilical stump, because it frequently appears in mixed culture. The following table (Table 14) shows the appearance of the stump in these eight cases; the purulent nature of the site in isolates number 234 and 235 may have been related to the presence of Staphylococcus pyogenes, but in the other four cases, 231, 232, 233 and 236, pure cultures of types 11, 16, 17 and 19 were isolated in association

with local signs of infection. Paradoxically, type 11 was found in heavy growth with Proteus in isolate number 229, and the cord remained healthy. We cannot in these findings associate any particular serotype with cord infection, and probably Klebsiella is of no great significance when localised to the umbilical stump. All cases showed normal temperatures. White counts were not done.

Table 14

Clinical Appearance of Cord	Number of Cases	Isolate Number	Days After Delivery	Serotype Isolated
Normal	2	229	6	11 (+Proteus)
		236	4	not 1-40 (+coagulase positive Staphylococcus.
Moist. Mildly infected.	2	231	3	16
		232	5	17
Purulent.	4	230	3	11
		233	4	19
		234	4	23(29) (+coagulase positive Staphylococcus.
		235	5	not 1-40 (+coagulase positive Staphylococcus.

Table 17 - Appendix shows the biotypes of Klebsiella isolated from the umbilicus. Four strains

had previously been classified in isolates obtained from throat or urine cultures, but two new strains designated W1 and W2 were encountered.

Klebsiella associated with abscess formation

Seven cases of abscess formation associated with heavy pure culture of klebsiella occurred. In no case was any other organism isolated, and secondary invasion seems unlikely. The sex ratio was five males: two females. Details are given in Table 18 - Appendix. Four cases were febrile (Table 19 - Appendix), and leucocytosis in excess of 13,000 cells/cu mm was a feature of 5/7 cases. Table 20 - Appendix shows the biotypes encountered in abscesses. No new strains were discovered.

Klebsiella from wound infections

Thirty-seven isolates of klebsiella in pure culture were collected in one year. Cases of mixed infection were excluded.

The incidence of klebsiella from wound swabs in pure culture was 37/428 (8%) and was much less common than Staphylococcus pyogenes (118/428), but only slightly less common than Beta haemolytic streptococcus (44/428). Other coliforms encountered in order of frequency were pseudomonas (39/428), E. coli

(35/428) and proteus (34/428). The remaining cultures were mixed. Therefore, during the year of study, klebsiella, E. coli, proteus and pseudomonas collectively occurred in 145 wound isolates, so that the gram negative organisms were commoner than Staphylococcus pyogenes.

Consideration will be made of the serotypes of klebsiella isolated, and the types of wound infection found in association with klebsiella isolation. These 37 cases comprised 18 males and 19 females. The age distribution was two days to 78 years.

There were three cases of infected burns (Table 21 - Appendix) - numbers 249, 269 and 273 - klebsiella infection occurring on the first, second and eleventh day after injury.

Twenty cases of post-operative wound infection occurred (Table 21 - Appendix). These were numbers 244, 245, 251, 252, 253, 254, 258, 257, 259, 260, 261, 262, 263, 264, 267, 272, 274, 278, 279 and 280. Of these 20 post-operative wound infections, seven cases occurred in the first post-operative 48 hours. Eight occurred on the third to fourth post-operative day. Two occurred on the fifth to ninth post-operative day. Three occurred more than ten days post-operatively.

The wound was classified as:

(a) Normal - 2 cases

(b) Inflamed - 8 cases (i.e. red and moist, but not purulent).

(c) Purulent - 10 cases

The remaining 14 cases were found in skin lesions associated with pustule formation or ulceration - numbers 247, 248, 250, 256, 266, 270, 275, 276, 255, and 277, or from draining sinuses or drainage tubes - four cases, numbers 246, 265, 268 and 271.

Seven cases were associated with defective blood supply (vis. 247, 255, 259, 262, 266, 272 and 275). Number 277 was a case of chronic leg ulcer of unknown aetiology (see Table 21 - Appendix). Table 22 - Appendix gives the temperature range in 37 cases of klebsiella wound infection. Eighteen cases showed pyrexia in excess of 100°F.

Peripheral white counts were done on 28 patients and 15/28 showed a leucocytosis exceeding 11,000. However, only seven of these had no other signs of infection apart from klebsiella wound infection - these were isolates 249, 253, 259, 260, 262, 273 and 274 (Table 21 - Appendix). Table 23 - Appendix shows the biotypes and serotypes of klebsiella found in association with wound and skin infection.

No especial serotype of klebsiella is seen in association with infection. Types 5, 9 and higher serotypes were found both commensally and in association with infection (Table 15).

Table 15

SEROTYPES ASSOCIATED WITH INFECTION

Appearance of wound, ulcer or drainage aspirate.	Number of Cases.	Isolate Numbers	Serotype
Normal	3	249	Type 5
		251	Type 9
		267	not 1-40
Inflamed	15	245 & 247	Type 3
		250	Type 7
		254	Type 11
		256	Type 16
		259	Type 26
		260 & 262	Type 35
		264, 266, 269, 270, 272, 274, and 275.	not 1-40
Purulent	19	244 & 246	Type 3
		248	Type 5
		252	Type 9
		253	Type 11
		255	Type 15(x17)
		257	Type 17
		258	Type 19
		261	Type 35
		263, 265, 268, 271, 273, 276, 277, 278, 279, 280.	not 1-40

(VIII) Klebsiella from Autopsy Specimens

Ten isolates of Klebsiella aerogenes were grown from autopsy swabs (Table 24 - Appendix). All occurred in pure culture. Isolates 281 - 286 (Table 24 - Appendix) were taken from the same patient at autopsy, a case of klebsiella meningitis and septicaemia. Isolates number 287 - 290 were from a fatal case of klebsiella liver abscess. Both of these cases, and the significance of the autopsy bacteriology will be discussed under the heading of multiple klebsiella infections. No new strains were designated from autopsy specimens.

Investigation of Biochemical Heterogeneity of Respiratory Strains

Having now described the various serotypes and biotypes of Klebsiella aerogenes isolated from a variety of clinical sources, irrespective of pathogenicity, consideration will now be made of whether strains isolated from the respiratory tract are biochemically more heterogeneous than strains isolated from other sources.

The typical Klebsiella aerogenes as described by Cowan et al (1960) has previously been defined. All strains differing in one to four biochemical tests from the typical strain are defined as heterogeneous.

Previous description has been made of 111 respiratory strains isolated during life. One hundred and seventy-nine other strains, 79 from urine and 100 from other sources were also studied. An analysis follows, of whether strains from any particular source are biochemically more heterogeneous.

Table 16

	Respiratory Strains	Others*	Urines
Total Number of Observations	111	100	79
Biochemically typical	82	82	51
Biochemically variable	29	18	28

($x_2^2 = 7.01$) x^2 overall shows a significant difference but does not specify which group.

*all isolates except respiratory and urines.

Table 17

COMPARISON OF RESPIRATORY STRAINS WITH ALL OTHER SOURCES

	Respiratory	All Other Sources
Total Number of Observations	111	179
Biochemically typical	82	133
Biochemically variable	29	46

t test of comparison of respiratory with all other sources shows 0.08. x^2 test on respiratory and other sources shows no difference between them. p value = 0.94. Significance none.

Table 18

COMPARISON OF URINARY KLEBSIELLA WITH ALL OTHER SOURCES

	Urines	All Other Sources
Total Number of Observations	79	211
Biochemically typical	51	164
Biochemically variable	28	47

t test on comparison of urines and All other sources shows biochemically typical t 2.16, p 0.03, significant at 5%.

x^2 shows a significant difference at 5% level.

In the series of Klebsiella aerogenes isolates from the respiratory tract 70 were regarded as pathogenic and 41 as commensal. Although none of the typical K. edwardsii edwardsii, K. edwardsii atlanta strains of Cowan (1960) were encountered, an analysis was done to see if biochemically atypical strains of Klebsiella aerogenes were more pathogenic in the respiratory tract, as suggested by Wilson and Miles (1964). (table 19).

Antibiotic Resistance Patterns for Klebsiella aerogenes

Antibiotic resistance of all 290 isolates was measured by the multidisc method, as described in

Table 19

ARE BIOCHEMICALLY ATYPICAL STRAINS MORE VIRULENT IN THE RESPIRATORY TRACT?

	Pathogenic Strains	Commensal Strains
Total Number of Observations	70	41
Biochemically typical	53	31
Biochemically variable	17	10

t test done shows no difference between pathogens or commensals for either group ($x_1^2 = 0.0002$).
x^2 shows no difference for pathogens and commensals overall.

the Materials and Methods section. Urinary strains will be considered separately, as higher concentration discs were used for this series. The resistance patterns of 111 respiratory strains of Klebsiella aerogenes, and 100 isolates from other sources (excluding urines) are shown as follows (Table 20). (Page 118)

Consideration will now be made of the number of antibiotics to which these 111 respiratory and 100 non-respiratory strains are resistant (Table 21) (page 119).

Table 22 (page 120) compares the differences in the resistance patterns on the commensal and pathogenic respiratory strains.

Table 20

COMPARISON OF ANTIBIOTIC RESISTANCE IN RESPIRATORY AND NON-RESPIRATORY KLEBSIELLA (URINES EXCLUDED)

	Respiratory	Non-Respiratory	p Value	Value of t	Significance
Total Number of Observations	111	100			
Resistant to Penicillin	111(100%)	100(100%)	-	-	None
Resistant to Streptomycin	46(41.5%)	46(46%)	0.50	0.67	None
Resistant to Tetracycline	43(39%)	45(45%)	0.36	0.92	None
Resistant to Chloramphenicol	30(27%)	26(26%)	0.87	0.17	None
Resistant to Sulphonamide	85(77%)	74(74%)	0.66	0.43	None
Resistant to Albamycin T	29(26%)	22(22%)	0.48	0.70	None
Resistant to Ampicillin	44(39.8%)	46(46%)	0.35	0.93	None
Resistant to Framycetin	13(11.8%)	6(6%)	0.14	1.48	None
Resistant to Ceporin	29(26.2%)	21(21%)	0.38	0.88	None
Resistant to Polymyxin B	8(7.25%)	8(8%)	0.83	0.22	None

Table 21

COMPARISON OF NUMBER OF ANTIBIOTICS TO WHICH RESPIRATORY AND NON-RESPIRATORY STRAINS ARE RESISTANT

	Respiratory	Non-Respiratory	P Value	Value of t	Significance
Total Number of Observations	111	100			
Resistant to 1 Antibiotic	17	12	0.48	0.70	None
Resistant to 2 Antibiotics	22	27	0.22	1.23	None
Resistant to 3 Antibiotics	19	15	0.68	0.41	None
Resistant to 4 Antibiotics	12	11	0.96	0.04	None
Resistant to 5 Antibiotics	11	10	0.98	0.02	None
Resistant to 6 Antibiotics	9	10	0.63	0.48	None
Resistant to 7 Antibiotics	7	6	0.93	0.09	None
Resistant to 8 Antibiotics	6	5	0.89	0.13	None
Resistant to 9 Antibiotics	6	4	0.63	0.48	None
Resistant to 10 Antibiotics	2	0	0.15	1.43	None

$x^2_9 = 4.00$). x^2 overall shows no difference between respiratory and non-respiratory in Table 21.

Table 22

RESISTANCE OF RESPIRATORY KLEBSIELLA

	Commensal	Pathogenic
Total Number of Observations	41	70
Resistant to 1 Antibiotic	9	8
Resistant to 2 Antibiotics	3	19
Resistant to 3 Antibiotics	5	14
Resistant to 4 Antibiotics	5	7
Resistant to 5 Antibiotics	4	7
Resistant to 6 Antibiotics	6	3
Resistant to 7 Antibiotics	2	5
Resistant to 8 Antibiotics	2	4
Resistant to 9 Antibiotics	4	2
Resistant to 10 Antibiotics	1	1

x^2 overall shows no difference between the groups (analysis of Table 22).

Finally, in the following table (Table 23) an overall comparison is made in resistance patterns between biochemically typical and biochemically heterogeneous isolates, from all sources except urine. (Page 121).

Hospital Acquired Strains of Klebsiella

Antibiograms of hospital and non-hospital acquired strains. One hundred and seven of the 290 isolates were

Table 23

ARE BIOCHEMICALLY ATYPICAL STRAINS MORE RESISTANT TO ANTIBIOTICS?

	Total	Typical Klebsiella	Atypical Klebsiella
Number of Strains	211	164	47
Resistant to 1 Antibiotic	29	19	10
Resistant to 2 Antibiotics	49	41	8
Resistant to 3 Antibiotics	34	27	7
Resistant to 4 Antibiotics	23	18	5
Resistant to 5 Antibiotics	21	15	6
Resistant to 6 Antibiotics	19	17	2
Resistant to 7 Antibiotics	13	11	2
Resistant to 8 Antibiotics	11	9	2
Resistant to 9 Antibiotics	10	7	3
Resistant to 10 Antibiotics	2	0	2

(x^2 = 10.35) x^2 shows no difference between typical Klebsiella and biochemically atypical Klebsiella overall (Table 23).

definitely hospital acquired infections.

The source of these hospital acquired infections were :

Respiratory tract	46
Urines	26
Female genital tract	2
E.N.T. swabs	2

Blood	1
Umbilical swabs	8
Wound swabs	22
total	107

These strains will be discussed later.

One hundred and sixty-four cases were definitely not hospital acquired and the remaining 19 were impossible to assess, as the first specimens was taken more than three days after admission to hospital.

The following table compares the resistance patterns of the 107 hospital acquired strains with strains present in out-patients or on admission, to determine whether there is any significant increase in resistance in hospital strains. (Table 24 - page 123).

The number of the hospital acquired strains were compared statistically with the non-hospital acquired strains. For streptomycin and chloramphenicol (p value in each case 0.003) the hospital acquired strains were highly significantly more resistant than the non-hospital acquired.

(x) Details of Hospital Acquired Isolates

We defined as hospital acquired infection :

1. Any neonatal infection following hospital delivery.
2. Any case where a previous negative culture had been obtained from the same site since admission.

Table 24

RESISTANCE OF ANTIBIOTICS EXPRESSED AS PERCENTAGE

Antibiotic	Total Series	Hospital Acquired (107 isolates)	Non-Hospital Acquired (164 isolates)
Penicillin	100%	100%	100%
Streptomycin	45%	56%	39%
Tetracycline	41%	43%	39%
Chloramphenicol	29%	39%	23%
Sulphonamide	73%	70%	74%
Albamycin T	29%	34%	26%
Coperin	23%	18%	26%
Polymyxin B	9%	6%	10%
Framycetin (excluding urines)	9%	7%	11%
Furadantin (urines only)	7%	8%	6%
Ampicillin	45%	45%	45%

3. When symptoms and signs of infection clearly developed after admission.

Of 248 patients under study, 94 (37%) developed hospital acquired Klebsiella infections and 107 different isolates were obtained from these 94 patients. Table 25 shows the ward distribution of the patients showing Klebsiella infections and of hospital acquired Klebsiella infections.

Table 25

PATIENT DISTRIBUTION OF KLEBSIELLA INFECTIONS

Ward Number	Type of Ward	Patients showing Klebsiella Infection.	Patients showing hospital acquired Infections	Total hospital acquired isolates
1	Female surgical	2	1	1
2	Female surgical	7	2	4
3	Female medical	11	5	5
4	Male medical	16	0	0
5	Male surgical	7	5	5
6	Male surgical	22	8	11
7	Female medical	12	5	6
8	Male medical	20	7	7
9	Gynaecology	15	8	8
10	Gynaecology	6	3	3
11	Obstetrics	13	7	7
12	Obstetrics	8	3	3
	Nursery	12	11	14

Continued...../

Table 25 (Continued)

PATIENT DISTRIBUTION OF KLEBSIELLA INFECTIONS

Ward Number	Type of Ward	Patients showing Klebsiella Infection	Patients showing Hospital acquired Infections	Total Hospital Acquired Isolates
14	Surgical Paediatrics	11	4	4
15	Medical Paediatrics	8	1	1
16	Medical Paediatrics	23	13	15
17	Orthepaedics	2	2	3
18	Ophthalmology	1	0	0
19	E.N.T.	2	1	1
20	Dermatology	1	0	0
	Recovery ward	14	8	9
	Observation ward	1	0	0
	Casualty	10	0	0
	Out Patients	24	0	0
	TOTAL	248	94	107

The highest incidence of overall klebsiella isolation came from Medical Paediatrics and Male Medical and Male Surgical wards. The highest incidence of hospital acquired infection came from Ward 16 - Medical Paediatrics (13.8% of all hospital acquired infections). Other wards showing a high incidence of hospital acquired infections were the Nursery, Ward 6 (Male Surgical), Ward 9 (Gynaecology) and Ward 11 (Obstetrics).

In the Nursery, Wards 2, 6, 7, 16, and in the Recovery Ward sometimes more than one hospital acquired klebsiella occurred in the same patient, giving a greater number of total hospital acquired klebsiella isolates than patients showing hospital acquired infection.

One hundred and seven isolates of hospital acquired klebsiella were cultured from 94 patients. The serotypes of these 107 isolates of hospital acquired infections were as follows:

Serotype	Number of Specimens
1	3
2	5
3	5
7	4
8	2
9	8
10	1

Serotype	Number of Specimens
11	7
12	1
13	1
14	1
15	4
16	2
17	2
19	3
21	1
23(x29)	1
24	3
26	2
29	8
35	10
not 1-40	33

Excluding the untypable strains, the commonest hospital acquired serotypes were types 35, 29, 9, 11, 3 and 2. All the other biotypes encountered were represented by less than five isolates.

It is seen (Table 26) that in the hospital acquired serotypes there is considerable biochemical variation within the same serotype, indicating that these represent different strains. Thus the three specimens represented by serotype 1 show two different

Table 26

Table 26 shows the source of infection and the biotype of each of these serotypes, and also the ward of isolation of each strain.

Serotype	Isolate Number	Type of Specimen	Biotype	Ward
1 (3 specimens)	1	Throat swab	KA dulcitol +	Nursery
	3	Throat swab	KA dulcitol +	Recovery ward
	51	Tracheotomy	KA dulcitol -	Recovery ward
2 (5 specimens)	4	Throat swab	MR+ indole+	8
	6	Throat swab	Malonate -	16
	77	Throat swab	KA dulcitol -	8
	112	Urine	KA dulcitol -	Nursery
	113	Urine	MR+ VP-	Nursery
3 (5 specimens)	49	Tracheotomy	KA dulcitol -	Recovery ward
	114	Urine	KA dulcitol +	10
	225	Blood	MR+	6
	244	Wound	KA dulcitol -	16
	245	Wound	KA dulcitol -	6
7 (4 specimens)	58	Sputum	Indole +	5
	117	Urine	(MR+ VP- (gluconate - (malonate -	6
	216	Eye swab	KA dulcitol +	Nursery
	250	Wound	KA dulcitol -	14
8 (2 specimens)	53	Tracheotomy	KA dulcitol -	Recovery ward
	16	Throat swab	KA dulcitol -	Nursery
9 (8 specimens)	52	Tracheotomy	KA dulcitol -	5
	59	Sputum	KA dulcitol +	7
	61	Sputum	KA dulcitol -	7
	83	Sputum	MR+ VP-	3
	87	Sputum	KA dulcitol +	7
	120	Urine	MR+ VP- indole+	16
	251	Wound	KA dulcitol -	19
	252	Wound	KA dulcitol -	17
10 (1 specimen)	125	Urine	KA dulcitol -	2

Continued...../

Table 26 (Continued)

Serotype	Isolate Number	Type of Specimen	Biotype	Ward
11 (7 specimens)	20	Throat swab	KA dulcitol -	Nursery
	21	Throat swab	KA dulcitol +	16
	50	Tracheotomy	KA dulcitol -	Recovery ward
	229	Umbilicus	KA dulcitol +	11
	230	Umbilicus	KA dulcitol -	11
	253	Wound	KA dulcitol -	2
	254	Wound	KA dulcitol +	5
12 (1 specimen)	23	Throat swab	Malonate -	Recovery ward
13 (1 specimen)	64	Sputum	KA dulcitol -	5
14 (1 specimen)	128	Urine	KA dulcitol +	9
15 (4 specimens)	92	Sputum	KA dulcitol +	7
	134	Urine	(MR+ VP- (gluconate -	3
	135	Urine	KA dulcitol -	7
	138	Urine	(MR+ VP- (gluconate -	10
16 (2 specimens)	231	Umbilicus	KA dulcitol -	Nursery
	256	Wound	KA dulcitol +	6
17 (2 specimens)	232	Umbilicus	(adonitol - (starch - (glycerol -	Nursery
	257	Wound	KA dulcitol -	15
19 (3 specimens)	143	Urine	KA dulcitol -	6
	233	Umbilicus	KA dulcitol -	12
	258	Wound	KA dulcitol -	9
21 (1 specimen)	48	Tracheotomy	KA dulcitol +	8
23 x 29 (1 specimen)	234	Umbilicus	KA dulcitol -	12
24 (3 specimens)	29	Throat swab	KA dulcitol -	16
	47	Tracheotomy	KA dulcitol -	16
	148	Urine	KA dulcitol +	11

Continued..../

Table 26 (Continued)

Serotype	Isolate Number	Type of Specimen	Biotype	Ward
26 (2 specimens)	151	Urine	KA dulcitol +	1
	259	Wound	MR+ VP-	6
29 (8 specimens)	30	Throat swab	KA dulcitol -	16
	31	Throat swab	KA dulcitol -	16
	32	Throat swab	adonitol - glycerol -	Recovery ward
	33	Throat swab	KA dulcitol -	16
	67	Sputum	KA dulcitol +	8
	68	Sputum	MR+ VP- gluconate -	6
	69	Sputum	KA dulcitol -	8
	152	Urine	KA dulcitol -	9
35 (10 specimens)	34	Throat swab	KA dulcitol -	Nursery
	35	Throat swab	starch - glycerol -	Nursery
	36	Throat swab	adonitol - starch - glycerol -	Recovery ward
	37	Throat swab	starch - glycerol -	16
	38	Throat swab	malonate -	Recovery ward
	39	Throat swab	KA dulcitol -	Nursery
	40	Throat swab	KA dulcitol -	16
	70	Sputum	KA dulcitol -	8
	260	Wound	MR+ VP- indole-	14
	262	Wound	KA dulcitol -	10
not 1-40 (33 specimens)	41	Throat swab	KA dulcitol -	16
	45	Throat swab	lysine -	16
	101	Sputum	MR+ VP-	7
	102	Sputum	KA dulcitol -	16
	106	Sputum	KA dulcitol -	16
	107	Sputum	KA dulcitol +	14
	109	Sputum	adonitol -	8
	157	Urine	KA dulcitol +	6
	158	Urine	KA dulcitol -	Nursery
	161	Urine	KA dulcitol +	9
	163	Urine	indole +	6
	165	Urine	KA dulcitol +	6
	167	Urine	adonitol -	2
	168	Urine	KA dulcitol +	11
	175	Urine	KA dulcitol -	9
	178	Urine	indole +	3

Continued..../

Table 26 (Continued)

Serotype	Isolate Number	Type of Specimen	Biotype	Ward
not 1-40 (33 specimens)	179	Urine	KA dulcitol -	6
	182	Urine	gluconate - malonate -	3
	186	Urine	KA dulcitol +	12
	204	H.V.S.	KA dulcitol -	9
	205	H.V.S.	KA dulcitol -	9
	217	Eye swab	KA dulcitol +	Nursery
	235	Umbilicus	adonitol -	11
	236	Umbilicus	KA dulcitol -	11
	263	Wound	KA dulcitol +	9
	264	Wound	KA dulcitol +	5
	266	Wound	KA dulcitol -	17
	267	Wound	KA dulcitol +	17
	272	Wound	MR+ VP- adonitol -	14
	273	Wound	KA dulcitol -	2
	274	Wound	KA dulcitol -	Nursery
	276	Wound	KA dulcitol -	11
	277	Wound	KA dulcitol -	3

biotypes; the five of type 2 show four different biotypes; the five of type 3 show three different biotypes, and so on. The 74 hospital acquired infections that were types 1-40 in fact are represented by 47 different biotype and serotype combinations as shown below (Table 27). (Page 132)

The 33 isolates that were not serotyped were represented by eight different biotypes. Hospital acquired strains encountered on two or more occasions were the following (Table 28) (Page 133).

Table 27

SEROTYPES OF KLEBSIELLA ENCOUNTERED IN HOSPITAL ACQUIRED INFECTION

Sero-type	Number of Isolates	No. different biotypes within serotype
1	3	2
2	5	4
3	5	3
7	4	4
8	2	1
9	8	4
10	1	1
11	7	2
12	1	1
13	1	1
14	1	1
15	4	3
16	2	2
17	2	2
19	3	1
21	1	1
23x29	1	1
24	3	2
26	2	2
29	8	4
35	10	5

Table 28

HOSPITAL ACQUIRED STRAINS ISOLATED ON TWO OR MORE OCCASIONS

Serotype	Biotype	Ward Isolated
1	KA dulcitol +	Nursery, Recovery ward
2	KA dulcitol -	8 and Nursery
3	KA dulcitol -	Recovery ward, 16 and 6
8	KA dulcitol -	Recovery ward, Nursery
9	KA dulcitol - KA dulcitol +	5, 7, 19, 17, 7, 7.
11	KA dulcitol - KA dulcitol +	Nursery, Recovery ward, 11 and 2. 16, 11, 5
15	MR+ VP- gluconate -	3, 10
19	KA dulcitol -	6, 12, 9
24	KA dulcitol -	16, 16
29	KA dulcitol -	16, 16, 16, 8, 9
35	KA dulcitol - Starch -) Glycerol -)	Nursery, Nursery, 16,8,10 Nursery, 16

From the above table there would appear to be a spread of hospital acquired strains from one ward to another, the hospital acquired strains appearing to be relatively ubiquitous. Only in the cases of certain biotypes belonging to serotypes 9, 24, 29 and 35 did the same strain appear as a cause of hospital acquired infection in the same ward on more than one occasion within the year.

Also, several wards showed the presence of different hospital strains - most notably Ward 16 (Paediatrics) in which serotypes 2, 3, 9, 24, 29 and 35 and several other untyped strains occurred as hospital acquired infections; the Nursery, where serotypes 1, 2, 7, 8, 11, 16, 17 and 35 occurred; the Recovery ward, where types 1, 3, 8, 11, 12, 29 and 35 occurred; Ward 6 (male surgical), where types 3, 7, 16, 19, 26 and 29 were encountered.

The following table (Table 29) shows the ward distribution of the various hospital acquired serotypes, and Table 30 (page 136) shows the distribution of serotypes from the various clinical sources (290 specimens).

Table 29

WARD DISTRIBUTION OF THE VARIOUS HOSPITAL ACQUIRED SEROTYPES

Ward	1	2	3	7	8	9	10	11	12	13	14	15	16	17	19	21	23	24	26	29	35	not 1-40
Recovery Ward			1		1			1	1											1	2	
20																						
19						1																
18																						
17						1																2
16		1	1			1		1										2		3	2	4
15														1								
14				1																	1	2
Nursery	1	2		1	1			1					1	1							3	3
12															1		1					1
11								2										1				4
10			1									1									1	
9											1				1					1		5
8		2														1				2	1	1

Continued...../

Table 29 (Continued)

WARD DISTRIBUTION OF THE VARIOUS HOSPITAL ACQUIRED SEROTYPES

Ward	1	2	3	7	8	9	10	11	12	13	14	15	16	17	19	21	23	24	26	29	35	not 1-40
7						3						2										1
6			2	1									1		1				1	1		4
5				1		1		1		1												1
4																						
3						1						1										3
2							1	1														2
1																			1			

Table 30

DISTRIBUTION OF SEROTYPES FROM THE VARIOUS CLINICAL SOURCES (290 SPECIMENS)

	SEROTYPES																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
Autopsy							7						1									C
Blood		1	1				1		1								1					O
C.N.S.							2		1													N
Wounds, Abscesses, Umbilicus	1	2	6		2		2		2		4				1	2	2		2			T
H.V.S.			2				1		1						1		1					I
Urine		2	1				3	1	3	6				2	9	1	2		3	1		N
Sputum	1	6	1		1		2	1	10	1	3		2			3			1	1		U
Tracheotomy	1		1					1	1		1										1	E
Throat swabs	3	5	5	2				1	2		4	1	1		2	1						D

Table 30 (Continued)

DISTRIBUTION OF SEROTYPES FROM THE VARIOUS CLINICAL SOURCES (290 SPECIMENS)

	SEROTYPES																			
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	not 1-40
Autopsy										1										1
Blood																				1
Ear and Eye swabs																				2
Wounds, Abscesses, Umbilicus		1		1	1									3						23
H.V.S.																				18
Urine		2	1		3			2												37
Sputum	1							3		2				2						15
Tracheotomy	1		1																	
Throat swabs		1	1					4						7						5

(XI) Effect of Chemotherapy on Infection

A history of antibiotics given immediately preceeding the first isolation of klebsiella from the patient, was taken. Other authors have commented on the frequency of gram negative infections since the onset of chemotherapy, and it seemed worthwhile to establish whether this klebsiella group was receiving significantly more antibiotics than matched controls showing no evidence of klebsiella infection.

Details were taken of the type of antibiotic prescribed, the duration of therapy, and the number of antibiotics prescribed immediately preceeding the first isolation of klebsiella.

These data were statistically compared with data from a control group of hospital patients showing no evidence of klebsiella infection - routine throat, ear swabs and urine cultures were taken; where sputum could be obtained it was cultured three times, and any skin lesions were cultured. This klebsiella-free group were exactly matched for days of hospital admission, ward distribution, age and sex, and approximately matched for admission diagnosis.

The antibiotic history of the klebsiella group of patients was then compared to the above control group.

Of the 248 patients in this series, 89 were on antibiotics at the time of first isolation of Klebsiella (i.e. 35% of total).

Of these 89 patients on chemotherapy:

No. of patients on 1 antibiotic = 37 (14.9% of series)

No. of patients on 2 antibiotics = 39 (15.7% of series)

No. of patients on 3 or more antibiotics = 13 (5.35% of series)

The question was then asked -

How often was the infecting Klebsiella resistant to the antibiotic to which the patient was exposed at the time of infection?

	Group	Total Number	No. resistant to prescribed antibiotic	%
A	Patients on 1 antibiotic	37	28	75
B	Patients on 2 antibiotics	39		
	Number resistant to one of the prescribed antibiotics		11	28
	Number resistant to both prescribed antibiotics		20	51
C	Patients on 3 antibiotics	13		
	Resistant to 1 antibiotic prescribed		1	7
	Resistant to 2 antibiotics prescribed		1	7
	Resistant to 3 antibiotics prescribed		10	76

From the above table we see that multiple chemotherapy leads to infection by multi-resistant strains, i.e. 10 out of 13 of the patients receiving three antibiotics developed klebsiella infections resistant to all three prescribed antibiotics. However, even when only one antibiotic was prescribed, the strain of klebsiella was resistant to it 75% of the time.

In these 89 cases the time interval between onset of antibiotic therapy and development of klebsiella infection was examined. Only 15 cases developed infection during the first three days of therapy. The peak incidence of infection was at the fifth day after onset of chemotherapy, with little decline after the seventh day.

Consideration will now be made of the type of antibiotic prescribed and the frequency of prescription, either alone or in combination, in the 248 patients showing klebsiella infection (Table 31).

The most striking feature is that 20% of all the series were on penicillin alone or in combination at the time of infection. The other most commonly prescribed antibiotics were streptomycin (11%), tetracycline (9.6%) and chloramphenicol (8.8%).

Table 31

TYPE OF ANTIBIOTIC PRESCRIBED AND FREQUENCY OF PRESCRIPTION, ALONE OR IN COMBINATION, IN 248 PATIENTS SHOWING KLEBSIELLA INFECTION

Antibiotic Prescribed	Prescribed Alone		In Combination		Total	%
	No. cases	%	No. cases	%		
Penicillin	15	6	36	14	51	20
Cloxacillin or Methicillin	1	0.4	2	0.8	3	1.2
Ampicillin	1	0.4	5	2	6	2.4
Streptomycin	0	-	28	11	28	11
Tetracycline	10	4	14	5.6	24	9.6
Chloramphenicol	5	2	17	6.8	22	8.8
Sulphonamide	3	1.2	8	3.2	11	4.4
Furadantin	2	0.8	2	0.8	4	1.6
Neomycin	0	-	1	0.4	1	0.4
Polymyxin	0	-	1	0.4	1	0.4

Control Group

The control group showing no evidence of klebsiella infection had an incidence of antibiotic prescription of 94 out of 248 = 38%.

Incidence of antibiotic prescription among the klebsiella group was 89 out of 248 = 35%. (p value = 0.49, not significant).

As there was such a close correlation between the incidence of antibiotic prescription in the two groups, consideration was then made of the types of antibiotic prescribed in the two groups and the duration of prescription - (Table 32).

There was a remarkably close correlation between the incidence of prescription of the various antibiotics in the two groups. The only significant difference was the more frequent prescription of chloramphenicol in the klebsiella group (p = 0.016, significant).

It therefore does not appear that antibiotic prescription per se predisposes to klebsiella infection. However, when an analysis is made of the duration of prescription of antibiotics in the two groups, some differences emerge. (Table 33).

Table 32

FREQUENCY OF PRESCRIPTION OF EACH ANTIBIOTIC PER 248 PATIENTS

Antibiotic	Klebsiella Group	Control Group	p value
Penicillin	20.0%	17.4%	0.46
Cloxacillin or Methicillin	1.2%	1.2%	1.0
Ampicillin	2.4%	4.0%	0.31
Streptomycin	11.0%	10.1%	0.74
Tetracycline	9.6%	8.1%	0.56
Chloramphenicol	8.8%	3.6%	0.016*
Sulphonamide	4.4%	4.4%	1.0
Furadantin	1.6%	1.6%	1.0
Neomycin	0.4%	2.4%	0.06
Polymyxin B	0.4%	0.8%	0.56

*(p = 0.016, significant)

Table 33

A COMPARISON OF THE DURATION OF CHEMOTHERAPY IN 89 CASES OF KLEBSIELLA INFECTION AND 94 CONTROL CASES ON ANTIBIOTICS, EACH OUT OF A TOTAL SAMPLE OF 248 CASES

(Number of cases expressed as %)

Duration of Chemotherapy	Klebsiella Group (89 cases)	Control Group (94cases)	p value
Less than three days			
(1) One drug prescribed	7.8%	13.8%	0.19
(2) Multiple chemotherapy	9.0%	10.6%	0.72
Three to six days chemo-therapy			
(1) One drug prescribed	19.1%	38.3%	0.003*
(2) Multiple chemotherapy	23.5%	19.2%	0.47
Seven days or more chemotherapy			
(1) One drug prescribed	16.9%	12.8%	0.44
(2) Multiple chemotherapy	23.6%	5.3%	0.0033*

*(significant)

Thus the control group (which matched the group showing klebsiella infection closely, when we consider incidence of antibiotic prescription), significantly had more patients on antibiotics for three to six days; but after seven days or more many more patients in the klebsiella group were on multiple chemotherapy, compared to the control group, and this difference was significant.

Thus the duration of antibiotic therapy is significantly longer in patients exhibiting klebsiella infection.

Incidence of Diabetes

The incidence of clinically diagnosed diabetes mellitus was taken in the control group and in the klebsiella group.

	Total 248 patients in each group	
	Incidence of diabetes	
Klebsiella group	8.0%	p value 0.87
Control group	7.6%	

Therefore diabetes mellitus does not predispose to klebsiella infection in hospitalised patients.

(XII) Multiple Klebsiella Infection

This section considers 290 isolates of Klebsiella aerogenes from 248 patients. It is interesting to consider in some detail some of the patients who developed -

(1) klebsiella infection at different sites, and to consider whether the same strain is involved at each site;

(2) klebsiella infection in the same site showing a change in infecting biotype or serotype.

It is tempting, in clinical medicine, when an organism is isolated from a number of different sites, to attribute one common source of infection in the body. It can be demonstrated that in some cases in this series several different species of klebsiella have been co-existing in the same individual, and to imply spread of infection from one site to the other would be fallacious.

Twenty-five patients showed multiple klebsiella infection. Consideration will first be made of 16 of these patients from whom Klebsiella aerogenes was isolated from different sites in the same patient.

Case 1. B.B. A female child of five years developed multiple klebsiella liver abscesses, serotype 7, biochemically typical Klebsiella aerogenes, dulcitol + (isolate 241). Clinical details of this case are described in the Appendix.

At autopsy, Klebsiella aerogenes was recovered from the liver (isolate 287), the spleen (isolate 288),

the kidneys (289) and from mesenteric lymph nodes (290). Isolates 287-290 were identical biotype and serotype to isolate 241, and showed identical antibiotic resistance, i.e. resistant to penicillin, streptomycin, tetracycline, chloramphenicol and sulphonamide. These isolates can therefore be regarded as arising from one common infective focus.

Case 2. E.P. Male, 46 years. Past history of scrotal abscesses seven years ago, leading to multiple scrotal fistulas. Also history of chronic prostatitis and urethral stricture.

Urine culture on admission showed pus cells and Klebsiella aerogenes which failed to ferment adonitol or starch and was not types 1-40 (isolate 162). This strain was sensitive to chloramphenicol, albamycin T, furadantin and polymyxin B. After excision of urethral stricture and suprapubic cystostomy catheter drainage yielded K. aerogenes which was indole +. but which, unlike the previous strain, fermented starch and adonitol. It was also not types 1-40. This strain (isolate 163) was sensitive only to chloramphenicol and furadantin, and can be considered as a case of Klebsiella super-infection following Foley catheterisation, and a different strain to the original urinary tract infection.

Case 3. M.H. This was one of the three fatal cases in the series. Female, aged 43 years, para 1+0, developed fever, vomiting and abdominal distention four days after Caesarian section. On the eighth post-operative day a laparotomy revealed massive haematoma of the anterior abdominal wall, and paralytic ileus. The patient, who was diabetic, developed thrombophlebitis of the left leg, remained pyrexial, and died on the tenth post-operative day. Permission for autopsy was refused.

Three klebsiella isolates were obtained during life. Isolate 140 from urine and isolate 222, recovered three times from blood cultures, were K. aerogenes, serotype 17, showing typical klebsiella biochemistry and dulcitol +. Isolates 140 and 222 were sensitive only to framycetin and polymyxin. A high vaginal swab was taken two days after admission (isolate 196) and this also grew Klebsiella aerogenes type 17, but of a different biotype, i.e. adonitol, starch and glycerol were not fermented, and also differing from the urine and blood cultures in being sensitive to streptomycin, tetracycline, chloramphenicol, albamycin T, framycetin, ceporin and polymyxin B. The infant of this mother also developed a klebsiella infection of the umbilical stump (isolate 232) identical in antibiotic sensitivity, serotype and biotype to the strain of K. aerogenes recovered from the high vaginal swab of the mother.

It seems reasonably certain that the klebsiella septicaemia, which contributed to the cause of death, was the same strain as that causing the urinary tract infection, while the vaginal strain appeared to contribute in no way to the septicaemia. It may also be assumed that by some means the maternal vaginal klebsiella strain was transmitted to the infant cord (the child was nursed by the mother for the first two days after Caesarian section) but the mode of transmission can only be speculative.

Case 4. R.S. Female, aged 75 years. Presented with obstructive jaundice due to cholelithiasis. This patient developed a klebsiella urinary infection, seretype 17, which failed to ferment adonitol (isolate 141). This strain was sensitive to polymyxin B only. The patient also showed clinical and radiological evidence of acute bronchitis. Sputum culture (isolate 81) yielded pure K. aerogenes, type 9, which was MR +, indole +, and sensitive to a wider range of antibiotics, viz. streptomycin, tetracycline, chloramphenicol, albamycin T, ampicillin, framycetin, ceporin and polymyxin. Blood cultures at this time (isolate 223) showed a type 9 klebsiella septicaemia of the same biotype and sensitivity as the respiratory strain - implying a common origin. The patient responded well to chemotherapy.

Case 5. C.C. Female, aged 16 years. A case of chronic pyelonephritis with oliguria and uraemia. Urine culture (isolate 139) yielded a pure growth of K. aerogenes, type 16, which was malonate negative and resistant to penicillin, sulphonamide, ampicillin and ceporin. The throat swab on admission grew K. aerogenes type 23 (isolate 28), which was biochemically typical klebsiella and resistant only to penicillin. The throat was normal, and there seems to be no connection between the commensal klebsiella throat carriage, and the significant klebsiella bacteruria of a completely different serotype and biotype.

Case 6. R.C. Male, aged 21 months. Diagnosis haemolytic uraemic syndrome. Admitted initially as a case of acute nephritis, the throat swab on admission grew pure klebsiella type 3 (isolate 11), biochemically typical (dulcitol +), and sensitive to albamycin T, framycetin, ceporin and polymyxin. Three days later, the throat swab (isolate 12) had been replaced by a dulcitol negative type 3 klebsiella, sensitive to ceporin and polymyxin only. The throat remained normal, and could not be regarded as the focus for a urinary tract infection due to K. aerogenes (isolate 127) which was type 10 and sensitive to chloramphenicol, furadantin, ceporin and polymyxin, although the biotype

of isolate 127 from urine was the same as the biotype of the original throat swab.

Case 7. A.B. Female, aged 50 years. Diagnosis - diabetes mellitus, peripheral neuropathy.

On admission, sputum (isolate 72) yielded pure culture of K. aerogenes not types 1-40, which was biochemically typical, sensitive to albamycin T, framycetin, ceporin and polymyxin. One day later a urine sample (isolate 147) showed significant culture of Klebsiella aerogenes type 23, which was MR +, VP -, sensitive to streptomycin and chloramphenicol only, and associated with signs of acute urinary tract infection. Therefore, a common origin for these two isolates cannot be assumed.

Case 8. C.B. Female, aged 16 months. A child with clinical and radiological evidence of bronchopneumonia. Ear and throat swabs both yielded a dulcitol negative, biochemically typical K. aerogenes, not types 1-40, both isolates (219 and 43) being sensitive to all antibiotics except penicillin and sulphonamide. Sputum could not be obtained, so the aetiology of the pneumonia remained uncertain.

Case 9. R.J. This case is of particular interest since nine cultures of Klebsiella were obtained from various

sources. R.J. was a male of 62 years and a known diabetic, admitted in a deeply comatose condition, with flaccid paralysis, neck stiffness, dehydration, fever and a peripheral white count of 25,450 cells/cu.mm (75% polymorphs).

Lumbar puncture showed a turbid CSF, and a pure growth of K. aerogenes type 7, biochemically typical and dulcitol negative (isolate 215). Three blood cultures (isolate 220) yielded K. aerogenes type 7, of identical biotype to the CSF. Urine culture (isolate 115) yielded Klebsiella type 7, biochemically identical to isolates 215 and 220.

The patient died 13 hours after admission.

At Autopsy (see Appendix for photographs).

CNS. Meninges diffusely inflamed with a green-grey exudate, mostly localised to the base. Swab from meninges yielded K. aerogenes type 7, biochemically typical dulcitol negative strain (isolate 282).

Renal system. Both kidneys had perirenal fibrosis with densly adherent capsules. The surface showed focal yellow abscesses varying in size from 2 mm to 16 mm. Parenchyma showed numerous green abscesses. (Photograph, see Appendix).

Severe acute pyelonephritis, mainly restricted

to the medulla, showed papillitis necrotans (see Appendix).

Prostate. Large but not nodular. Right lobe filled by an abscess (2 x 3 cms) filled with green-grey pus.

Histology. Showed a large pyogenic abscess cavity in the lateral lobe.

Swab from prostatic abscess (isolate 285) showed K. aerogenes type 7, biochemically typical and dulcitol negative.

Swab from spleen, which was unremarkable on section, yielded Klebsiella aerogenes type 7, biochemistry as above (isolate 286).

Here is a case of klebsiella type 7 septicaemia, arising from a klebsiella prostatic abscess, causing acute pyelonephritis and klebsiella meningitis. The identical nature of the biotype and serotype of klebsiella obtained from the sources described above, coupled with the fact that all the above isolates were sensitive to all antibiotics except penicillin and ceporin, would confirm that the autopsy swabs were not contaminants. These can be separated from three other swabs obtained at autopsy.

Isolate 281 - swab from bladder wall, which was slightly dilated with minimal trabeculation = Klebsiella aerogenes type 13, biotype MR +, VP -, indole +, sensitive to sulphonamide, framycetin and polymyxin.

Isolate 283 - swab from lung at autopsy. Klebsiella not types 1-40. Indole +. Sensitive to all antibiotics except penicillin.

Isolate 284 - swab from right main bronchus at autopsy. Type 31. Indole +. Sensitive to all antibiotics except penicillin.

It is likely that the three latter isolates of klebsiella were in no way related to the klebsiella causing fatal septicaemia. At autopsy, apart from congestion and oedema, no gross pulmonary lesions were seen. Probably the isolates from the lung and bladder wall are examples of post-mortem contaminants.

Case 10, G.B. Female, aged 28 years.

Caesarian section for pelvic disproportion. Post-operative urinary infection - Klebsiella aerogenes type 3, biochemically typical (isolate 114) was hospital acquired. A high vaginal swab (isolate 195) yielded K. aerogenes type 15. The urinary tract infection had therefore not arisen from the vaginal focus.

Case 11, Baby W. Aged 21 days.

Diagnosis - posterior urethral valve. Bilateral cutaneous loop uretostomies performed. Post-operatively the urine (isolate 158) grew K. aerogenes, biochemically typical, not serotype 1-40.

Wound swabs also repeatedly grew the same serologically untypable biotype (isolate 274) and all isolates were sensitive to ampicillin, albamycin T, furadantin and polymyxin. In the absence of serological confirmation we can only say that the wound infection may have arisen from contamination from infected urine from the drainage tubes.

Case 12, H.B. Female, aged 41 years.

Renal calculus, with recurrent urinary tract infections over 20 years. Pyelolithotomy performed. Ten days after operation the patient developed a klebsiella urinary tract infection (isolate 125), serotype 10, biochemically typical and sensitive only to furadantin.

The wound became infected and subsequently the nephrectomy tube began to drain foul smelling pus, which grew K. aerogenes type 11, resistant to

all antibiotics. Therefore, this patient showed two independent klebsiella hospital acquired infections, in contrast to Case 11.

Case 13. O.G. Male, aged 61 years.

Prostatic hypertrophy. When seen at Out Patients, urine (isolate 136) yielded K. aerogenes type 15, indole +, gelatin liquefied. This strain was sensitive to chloramphenicol, ceporin and polymyxin. This strain was subsequently replaced by a biochemically typical K. aerogenes in the urine (isolate 183) which was sensitive to chloramphenicol, sulphonamide, furadantin, ceporin and polymyxin.

After hospital admission, swabs from the area of suprapubic cystostomy (isolate 216) yielded a third klebsiella, type 35, sensitive to chloramphenicol, albamycin T, ampicillin, framycetin, ceporin and polymyxin.

This patient apparently became infected with three different strains of klebsiella, or may even at one time have had multiple infection.

Case 14. C.C. Male, aged 49 years.

A diabetic, admitted with perforated gastric ulcer. At laparotomy a large subphrenic abscess was found which yielded type 3 K. aerogenes which was MR +

(isolate 246). Blood cultures at this time (isolates 224 and 225) yielded two different types of Klebsiella. Isolate 225 was recovered on six occasions from the blood, was type 3, MR +, and antibiotic resistance patterns were identical to the pus from the subphrenic abscess.

One blood culture grew a citrate negative, untypable Klebsiella with a completely different sensitivity, and should be regarded as a contaminant.

Case 15. L.J. Female, aged 54 years.

Diagnosis - carcinoma of uterus and left tubo-varian abscess. Swab from purulent vaginal discharge (isolate 205) yielded a biochemically typical Klebsiella, dulcitol negative, sensitive to chloramphenicol, sulphonamide, ampicillin, framycetin, ceporin and polymyxin.

After hysterectomy and bilateral salpingoophorectomy the wound drained foul-smelling pus, and this yielded a few E. coli, but predominantly a heavy culture of K. aerogenes, with the same biotype and sensitivity as the HVS. It is unfortunate that neither of these isolates could be typed, but with the data available they seem to represent the same strain.

Case 16. J.C. Female, aged 56 years.

Admitted with 17% burns. Forty-eight hours after admission, swabs from the burned areas were sterile, but five days after admission became colonised with K. aerogenes (isolate 273), biochemically typical, dulcitol negative. Serotype was not 1-40 and sensitive only to polymyxin.

Thrice weekly swabs during the following two weeks showed persistence of isolate 273, and superinfection with pseudomonas and proteus in addition. On the sixth day of hospitalisation the patient developed a klebsiella urinary tract infection of a different biotype, i.e. adonitol negative, ceporin, furadantin and also untypable. It would seem, therefore, that cross infection between the two sites had not occurred, but rather that this represents two separate incidences of hospital acquired infection in the same patient.

The remaining ten cases represent changes in serotype or biotype or resistance patterns in specimens taken from the same site.

Case 17. J.C.F. Male, aged 66 years.

Diabetic gangrene of heel, below knee amputation

performed. Discharged home but stump failed to heal. First swab (265) yielded K. aerogenes not 1-40, biochemically typical, sensitive to tetracycline, chloramphenicol, sulphonamide, albamycin T, ampicillin, framycetin, ceporin and polymyxin.

One week later, after tetracycline had been given for seven days, a strain sensitive only to chloramphenicol, albamycin T, ampicillin, framycetin and ceporin was grown (isolate 275) which was biochemically identical to isolate 265 and not typable. It is not known whether this represents the emergence of a resistant mutant or superinfection with another klebsiella species.

Case 18, I.L. Female, aged 29 years.

This lady was admitted with tetanus following compound fractures of radius and ulna. Tracheotomy was performed, and it is of interest that the first tracheotomy klebsiella culture (isolate 50) was type 11, sensitive only to polymyxin. Two days later that strain had been replaced by a type 1 klebsiella which was isolated on several occasions thereafter, and which was sensitive to tetracycline, streptomycin, chloramphenicol, albamycin T, ampicillin and polymyxin. Neither strain was associated with clinical signs of infection, but it is of interest that this patient, who had already had four days streptomycin and chloramphenicol therapy, and 14 days tetracycline therapy could

acquire a type 1 organism sensitive to these antibiotics.

Case 19. D.W. Male, aged 74 years.

Prostatectomy four months previously, following one episode of acute retention. Patient developed vesico-cutaneous fistula draining purulent material. Isolates 268 and 271 represent Klebsiella aerogenes obtained from pus draining from the fistula. Neither were types 1-40 and both were biochemically typical dulcitol positive strains.

However, isolate 268 was sensitive to streptomycin, tetracycline, chloramphenicol and framycetin, while strain 271 had developed further resistance to albamycin T. There was no interval therapy on antibiotics so this may represent either a resistant mutant of 268, or a separate strain, since neither could be serotyped.

Case 20. E.S. Female, aged 73 years.

Crush injury to left leg. Wound swab (252) initially grew Klebsiella type 9, resistant to penicillin, sulphonamide and ampicillin. Three days later the wound swab grew Klebsiella, not types 1-40, resistant to the above antibiotics and to tetracycline. The patient had had penicillin and tetracycline therapy since admission.

This seems a clear cut case of prolonged administration of an antibiotic causing replacement by more resistant flora.

Case 21, A.C. Male, aged 65 years.

This also represents an example of replacement by more resistant flora, but this time the patient was not receiving antibiotics.

The patient had had first stage urethroplasty and supra pubic cystostomy performed two months previously, and was readmitted for revision of perineal urethrostomy. On admission, the patient had a Klebsiella infection of the urine (isolate 156) which could not be typed, but which was sensitive to furadantin and polymyxin. Five days later this had been replaced by a totally resistant strain of the same biotype and unknown serotype.

Case 22, T.C. Male, aged 55 years.

Diagnosis - Mycosis fungoides. Patient on steroid therapy, and had glycosuria at the time of developing a hospital acquired urine infection (112) - type 2, biochemically typical and sensitive to chloramphenicol, albamycin T, furadantin, ceporin and polymyxin. He was given four days furadantin

and thereafter the urine grew a type 2 strain but this was MR +, VP -, and sensitive only to ceporin. Here is an example of reinfection with more resistant strains during antibiotic therapy.

Case 23. M.G. Female, aged 64 years.

Diagnosis - epilepsy. Two separate strains of K. aerogenes isolated from urine at a five day interval, isolate 190; not 1-40, biochemically MR + VP -, malonate negative, resistant only to penicillin and sulphonamide.

After five days hospitalisation, isolate 151, Klebsiella type 26, biochemically typical, resistant only to penicillin. No chemotherapy given in the interim.

Case 24. J.G. Female, aged five months.

Pierre Robin syndrome. Admission throat swab (isolate 44) was typical K. aerogenes not types 1-40, resistant to penicillin, sulphonamide and polymyxin. After four days hospitalisation and no chemotherapy this was replaced by isolate 45, which did not produce a lysine decarboxylase and was resistant to penicillin, streptomycin, tetracycline, chloramphenicol and sulphonamide. The throat was inflamed throughout.

Finally, Case 25. S.A., was a child of six years with

acute stem cell leukaemia. The throat was clinically normal, but this child changed the Klebsiella carried in her throat over a period of ten days. Strain 14 was type 4, dulcitol +, sensitive to chloramphenicol, framycetin and polymyxin. Strain 15, also type 4, was dulcitol negative and sensitive to all the test antibiotics except penicillin. No antibiotics were given in the interim.

PART II

The preparation of Klebsiella typing sera in the rabbit and mouse, with a comparison of titres and cross reactions obtained in the two species.

3B

Review of the Literature - Part II

This is divided into five parts :

a) Development of serotyping in the identification of Klebsiella capsular serotypes.

b) Immunological specificity of the Klebsiella serotypes.

c) Antibody production in rabbit and mouse.

d) Effect of adjuvants on antibody production.

e) Production of antibody-containing ascites fluid in mice.

PART II

3B. REVIEW OF THE LITERATURE

(a) **The development of serotyping in the identification of Klebsiella capsular serotypes.**

The first attempt to separate the Klebsiella group serologically was made by Clairmont (1902) but he found agglutination reactions to be unsatisfactory in separating the various species. Porges (1905) recorded that agglutination and floccoulation tests depended on the properties of the "mucoid envelope", and he immunised rabbits with heat killed bacteria and demonstrated somatic agglutination with suspensions heated above 100°C. Toeniessan in 1914 demonstrated that the mucoid capsule was not important in agglutination and the bacterial membrane was of prime impertance in agglutinin production. Toeniessen (1921) stated that Friedlanders bacillus was composed of three parts - (a) endoplasm, (b) ectoplasm, (c) gelatinous shell, and the latter was protein free and composed of galactose.

Fitzgerald (1914) found that only strains which had lost their capsule were agglutinable, so that with most strains agglutination reactions were valueless in serological differentiation. Small and Julianelle (1923) eliminated capsules by prolonged culture on

agar and prepared nine agglutinating sera, and in their experiments noted that in strains which retained capsules precipitin reactions were observed; they were the first to postulate that precipitating antigens may be present in the capsular material.

Julianelle (1926a) studied 30 strains of Friedlanders bacillus and demonstrated three groups, A, B and C, and one heterogeneous group, X. Precipitation reactions were found to be highly specific for these groups in addition to agglutination and agglutinin adsorption reactions. In the same year, Julianelle (1926b) made the important observation that capsulate strains produced a specific soluble substance, and that these strains reacted only with the type specific antibodies of the homologous type. He also demonstrated that immunisation with capsulate strains induced antibody production which agglutinated type specifically, precipitated the soluble specific substance and protected white mice against infection caused by organisms of the same type. Rough strains were acapsulate and produced no soluble specific substance. Julianelle also observed (1926c) that the soluble specific substance of Friedlanders bacillus was non-antigenic when dissociated from

the cell, and this substance is demonstrable in filtrates of actively growing cultures, and in the blood and urine of infected animals.

Four years later, Julianelle (1930) reported on 80 strains isolated from various disease sites, and of these 42 were Group A, 12 Group B, 7 Group C and 19 Group X. More than 70% of his Group A strains were associated with pneumonia in man and 13 of these were non-lactose fermenters. He found no correlation between the serological type and fermentation reactions.

Price (1930) was mainly concerned with differentiating between rhinoscleroma and other groups of Klebsiella, but he, too, confirmed that specific antibodies could be induced in rabbits to all capsulate strains and these could be demonstrated by reactions of agglutination, precipitation and complement fixation.

Prasek and Price (1933) demonstrated for the first time the presence of a specific soluble carbohydrate in the capsules of rhinoscleroma and ozena strains, and Neuber (1934) showed that K. rhinoscleroma strains represented an antigenically distinct type from Friedlanders strains.

Morris and Julianelle (1934) reviewed the literature to 1934 on the serological reactions of rhinoscleroma and showed cross agglutination with type C of Friedlanders bacillus. Goslings and Snijders (1936) confirmed this close relationship and described the D, E and F capsular types in ozena.

Julianelle (1937), in a series of experiments, demonstrated common serological reactions between pneumococcus type II and Friedlanders bacillus type B and a strain of B. aerogenes, and confirmed these cross serological reactions by demonstrating protection in white mice. He noted that decapsulation of these strains "deprived them of the immunological relationships" and type specificity, rendering them species reactive, thus indicating a difference in somatic antigens within the three strains.

Osterman and Rettger (1941) compared the biochemistry and serology of 20 strains of Friedlanders bacillus and found biochemical heterogeneity among the type A organisms, thus confirming previous work by Julianelle (1930) and by Edwards (1929).

In 1948, interest arose in the morphology of

the capsulate bacteria. Klieneberger-Nobel (1948) studied capsulate organisms of many different species, and found that while the capsules had definite shape, slime was amorphous, and in Friedlanders bacillus the capsulated bacteria were embedded in slime, although loss of either could occur. By capsule and slime staining methods she demonstrated the cell wall and adjoining cytoplasmic layer. Duguid (1948) demonstrated that various nutritional deficiencies (such as nitrogen or phosphate) led to formation of large capsules, since carbohydrate was conserved for polysaccharide synthesis when growth was arrested.

In 1949, Kauffmann demonstrated that Klebsiella contain O antigens and can be divided into O groups. By capsular antigens these O groups can be divided into types, with the same capsules occurring in different groups. Klebsiella K10 was almost identical with Escherichia K39, and antigenic relationships were also found between :

Klebsiella K7 and Escherichia K55
Klebsiella K8 and Escherichia K34
Klebsiella K11 and Escherichia K37

Kauffmann also drew attention to the pathogenic significance of the Klebsiella group, especially in

urinary tract infections, where types 8, 9 and 10 were demonstrated in 90% of cases. Kauffmann stated "We must put the Klebsiella group .... on a par with the Escherichia group, which likewise is divided into seretypes without specific names".

Henriksen (1949a and b) reviewed the literature to date but claimed that the main source of infection was the respiratory tract.

Duguid (1951) recorded the value of the wet India ink film in demonstrating capsule formation, and showed an incidence of 37/40 cases of capsule production in B. aerogenes.

Two remarkable papers by Brooke (1951a and b) demonstrated 27 new capsular types of Klebsiella - designated 15 to 41. Factor sera were prepared for types 1 to 14 and the new seretypes 15 to 41. Brooke also showed that biochemically typical, and biochemically aberrant (i.e. adonitol negative, indole positive, gelatin positive, etc.) strains could possess identical capsules. Three hundred and eleven out of 324 strains isolated from human material were capsulate. None of the new capsular types were pathogenic for mice.

Edwards and Fife (1952) studied 256 non-motile encapsulated cultures classed as Klebsiella, confirmed

their biochemical heterogeneity and divided the organisms into 57 different capsular types. They noted a marked difference in the distribution of capsular types in the USA and Denmark. Edmunds (1954) described types 64 to 69.

Henriksen (1952) commented on results obtained mainly from the literature, and proposed that the Kauffmann classification be supplemented by dividing the Klebsiella group into rhinoscleroma, ozaena, pneumonia or Friedlander and aerogenes subgroups, as these play different roles in human pathology.

In 1953, two articles contributed to our knowledge of polysaccharide production. Knoll (1953) showed that addition of sucrose or galactose to the medium did not influence capsule production, but this was enhanced by serum. Vaccination with polysaccharide protected mice against infection with homologous strains, in contrast to Julianelle's observations (1926c) that the specific soluble substance alone was not antigenic. Duguid and Wilkinson (1953) related polysaccharide production to growth, and noted that the rate of production per cell was greatest in the log phase, although most polysaccharide was produced after growth cessation. Capsule production was increased ten to 20 fold when growth cessation was due to limitation of nitrogen, phosphorus or sulphur, and this was paralleled by increased intracellular polysaccharide and capsule diameter.

By 1954, 61 seretypes of Klebsiella had been described. Henriksen (1954a,b,c and d), in a series of papers, compared the cross reactions obtained by Brooke in Denmark (1951a), and by Edwards and Fife in the USA (1952) with his own results and found considerable discrepancy in reaction patterns of strains and sera. He raised the very pertinent question of whether the antigenic constitution of the Klebsiella types could be constant, and the question has still not been answered today.

Henriksen (1954d) showed a wide variety of biochemical patterns, and showed similar serological reactions in different biotypes, and even in a typical. He suggested that the Klebsiella group should be defined biochemically and not serologically, since cross reactions between types could also be demonstrated, e.g. five of Henriksen's strains showed a combination of types 29 and 42, suggesting there may be gradual transition between some types.

In the Report of the Enterobacteriaceae Sub-committee (1954) the subdivision of the Klebsiella group as proposed by Kauffmann (1949) was supported; and recommended that the single seretypes have no special names, but should be characterised by their antigenic formula, as recommended by Kauffmann, i.e.:

O Group	Capsule Type	Earlier Designation
1	1	A
	2	B
	3	C
	7,8,10,12.	--
2	2	B
	3	C
	4	D
	5	E
	6	F
	8	-
3	11	-
	9,13,14.	-

Ørskov (1954a) reported her work which comprised an O antigen analysis of capsule types 15 to 62. By agglutination and absorption experiments 39 strains were designated to the previous three O groups described by Kauffmann, and two new O groups, 4 and 5 were described, each including two strains. O5 was completely identical to Escherichia O8, and O4 was closely related to Escherichia O8.

Ørskov (1954b) investigated 104 Klebsiella strains from the upper respiratory tract and 76 from the intestinal canal of infants, and only six strains could not be typed completely by 69 sera. These were designated types 70, 71 and 72.

Edwards and Fife (1955) studied 626 capsulated

non-motile Klebsiella cultures, and gave details of serotype and source, and stated "As would be expected, the majority of cultures of types 1 to 6 inclusive were derived from the respiratory tract. However, their presence was by no means confined to the respiratory organs; they appeared in localised infections, urinary infections and stools and intestinal contents, blood, and infections of lower animals ..... Types 7 to 69 were found as frequently in respiratory diseases as were types 1 to 6, and were almost three times as frequent as the latter among cultures from blood and localised infections."

Cowan (1956) considered the value of the taxonomy of the different groups with the family Enterobacteriaceae, and stated that the aerogenic forms consisted of strains of typical Aerobacter aerogenes and Klebsiella pneumonia and are now usually grouped together. Strains differing from the typical IMViC reactions --++ are distributed among many capsule serotypes, especially 1, 2, 3, 4 and 5.

Kauffmann (1956a) investigated 20 Klebsiella cultures belonging to various serotypes and described a special subgroup which was indole +, gelatin + in

serotypes K26, 29 and 41. In a further paper (1956b) Kauffmann stressed the need for uniform evaluation and reporting of biochemical findings.

Ørskov (1957) examined biochemically the established 72 Klebsiella capsular types and an additional 158 strains belonging to 49 different capsular types. She was able to confirm the biochemical heterogeneity existing within the serotypes by setting up 36 biochemical types.

Sakazaki and Namioka (1958), working in Japan, were able to type 77.8% of 396 cultures with types 1 to 72 antisera. Of 107 sputum samples 55% were types 1, 2 and 4, and the rest belonged to 20 different types. Of 58 cultures of urine, ten were type 10; types 1, 4 and 25 were recovered from abscesses, and types 7 and 35 from CSF.

Gourden et al (1959) studied 34 strains, and 28 strains belonged to 12 of the biochemical types defined by Ørskov, and six new biotypes were described.

Bhattacharya, Gupta and Gupta (1960) studied 200 urinary strains of which only 156 were capsulate. No particular type was found responsible for urinary infection (ten were type 23), but the authors did mention that 14 were types 1 - 4, types generally respiratory in origin.

Durlakowa et al (1960) studied 558 strains and typed 443. They could find no biochemical criteria for the determination of serotype on the results of 15 biochemical tests, nor could they demonstrate correlation between the biochemical properties and the O antigen.

The problem of correlation of biotype and serotype was studied by Cowan, Steel, Shaw and Duguid (1960). The authors studied 182 strains and on the basis of biochemical and serological findings proposed five specific categories and one sub-specific category, and named a new species Klebsiella edwardsii and a new sub-species Klebsiella edwardsii var atlantae.

The biochemical characters of the six categories of Klebsiella were as follows (see page 178).

In Cowan and Steel (1965) the serotypes of these various biotypes were defined as follows:

Biotype	Serotype
K. aerogenes	1 - 72
K. edwardsii var edwardsii	1 and 2
K. pneumonia	3
K. edwardsii var atlanta	1

DISTINGUISHING CHARACTERS OF SIX CATEGORIES

(From Cowan, Steel, Shaw and Duguid (1960))

Test	K. aerogenes	K. edwardsii var edwardsii	K. pneumonia	K. edwardsii var atlantae	K. rhinoscleroma	K. ozaenae
Fimbriae	-	-	+	-	-	-
Gas from glucose	+	-	+	+	-	+
Acid from lactose	+	(+)	+	(+)	-	(+)
MR	-	d	+	+	+	+
VP	+	+	-	d	-	-
Citrate	+	d	+	+	-	d
Urease	+	+	+	+	-	d
Gluconate	+	+	d	d	-	-
Malonate	+	d	+	-	+	-
Lysine decarboxylase	+	+	+	+	-	-
44° test (gas from MacConkey)	d	-	-	-	-	-
Dulcitol (acid)	d	-	+	-	-	-
KCN	+	+	-	+	+	+

Biotype	Serotype
K. rhinoscleromatis	3
K. ozaenae	3,4,5 and 6.

Durlakowa (1960) studied 897 Klebsiella strains and determined both K and O antigenicity. Approximately one third of respiratory strains were K4, K5 and K6, and the O1, O2 or O3 antigen was determined in 85% of cases.

In 1962, four new capsular antigens were described by Marosz-Babczyszyn, designated K73, K74, K75 and K76.

K73 showed no cross reactions with K1 to K72;
K74 showed weak reaction with K10;
K75 cross reacted with undiluted K3 and K8;
K76 showed weak cross reaction with K24 and K43.

Tomisawa (1962a and b), working in Japan, studied cross precipitation reactions of 72 type sera of the Klebsiella group. Antigens which showed weak and retarded cross reactions and very low antigen titres reacted with many type sera, and this he attributed to reaction with O antigen. In his second paper (1962b) Tomisawa showed that precipitin reactions with O antibody and O antigens were very low, and that the K antibody titres were much higher than the O titres.

Durlakowa et al (1962) further investigated the

new capsular types K73 and 75 and found that many of these would decarboxylase arginine or ornithine as well as lysine. The other serotypes generally produced only a lysine decarboxylase, except K3 and some K4 and K5. The following year Durlakowa et al (1963) studied the capsular and somatic antigens of 20 strains of Klebsiella isolated from human secretions, and described four new capsular antigens, K77, K78, K79 and K80, and two new somatic antigens O11 and O12.

However, Katuszewski (1965) studied the capsular types K63 to K72 which had not been considered in the Kauffmann scheme of 1954, and showed that, except for strain 67, the rest belonged to groups O1 to O5, viz:

O1 = Strains 63, 64, 65, 66, 68 and 70.

O2 = Strains 69 and 72.

O5 = Strain 71.

The conclusions of Durlakowa et al (1963) that somatic antigens in strains 64, 69 and 72 were new was therefore not confirmed.

(II) Review of the literature dealing with the immunological specificity of the klebsiella serotypes

The specific sugar composition of the klebsiella antigens has not been worked out in the detail available for the salmonella and E. coli antigens, nor has the basal core or specific side chain sugar sequence been elucidated, which in the salmonellae give the characteristic O antigen determinants. This classification of the constituent sugars of the salmonellae into a series of chemotypes closely related to the serological properties is very thoroughly reviewed and explained by Lüderitz, Staub and Westphal (1966). Like most enterobacteriacae, the reactivity of klebsiella antigens extends beyond the klebsiella group (Cowan and Steel) (1965).

Kabat and Mayer (1967), in describing the cross reactions of the pneumococcal polysaccharide, states that the Friedlander type 2 polysaccharide cross reacts extensively with type 2 anti pneumococcal antisera, and this cross reaction is more readily inhibited by glucoronic acid than by glucose. The specific capsular polysacoharides of type II pneumococcus are :

(a) neutral sugars: L.rhamnose (50%)
D glucose

(b) sugar acids: D glucuronic acid

Heidelberger and Avery (1923) first extracted the soluble specific substance from type 2 pneumococcus and showed it to be a polysaccharide, built up of glucose molecules. The same authors (1924) demonstrated marked chemical differences between the specific soluble substances of types 2 and 3 pneumococcus, although both reacted as polysaccharides. Goebel (1927) demonstrated an isomeric relationship between the products of hydrolysis of the specific soluble carbohydrate in the capsule of Friedlanders type A and pneumococcus type 2, although he noted no immunological resemblance. Rake (1948) by complement fixation studies with antigens from Klebsiella pneumonia, Aerobacter, Granuloma bacilli and E. coli demonstrated antigenic relationships.

MacPherson, Wilkinson and Swain (1953) showed that K. aerogenes polysaccharide after NaCl treatment could inhibit haemagglutination by viruses of the influenza-mumps-NDV group due to the polysaccharide causing increased surface charge on the RBC.

Knoll (1954) showed that the pathogenicity of K. pneumoniae in animal experiments was increased by simultaneous injection of vegetable or bacterial polysaccharides. The pathogenicity of pathogenic strains was increased, and apathogenic strains were rendered pathogenic, thus establishing the importance of capsular

polysaccharides in pathogenicity. Read, Keller and Cabelli (1957) showed loss of mucoid consistency in some Klebsiella strains on subculture - so-called "suicide" or decapsulation phenonemon, and this was related to loss of capsule.

Barber, Wisnak and Ciuca (1957) used phenol to extract capsular material from the Klebsiella organism and this soluble complex represented 10% of the dry weight of the bacteria. Capsular material was extracted in buffered phosphate and somatic polysaccharide was shown to contain glucose, galactose, fucose, xylose, glucuronic acid and galacturonic acid.

Henriksen, Eriksen and Kaptein (1959) showed that the composition of types A, AE and E (modern nomenclature 1, 1x5 and 5) had a polysaccharide containing four monosaccharides. A common uronic acid was present in all three types.

The monosaccharides found were as follows :

Galactose in all three in very small amounts.
Glucose in A and E (1 and 5).
Fucose in A and AE (i.e. in 1 and 1x5).
Mannose in AE and E (i.e. in 1x5 and 5).

There was evidence to show that fucose was mainly

responsible for the cross reaction between types A and AE.

Eriksen and Henriksen (1961) studied the effect of purified Klebsiella polysaccharide on mice, in an attempt to compare the effect of Klebsiella polysaccharide with the prolonged pneumococcal paralysis produced in mice by Felton and Ottinger (1942 and 1949). Eriksen and Henriksen (1961) showed that Klebsiella type 1 purified polysaccharide was antigenic to mice, and this was probably protein free. The immunity was not of a high order and only gave protection against a range of 6 to 20,000 LD_{50} in different experiments. Precipitating antibodies could not be demonstrated in the blood of surviving mice by gel precipitation. Immunity was still detectable after six weeks, but unlike the work of Felton and Ottinger (1942) with pneumococcal polysaccharide, there was not true immunological paralysis but only a reduction in the level of immunity. The results suggest that the Klebsiella polysaccharide does not remain as long in an active form as the pneumococcal polysaccharide. Also, no cross protection could be demonstrated in mice immunised with type K1 polysaccharide and with type AE polysaccharide when challenged with a type 1 polysaccharide. The authors concluded that the cross reactivity between the two types is insufficient to cause cross protection

such as is found in the cross reactivity between Klebsiella type B and Pneumococcus type 2 by Avery, Heidelberger and Goebel (1925).

Henriksen and Eriksen (1962a) showed that fucose was present in the capsular polysaccharide of a type AE Klebsiella which had previously been regarded as fucose free, and the appearance of fucose reduced the cross reactivity of the strain with other fucose containing strains of type AE. Fucose in this polysaccharide disappeared after oxidation with periodic acid unlike fucose of other strains, suggesting that fucose might have a different location. The same authors proceeded to show (1962b) that capsular polysaccharides from strains of K. rhinoscleromatis, K. ozena, K. pneumoniae and K. aerogenes, all classified as type 3, had the same serological specificity. Eriksen, Henriksen and Kaptein (1963) studied the capsular antigens of three cross reacting Klebsiella strains and could detect two separate antigens in each of the three strains.

Eriksen - working in Oslo - (1965a) compared the structure of the capsular polysaccharides from K. aerogenes, Enterobacter and Klebsiella type 3 and showed that the only demonstrated difference between them was the nature of the uronic acids. Eriksen (1965b) proceeded to investigate the serological reactions of four type 3 specific polysaccharides isolated from

four different species, K. ozaenae, K. pneumoniae, K. rhinoscleromatis and K. aerogenes. Quantitative precipitin determinations in immune sera against the four different species showed that the capsular polysaccharide precipitated the same quantity of antibody; the author concluded that this indicated that the polysaccharides were identical and was in agreement with the chemical investigations. Gel precipitation lines were also identical.

Jones and Linker (1965) extracted capsular polysaccharide from K. pneumoniae type 2 by phenol and weak alkali methods. The phenol extract had a higher MW 4.7×10^5 and after I.V. injection in rats both preparations were rapidly cleared from plasma, but more of the high MW polysaccharide was taken up by the liver and spleen than the lower.

Nimmich and Munter (1967) isolated the capsular K and somatic O antigens of 72 strains. They identified /and the O antigens as lipopolysaccharides. the K antigens as acidic heteropolysaccharides/ In a further paper Nimmich (1968) showed that the capsular acidic heteropolysaccharides were composed of seven different monosaccharides on paper chromatography. The main component was glucoronic acid, and galacturonic acid was present in only six antigens, but the capsular polysaccharides contained at least two other sugars as

well. Various combinations of monosaccharides were observed, some with the same qualitative sugar composition but differing in their serological specificity, (see Table 27 - Appendix).

Rojas-Espinosa and Estrada-Parra (1968a) investigated the capsular polysaccharide of K. rhinoscleromatis and isolated a purified polymer composed of 60% mannose, part of which was destroyed on periodate oxidation after reduction and hydrolysis. From the end products produced it was concluded that mannose was linked 1,3 1,4 and 1,2 and that uronic acid, glucose and galactose are linked 1,3. The importance of mannose in specificity was demonstrated by showing that intact polysaccharide would precipitate with sera from rabbits hyperimmunised with K. rhinoscleromatis and also with serum from a patient suffering from rhinoscleroma, whereas the periodate treated polymer would not precipitate with the same sera. In a subsequent paper the same authors (1968b) showed that the K. rhinoscleromatis capsular polysaccharide polymer consisted of 52% galactose, 20% mannose, 12% rhamnose, 17% uronic acid and traces of glucose.

Eriksen and Kapitein (1968) isolated the polysaccharides from K. pneumoniae types 1, 2 and 3. Type 1 was shown to contain in the hydrolysate, the monosaccharides D glucose, L fucose and glucuronic acid.

Type 1 contained D glucose, mannose and glucuronic acid and type 3 D mannose, D galactose and galacturonic acid. Specific antibodies against the oxidised type specific polysaccharide were demonstrated in antisera to types 1 and 3, and a cross reaction with type 2 was observed.

Two recent papers dealing with virulence of Klebsiella from a different approach are worth noting at this stage. Hall and Humphries (1958) showed a high correlation between mouse virulence of three mucoid type A strains and one smooth strain of K. pneumoniae and the insusceptibility of these strains to phagocytosis by mouse polymorphs. The most virulent mucoid strain was insusceptible to phagocytosis and the avirulent smooth strain was readily phagocytosed.

Knoll (1958) described a "pyrogenic factor" in virulent Klebsiella strains, which was identified as a lipid bound to polysaccharide. The somatic lipopolysaccharides differed in composition of their polysaccharides from the filtrate containing the pyrogen factor, and the former were less pyrogenic. The authors postulated that carbohydrates were responsible for serological specificity whereas the lipid-polysaccharide complex mediated pyrogenicity.

(c) Review of the literature dealing with antibody production in the rabbit and the mouse

Since a comparison will be made of antibody levels to killed K. aerogenes cultures in the rabbit and the mouse, a review of the literature dealing with antibocy production in these two species follows.

As far back as 1883, Friedlander inoculated live cultures of Klebsiella suspended in distilled water - his "misrococci der Pneumonie", into the thoracic wall of rabbits, mice, guinea pigs and dogs. Only the mice, and 50% of the guinea pigs developed positive signs of pneumonia and one out of five dogs died of dyspnoea and positive lung culture.

Hoyle (1935) induced pneumonic lesions in mice to a variety of bacteria and viruses, and by intranasal insufflation of 0.05 cc broth culture of K. pneumonia produced patchy consolidation and pleural effusion. Hoyle and Orr (1945) did further histology on these lesions, and demonstrated multifocal collapse as early as the first day, and extensive haemorrhagic consolidation by the second to fourth day.

Fink and Quinn (1953) studied antibody production to a protein antigen - Egg Albumin, and to pneumococcus polysaccharide. For both antigens, the intra abdominal

route produced a higher antibody response than the intramuscular route. However, of the total number of animals tested only 4.7% failed to produce anti egg albumin, whereas 57.2% failed to produce anti-pneumococcal polysaccharide. Five inbred strains of mice were studied and genetic variation in the ability of the strains to produce antibody to either polysaccharide or protein antigen was demonstrated, and a maximum antibody response was not demonstrated to both antigens in those mice geared to high antibody production. The authors suggested that the mouse does not form antibody to antigens of different chemical composition in an identical manner. Age was highly significant; animals aged four to five months produced much more antibody than two month old mice; the sex of the animals had no effect. Oren and Olitzki (1953) tested 55 Klebsiella strains for mouse pathogenicity by the intraperitoneal and respiratory routes and found only one strain (O1K2) to be pathogenic in high dilutions. The others, which included type 1 and other type 2 strains were only slightly, or non pathogenic, concluding that there was no correlation between antigenic formulae and pathogenicity.

Marcus, Donaldson and Esplin (1955) showed reduced host resistance to K. aerogenes infection in inradiated compared to normal mice as demonstrated by higher mortality rate in the former group.

Immunological paralysis in rabbits after infection of Klebsiella type 5, and the fact that excess of capsular substance decreased the level of detectable homologous antibody was first demonstrated for the Klebsiella genus by Ørskov (1956)

Ehrenworth and Baer (1956) gave four strains of Klebsiella pneumoniae type II intraperitoneally to mice and although a non-pathogenic strain had the smallest capsule, this capsule increased in size after 24 hours in broth culture.

Epstein (1959b) showed that the ability of a given strain to evoke a periarterial reaction depended on type, virulence, concentration, viability and route of inoculation although none of these appeared to be obligate determinants. In a further paper, Epstein and Payne (1959) showed that young mice had shorter survival periods than older mice at comparable dose levels. Sixteen hour cultures were more virulent than older cultures inoculated intraperitoneally but not orally. Seven of the strains were more virulent by the oral route, and virulence was regarded as an unsatisfactory taxonomic characteristic.

In a paper dealing with the levels of circulating antibody and its relation to anaphylaxis in mice, Rothberg and Talmage (1961) demonstrated that different genetic strains of mice varied greatly in the antibody

response to bovine serum albumin in incomplete Freunds adjuvant. The presence of naturally occurring antibodies for staphylococci in non-immunised germ free mice was demonstrated by Cohen, Newton, Cherry and Updyke (1963) although the stimulus for antibody formation was not determined. The work of Rothberg and Talmage (1961) was extended by Farr, Grey, Dickenson and Rothenstein (1963), who showed that the capacity of different genetic strains of mice to produce antibodies to B.S.A. varied inversely with the amount of B.S.A. needed to produce detectable antibody synthesis in each strain, confirming that the parameters of antibody production tested were under some genetic influence.

Batshon, Baer and Shaffer (1963) demonstrated the ability of large doses of Klebsiella pneumoniae type 2 to produce immunological paralysis in mice. The dose required to produce paralysis was 750 ug of polysaccharide, and this persisted for 60 days. Mice given only 2.5 ug polysaccharide showed serum antibody production within five days.

Asherson and Holborrow (1966) demonstrated autoantibody production to colon in rabbits inoculated with various gram negative bacteria, including 212 animals inoculated with Klebsiella and complete Freunds adjuvant. Wu and Trice (1967) did further work on immunological paralysis in Swiss albino mice with 5 ug of type 2

Klebsiella capsular polysaccharide and paralysis with 1,000 ug, and this declined after three months.

(d) Review of the literature dealing with the effect of adjuvants on antibody production.

Freund and McDermott (1942) showed increased antigenicity of mice serum when combined with a mixture of lanolin, parrafin oil and killed tubercle bacilli, and guinea pigs injected with this combination showed increased sensitisation and precipitin formation.

Landsteiner and Chase (1942) and Chase (1943) used Freund's adjuvants to induce sensitisation and antibody formation towards chemicals, protein conjugates, horse serum and protein. Kopeloff and Kopeloff (1943) used Freund's complete adjuvant to demonstrate antibody formation against brain extracts in the monkey, and as a result of this work it seemed desirable to extend studies to other antigens. Freund and Bonnato (1944) therefore selected a water soluble antigen (diphtheria toxoid) and a particulate antigen (killed Salmonella typhi) and showed that lanolin and diphtheria toxoid enhanced and prolonged antitoxin production compared to injection of plain toxoid. The authors also showed that paraffin oil enhanced and sustained antibody formation against S. typhi, and that killed tubercle bacilli had an additional synergistic effect

Fox, Rickard and others (1949) showed more prolonged immunity to Murine typhus when the vaccine was given in a water in oil emulsion, and Lipton and Freund (1950) showed that complement fixing and neutralising antibodies could be produced with inactiviated rabies virus in paraffin oil.

Ward and others (1950) showed enhanced production of neutralising antibodies to poliovirus when injected with either Freund's incomplete or complete adjuvant, but the latter gave a higher titre. Salk et al (1951) confirmed the enhanced immunogenicity of virus antigen with adjuvant in his work on influenza viruses.

Freund (1951) noted that paraffin oil alone enhanced and prolonged antibody formation, but had little effect on sensitisation. The addition of mycobacteria had a marked increase in sensitisation but, depending on the antigen, may or may not increase antibody production above that effected by oil. This potentiating quality was independent of whether the organism was pathogenic (Mycobacterium tuberculosis) or saprophytic (Nocardia asteroides). Uchitel and Khasman (1965) reviewed the various theories on the mode of action of adjuvants, and by working with rabbits showed that non-specific stimulators of

antibody formation (endotoxin, pyrogen, Freund's adjuvant) rapidly increased incorporation of labelled amino acids in serum proteins and to regional lymph nodes, spleen and adrenal glands, and this was coincidental to a marked rise in the number of plasma cells in the spleen and regional lymph nodes. The authors therefore concluded that the adjuvant action of substances causing non-specific stimulation of antibody formation was associated with their capacity to stimulate protein synthesis in the animal, this being of paramount importance in the inductive stage of antibody formation.

Ramon (1957), in studying the mechanism of adjuvant effect, discussed the effect of slow release of antigen from the depot site. He gave very small amounts of antigen to an animal at short intervals, and noted a stimulating effect only when the injection was repeatedly given at the same site, and not when it was applied at different sites. Ramon concluded that inflammation was effective in antibody production.

Bernstein and Malkeil (1964) studied humoral antibody response in 31 patients before and after treatment with one injection of water in oil emulsion. There was no significant alteration in viral, bacterial,

or pollen antibody titres following this injection, nor were liver function tests altered, nor the presence of anti-tissue antibodies demonstrated. These results demonstrated that the clinical effects of water in oil adjuvants were not mediated by a non-specific, anamnestic response, nor are they particularly dangerous to such organs as the liver.

Lowe (1964) studied serum protein changes in adjuvant induced arthritis in rats and showed that the majority of changes were associated with inflammation - such as elevation of alpha and beta globulins, and the appearance of a pre-albumin band and fall in total serum albumin.

The mouse has been shown to possess four major immunoglobulin classes, IgG_1, IgG_2, IgA and IgM. Barth, McLaughlin and Farley (1965) investigated ten strains of mice with DNP-haemocyanin and complete Freund's adjuvant. The authors found no change in serum immunoglobulins after one injection of antigen alone. A single injection of antigen in Freund's adjuvant showed a two to five fold rise in IgG_1 and a second injection a ten fold increase. There was also lesser absolute increases in the other three immunoglobulins, but the interesting feature was

that the response of specific immunoglobulins to antigenic challenge was strain dependent. C57BL mice showed marked increases in IgM and IgA, while the white Swiss mice showed highest IgG_1 levels.

Coe (1966) studied the qualitative aspect of the IgG(7S) antibody response in the mouse and showed that the production of IgG_1 and/or IgG_2 would be influenced by the antigen, the adjuvant, the strain of mouse, and previous exposure to antigen.

White (1968), in his presidential address to the Royal Society of Medicine, pointed out that an adjuvant may even start off an immunological response which would not occur in the presence of antigen alone; in addition, adjuvants act by enhancing both humoral and cell mediated immunity, but in guinea pigs such adjuvants as water in oil emulsions have not produced delayed hypersensitivity responses.

Stavitsky and co-workers (1965) reviewed the evidence to show that the rabbit synthesised first 19S then 7S antibodies and they studied the intravenous injection of diphtheria toxoid and complete

Freund's adjuvant and showed that 19S antibody was related to the appearance of a non-phagocytic mononuclear cell, while levels of circulating 7S (IgG) paralleled antibody and γ globulin in the plasma cell. The authors reviewed the controversial literature which exists on whether the two responses can be effected by the same cell. The observations on the type of antibody made may not apply to other antigens given with or without adjuvant.

Youmans and Youmans (1967) tested adjuvant activity of several incomplete adjuvants in mice to virulent tubercle bacilli and found that Freund's incomplete adjuvant consisting of aquaphor and heavy mineral oil was the best adjuvant tested. Using minimal vaccinating doses it was essential to produce uniform and complete emulsification for maximal adjuvant activity.

Herbert (1968) produced peak antibody titres in mice given a single subcutaneous inoculation of ovalbumin in incomplete Fr$unds adjuvant, and antibody titres were 500 times those produced by the same dose of ovalbumin without adjuvant. Incomplete adjuvant alone, or when injected at a separate site from the antigen produced an antibody response. Ovalbumin was

was detected in emulsion recovered from injected mice for up to 544 days after inoculations, and its half life within the emulsion was found to be about 90 days. This indicated that incomplete adjuvant exerts its effect solely by the slow and even release of small quantities of antigen over a prolonged time. Creach et al (1968) studied the effects of complete Freund's adjuvant, incomplete Freund's adjuvant, lipopolysaccharide and purified phospholipid. The highest titres were obtained by lipopolysacoharide in incomplete Freund's adjuvant, but the rise was quicker with phospholipid. There was an optimal amount of adjuvant above which the immunological response was weaker - this was 40 µgm for lipopoly-saccharides from S. typhi and 40 - 80 µgm for phospholipid extracts.

(e) Review of the literature dealing with production of antibody containing ascites fluid in mice

The production of antisera in mice is limited by the small volumes of blood obtainable from each animal. Peritoneal fluid contains specific antibody in high concentration, and Munoz (1957) inoculated Swiss Webster female mice with either bovine serum albumin or egg albumin in Freund's complete adjuvant. Each mouse received two 0.25 ml intraperitoneal injections of the

antigen/adjuvant mixture at two weekly intervals, then was allowed to develop ascites for at least three weeks. The volumes of ascitic fluid obtained ranged from 0.5 ml to 14 ml per mouse and up to three tappings were obtained. About 50% of all mice responded with accumulation of peritoneal fluid.

Herrmann and Engle (1958) worked with Influenza A virus and Newcastle Disease virus, and showed that following twice weekly injections on alternate weeks for six weeks, the Webster strain of Swiss mice would produce an ascites following the injection of a 1/20 saline solution of Sarcoma 180 cells. The antibody levels in the sarcoma induced ascites were comparable to blood levels by haemagglutination inhibition, and virus neutralisation tests were positive with the specific immune ascitic fluid.

Kasel, Lieberman and Smith (1959) obtained ascitic fluid in adult female Swiss albino mice by giving intramuscular injections of Influenza A or Caxsackie B viruses followed by several intraperitoneal injections of complete adjuvant.

Lieberman, Douglas and Humphrey (1959) produced ascitic fluid containing high titres of antibody in male Swiss stock strain of mice, after giving

two to three intraperitoneal injections of 0.3 ml of Staphylococcus aureus mixed with complete adjuvant. One hundred percent of the injected mice showed an ascitic response, and at each tap the volume obtained was two to 12 ml per animal, and up to three aspirates could be obtained. Serum and ascitic fluid antibody titres were similar, measured by the slide agglutination methods.

In an extension of the above work, Lieberman, Douglas and Mantel (1960) showed that ascitic fluid containing antibodies could be produced from 100% of mice injected with various strains of Staphylococcus aureus or Salmonella enteriditis mixed with incomplete Freund's adjuvant. Production of ascitic fluid was not, however, continuous for all mice and over a four month interval, the yield was 12 to 15 ml per mouse for persistant producers and four to six ml on an average. Increase in the volume of ascitic fluid produced was also obtained by adding hyaluronidase or trypsin to the antigen/adjuvant mixture. The authors found the average total protein of ascitic fluid to be 3.98, composed of 1.37 g/100ml albumin, 2.61 globulin and 0.86 γ globulin, compared to immune serum which contained an average of 5.3 gm/100 ml total protein, 1.97 g/100 ml albumin, 3.57 globulin and 1.27 γ globulin. Sommerville (1967) used Freund's complete

adjuvant and Mycobacterium smegmatis as the adjuvant in obtaining hyperimmune anti-species globulin to human or rabbit IgG and obtained titres of up to 1:64 by gel diffusion.

Sartorelli and Booth (1961) showed enhanced inhibition of Sarcoma 180 ascites tumour using combinations of azaserine and purine analogs, and further work on the modification of Sarcoma 180 tumour was done by Fodor, Clarke and Bodansky (1961).

A comprehensive review of the literature on metabolic factors affecting tumour inhibition was presented by Sartorelli, Upchurch, Bieber and Booth (1964), who studied the biochemical mechanisms in the anti neoplastic activity of azerine and various purine analogs such as 6 chloropurine and 6 mercapto-purine on Sarcoma 180. They described the metabolic features of the modified purine resistant neoplasm Sarcoma 180 TG, and this work was extended in a further article by Bieber and Sartorelli (1964).

The use of this modified relatively non-virulent subline Sarcoma 180 TG was described by Sartorelli, Fischer and Downs (1966) to produce ascites fluids containing antibodies against several arboviruses, proteins (albumin and γ globulins), whole

human serum, and a cellular antigen (sheep erythrocytes). Quantitative precipitin tests using mouse ascitic fluid as antibody and human serum albumin as antigen showed the zone of equivalence to be a broad plateau at 19 mgm/ml of protein antibody in one animal and to be 11 mgm/ml in a second. The authors comment that the quantitative precipitin curve of mouse antibody is very similar to rabbit antibody.

The same principles of immunisation were applied by Tikasingh, Spence and Downs (1966) and produced large volumes of mouse ascitic fluids with high antiviral titres to a range of arboviruses. The ascitic fluids produced reacted specifically with the viruses to which they were prepared in cross complement fixation and haemagglutination inhibition tests.

PART II

4B. MATERIALS AND METHODS

The immunisation schedule in the rabbits has already been described (Materials and Methods, Part I).

Immunisation Schedule in Mice

STRAIN Swiss strain of white mice

Sex: female **Weight**: 18 - 22 gms.

Adjuvant: (Difco) Freund's incomplete.

Sarooma: modified suckling 180 TG (TRVL 53886) obtained from Trinidad Regional Virus Laboratory (Sacterelli et al - 1964)

Preliminary screening for naturally occurring antibodies in ascitic fluid

Fifty mice were inoculated as follows:

Day 1

0.2 ml sterile physiological saline + 0.2 ml Freund's incomplete adjuvant (Difco) well mixed and injected intraperitoneally.

Day 8

0.3 ml saline + 0.3 ml Freund's incomplete adjuvant intraperitoneally.

Day 15

0.5 ml saline + 0.5 ml Freund's incomplete adjuvant intraperitoneally.

Day 22

0.5 ml of 1/10 suspension in physiological saline of Sarcoma 180 TG.

Sarcoma 180 TG was maintained by serial passage through adult Swiss mice. These mice were tapped and Sarcoma dilutions made immediately prior to inoculation for immunisation purposes.

When abdominal distension was marked, mice were tapped intraperitoneally and ascitic fluid collected in sterile test tubes (see photograph - Appendix).

Ascitic fluid was centrifuged three times at 5,000 rpm for 30 minutes, and clear supernatant collected. Ascitic fluid obtained from the 50 mice inoculated above were tested neat, and at dilutions of one quarter against formol saline suspensions of each of the 72 Klebsiella serotypes, for Quellung reaction. None of these 50 mice showed naturally occurring Klebsiella antibodies in ascitic fluid at these dilutions. Each mouse was then exsaguinated, but the small amount of serum obtained from each made it necessary to test for circulating antibodies to the 72 serotypes at a serum dilution of one quarter only. No evidence of naturally occurring serum antibodies to any of the Klebsiella serotypes could be detected by this technique.

Immunisations

Batches of six mice were caged together and each batch was immunised with a mixture of homologous antigen prepared as described for the rabbit schedule, and Freund's incomplete adjuvant. The immunisation schedule was as previously described in the screening technique, except that antigen was substituted for saline. Immediately prior to each inoculation equivolumes of antigen and Freund's incomplete adjuvant were well shaken in a sterile Bijou bottle and this mixture used for intraperitoneal injections.

Mice were tapped intraperitoneally when abdominal distension was marked - this varied from day 30 to day 42 of the immunisation schedule. Ascitic fluid obtained from each batch of mice was pooled and centrifuged as above, and stored in 5 ml aliquots at -20°C. Ascitic fluid was then tested for homologous antibody levels by the capsular Quellung reaction and for cross reactions, as described in Materials and Methods, Part I.

Batches of mice were immunised to 40 Klebsiella serotypes (types 1-40) by this method, and antibody titres and cross reactions investigated. The volumes of ascitic fluid obtained in each batch of six mice, and the number of surviving mice are listed in Table 26 of the Appendix.

A proportion of mice survived to produce a second ascitic response. Ascitic fluid collected from the second aspiration was tested for cross reactions independently of the first aspirate. No changes in cross reaction were seen in the two aspirates..

PART II

5B. RESULTS

The following table (Table 1 - Part II) (page 209) shows the antibody levels obtained in rabbit and mouse, tested by Quellung reaction with homologous antigen.

From 40 antisera prepared to different Klebsiella serotypes in rabbit and mouse :

Rabbit titre exceeded mouse titre in 17.

Rabbit titre same as mouse titre in 8.

Rabbit titre less than mouse titre in 15.

It can therefore be assumed that the method used for hyperimmunisation of the mouse against Klebsiella species is as effective as the standard method described in the rabbit, in obtaining homologous antisera.

The Klebsiella serotypes which were poor antigens in the rabbit, i.e. gave titres of 1/8 or less, were serotypes 5, 6, 11, 13, 14, 16, 20, 22, 23 and 39. Of these ten serotypes three of them were also poor antigens in the mouse, viz. 5, 16, 22. Serotypes 11 and 14 gave much the better responses in the mouse (titres 1/128) as did serotype 23 (titre 1/64), demonstrating that a serotype may be only weakly antigenic in one animal and strongly antigenic in another.

Table 1 - Part II

ANTIBODY TITRES

Rabbit serum	1/16	1/32	1/64	1/64	1/8	1/8	1/64	1/64	1/64	1/32	1/4	1/32
Mouse ascitic fluid	1/16	1/16	1/8	1/128	1/4	1/16	1/16	1/64	1/16	1/8	1/128	1/64
Klebsiella serotype	1	2	3	4	5	6	7	8	9	10	11	12

Rabbit serum	1/8	1/8	1/32	1/8	1/16	1/16	1/16	1/8	1/16	1/4	1/8	1/32
Mouse ascitic fluid	1/32	1/128	1/32	1/8	1/64	1/64	1/32	1/16	1/64	1/4	1/64	1/8
Klebsiella serotype	13	14	15	16	17	18	19	20	21	22	23	24

Rabbit serum	1/64	1/32	1/64	1/64	1/32	1/16	1/64	1/32	1/64	1/64	1/32	1/16
Mouse ascitic fluid	1/8	1/8	1/4	1/128	1/8	1/16	1/16	1/32	1/8	1/32	1/8	1/16
Klebsiella serotype	25	26	27	28	29	30	31	32	33	34	35	36

Rabbit serum	1/16	1/32	1/8	1/64
Mouse ascitic fluid	1/64	1/8	1/16	1/8
Klebsiella serotype	37	38	39	40

Each of the 40 antisera prepared were tested for cross reactions with antigen suspensions of serotypes 1 - 72. The serum dilution to which a positive Quellung reaction was given is shown in brackets (Table 2 - Part II) (page 211).

A very striking difference in the cross reactions is seen in antisera prepared in the rabbit and hyperimmune ascitic fluid prepared in the mouse, despite the fact that the antigens used were identical.

The only common cross reactions found were in antisera prepared against type 11 which gave a common cross reaction to type 24, and here the cross reaction in the mouse was positive to a very high titre (1/128) compared to the rabbit (1/4); to antisera to type 12 where cross reactions to types 10 and 26 were present in both (each to 1/4); and to type 15 where common cross reactions to types 5 and 67 were observed (both to 1/4).

In the 40 antisera prepared in the rabbit there was a total of 58 cross reactions to heterologous antigens, and in hyperimmune ascitic fluid a total of 99 cross reactions, of which only five were common to both animals.

It appears that the mouse recognises many more

Table 2 - Part II

CROSS REACTIONS AGAINST OTHER KLEBSIELLA ANTIGENS OBTAINED IN RABBIT ANTISERA AND MOUSE ANTIASCITIC FLUID

Antisera to Klebsiella Serotype.	Cross Reactions obtained with antigens 1-72	
	RABBIT	MOUSE
1	12(1/8),37(1/4)	41(1/4)
2	16(1/8),26(1/4)	24(1/128), 29(1/128)
3	59(1/16)	13(1/64),24(1/64)
4	21(1/16),66(1/16)	1(1/128),5(1/16), 14(1/64)
5	None	None
6	34(1/4)	27(1/4),59(1/16)
7	23(1/16),48(1/8)	1(1/4),10(1/4), 18(1/4),22(1/4)
8	22(1/8),37(1/8)	None
9	18(1/4),25(1/4), 36(1/4).	63(1/4)
10	11(1/4),13(1/4), 17(1/4),37(1/4)	5(1/8),21(1/4), 49(1/4),60(1/8)
11	12(1/4),24(1/4)	4(1/32),9(1/32), 24(1/128.
12	10(1/4),26(1/4), 29(1/4),41(1/4).	1(1/4),5(1/8), 10(1/4),26(1/4), 53(1/4).
13	8(1/4)	47(1/4),48(1/4)

Continued...../

Table 2 - Part II

CROSS REACTIONS AGAINST OTHER KLEBSIELLA ANTIGENS OBTAINED IN RABBIT ANTISERA AND MOUSE ANTIASCITIC FLUID

Antisera to *Klebsiella* serotype	Cross Reactions Obtained with Antigens 1-72	
	RABBIT	MOUSE
14	None	4(1/64), 15(1/128), 24(1/64), 47(1/64)
15	5(1/4), 59(1/4), 67(1/4)	4, 5, 19, 24, 30, 31, 33, 38, 43, 62, 67 and 69 (all at 1/4)
16	43(1/4)	23, 34, 40, 53, 58, 62, 63 (all at 1/4)
17	21(1/4)	13(1/4)
18	5(1/8), 9(1/4), 6(1/4), 16(1/4)	None
19	9(1/4), 60(1/4)	None
20	21(1/4)	5(1/4), 11(1/4), 30(1/4), 67(1/4)
21	56(1/4), 62(1/4)	19(1/8), 65(1/4)
22	29(1/4), 30(1/4)	None
23	22(1/4), 25(1/4), 32(1/4)	None
24	43(1/4), 46(1/4)	6, 34, 35, 55, 65 (all at 1/4)
25	None	9(1/4), 17(1/4), 23(1/4)
26	None	None

Continued..../

Table 2 - Part II (Continued)

CROSS REACTIONS AGAINST OTHER KLEBSIELLA ANTIGENS OBTAINED IN RABBIT ANTISERA AND MOUSE ANTIABSETIC FLUID

Antisera to *Klebsiella* serotype	Cross Reactions obtained with Antigens 1-72	
	RABBIT	MOUSE
27	None	1(¼), 4(¼), 24(¼)
28	None	4(1/16), 10(¼), 14(⅛)
29	None	None
30	16(⅛)	13(¼)
31	None	70(¼)
32	None	12(⅛)
33	None	18, 19, 20, 24, 34, 51 (all at ¼).
34	None	12, 24, 38, 50 (all at ¼)
35	6(¼), 9(¼)	17(¼), 18(¼), 23(¼)
36	20(¼)	23(¼)
37	8(¼), 17(⅛)	27(¼), 34(¼)
38	8(¼), 9(¼)	45(¼), 48(¼)
39	7(¼), 23(⅛)	19(¼), 23(¼)
40	11(¼), 27(¼), 70(⅛)	24(¼), 26(⅛), 29(⅛)

antigenic determinants in the Klebsiella capsular polysaccharide as foreign, than does the rabbit, although the extent of response to the homologous antigen is comparable in both animals.

Nimmich (see Table 27 - Appendix) characterised sugar components of the capsular polysaccharides of the 72 capsular serotypes of Klebsiella. Type 1 has the relatively simple structure of glucuronic acid, glucose and fucose. In antisera to type 1 prepared in the rabbit, this cross reacted with type 12, glucuronic acid, galactose, glucose and rhamnose, and type 37 galactose and glucose.

The common component sugar in these three serotypes is glucose, and glucuronic acid is common to types 1 and 12 but not to type 37. Type 41, which cross reacted in the mouse also has glucuronic acid and glucose in common with type 1. However, glucuronic acid and glucose are present in a large number of the other serotypes (see Table 27 - Appendix) where no cross reactions were shown. The very high order of cross reactions obtained in hyperimmune ascitic fluid prepared against serotype 2 (immunodominant sugars — glucuronic acid, galactose, glucose and mannose), which cross reacted to a titre of 1/128 to types 24

and 29 may be explained by the fact that type 24 is composed of three out of four of these sugars, i.e. glucuronic acid, glucose and mannose, and type 29 of two out of four of these sugars; i.e. galactose and mannose. This does not explain why no cross reactions to other serotypes containing these sugars occurred.

Galacturonic acid appears to play no part in immunological specificity, since no cross reactions were observed between the serotypes of which it is a component, i.e. types 3, 29, 34, 48, 49, 57 and 63.

In considering the position of the 6-desoxy-hexoses in immunological specificity, no cross reactions were observed between serotypes containing fucose, i.e. serotypes 1, 6, 16, 54, 58, 60, 63 and 68 . In the 40 antisera studied, rhamnose is contained in the capsular polysaccharide of types 9, 18, and 63 and antisera to type 9 cross reacted with type 18 in the rabbit and type 63 in the mouse, so rhamnose may play some part in immunological specificity, but this does not explain the cross reactions in rabbit antisera to type 9 with antigenic types 25 and 36.

It would appear from Nimmich's qualitative analysis that some differences in the three dimensional

arrangement of glucuronic acid, galactose, glucose and mannose determine the immunological specificity of the Klebsiella capsular antigens. An important fact is that antisera to the various Klebsiella serotypes prepared by different workers show different cross reactions (Edwards and Ewing (1962)), the Klebsiella serotypes cannot be grouped for standard cross reactions, and it is absolutely necessary that each capsular antiserum prepared be tested against all known capsular types.

The immunisation schedule used in the mouse involved the use of Freund's incomplete adjuvant. The differences in cross reactions in the two species may have been influenced by adjuvant. The incomplete adjuvant may have modified the structure of the immunodominant sugar; e.g. mineral oil can extract lipopolysaccharide from mycobacteria. However, as pointed out by White (1967) it is unlikely that adjuvant effect is due to this factor. However, modification of capsular polysaccharide materials by mineral oil should be considered as a possible factor. One other explanation of the differences in the cross reactions in the two immunisation schedules is that adjuvant, by prolonging antigen retention in the body has reduced the specificity of the reaction.

An argument against the latter explanation is that the cross reactions observed in the mouse would have been <u>additional</u> to those observed in the rabbit, rather than almost completely different, as was the case. Also the mice which were tapped for ascitic fluid a second time, showed no increase in the number of cross reactions detected.

6. GENERAL DISCUSSION

Although the literature on clinical infective syndromes due to klebsiella species is extensive, no previous publications compare the incidence of isolation of K. aerogenes from different clinical sites in health and disease. This survey was undertaken in an attempt to elucidate the significance of isolating K. aerogenes from clinical specimens, and to investigate the role of antibiotics in establishing or facilitating infection.

The wide variety of sources from which klebsiella was obtained well indicate the ubiquitous nature of K. aerogenes in the body, apart from its normal commensal role in the intestinal flora. (No intestinal isolates of K. aerogenes were included in this work).

Respiratory Tract Infections (pp 63 - 94)

Eisenberg and others (1958) noted the presence of klebsiella organisms in respiratory secretions in the absence of disease. This present study shows that klebsiellae appear to be of no significance in the upper respiratory tract, with a similar incidence in both normal and infected throats.

Bloomfield (1921) found a 5.8% incidence of klebsiella isolation from the upper respiratory tract of 85 unselected hospital patients, and Hyde (1943) had only

a 1% isolation rate of K. aerogenes from the normal upper respiratory tract. A much higher incidence was found in Jamaica - 11.5% from normal throats and 13.5% from infected throats of hospitalised patients. Furthermore, there appears to be no particular biotype or serotype related to throat infection, and a wide variety of each was found in this series.

No previous reports could be found relating to the significance of klebsiella in tracheostomes. Klebsiella, while frequently colonising tracheotomy sites in this hospital (60% of cases), was related to infection in four out of six cases, but two of these were complicated by a mixed flora with Pseudomonas pyocyanea. Nevertheless, attempts should be made to eradicate the organism from the tracheotomy site because of the potential danger of descending infection to the lower respiratory tract in patients who are usually severely ill.

In contrast to the findings in the upper respiratory tract, isolation of K. aerogenes in the sputum is a significant finding. There is no particular serotype associated with lower respiratory tract infection in Jamaica. Types 1 and 2, which are frequently regarded as respiratory pathogens, were isolated from asymptomatic patients, and the higher serotypes appear to be almost as common as the lower serotypes in respiratory tract infections.

The thin walled abscess formation usually associated

with Friedlander's pneumonia has been described by many authors including Sisson and Thompson (1915), Berglund (1925), Belk (1926) and Westermark (1926). A variety of respiratory syndromes were associated with K. aerogenes in Jamaica. This series shows an association with acute bronchitis - 13 cases, bronchopneumonia - nine cases, or lobar pneumonia - 13 cases. Only three out of 58 cases progressed to lung abscess formation, and none of these was fatal. The isolation of K. aerogenes serotypes 2, 10 and 31 in pure culture from cases of lung abscess indicated that Friedlander's pneumonia is not restricted to types 1 and 2 as is commonly supposed, and does not confirm the findings of Weiss et al (1956) that destructive lung disease was commoner with lower type infections.

The diversity of the respiratory signs found in Jamaica correspond to the descriptions by Colins and Kornblum (1929), Foster and Bragg (1962) and Steinhauser et al (1966). As would be expected, there was frequently a predisposing cause for respiratory infection, such as bronchiectasis, chronic bronchitis and emphysema, and carcinoma of the lung. Nevertheless, in eight of the cases of lobar pneumonia and all of the cases of lung abscess, infection occurred in previously healthy patients, not on antibiotics, indicating that K. aerogenes must be regarded in some situations as a primary invader. Three additional cases of lobar pneumonia were secondary to other respiratory infections after antibiotic therapy had suppressed the primary invader.

Alcott (1933) showed that males above 40 years of age were the group most susceptible to Friedlander's pneumonia. Eighty-eight percent of the series described by Hyde (1943) were over 40 years with a male/female ratio of 5:1. The predominance of klebsiella respiratory infections in older age groups was confirmed by Solomon (1940) and Pearlman and Bullowa (1941). In this survey the male/female ratio was 26:12, with 50% of the cases occurring in patients over 40 years of age. However, in Jamaica in one year, eight cases of lower respiratory infection occurred in the under 20 age group (about 20% of all klebsiella respiratory infections).

Commensal klebsiella carriage in the lower respiratory tract was a feature of the over 40 age group, 75% (15/20) of lower respiratory carriers without clinical or radiological evidence of infection being in this age group. No cases of commensal carriage occurred in the lower respiratory tracts of persons under 20 years, but the incidence of structural respiratory abnormalities was, of course, much lower in the younger age groups. Types 1, 2 and 3, as well as higher serotypes, were shown to be commensal and both higher and lower serotypes can be associated with infection. Furthermore, not all patients with klebsiella respiratory infection showed the expected leucocytosis (13/38 cases, or 34%), although fever was more often present (21/38 cases or 55%

Urinary Tract Infections (pp 94 - 101)

Urinary tract cross-infections acquired in hospital, as described in Denmark by Ørskov (1954b) were not seen in Jamaica, and 42/79 isolates of K. aerogenes from urines, which could be serotyped, belonged to 16 different serotypes, further confirming that cross infection was not very important in this situation. The wide variety of seretypes encountered in urinary tract infections in Jamaica confirms the observations of Steinhauser, Eichoff et al (1966), who described many serotypes from this source. Apart from the absence of cross infection, studies of K. aerogenes in the urinary tract in Jamaica show little difference from studies elsewhere. Klebsiella is second only to Escherichia coli as a urinary tract pathogen, confirming the studies of Hill et al (1929) and Coleman and Taylor (1949). Klebsiella urinary infection occurs predominantly in older age groups, and in relation to urethral stricture, as previously shown by Coleman and Taylor (1949). Other predisposing factors to urinary tract infection, such as calculus formation and catheterisation were found just as frequently in an E. coli age and sex matched control group.

Again, a peripheral leucocytosis was the exception rather than the rule, and occurred in only five out of 74 patients with klebsiella urinary tract infection. Less than half the patients were febrile on the day of submitting a positive urine culture for K. aerogenes.

Female Genital Tract Infections (pp 101 - 104)

Baehr et al (1937) reported a 3.5% incidence of klebsiella in the genital tract. In normal healthy controls in Jamaica an incidence of 6% was found, compared to 12% in cases of vaginal discharge. An association with Trichomonas vaginalis infection was seen in the group presenting with vaginal discharge, confirming the observations of Gordon, Hughes and Barr (1966), who noted an increase in enterobacteriacae in their T. vaginalis infections. In the female genital tract K. aerogenes was most frequently encountered in the puerperium but was not more frequently associated with an offensive than with a normal lochia. It seems unlikely that K. aerogenes alone could act as a primary pathogen in a normal female genital tract. The higher serotypes (41-72) predominate in the female genital tract, since 18/24 isolates were not types 1-40, but were capsulate.

Abscesses and Wound Infections (pp 107 - 113)

The literature on klebsiella associated with abscess formation is sparse, but the two cases in the Jamaican series coming to autopsy, and showing multiple pyaemic abscesses are comparable to the clinical syndrom described by Tennenbaum and Ravid (1936) and by Pfeiffer (1937). Since neither of these published reports gave details of the serotypes involved, the serotype results in the present survey demonstrate spread of infection by the same serotype.

This series includes seven cases of abscess formation when pure cultures of klebsiella were isolated. One case of

prostatic abscess who ultimately came to autopsy with klebsiella meningitis, shows a very similar pathology to the case described by Tennenbaum and Ravid (1936) and by Pfeiffer (1937).

A case of type 7 liver abscess who came to autopsy is included in the Appendix.

Quick and Brogan (1968) showed that more than half of their post-operative wound infections were due to gram negative rods with E. coli as the predominant pathogen, but with many strains involved. In the Jamaican series the gram negative rods occurred in 145/428 (32%) wound infections and collectively were more commonly isolated than Staph. pyogenes (118/428 cases, or 17%). Thirty-seven cases of klebsiella isolation from wounds occurred in this series, and 34 cases were associated with signs of local infection (p 113). A wide variety of serotypes were involved.

K. aerogenes appeared to be the primary pathogen in two cases of neonatal sticky eye but again different serotypes were involved and the lesions healed spontaneously. K. aerogenes is probably of little significance when isolated from the umbilical stump, as it was only isolated in pure culture in four out of eight cases, and cleared spontaneously.

Association with diabetes mellitus (page 146)

This is the first large scale survey to demonstrate that, unlike staphylococcal infections, diabetes mellitus is not a predisposing factor to K. aerogenes infection; the incidence of diabetes in the klebsiella group being the same as for the hospital as a whole.

Klebsiella Septicaemia (pp 106 - 107)

Klebsiella septicaemia was invariably restricted to the over 40 age group, with deaths in two out of five cases. Ten percent of all cases of septicaemia in the University Hospital were due to K. aerogenes, in comparison to 28% in the series by McCabe and Jackson (1962a) and 30% due to 16 different serotypes in the series by Maizetegui et al (1965). The five cases of klebsiella septicaemia were caused by different serotypes, so no relation between serotype and virulence could be demonstrated.

Klebsiella in Cerebrospinal Fluid

The incidence of klebsiella menigitis in this series was less than 2% (page 104). There is no doubt as to the grave significance of any repeatedly pure culture of klebsiella from cerebrospinal fluid - a type 7 klebsiella meningitis came to autopsy and was isolated from various other sites in the body (Case R.J. - Appendix photographs) and (Results, Part I, page 162, Case 9). The low incidence of klebsiella meningitis is comparable to the 0.01% incidence reported by Neal (1931), 0.14% by Fothergill and Sweet (1933) and 0.02% by Jacob and Topp (1948). The case reported in

the Jamaica series is similar to that reported by Ramsmeier (1943) except that in the Jamaican case the primary lesion was a klebsiella prostatic abscess.

Biotype and Serotype Combinations (Table in Appendix, 2, 3, 4, 6, 8, 10, 12, 14, 16, 17, 20 and 23).

In order to estimate the extent to which different biotype and serotype combinations occurred in clinical material, each new strain isolated was given a number, since within each serotype, several different biochemical reactions were encountered, indicating different strains.

Thirty different serotype and biotype combinations were encountered in 53 specimens from throat swabs and tracheostomes and this did not include six additional strains which could not be serologically identified. Twenty additional serotype and biotype combinations were encountered in sputum. An additional 22 serotype and biotype combinations were found in the urine specimens, and a further eight in wound and skin infections; and a further one in the genital tract and ascitic fluid.

Of the 290 cultures studied, 196 were serotypes 1 - 40 and were biochemically classified. Within these 196 cultures no less than 82 different biotype and serotype combinations were found. Strains present in the respiratory tract were frequently encountered at other sites, such as urine and wound infections, and vice versa. If the 94 strains which were not serotypes 1 - 40 could have been typed, an even

greater heterogeneity would have been seen, because many different biotypes were seen among this group.

Furthermore, 21 different serotypes caused hospital acquired infections (Table 26), and within these serotypes many different biotypes were found, so that there could not be said to be any particular 'hospital' strain, although types 2, 3, 9, 11, 29 and 35 were encountered as hospital acquired infections rather more frequently than other serotypes. Hospital acquired infections occurring in any one particular ward showed a great variety in serotype and biotype, and cross infection did not appear to be an important characteristic of K. aerogenes in hospital.

Effect of Antibiotics (pp 139 - 146)

Eighty-nine out of 248 patients were on antibiotics at the time of first isolation of klebsiella, and this was not a significantly higher proportion than a carefully matched control group of hospital patients showing no evidence of klebsiella infection. When the duration and type of antibiotics given were analysed, it was seen that there was a predisposition to develop klebsiella infection after seven or more days on multiple chemotherapy. Chloramphenicol had been prescribed significantly more often in patients showing klebsiella infection than in the controls but the incidence of prescribing the other antibiotics was similar.

Wilson and Miles (1964) state that serotypes 1 - 6

occur more frequently in the respiratory tract, and it was found that 26/44 (59%) of strains of serotypes 1 - 6 came from the respiratory tract, while only 38% of all cultures investigated came from this source. However, as seen in Table 30 there was a scatter of serotypes from all sources.

Klebsiella edwardsii edwardsii and Klebsiella edwardsii atlanta strains described by Cowan and others (1960) were not encountered in Jamaica during this survey. Serotypes 1 - 6 show great biochemical heterogeneity (see Wilson and Miles, 1964) and an attempt was made to see if biochemical heterogeneity was a feature of respiratory tract isolates; this was found not to be the case. Strains of K. aerogenes isolated from urines were found to be more biochemically heterogeneous than strains from all other sources. Biochemical heterogeneity was not related to pathogenicity in any way, nor were the strains that were multiresistant to antibiotics any more pathogenic than less resistant strains. Respiratory strains and non-respiratory strains showed no significant difference in resistance patterns to the ten antibiotics used in testing.

Multiple Klebsiella Infections (pp 146 - 164)

In a detailed study of multiple klebsiella infection or isolation, in a group of patients it is shown that one patient may carry a variety of serotypes and biotypes at any one time, and that during the period of hospital stay, these may alter. In these cases, determination of serotype

and biotype can be of great importance in tracing the course of an infection in the patient, and without which there would be considerable confusion. This, and the study of cross infections are perhaps the two justifications for serotyping isolates of klebsiella.

Comparison with Other Pathogens

The role of K. aerogenes as an infecting organism must be compared with other bacterial species. Although K. aerogenes is frequently isolated from hospital patients its pattern of infectivity can bear no comparison to the streptococcal epidemics which were common in hospitals in the 19th century and caused wound infection in World War I. However, improved aseptic techniques and introduction of antibiotic therapy have not resulted in a decline of infection, as has been the case with streptococcus. Furthermore, virulent streptococci in general belong to Lancefield Group A, and much less commonly to Group C, but type identification does not usefully serve to distinguish strains with different clinical propensities (Williams et al, 1966). This, and tha fact that potentially virulent streptococci may be carried in normal throats are about the only common denominators in comparing the two groups of organisms.

Virulence can be correlated with coagulase production in the staphylococcus, and various phage types have been associated with various diseases. Unlike staphylococci, hospital epidemics due to a particular strain of klebsiella

do not occur as a rule in Jamaica or elsewhere, although Ørskov (1954c) has demonstrated cross infection in Danish hospitals. Nasal and skin carriage are important factors in spread of staphylococcal infection in hospitals but work currently underway in Jamaica shows that nasal and skin carriage of K. aerogenes is extremely rare. Lowbury and Fox (1953) have shown that the gram negative bacilli survive for a much shorter time in the atmosphere than do the staphylococci and streptococci, so this may be a reason why hospital epidemics due to gram negative bacteria are not so common. The Public Health Laboratory survey in 1960 showed that coliforms commonly affected wounds after six days stay in hospital, and could produce septicaemia in debilitated patients. Williams and others (1966) have pointed out the great gap in our understanding of the epidemiology of the gram negative infections and our ignorance of the changes, if any, that occur in the carriage of these organisms in patients after admission to hospital.

Like other gram negative organisms, klebsiella has been shown in this survey to be important in surgical wounds, burns and in urinary infections. Unlike Pseudomonas pyocyanea it has not been shown to cause serious eye infection, although it is probably a commoner cause of respiratory tract infection in hospitalised patients than any other gram negative bacillus.

Klebsiella species shares with the other gram

negative bacilli the ability to produce bacteraemic shock due to action of the lipopolysaccharide endotoxin, and Weil et al (1964) have noted this complication in 24% of cases of gram negative bacteraemia.

In the Lancefield Group A streptococci, protein antigens are most important in characterising the type specificity. In contrast, the klebsiella and pneumococcal capsular antigenic types are determined by variations in the composition of the capsular polysaccharide. Indeed, cross reactions occur between type 2 pneumococcus and type 2 K. aerogenes, presumably due to common sugars in the capsular polysaccharide of each. Klebsiella and pneumococcal organisms share other features in common. Both can produce immunological paralysis in mice. Both have well defined capsular polysaccharide antigens, and Austrian and Gold (1964) have shown that the biology of infections caused by the several pneumococcal types varies due to differences in invasiveness.

The absence of leucocytosis in many of the cases of klebsiella infection described in this survey, and the low grade inflammatory response seen locally around the lesions of at least one case coming to autopsy (Talerman, Finnie and Wontumi, 1968) is very similar to the resistance of pneumococcus type 3 to surface phagocytosis described by Wood and Smith in two papers (1949 and 1950). Indeed, the antiphagocytic action of the capsule in the two organisms

is very similar. Therefore, the host response to infection with klebsiella species and to pneumococcus type 3 organisms appear to be very similar. However, this study does not show any particular association with serotype and virulence, unlike the enhanced virulence of type 3 pneumococcus due to antiphagocytic action. Austrian and Gold (1964) have re-introduced the typing of infective strains of pneumococci and found that pneumonia caused by type 1 pneumococcus had a case fatality rate of 6%, compared to 48% for infections caused by type 3.

A recent study from Edinburgh by Calder, McHardy and Schonell (1970) reported on 137 cases of pneumococcal pneumonia. Type 3 occurred in 36% and type 8 in 15% of cases. 15% had bacteraemia and the mortality rate in this group was 25%. The wide distribution of pneumococcal serotypes in the Edinburgh series compares to the heterogeneity of serotypes encountered in the Jamaican klebsiella series. Unlike type 3 pneumococcus, no particular serotype of K. aerogenes can be said to have been found to be especially virulent in the respiratory tract - lobar pneumonia and lung abscess being caused by a variety of serotypes. This does not confirm the work of Julianelle (1941) who showed a high mortality in type 1 infections. Cowan et al (1960) have postulated the highly virulent nature of K. edwardsii edwardsii and K. edwardsii atlanta (serotypes 1 and 2) and this has been confirmed by Foster and Bragg (1962) and

Darrell and Hurdle (1964). These biotypes were not encountered in Jamaica, and K. aerogenes serotypes 1 and 2 (Table 8, page 85) appear to be capable of occurring commensally in sputum.

The two fatal cases were both due to K. aerogenes serotype 7, but this may be fortuitous, and further studies on case fatality rates in infections due to different serotypes are necessary. Death occurred in two out of five cases of positive klebsiella blood culture, but since not all cases of klebsiella infection had specimens submitted for blood culture, the incidence of septicaemia in this group of 248 patients may have been higher, and comparison cannot be made with the case fatality rates of pneumococcal septicaemia from Edinburgh.

The second part of this study deals with a comparison of humoral antibody responses to klebsiella in rabbits and mice. Induction of an ascitic response in mice was demonstrated by Munoz (1957) using protein antigen, Herrmann and Engle (1958) and Kasel et al (1959) using viruses. The only attempt to produce antibodies to bacteria by this method was by Lieberman et al (1959) using Staphylococcus aureus but Cohen et al (1963) showed naturally occurring staphylococcal antibodies in germ free mice. No such naturally occurring klebsiella antibodies were demonstrated in this series, although an extensive screening was done within the limitations of the Quellung reaction. The use of Sarcoma 180 TG to produce an ascitic

response as described by Sartorelli et al (1966) has previously been confined to arboviruses, proteins, and sheep erythrocytes. This study shows that this technique can also be applied to produce antibodies to the capsular polysaccharides. Sartorelli et al (1966) have already demonstrated that quantitative precipitin curves of mouse antibody to human serum albumin is very similar to rabbit antibody, and although quantitative precipitation curves were not done here the titres obtained by Quellung reaction were similar in the two animals.

The volumes of ascitic fluid obtained in this survey (average 9.6 ml) are comparable to the results obtained by Munoz (1957), and Lieberman et al (1959).

This study shows that the capsular polysaccharide substance of the klebsiella species produces a different antigenic response in two species of laboratory animals. It can be assumed that these differences are genetically engendered. A possible explanation for the differences in cross reactions in the rabbit and mouse is that the capsular surface of the klebsiella organism possesses large numbers of antigenic determinants, and the mouse has the ability to recognise more of these than the rabbit, and so produces a less specific antisera.

It is noted that the mouse will select different antigenic determinants from the rabbit, and that both species will react to high concentrations of the homologous type, but in different degrees.

Cross reactions can be removed by adsorption without a fall of more than one or two dilutions in the homologous titre, in most cases. Therefore, it may be postulated that true cross reactions are not really occurring, but that there is a lack of antigenic specificity in the different serotypes.

It is apparent from both sections of this study, that further work is needed on the epidemiology of the gram negative organisms. Present work underway in Jamaica is concerned with the role of faecal and throat carriage of klebsiella in auto infection and cross infection in the hospital environment, and faecal carriage of the infecting serotype and biotype seems to be an important factor (Finnie, MRC Grant 1826, in progress).

The chemical structure and nature of the antigenic determinants of the klebsiella serotypes have to be more clearly elucidated, and the way (if any) by which these are modified by adjuvants. Paired experiments with rabbits, immunised with and without adjuvants and a comparison of cross reactions obtained in the two groups might be useful, except that cross reactions can vary within the same species as noted by Henriksen (1954a, b, c, d).

Any work on the gram negative organisms, which are assuming increasing importance in the hospital environment, is inevitably complicated by the large numbers of serotypes

and biotypes existing not only in klebsiella species, but also in the escherichia and pseudomonas groups. Henriksen's proposal (1954e) that the klebsiella group should be defined biochemically and Kauffmann's (1949) characterisation of the klebsiella serotypes by antigenic formulae alone cannot be fully accepted in any survey of an epidemiological nature, since the number of biotypes within each serotype has been shown to be large.

The above work shows that it is not possible to be dogmatic about the significance of isolation of any particular serotype of klebsiella from any source, except that this organism is unlikely to have any significance when isolated from throat swabs or from high vaginal swabs, and is probably not an important pathogen in the umbilical stump. It has been demonstrated that previous assumptions made on the association of pathogenicity with certain serotypes cannot be upheld. Antibiotic prescription in a klebsiella group was very similar to a matched hospital control group, except after seven or more days chemotherapy when a significant increase in klebsiella isolation occurred.

The animal studies show that an alternative but less specific means of preparing klebsiella antisera in mice is available, but our knowledge of the immunogenicity of the different klebsiella polysaccharides requires further investigation.

It would seem relevant to conclude with the words of neither a Bacteriologist nor an Immunologist, but to seek wisdom from the Law and be reminded that "The logical method and form flatter that longing for certainty and repose which is in every human mind. But certainty is an illusion, and repose is not the destiny of man".

(O.W. Holmes - Collected Legal Papers)

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BIBLIOGRAPHY

APPLEBAUM. E. LEFF, W.A. (1948).

J.A.M.A. 138. 119

Occurrance of superinfections during antibiotic therapy.

ASHERSON. G.L. and HOLBORROW. E.J. (1966).

Immunology. 10. 161.

Autoantibody production in rabbits. VIII. Autoantibodies to gut produced by the injection of bacteria.

AUSTRIAN. R. and GOLD. J. (1964).

Ann. Int. Med. 60. 759.

Pneumococcal Bacteremia with especial reference to Bacteremic pneumococcal pneumonia.

AVERY. O.T.. HEIDELBERGER. M.. and GOEBEL. W.F. (1925).

J. expt. Med. 42. 709.

The soluble specific substance of Friedlanders bacillus. II. Chemical and Immunological relationships of Pneumococcus type II and a strain of Friedlanders bacillus.

BAEHR. G.. SCHWARTZMAN. G.. GREENSPAN. E.B. (1937).

Ann. Int. Med. 10. 1788.

Bacillus Friedlander Infections.

BARBER, G., WISNER, B., CIUCA, M. (1957)

Arch. Roum. Path. Exp. Microbiol. 16/1. 150.

A new method for separation of capsular material from the somatic polysaccharide of a strain of K. pneumoniae.

BARRITT, M.M. (1936)

J. Path. Bact. 42. 441.

The intensification of the Voges Proskauer reaction by the addition of ∝ naphthol.

BARTH, W.L., McLAUGHLIN, C.L., FAHEY, J.L. (1965)

J. Immunol. 95. 781.

The immunoglobulins of mice.

BATSHON, B.A., BAER, H., SHAFFER, M.F. (1963)

J. Immunol. 90/1. 121.

Immunological paralysis produced in mice by Klebsiella pneumoniae type 2 polysaccharide.

BAUER, A.W., KIRBY, W.M.M., SHERRIS, J.C., TURCK, M. (1966)

Am. J. Clin. Path. 45. No. 4. 493. (Reprinted from Technical Bulletin of the Registry of Medical Technologists. 36. No. 3.)

Antibiotic susceptibility testing by a standardised single disc method.

BELK, W.P. (1926)

J. Infect. Dis. 38. 115.

Pulmonary infections by Friedlanders bacillus.

BENNER, E.G., MICKLEWAIT, J.S., BRODIE, J.L., KIRBY, W.M.M (1963). Proc. Soc. Exptl. Biol. and Med. 119. 536. Natural and acquired resistance of Klebsiella aerobacter to Cephalothin and Cephaloridine.

BERGLUND, N. (1925 - 1926)
Beitr. z. Klin. d. Tuberk. 62. 745.
Fall von Friedlander pneumonie unter dem Bilde einer Lungentuberkulose Terminale Affektion der Harnwege.

BERNSTEIN, I.L., MALKIEL, S. (1964)
J. Allergy. 35/5. 390.
A study of diverse humoral antibody systems following a single injection of Freunds incomplete adjuvent.

BHATNAGAR, S.S., SINGH, K. (1935)
Ind. J. Med. Res. 23. 337.
Bacteriological studies in acute lobar pneumonia due to pneumococcus and B. pneumoniae Friedlander.

BHATTACHARYA, K., GUPTA, N.P., GUPTA, S.P. (1960)
Ind. J. Med. Res. 48/2. 127
Klebsiella strains isolated from cases of urinary infection. II. Serological investigation.

BIESER, A.L., SARTORELLI, A. (1964)
Cancer Research. 24. 1210.
The metabolism of Thioguanine in Purine Analog-resistant cells.

BLOOMFIELD. A.L. (1920).

Bull. John Hopkins Hosp. 31. 203.

The significance of the bacteria found in the throats of healthy people.

BLOOMFIELD. A.L. (1921a)

Ibid. 32. 33.

BLOOMFIELD. A.L. (1921b)

Am. Rev. Tubercle. 4. 847.

The mechanism of the Bacillus carrier state with special reference to Friedlanders bacillus.

BOARDMAN. D.L. and O'BRIEN. T.D. (1961)

Geront. Clin. 3. 183.

A case of Klebsiella pneumoniae meningitis in association with liver abscess.

BOETTIGER. C.. WEINSTEIN. M.. WERNE. M. (1940)

J.A.M.A. 114. 1050.

Primary suppuration of liver due to Friedlanders bacillus.

BORDES, L.A. (1949)

Medicine Tropicale. 9. 802.

Colo hépatité Amibienne. Abcès du foie a bacille de Friedlander.

BOTSFORD. T.W. and KINNEY. T.D. (1946)

New England J. Med. 235. 539.

Acute salpingitis due to Friedlanders bacillus.

BROOKE, M.S. (1951a)

Acta Path. Micro. Scand. 28. 313.

Further capsular antigens of Klebsiella strains.

BROOKE, M.S. (1951b)

Acta Path. Micro. Scand. 28. 328.

The biochemical properties of Klebsiella strains with new capsular antigens.

BROWN, T.R. (1902)

John Hopkins Hosp. Rep. 10. 11.

"The bacteriology of cystitis, pyelitis and pyelonephritis in women, with a consideration of the accessory etiological factors in these conditions, and of the various chemical and microscopical questions involved."

BRULÉ, M., HUGUENIN, R., GILBERT DREFUS. (1927)

Bull. et Mém. Soc. Med. d. Hôp. de Paris. 51. 1370.

Pneumopathie chronique à bacilles de Friedlander.

BRUNNER, C. (1896).

München med Wchnschr. 43. 286 and 318.

Zur pathogenen Wirkung des Bacillus Friedländer. Ein Fall von akut metastiskerender allgemeininfektion nach otitismedia und empyem des Processus mastoides.

BUGGS, C.W., BRONSTEIN, B., HIRSCHFELD, J.W., PILLING, M.A. (1946). J.A.M.A. 130. 64.
In vitro action of streptomycin on bacteria.

BULGER, R.J. (1967)
Ann. Int. Med. 67/3. 523.
In vivo effectiveness of Kanamycin and Kanamycin/Cephalothin against Klebsiella. Comparison with other antibiotics.

BULLOWA, J.S.M., CHESS, J., FRIEDMAN, N.B. (1937)
Arch. Int. Med. 60. 735.
Pneumonia due to Bacillus Friedlanderi.

BURKE-GAFFNEY, H.J.O'D. (1933)
J. Hyg. 33. 510.
The type of coliform bacilli prevalent in urine and their significance, with special reference to the sanitary aspects.

BURNS, M.W. (1968)
Lancet. Feb. 24th. 383.
Precipitins to Klebsiella and other enterobacteria

OMISSION

CALDER, Margaret, McHARDY, V.U., SCHONELL, M.E. (1970)
Lancet. Jan. 3rd. page 5.
Importance of pneumococcal typing in pneumonia.

to Shigella boydii 14.

CHASE, M.W. (1943)

Proc. Soc. Exp. Biol. and Med. 52. 238.

Production of local skin reactivity by passive transfer of antiprotein sera.

CLAIRMONT, P. (1902).

Z. Hyg. Infektkr. 39. 1.

Differential diagnostische unter suchungen uber Kapselbakterien.

CLEMENSHA, W.W. (1912)

J. Hyg. 12. 463.

A criticism of Dr. A.C. Houston's report on the biological characters of B. coli isolated from (1) raw (2) stored river water (3) stored and filtered water.

COE, J.E. (1966)

J. Immunol. 96. 744.

$7S_1$ and $7S_2$ antibody response in the mouse. Influence of strain, antigen and adjuvent.

COFFEY, G.L., SCHWAB, J.L., EHRLICH, J. (1950)

J. infect. Dis. 87/2. 142.

In vitro studies of bacterial resistance to chloramphenicol.

COHEN, J., NEWTON, W., CHERRY, W., UPDYKE, E. (1963)

J. Immunol. 90. 358.

Normally occurring Staphylococcal antibodies in germ free mice.

COLEMAN, P.N., TAYLOR, S. (1949)

J. Clin. Path. 2. 134.

Coliform infection of the urinary tract.

COLLINS, L.H. and KORNBLUM, K. (1929)

Arch. Int. Med. 43. 351.

Chronic pulmonary infection due to the Friedlanders bacillus.

CONBA, C. (1896)

Arch. de Biol. 1. 112.

Un case di setticaemia da bacillo de Friedlander in un neonate associata a scleroma.

COPPO, O. (1957)

Antibiot. and Chemotherapy. 7. 297.

The in vitro antibiotic activity of Novobiocin on Staphylococcus pyogenes and some intestinal gram negative bacteria.

COWAN, S.T., (1956)

J. Gen. Microbiol. 15. 345.

Taxonomic rank of Enterobacteriacae groups.

COWAN, S.T., STEEL, K.J., SHAW, C., DUGUID, J.P. (1960)

J. Gen. Microbiol. 23. 601.

A classification of the Klebsiella group.

COWAN, S.T., STEEL, K.J. (1965)

Manual for the identification of Medical Bacteria.

1st Edition pub. Camb. Univ. Press.

CREACH, O., GAJA, R., FONTANGES, R. (1968)

Ann. Inst. Pasteur. 114/6. 786.

A comparison in mice between the adjuvent action of lipopolysaccharides from gram negative bacteria and of related phospholipids.

DARRELL, J.H. and HURDLE, A.D.F. (1964)

J. Clin. Path. 17. 617.

Identification and clinical significance of Klebsiella species in chest infections.

DAVIDSON, Jane. (1948)

J. Path. Bact. 60. 51.

Massive colony formation of Bacterium Friedlander in the liver in agranulocytosis.

DAVIS, G., WOOLHOUSE, N.M. (1966)

West Afr. Med. J. 15/2. 69.

Taxonomy of Klebsiellas and non-fermenting gram negative bacilli isolated from clinical material.

DENYS, J. (1892)

Bull. de l'Acad. Royale de Méd. de Belgique. 6. 114.

Etude sur les infections urinaires.

DIFIGLIA, S.E., CRAMER, C. (1951)

New York State J. Med. 51/6. 761.

Friedlanders bacillus meningitis in a case with liver abscess and recurrent Bacteriaemia and analysis of cases receiving specific therapy.

DUBAY, L. (1960)

Cas. Lek. Ces. 99/15. 449.

The incidence of Klebsiella in the upper respiratory passages.

DUDGEON, L.S. (1926)

J. Hyg. 25. 119.

A study of intestinal flora under normal and abnormal conditions.

DUGUID, J.P. (1948)

J. Path. Bact. 60. 265.

The influence of cultural conditions on the morphology of B. aerogenes with reference to nuclear bodies and capsule size.

DUGUID, J.P. (1951)

J. Path. Bact. 63. 673.

The demonstration of Bacterial capsules and slime.

DUGUID, J.P., WILKINSON, J.F. (1953)

J. Gen. Microbiol. 9/2. 174.

The influence of cultural conditions on polysaccharide production by Aerobacter aerogenes.

DURLAKOWA, I. (1960)

Arch. Immunol. Ter. Dosw. 8/4. 631.

Studies on the antigenic structure of Klebsiella bacilli.

DURLAKOWA, I., MARESZ-BABCZYSZYN, J., PRZONDO-HESSEK, A. (1960). Arch. Immunol. Ter. Dosw. 8/2. 213. Biochemical properties of Klebsiella strains.

DURLAKOWA, I., MARESZ-BABCZYSZYN, J., PRZONDO-HESSEK, A. (1962). Arch. Immunol. Ter. Exp. 10/3. 619. Studies on amino acid decarboxylases and the influence of KCN on the growth of Klebsiella bacilli.

DURLAKOWA, I., MARESZ-BABCZYSZYN, J., PRZONDO-HESSEK, A., LUSAR, Z. (1963) Arch. Immunol. Ther. Exp. 11/4. 549. New K: antigenic types of bacilli of the genus Klebsiella.

DUTTON, A.A.C., RALSTON, M. (1957) Lancet. 1. 115. Urinary tract infection in a male urological ward.

EDMUNDS, P.N. (1954) J. Infect. Dis. 94/1. 63. Further Klebsiella capsule types.

EDWARDS, P.R. (1929) J. Bact. 17. 339. Relationships of the encapsulated bacilli with especial reference to Bact. aerogenes.

EDWARDS, P.R. and BRUNER, D.W. (1942)

Circ. Ky. Agric. Exp. Sta. No. 52.

Serological identification of Salmonella cultures.

EDWARDS, P.R. and EWING, W.H. (1962)

"Identification of Enterobacteriacae"

2nd Ed. pub. Burgess, Minneapolis.

EDWARDS, P.R. and FIFE, M.A. (1952)

J. Infect. Dis. 91: 92.

Capsule types of Klebsiella.

EDWARDS, P.R. and FIFE, M.A. (1955)

J. Bact. 70/4. 382.

Studies on the Klebsiella-Aerobacter group of bacteria.

EHRENWORTH, L., BAER, H. (1956)

J. Bact. 72/5. 713.

The pathogenicity of Klebsiella pneumoniae for mice; the relationship to the quantity and rate of production of type specific polysaccharide.

EICKHOFF, T.C., STEINHAUSER, B.W., FINLAND, M. (1966)

Ann. Int. Med. 65. 1163.

The Klebsiella-Enterbacter-Serratia division. Biochemical and Serologic Characteristics and Susceptibility to antibiotics.

EISENBERG.G.M., O'LOUGHLIN, J.M., FLIPPIN, H.F. (1954)
J. Lab. Clin. Med. 43/5. 707.
Distribution and in vitro antibiotic susceptibility of Klebsiella.

EISENBERG, G.M., WEISS, W., FLIPPIN, H.F. (1958)
Ann. Int. Med. 49/2. 310.
The influence of penicillin therapy on the emergence of Klebsiellae in sputum.

EPSTEIN, S.S. (1959a)
J. Clin. Path. 12/1. 52.
The biochemistry and antibiotic sensitivity of the Klebsiellae.

EPSTEIN, S.S. (1959b)
J. Path. Bact. 78/2. 389.
Experimental Klebsiella pneumonia in mice with particular reference to periarterial changes.

EPSTEIN, S.S., FRIEDMANN, I. (1958)
J. Clin. Path. 11/4. 359.
Klebsiella serotypes in infections of the ear and upper respiratory tract.

EPSTEIN, S.S., PAYNE, P.M. (1959)
J. Hyg. (Lond.). 57/1. 68.
The effects of some variables on experimental Klebsiella infections in mice.

ERASMUS, L.D. (1956)

Q. J. Med. 25. 507.

Friedlander Bacillus infection of the lung, with special reference to classification and pathogenesis.

ERIKSEN, J. (1965a)

Acta Path. et Microbiol. Scand. 64. 511.

Immunological studies on some serological cross reactions in the Klebsiella group 11, 12 and 13.

ERIKSEN, J. (1965b)

Acta Path. et Microbiol. Scand. 64/4. 522.

Immunochemical studies on some serological cross reactions in the Klebsiella group 12. Serological reactions with the acidic capsular polysaccharides from Klebsiella type 3 as antigen.

ERIKSEN, J., HENRIKSEN, S.D. (1961)

Acta Path. Micro. Scand. 52. 163.

Immunochemical studies on some serological reactions in the Klebsiella group. (4) Immunisation of mice with purified capsular polysaccharide.

ERIKSEN, J., HENRIKSEN, S.D., KAPTEIN, W. (1963)

Acta Path. Microbiol. Scand. 58/2. 245.

Immunochemical studies on some serological cross reactions in the Klebsiella group. IX. Cross reactions between Klebsiella type 11, 21, and a closely related strain.

ERIKSEN, J., KAPTEIN, W. (1968)

Arch. Immunol. Ther. Exp. 16/2. 260.

Comparison of the capsular polysaccharides isolated from Klebsiella pneumoniae types 1, 2 and 3.

ESCHERICH, T. (1885)

Fortschr. Med. 3. 515.

Die Darmbacterien des Neugeborenen und Säuglings.

ÉTIENNE, G.. (1895)

Arch. de Méd. expér. et d'anat. path. 7. 124.

Le pneumo-bacille de Friedlander. Son role en pathologie.

FARR, R.S., GREY, H.M., DICKINSON, W., ROSENSTEIN, D.H. (1963)

Fed. Proc. (No. 629). 22. 265.

Genetic influences on anti BSA produced in mice.

FAUCETT, R.L., MILLER, H.C. (1948)

Paediatrics. 1. 458.

Stomatitis in infants caused by B. mucosis capsulatis.

FEDER, J.G. (1938)

Bull. Ayer. Clin. Lab. Penn. Hosp. 3. 231.

Review of Friedlanders bacillus pneumonia in the Pennsylvania hospital from 1927 - 1938.

FELTON, L.D., OTTINGER, B. (1942)

J. Bact. 43. 94.

Pneumococcus as a paralysing agent on the mechanism of immunity in white mice.

FELTON, L.D. (1949)

J. Immunol. 61. 107.

The significance of antigen in animal tissues.

FERGUSON, J.A., TOWER, A.A. (1933)

Am. J. Dis. Child. 46. 59.

Pneumonia in infants due to bacillus mucosus capsulatus.

FINK, M.A., QUINN, V.A. (1953)

J. Immunol. 70. 61.

Antibody production in inbred strains of mice.

FINLAND, M., JONES, W.F., BARNES, M.W. (1959)

J.A.M.A. 170. 2188.

Occurrence of serious bacterial infections since introduction of antimicrobial agents.

FINLAND, M., MURRAY, R., HARRIS, H.W. (1946)

J.A.M.A. 132. 16.

Development of Streptomycin resistance during treatment.

FITZGERALD, J.G. (1914)

J. Infect. Dis. 15. 268.

A biometrical study of the mucosis capsulatis group.

FLEMING, P.C., GOLDNER, M., GLASS, D.G. (1963)

Lancet. 1. 1399.

Observations on the nature, distribution and significance of cephalosporinase.

FODOR, P.J., CLARKE, D.A., BODANSKY, O. (1961)

Fed. Proc. 20. 156.

Glycolysis by sarcoma 180 of mice treated with 6-mercaptopurine.

FORD, W.W. (1927)

"Textbook of Bacteriology" p. 562, pub. W.B. Saunders.

FOSTER, W.D., BRAGG, Jean. (1962)

J. Clin. Path. 15. 478.

Biochemical classification of Klebsiella correlated with the severity of the associated disease.

FOTHERGILL, L.D., SWEET, L.K. (1933)

J. Paed. 2. 696.

Meningitis in infants and children.

FOX, J.P., RICKARD, E.R., VAN DER SCHEER, J., COX, H.R. (1949)

Am. J. Hyg. 49. 321.

Antibody response to vaccination against murine typhus.

FRAENKEL, A. (1886)

Ztschr. f. Klin. Med. 2. 437.

Weitere Beiträge zur Lehre van den Mikrococcen der fibrinösen pneumonie.

FREUND, J. (1951)

Am. J. Clin. Path. 21. 645.

The effect of paraffin oil and Mycobacterium on antibody formation and sensitisation.

FREUND, J., BONATO, M.V. (1944)

J. Immunol. 48. 325.

The effect of paraffin oil, lanolin-like substances and killed tubercle bacilli on immunisation with diphtheric toxoid and B. typhosum.

FREUND, J., McDERMOTT, K. (1942)

Proc. Soc. Exp. Biol. and Med. 49. 548.

Sensitisation to horse serum by means of adjuvents.

FRIEDLANDER, C. (1882)

Virchows. Arch. f. Path. Anat. 87. 319.

Uber die Schizomyceten bei der acuten fibrösen pneumoniae.

FRIEDLANDER, C. (1883)

Fortschr. Med. 1. 715.

Die Mikrokokken der Pneumonie.

FRISCH, A.W. (1940a)

Am. J. Clin. Path. 10. 472.

Sputum studies in pneumonia as an aid in prognosis.

FRISCH, A.W. (1940b)

Am. J. Clin. Path. 10. 873.

Sputum studies in type 3 pneumonia.

FRISCH, A.W. (1943)

Am. J. Clin. Path. 13. 69.

Autopsy studies in type III and Friedlanders pneumonia.

GEURDEN, L., DEVOS, A., VAN DEN WYNGAERT, M. (1959)

Vlaams. Dierengeneesk. T. 28/6. 161.

Subsequent investigations on Klebsiellae.

GILL, F.A., HOOK, E.W. (1965)

Am. J. Med. 39. 780.

Changing patterns of bacterial resistance to antimicrobial drugs.

GOEBEL, W.F. (1927)

J. Biol. Chem. 74. 619.

The soluble specific substance of Friedlanders bacillus. IV. On the nature of the hydrolytic products of the specific carbohydrate from type A Friedlanders bacillus.

GOLDBERG, B. (1895)

Centralbl. f. d. Krankheiten d. Harn u. Sexual-Organe. 6. 349. "Uber Bakteriurie"

GONDOLF, H.J., STRINGER, M.S. (1929)

New Orleans M. and S. J. 81. 735.

A bacteriological study of the gram negative bacilli found in 50 cases of U.T.I.

GORDON, A.M., HUGHES, H.E., BARR, G.T.D. (1966)

J. Clin. Path. 19. 429.

Bacterial flora in abnormalities of female genital tract.

GOSLINGS, W.R.O., SNIJDERS, E.P. (1936)

Zbl. Bakt. 136. 1.

Untersuchungen uber das scleroma respiratorum.

GRANT, L.S. (1958a)

West Ind. Med. J. 7. 183.

A survey of Klebsiella infections at the University College Hospital of the West Indies.

GRANT, L.S. (1958b)

West Ind. Med. J. 7. 285.

A bacteriological analysis of urinary infections (University College Hospital of the West Indies)

GRAY, J.D.A. (1932)

J. Hyg. 32. 132.

The significance of B. aerogenes in water.

GROTTS, B.F. (1949)

J. Paed. 34. 174.

Pneumonia in infancy caused by Friedlanders bacillus.

GUYON, F. (1889)

C.R. de l'Acad. des Sc. 108. 884.

"Sur les conditions de receptivité de l'appareil urinaire a l'invasion microbienne"

HALL, H.E., HUMPHRIES, J.C. (1958)

J. Infect. Dis. 103/2. 157.

The relationship between insusceptibility to phagocytosis and virulence of certain Klebsiella pneumoniae strains.

HARRIS, A.H., COLEMAN, M.B. (1963)

"Diagnostic Procedures and Reagents" Pub. Am. Pub. Health Ass. Inc. 4th Ed.

HARTMAN, M.M. (1940)

Ann. Int. Med. 14. 513.

Recovery from acute Friedlander pneumonia. Report of case and review of literature.

HEIDELBERGER, M., AVERY, O.T. (1923)

J. Expt. Med. 38. 73.

"The soluble specific substance of Pneumococcus"

HEIDELBERGER, M., AVERY, O.T. (1924)

J. Expt. Med. 40. 301.

The soluble specific substance of the Pneumococcus.

HEILMAN, F.R. (1945)

Proc. Staff Meetings, Mayo Clinic. 20. 33.

Streptomycin in the treatment of experimental infections with microorganisms of the Friedlander group.

HELMHOLZ, H.F. and SUNG, C. (1944)

Am. J. Dis. Child. 68. 236.

Bacterial action of Penicillin on bacteria commonly present in infections of the urinary tract.

HENRIKSEN, S.D. (1949a)

Acta Path. Microbiol. Scand. 26. 424.

Serotypes of a collection of mucoid gram negative rods.

HENRIKSEN, S.D. (1949b)

Acta Path. Microbiol. Scand. 26/3. 436.

Taxonomy and classification of the genus Klebsiella.

HENRIKSEN, S.D. (1952)

Acta Path. Microbiol. Scand. 30. 230.

Classification of the Klebsiella group.

HENRIKSEN, S.D. (1954a)

Acta Path. Microbiol. Scand. 34/3. 249.

Studies on the Klebsiella group. I. Serotypes of a collection of strains from human sources and from water.

HENRIKSEN, S.D. (1954b)

Acta Path. Microbiol. Scand. 34/3. 266.

Studies on the Klebsiella group. III. Demonstration of the M antigen of Escherichia coli in Klebsiella (Aerobacter).

HENRIKSEN, S.D. (1954c)

Acta Path. Microbiol. Scand. 34/3. 271.

Studies on the Klebsiella group. IV. Cross reactions of Klebsiella types 8, 11, 21, 26 and 35 and the M antigen of E. coli.

HENRIKSEN, S.D. (1954d)

Acta Path. Microbiol. Scand. 34/3. 276.

Studies on the Klebsiella group. V. Serological reactions of type AE and of strains related to types 14, 29, 42 and 41.

HENRIKSEN, S.D. (1954e)

Acta Path. Microbiol. Scand. 34/3. 281.

Studies on the Klebsiella group. VI. Conclusions.

HENRIKSEN, S.D., ERIKSEN, J., KAPTEIN, W. (1959)

Acta Path. Microbiol. Scand. 45/4. 381.

Immunochemical studies on some serological cross reactions in the Klebsiella group. I. A quantitative study of the cross reactions between type A (1) E (5) and AE. II. Chemical composition of the capsular polysaccharides.

HENRIKSEN. S.D., ERIKSEN. J. (1962a)

Acta Path. Microbiol. Scand. 54/4. 382.

Immunochemical studies on some serological cross reactions in the Klebsiella group.

HENRIKSEN. S.D., ERIKSEN. J. (1962b)

Acta Path. Microbiol. Scand. 54/4. 391.

Immunochamical studies on some serological cross reactions in the Klebsiella group.

7. Serological reactions of some strains of type 3 (C) and some cross reacting strains.

HEPP. J. (1936)

Ann. d'Anat. Path. 13. 116.

Gangrène utérine et septicémie à Friedlander post abortum.

HERBERT. W.J. (1968)

Immunology. 14/3

The mode of action of mineral-oil emulsion adjuvents on antibody production in mice.

HERRELL. W.E., NICHOLS. D.R. (1945)

Proc. Staff Meeting, Mayo Clinic. 20. 449.

The clinical use of streptomycin. A study of 45 cases.

HERRMANN, E.C., ENGLE, C. (1958)

Proc. Soc. Exper. Biol. and Med. 98. 257.

Tumour cell induced mouse ascites fluid as a source of viral antibodies.

HILL, J.H., SEIDMAN, L.R., STADNICHENKO, A.M.S., ELLIS, M.G. (1929). J. Bact. 17. 205.

A study of 200 cultures of gram negative bacilli isolated from cases of genitourinary infection.

HOJO, S. (1965)

Fukoshima Med. J. 15/2. 99.

Biological and serological studies on Klebsiella strains isolated from clinical and experimental specimens. (Japanese).

HOYLE, L. (1935)

J. Path. Bact. 41. 163.

The production of pneumonia in mice by bacteria and filtrable viruses.

HOYLE, L., ORR, J.W. (1945)

J. Path. Bact. 57. 441.

The histogenesis of experimental pneumonia in mice.

HUGHES, W.H. (1961)

J. Gen. Microbiol. 25/1. 157.

The diminution of variation in bacterial populations with special reference to Klebsiella pneumoniae and drug resistance.

HYDE. L., HYDE, B. (1943)

Am. J. Med. Sc. 205. 660.

Primary Friedlander pneumonia.

JACOB. S.S., TOP. F.H. (1948)

Ann. Int. Med. 28. 1003.

Friedlander bacillus meningitis. Report of 7 cases with two recoveries.

JAFFE. S.A. (1943)

J.A.M.A. 122. 292.

Extrapulmonary Klebsiella pneumoniae infections. An analysis of literature. Report of two unusual cases with recovery.

JAMES. O.B.O'L., GRANT. L.S., HULTQUIST. E. (1967)

West Indian Med. J. XVI. 129.

A bacterial survey of selected areas of a hospital.

JONES. R.S., LINKER. A. (1965)

Res. (N.Y.). 2/3. 210.

Molecular weight and tissue distribution of toxic Klebsiella polysaccharide.

JORDAN. E.O. (1903).

J. Hyg. 3. 1.

The kinds of bacteria found in river water.

JULIANELLE, L.A. (1926a)

J. Exp. Med. 44. 113.

A biological classification of encapsulatus pneumoniae (Friedlanders bacillus).

JULIANELLE, L.A. (1926b)

J. Exp. Med. 44. 683.

Immunological relationships of encapsulated and capsule-free strains of encapsulatus pneumoniae (Friedlanders bacillus).

JULIANELLE, L.A. (1926c)

J. Exp. Med. 44. 735.

Immunological relationships of cell constituents of encapsulatus pneumoniae (Friedlanders bacillus).

JULIANELLE, L.A. (1930)

J. Exp. Med. 52. 539.

The distribution of Friedlanders bacilli of different types.

JULIANELLE, L.A. (1937)

J. Immunology. 32. 21.

Immunological specificity of B. aerogenes and its antigenic relation to pneumococcous type 2 or Friedlanders bacillus type B.

JULIANELLE, L.A. (1941)

Ann. Int. Med. 15. 190.

The pneumonia of Friedlanders bacillus.

KABAT, E.A., MAYER, M.M. (1967)

"Experimental Immunochemistry" 2nd Ed., pub. Charles C. Thomas, Springfield, Illinois.

KAHLER, J. (1951)

Schweiz. Z. Allg. Path. Bakt. 14/6. 684.
Bacteria of the Klebsiella group as the cause of a pseudo-dysentery, in combination with a croupous pneumonia.

KASEL, J.A., LIEBERMAN, R., SMITH, H.A. (1959)

Virology. 9. 702.
Production of viral antibodies in ascitic fluid of mice.

KASS, E.H. (1955)

Am. J. Med. 18. 764.
Chemotherapeutic and antibiotic drugs in the management of infections in the urinary tract.

KASS, E.H. (1956)

Tr. A. Am. Physicians. 69. 56.
Asymptomatic infections of the urinary tract.

KATUZEWSKI, S. (1965)

Med. Dosw. Mikrobiol. 17/4. 283.
Somatic antigens of Klebsiella strains K63-K73.

KAUFFMAN, F. (1949)

Acta Path. Microbiol. Scand. 26. 381.
On the serology of the Klebsiella group.

KAUFFMAN, F. (1956a)

Acta Path. Microbiol. Scand. 39. 85.

On biochemical investigations of Enterobacteriaceae.

KAUFFMAN, F. (1956b)

Zbl. Bakt. 165. 344.

Zur gruppen und Typen einteilung der Entero-bakteriaceae.

KINDALL, L. (1941)

J. Urol. 46. 555.

Perirenal abscess with gas formation and metastatic pulmonary abscesses due to Friedlanders bacillus.

KINNEY, T.D., GINSBERG, H.S. (1943)

New Engl. J. Med. 228. 145.

Pyogenic liver abscess due to Klebsiella pneumoniae (Friedländer bacillus).

KIRBY, W.M.M., COPRON, D.O., TANNER, D.C. (1956)

J.A.M.A. 162. 1.

Urinary tract infection caused by antibiotic resistance coliform bacilli.

KISLAK, J.W., EICKHOFF, T.C., FINLAND, M. (1964)

New Engl. J. Med. 271. 834.

Hospital acquired infections and the antibiotic usage in the Boston City Hospital.

KLEINEBERGER-NOBEL, E. (1948)

J. Hyg. 46. 345.

Capsules and mucoid envelopes of bacteria.

KNOLL, K.H. (1953)

Zbl. Bakt. I. Abt. Orig. 159/6-7. 394.

The substance of the capsule of Klebsiella pneumoniae. I. Metabolic and serological abilities of Friedlanders bacteria.

KNOLL, K.H. (1954)

Zbl. Bakt. I. Abt. Orig. 160/6. 444.

The capsular substance of Klebsiella pneumoniae. II. Capsular substance and virulence.

KNOLL. K.H. (1958)

Z. Immun-Fprsch. 116/1-2. 2.

The comparative pyrogenic effect of polysaccharides from virulent and avirulent Klebsiella strains.

KNORR. G., PETZELT. K. (1953)

Z. Hyg. Infektkr. 138/2. 138.

Bacterium lactis aerogenes as the cause of sepsis with meningo-encephalitis.

KOCH. M.L. (1956)

Antibiot. Med. 2/2. 113.

Bacteremia due to bacterial species of the genera Aerobacter, Escherichia, Paracolobactrum, Proteus and Pseudomonas.

KOCH, M.L., ROSE, H.D. (1966)

Amer. J. Clin. Path. 46/5. 589.

Resistance of the Klebsiella-Aerobacter-Serratia division to cephalothin and ampicillin. Importance of identification and nomenclature.

KOLMER, J.A. (1934).

Ann. Int. Med. 8. 612.

Septicaemia.

KOPELOFF, L.M., KOPELOFF, N. (1943)

Fed. Proc. (Am. Soc. Exptl. Biol.) 2. No. 1. 99.

The production of brain antibodies in the monkey.

KOSSOWSKI, S., DURLAKOWA, I., AGOPSOWICZ, G., MARESZ-BABCZYSZYN, J., KUSTRZYCKA, H., PRZONDO-HESSEK, H., CYRULEWSKA, J., LUSAR, Z. (1964)

Arch. Immunol. Ther. Exp. 12/4. 483.

Clinical, bacteriological and serological studies on chronic atrophic fetid nasopharyngitis.

LACY, G.R., MURDOCH, A.C. (1922)

J. Inf. Dis. 31. 64.

Encapsulated non-gas forming bacilli.

LAMAR, R.V., MELTZER, S.J. (1912)

J. Expt. Med. 15. 133.

Experimental pneumonia by intrabronchial insufflation.

LANDSTEINER, K., CHASE, M.W. (1942)

Proc. Soc. Exp. Biol. and Med. 49. 688.

Experiments on transfer of cutaneous sensitivity to simple compounds.

LATTIMER, J.K., SENECA, M., ZINSSER, M. (1959)

J.A.M.A. 170. 938.

Increasing seriousness of resistant urinary infections with Aerobacter aerogenes.

LEMKE, C.E., GATES, L.E. (1951)

J. Lab. Clin. Med. 38/6. 889.

Distribution and antibiotic sensitivity in 55 strains of Klebsiella pneumoniae.

LENHART, Z. (1907)

Munch. Med. Woch. 54. 761.

Uber die akute und chronischen "Nierenbeckenentzündung".

LEVINE, M. (1921)

Bull. 62 Iowa State College of Agriculture and Mechanic Arts.

Bacteria fermenting lactose and their significance in water analysis.

LEVINE, S.Z. (1950)

Am. J. Dis. Child. 79. 377.

Prevention and control of infection in nurseries for premature infants.

LEVY-BRUHL, M., VIALA, M. (1935)

J. d'Urol. 39. 193.

Sur les infections genito-urinaires a pneumo-bacille de Friedlander.

LIEBERMAN, R., DOUGLAS, J.O.A., HUMPHREY, W. (1959)

Science. 129. 775.

Ascites induced in mice by Staphylococcus.

LIEBERMAN, R., DOUGLAS, J.O.A., MANTEL, N. (1960)

J. Immunol. 84. 514.

Production in mice of ascitic fluid containing antibodies induced by Staphylococcous or salmonella-adjuvent mixtures.

LIMSON, B.M., ROMANSKY, M.J., SHEA, J.G. (1956)

Ann. Int. Med. 44. 1070.

An evaluation of 22 patients with acute and chronic pulmonary infection with Friedlanders bacillus.

LIPMAN, M.O., COSS, J.A., BOOTS, R.M. (1946)

J. Bact. 51. 594.

Changes in the bacterial flora of the throat and intestinal tract during prolonged oral administration of penicillin.

LIPTON, M.M., FREUND, J. (1950)

J. Immunol. 64. 297.

The formation of complement fixing and neutralising antibodies after infection of inactivated rabies virus with adjuvents.

LOWBURY, E.J.L., FOX, Jean. (1953)

J. Hyg. (Lond.). 51. 203.

The influence of atmospheric drying on the survival of wound flora.

LOWE, J.S. (1964)

Biochem. Pharmacol. 13/4. 633.

Serum protein changes in rats with arthritis induced by mycobacterial adjuvent.

LUDERITZ, O., STAUB, O.M., WESTPHAL, O. (1966)

Bact. Rev. 30. 192.

Immunochemistry of O and R antigens of Salmonella and related enterobacteriae.

LUETSCHER, J.A. (1911)

Bull. John Hopkins Hosp. 22. 361.

Infection of the urinary tract by the bacillus lactis aerogenes with a consideration of the mode of entrance of bacteria into the bladder.

MacCONKEY, A. (1905)

J. Hyg. 5. 333.

Lactose fermenting bacteria in faeces.

MACKEY, L. (1927)

B.M.J. 1. 1004.

Nasal infection in children. Analysis of 85 cases treated by autogenous vaccine.

MacPHERSON. I.A.. WILKINSON. J.F.. SWANN. R.H.A. (1953)

J. Exp. Path. 34/6. 603.

The effect of K. aerogenes and K. cloacae polysaccharides on haemagglutination by, and multiplication of the influenza group of viruses.

MAIZTEGUI. J.I.. BIEGELEISEN. J.Z.. CHERRY. W.B.. KASS.E.H. (1965). New Eng. J. Med. 272. 222.

Bacteremia due to gram negative rods.

MAKITALO. R.. WIDHOLM. O. (1962)

Annales chirurgiae et gynaecologiae Fenniae. 51. 368.

Studies in enteric gram negative microorganisms isolated from nasopharynx of hospital personnel with specific reference to their occurrence in different wards and in different occupation groups.

MALIS. S. (1930)

Arch. otolaryng. 12. 162.

Pneumobacillus (Friedlanders) meningitis secondary to otitis media.

MARCUS. S.. DONALDSON. D.M.. ESPLIN. D.W. (1955)

J. Immunol. 74/6. 494.

Role of host defences in systemic Klebsiella pneumoniae infection : effect of antibiotics in normal and irradiated mice.

MARESZ-RABCZYSZYN, J. (1962)

Arch. Immun. Ther. Exp. 10/3. 589.

New antigenic types of Klebsiella bacilli.

MEYER, K.A., AMTMAN, L. (1939)

J.A.M.A. 113. 1641.

Treatment of Friedlanders septicaemia by Sulphapyrizine with recovery.

MEYER, K., HINMAN, F. (1920)

J. Inf. Dis. 27. 72.

A hemophilic anaerogenic paracolon bacillus, found in a case of infected bilateral hydronephrosis.

MILLER, B.W., ORRIS, H.W., TAUS, H.H. (1947)

J. Paed. 31. 521.

Friedlanders pneumonia in infancy.

MILLS, . (1892)

J. de Med. de Bruxelles. 94. 501.

Meningite a pneumocoques.

MILNER, P.F. (1963)

J. Clin. Path. 16. 39.

The differentiation of enterobacteriaceae infecting the urinary tract.

MIMICA, A., FRANCOVIC, A. (1951)

Acta Med. Jugoslav. 5/1.2. 82.

The aetiology of ozaena. III. The encapsulated bacteria of ozaena.

MOLLARI, M., RANDALL, W.A., REEDY, R. (1939)

J. Trop. Med. 42. 34.

The incidence of B. aerogenes and intermediates in the human faeces.

MØLLER, V. (1954)

Acta Path. Microbiol. Scand. 34. 115.

Diagnostic use of the KCN test within the entero-bacteriacae.

MØLLER, V. (1955)

Acta Path. Microbiol. Scand. 36. 158.

Simplified tests for some amino acid decarboxylases.

MONTT-SAAVEDRO, R. (1896)

Centrbl. Bakt. Abt. I. Orig. 20. 171.

Zwei Fälle von Cystitis mit Befund von Diplo-bacillus Friedlander.

MORI, G.E. (1943)

An. d'Hosp. Ninos e Inst. Puericuff de Rosario. 187. 196. Meningitis a neumobacilo de Friedlander en un nino de veintseis meses, curada.

MORRIS. G.L.. YATES. J.L. (1956)

Dis. Chest. 30/3. 298.

Respiratory infections due to Klebsiella pneumoniae.

MORRIS. M.C.. JULIANELLE. L.A. (1934)

J. Infect. Dis. 55. 150.

Biologic classification of bacillus of rhinoscleroma.

MUNOZ. J. (1957)

Proc. Soc. Exper. Biol. and Med. 95. 757.

Production in mice of large volumes of ascites fluid containing antibodies.

MUSCHENHEIM. C. (1940)

Internat. Clin. 3. 216.

Chronic Friedlander infections of the lung.

McCABE. W.R.. JACKSON. G.G. (1962a)

Arch. Int. Med. 110. 847.

Gram negative bacteremia (1) Etiology and Ecology.

McCABE. W.R.. JACKSON. G.G. (1962b)

Arch Int. Med. 110. 856.

Gram negative bacteremia. Clinical, laboratory and therapeutic observations.

McCURDY, R.S., NETER, E. (1952)

Paediatrics. 9. 572.

Effects of penicillin and broad spectrum antibiotics on emergence of gram-negative bacillary flora in upper respiratory tract of infants.

NAGESHA, C.N., LAXMAN PAI, M. (1966)

J. Ind. Med. Ass. 46/8. 418.

Klebsiella infection of the lungs.

NEAL, J.B. (1931)

Am. J. Public Health. 21. 147.

Experience of the meningitis division of the New York Department of Health.

NEUBER, E. (1934)

Arch. Derm. Syph. Wein. 170. 154.

Uber spezifische scutzstoffe des mit Kapselbakterien infizierten organisimes.

NICOLAIER, A. (1894)

Centralbl. Bakt. 16. 601.

Uber einen neuen pathogen Kapselbacillus bei eitriger nephritis.

NIMMICH, W. (1968)

Z. med. Mikrobiol. u. Immunol. 154/2. 117.

Zur Isolierung und Quantitativen Bausteinanalyse der K-antigene von Klebsiellen.

NIMMICH, W., MUNTER, W. (1967)

Experientia (Basel) 23/11. 907.

Immunochemistry of the Klebsiella antigens.

NORMAN, J.K., BINFORD, C.H. (1945)

Am. J. Clin. Path. 15. 534.

Liver abscess due to Friedlanders bacillus (K. pneumoniae) complicating adenocarcinoma of the ampulla of Vater.

OBRINSKY, W., DORMONT, R.E., FOWLER, R.E.L., RUHSTALLER, F. (1950). Am. J. Dis. Child. 80. 621.

Friedlander aerogenes infections in infancy.

OLCOTT, C.T. (1933)

Arch. Path. 16. 471.

Pneumonia due to Friedlanders bacillus.

OREN, R., OLITZKI, A.L. (1953)

Acta Med. Orient. (Tel Aviv) 12/4. 107.

Experimental infections produced by the intra abdominal and respiratory administration of Klebsiellae.

ØRSKOV, Ida. (1952)

Acta Path. Microbiol. Scand. Suppl. XCIII. 259.

Nosocomial infections with Klebsiella in lesions of the urinary tract.

ØRSKOV, Ida. (1954a)

Acta Path. Microbiol. Scand. 34. 145.

O antigens in the Klebsiella group.

ØRSKOV. Ida. (1954b)

Danish Med. Bull. 1/4. 110.

Nosocomial infections with Klebsiella in lesions of the urinary tract.

ØRSKOV. Ida. (1954c)

Acta Path. Microbiol. Scand. 35/2. 194.

Nosocomial infections with Klebsiella in lesions of the urinary tract II.

ØRSKOV. Ida. (1955a)

Acta Path. Microbiol. Scand. 36. 449.

Serological investigations in the Klebsiella group. I. New capsule types.

ØRSKOV. Ida. (1955b)

Acta Path. Microbiol. Scand. 36. 454.

Serological investigations in the Klebsiella group. 2. Occurrence in sputa.

ØRSKOV. Ida. (1955c)

Acta Path. Microbiol. Scand. 36/5. 461.

Serological investigations in the Klebsiella group. 3. Occurrence of Klebsiella strains in the faeces of normal infants.

ØRSKOV. Ida. (1956)

Acta Path. Microbiol. Scand. 38/5. 375.

Immunological paralysis produced in rabbits by a heavily capsulated Klebsiella strain.

ØRSKOV, Ida. (1957)

Acta Path. Microbiol. Scand. 40/2. 155.

Biochemical types in the Klebsiella group.

OSTERMAN, E., RETTGER, L. (1941)

J. Bact. 42. 721.

A comparative study of organisms of the Friedlander and Coli aerogenes groups. II. Pathogenicity, biochemical reactions and serological relationships.

OWEN, S.E., FINCH, E.P. (1949)

J. urol. 61. 258.

Bacteriological study of urine from paraplegic patients.

PERLMAN, E., BULLOWA, J.A.M. (1941)

Arch. Int. Med. 67. 907.

Primary bacillus Friedlander Klebsiella pneumonia.

PETROFF, B.P., LUCAS, F.V. (1946)

Ann. Surg. 123. 808.

Streptomycin in urinary infections.

PFEIFFER, D.B. (1937)

Ann. Surg. 106. 1115.

Klebsiella pneumoniae bacteraemia due to prostatic abscess.

PORGES, O. (1905)

Wien Klin. Wschr. 18. 691.

Agglutinability of capsulate bacteria.

PRASEK, E.V., PRICA, M. (1933)

Zblt. Bakt. 128. 381.

Uber die Kohlenhydratartige substans der Kapsil dee B. rhinoscleromatis, B. ozaenae abel und B. Friedlander.

PRICA, M. (1930)

Zblt. Bakt. 115. 334.

Studien über Kapselbakterien,

PUBLIC HEALTH LABORATORY SERVICE (1960)

Lancet, 2. 659.

Report: Incidence of surgical wound infection in England and Wales.

QUICK, C.A., BROGAN, T.D. (1968)

Lancet. 7553. 1163.

Gram negative rods and surgical wound infection.

RAKE, G.A. (1948)

J. Bact. 55. 865.

Further note on the antigen relationships of Donovania granulomatis.

RAMON, G. (1957)

Rev. Path. Gen/ Physiol. Clin. 57/684. 2.

Immunity - stimulating adjuvants, bases.

Experimental study. Applications.

RAMMELKAMP, J.C., MAJOR, J.W. (1943)
Arch. Int. Med. 72. 319.
Friedlanders bacillus septicaemia and meningitis. Report of a case and autopsy, with analysis of 29 cases collected from the literature.

RASKAI, D. (1905)
Monatsberichte fur Urol. 10. 1.
Untersuchungen über die Aetiologie der Cystitis.

READ, B.E., KELLER, R., CABELLI, V.J. (1957)
J. Bact. 73/6. 765.
The decapsulation phenomenon of Klebsiella pneumonia.

REICHERT, F. (1921)
Virchows. Arch. f. Path. Anat. 231. 453.
Uber zwei in aetiologischer Beziehung beimerkenswerte obduktionsbefunde.

REIMANN, H.A., PRICE, A.H., ELIAS, W.F. (1945)
Arch. Int. Med. 76. 269.
Streptomycin for certain systemic infections and its effect on urinary and faecal flora.

REPORT of the Enterobacteriacae sub-committee on the groups Salmonella, Shigella, Arizona, Bethesda-Ballerup, Escherichia, Klebsiella and Proteus. (1954)
Int. Bull. Bact. Nomen. 4. 37.

ROCHER, H.L., DUBAUKY, J.J. (1941)

Bull. Soc. Paediat. Paris. 38. 365.

Hépatite suppurée a pneumobacilles et entereccoques chez une fillette.

ROGERS, K.B., TAYLOR, J. (1961)

Bull. W.H.O. 24. 59.

Laberatory diagnosis of gastroenteritis due to Escherichia coli.

ROGERS, D.E. (1959)

New Eng. J. Med. 261. 677.

The changing pattern of life-threatening microbial disease.

ROGERS, D.E., KOENIG, M.G., HOLMES, K.K. (1965)

Southern Med. J. 58. 1391.

The problem of gram negative bacteremia and its management.

ROJAS-ESPINOSA, O., ESTRADA-PARRA, S. (1968a)

Rev. Lat. Amer. Microbiol. Parasit. 10/1. 7.

Immunochemistry of the capsular polysaccharide of Klebsiella rhinoscleromatis.

ROJAS-ESPINOSA, O., ESTRADA-PARRA, S. (1968b)

Rev. Lat. Amer. Microbiol. Parasit. 10/2. 89.

Immunochemistry pf the capsular polysaccharide of a gram negative bacillus isolated from four cases of rhinoscleroma.

ROSTOSKI, O. (1898)

Deut. Med. Woch. 24. 235.

Uber den baktericiden Einfluss der Aciditat des Harns auf die Cystitiserreger.

ROTHBERG, R., TALMAGE, D.W. (1961)

J. Immunol. 86. 302.

Circulating antibody and anaphylaxis in mice.

ROTHSCHILD, K. (1931)

J.A.M.A. 97. 1956.

Meningitis caused by Friedlanders bacillus.

SAKAZAKI, R., NAMOKA, S. (1958)

Jap. J. Exp. Med. 28. 85.

Serological typing of the Klebsiella group.

SALE, L., WOOD, W.B. (1947)

J. Exptl. Med. 86. 239.

Studies on the mechanism of recovery of pneumonia due to Friedlanders bacillus.

SALK, J.E. LAURENT, A.M., BAILEY, M.L. (1951)

Am. J. Public Health. 41. 669.

Direction of research on vaccination against influenza. New studies with immunologic adjuvants.

SANDISON, J.W., JAMES, O.B., GRANT, L.S., HULTQUIST, E. (1967)

West Ind. Med. J. XVI. 129.

A bacterial survey of selected areas of a hospital.

SARTORELLI, A.C., BOOTH, B.A., (1961)

Fed. Proc. 20. 156.

Studies on mechanisms of therapeutic synergy by combinations of azaserine and purine analogues.

SARTORELLI, A.C., UPCHURCH, H.F., BIEBER, A.L., BOOTH, B.A. (1964). Cancer Res. 24. 1202.

Some metabolic effects exerted by azaserine and purine analogs in vivo.

SARTORELLI, A.C., FISCHER, D.S., DOWNS, W.G. (1966)

J. Immunol. 96. 676.

The use of sarcoma 180/TG to prepare hyperimmune ascitic fluid in the mouse.

SCHEYER, H.E. (1926)

Zentralbl. f. Gynak. 50. 3186.

Der Bacillus pneumoniae Friedlander als Erriger gynakologischer Erkrankungen.

SCHEYER, H.E. (1933)

Zentralbl. f. Gynak. 57. 578.

Bacillus pneumoniae Friedlander als Erreger einer genital bedingten peritonitis.

SCHOENBACH, E.B. (1949)

J. Mt. Sinai Hosp. 16. 71.

The newer antibiotics polymyxin, chloramycetin and auromycin.

SHARMA. B.D., SINGH. R.P. (1966)
The Indian Practitioner. 19. 713.
Solitary pyogenic liver abscess.

SHAW. C., CLARKE. P.H. (1955)
J. gen. Microbiol. 13. 155.
Biochemical classification of Proteus and Providence cultures.

SHERIDAN. E.P. (1945)
New England J. Med. 233. 528.
Liver abscess, pneumonia and empyema due to Friedlanders bacillus and diabetes mellitus.

SIMMONS. J.S. (1926). J. Infect. Dis. 39. 209.
A culture medium for differentiating organisms of typhoid-colon aerogenes group, and for isolation of certain fungi.

SINCLAIR. F. (1941)
B.M.J. 1. 196.
Friedlander pneumonia successfully treated with sulphapyridine.

SISSON. W.R., THOMPSON. C.B. (1915)
Am. J. Med. Sc. 150. 713.
Friedlanders bacillus pneumonia with report of cases.

SMALL, J.C. and JULIANELLE, L.A. (1923)

J. Inf. Dis. 32. 456.

Biologic and serologic studies of Bacillus Mucosus group.

SMITH, D.T. (1952)

Ann. Int. Med. 37. 1135.

Ecology by administration of antibiotics with development of new clinical syndromes.

SMITH, M.R., WOOD, W.B. (1947)

J. Exp. Med. 86. 257.

Studies on the mechanism of recovery in pneumonia due to Friedlanders bacillus.

SOLOMAN, M.D. (1940)

J.A.M.A. 115. 1527.

Chronic Friedlander infection of the lungs.

SOLOMON, S. (1937)

J.A.M.A. 108. 937.

Primary Friedlander pneumonia. Report of 32 cases.

SOLOMON, S. (1947)

New Eng. J. Med. 237. 149.

Wound infections with Friedlanders bacillus followed by meningitis.

SOMMERVILLE, R.G. (1967)

Archiv. fur die Gesamte. virusforchung. 20. 445.

The production of fluerescent antibody reagents for virus diagnosis in the albino mouse.

SPINK, W.W. (1953)

J.A.M.A. 152. 585.

Clinical problems relating to management of infections with antibiotics.

STAVITSKY, A.B., SCHOENBERG, M.D., MOORE, R.D., FREEMAN, M.J. (1964). Symposium on Molecular and Cellular basis of antibody formation. Prague 1 - 5th June, 1964. Page 577. Pub. Academic Press, N.Y.

STEINER, B., FUTNOKY, G. (1956)

Arch. Dis. Child. 31. 96,

Klebsiella pneumoniae (Friedlanders bacillus) infections in infancy.

STEINHAUSER, B.W., EICKHOFF, T.C., KISLAK, J.W., FINLAND, M. (1966). Ann. Int. Med. 65. 1180.

The Klebsiella-Enterobacter-Serratia division. Clinical and Epidemiological characters.

STERNBERG, Y.M. (1885)

Am. J. M. Sc. 90. 106 and 435.

The pneumonia coccus of Friedlander.

SWARTZ, E.P., RHODE, P.A. (1946)

Am. J. Clin. Path. 16. 88.

Klebsiella infections in an army hospital.

SWEDIN, B., LILJESTRAND, A. (1945)

Acta Med. Scand. CXX. 73.

Splenic and hepatic abscesses due to B. friedlander in a patient with agranylocytosis.

TALAMAN, E. (1883)

Bull. Soc. Anat. de Paris. 7. 475.

Cpccus de la pneumonie.

TALERMAN, A., FINNIE, Evelyn, WONTUMI, J.A. (1968)

J. Med. Micro. 1. 164.

Multiple liver abscesses caused by Klebsiella aerogenes.

TARTAKOFF, S. (1946)

New Eng. Med. J. 235. 681.

Friedlander meningitis treated with streptomycin.

TENNENBAUM, J., RAVID, J.M. (1936)

Am. J. Med. Sc. 192. 494.

A fatal case of Bacillus Friedlander abscess of the prostate.

THALER, M.M. (1962)

Paediatrica. 30/2. 206.

Klebsiella aerobacter pneumonia in infants. A review of the literature and a report of a case.

TIKASINGH, E.S., SZENTE, L., DEVES, V.G. (1966)

Am. J. Trop. Med. Hyg. 15. 219.

The use of adjuvant and Sarcoma 180 cells in production of mouse hyperimmune ascitis fluids to arboviruses.

TOENIESSEN, E. (1914)

Zbt. f. Bakt. I. Orig. 73. 329.

Uber die Agglutination der Kapselbacillen.

TOENIESSEN, E. (1921)

Zbl. Bakt. 85. 225.

Untersuchungen uber die Kapsel der pathogenen Bakterien.

TOMISAWA, M. (1962a)

J. Bact. 17/3. 229.

Studies on the classification of the Klebsiella I. (Japanese)

TOMISAWA, M. (1962b)

J. Bact. 17/4. 255.

Studies on the classification of the Klebsiella II.

TRUMPP, J. (1896)

Munch. Med. Woch. 43. 1008.

Uber colicystitis in Kindesalter.

UCHITEL, I.Y., KHASMAN, E.L. (1965)

J. Immunol. 94/4. 492.

On the mechanism of adjuvant action of non-specific stimulators of antibody formation.

UNTERMAN, D., MILBERG, M.B., KRANIS, M. (1957)

New Eng. J. Med. 257/16. 773.

Evaluation of blood cultures after sigmoidoscopy.

U.S. DEPARTMENT OF HEALTH EDUCATION AND WELFARE (1965)

Morbidity and Mortality. 14. No. 31. 265.

Institutional outbreak of pneumonia.

VON FRISCH, A. (1882)

Wein Med. Wschr. 32. 969.

WARD, R., RADER, D., LIPTON, M.M., FREUND, J. (1950)

Proc. Soc. Exp. Biol. Med. 74. 536.

Formation of neutralising antibodies in monkeys injected with poliomyelitis virus and adjuvants.

WARNER, P.T.J.C.P. (1948)

B.M.J. 1. 146.

Chemotherapy of infected urines in paraplegia.

WEICHSELBAUM, A. (1886)

Med. Jahrb. 1. 483.

Uber die aetiologie der acuten lungen und Rippenfellentzündungen.

WEICHSELBAUM, A. (1888)

Monatschr. f. Ohrenh. 22. 200 and 229.

Uber eine von einer otitis media suppurativa ausgehende und durch den Bacillus pneumoniae (Friedlander) bedingte Allgemeininfektion.

WEIL. M.M., SPINK. W.W. (1958)

Arch. Int. Med. 101. 184.

The shock syndrome associated with bacteraemia due to gram negative bacilli.

WEIL. M.M., SHUBIN. H., BIDDLE. Marjorie. (1964)

Ann. Int. Med. 60. 384.

Shock caused by gram negative organisms.

WEINSTEIN. L. (1947)

Am. J. Med. Sc. 214. 56.

The spontaneous occurrence of new bacterial infections during the course of treatment with streptomycin or penicillin.

WEINSTEIN. L., GOLDFIELD. M., CHANG. T.W. (1954)

New Eng. Med. J. 251. 247.

Infections occurring during chemotherapy.

WEISS. G.M., EISENBERG. G.M., ALEXANDER. J.D., FLIPPIN. H.G. (1954). Am. J. Med. Sc. 228. 148.

Klebsiella pulmonary disease.

WEISS. W., EISENBERG. G.M., SPIVACK. A., NADEL. J., KAYSER. H.L., SATHAVARA. S., FLIPPIN. H.F. (1956)

Ann. Int. Med. 45/6. 1010.

Klebsiella in respiratory disease.

WESTERMARK, N., (1926)

Acta Radiol. 7. 626.

Ein tuberkulose vortäuschender Fall von Friedländers pneumenia mit lange sich Lönsiehendem verlauf.

WHITE, R.G. (1967)

"Modern Trends in Immunology" v. 2, page 41. Ed. Cruickshank, R. and Weir, D.M. Pub. Butterworths, London.

WHITE, R.G. (1968)

Proc. R.S.M. 61. 1.

Antigens and adjuvants.

WILHELM, S.F., ORKIN, L.A. (1949)

J. Urol. 61. 131.

The increased pathogenicity of B. lactis aerogenes in urinary tract infections.

WILHELM, S.F., SCHLOSS, W.A., ORKIN, L.A., SELIGMAN, E., WASSERMANN, M. (1949)

J.A.M.A. 141. 837.

A. aerogenes infection in the urinary tract.

WILLIAMS, R.E.O., BLOWERS, R., GARROD, L.P., SHOOTER, R.A. (1966)

"Hospital Infection" 2nd Ed. Pub. Lloyd Luke.

WILSON, G.S., MILES, A.A. (1964)

Topley and Wilson's "Principles of Bacteriology and Immunity" 5th Ed., v. I. pp 828-838. Pub. E. Arnold, Ltd.

WOOD, W.B., SMITH, M.R. (1949)

J. Exp. Med. 90. 85.

The inhibition of surface phagocytosis by the capsular slime layer of Pneumococcus type III.

WOOD, W.B., SMITH, M.R. (1950)

J. Exp. Med. 92. 85.

Host parasite relationships in experimental pneumonia due to Pneumococcus type III.

WORFEL, M.T., FERGUSON, W.W. (1951)

Am. J. Clin. Path. 21. 1097.

A new Klebsiella type (capsule type 15) isolated from faeces and urine.

WU, W.G., TRIGE, K.L. (1967)

J. Bact. 94/4. 896.

Influence of cellular factors on Immune unresponsiveness induced by Klebsiella pneumoniae capsular antigen.

WYLIE, R.M., KIRSCHNER, P.A. (1950)

Am. Rev. Tuberc. 61. 465.

Friedlanders pneumonia.

YOUMANS, A.S., YOUMANS, G.P. (1967)

J. Bact. 94/4. 836.

Preparation and effect of different adjuvants on the immunogenic activity of mycobacterial ribosomal fraction.

YOW, E.M. (1952)

J.A.M.A. 149. 1184.

Development of *Proteus* and *Pseudomonas* infections during antibiotic therapy.

YOW, E.M. (1955)

Post Grad. Med. 17. 413.

Clinical significance of rising incidence of infections due to gram negative bacilli.

ZANDER. (1919)

Deutsche Med. Wchnschr. 45. 1180.

Table 1 - Appendix

BIOTYPES AND SEROTYPES OF KLEBSIELLA ISOLATED FROM THROAT SWABS

STRAIN NO.	SOURCE	SEROTYPE	BIOTYPE
1	Infected throat	1	Typical K. aerogenes on 14 tests (dulcitol +)
2	Infected throat	1	Typical (dulcitol +)
3	Infected throat	1	Typical (dulcitol +)
4	Infected throat	2	MR + Indole +
5	Infected throat	2	Typical (dulcitol -)
6	Infected throat	2	Malonate negative
7	Infected throat	2	Typical (dulcitol -)
8	Infected throat	2	Typical (dulcitol -)
9	Infected throat	3	Typical (dulcitol -)
10	Infected throat	3	Indole +
11	Normal throat	3	Typical (dulcitol +)
12	Normal throat	3	Typical (dulcitol +)
13	Normal throat	3	Typical (dulcitol +)
14	Normal throat	4	Typical (dulcitol +)
15	Normal throat	4	Typical (dulcitol -)
16	Infected throat	8	Typical (dulcitol -)
17	Infected throat	9	Typical (dulcitol -)
18	Normal throat	9	MR +, V-P -, gluconate -
19	Infected throat	11	Typical (dulcitol +)
20	Infected throat	11	Typical (dulcitol -)
21	Normal throat	11	Typical (dulcitol +)

Cont'd...../

Table 1 (Cont'd.) - Appendix

BIOTYPES AND SEROTYPES OF KLEBSIELLA ISOLATED FROM THROAT SWABS

STRAIN NO.	SOURCE	SEROTYPE	BIOTYPE
22	Normal throat	11	Typical (dulcitol +)
23	Normal throat	12	Malonate negative
24	Infected throat	13	Typical (dulcitol -)
25	Normal throat	15	Typical (dulcitol +)
26	Infected throat	15	Acid and gas not produced from starch or glycerol
27	Infected throat	16	Typical (dulcitol +)
28	Normal throat	23	Typical (dulcitol -)
29	Infected throat	24	Typical (dulcitol -)
30	Infected throat	29	Typical (dulcitol -)
31	Infected throat	29	Typical (dulcitol -)
32	Normal throat	29	Acid and gas not produced from adonitol and glycerol
33	Normal throat	29	Typical (dulcitol -)
34	Normal throat	35	Typical (dulcitol -)
35	Normal throat	35	Acid and gas not produced from starch and glycerol
36	Normal throat	35	Acid and gas not produced from adonitol, starch and glycerol
37	Infected throat	35	Acid and gas not produced from starch and glycerol
38	Infected throat	35	Malonate negative
39	Infected throat	35	Typical (dulcitol -)
40	Infected throat	35	Typical (dulcitol -)
41	Normal throat	Not 1-40	Typical (dulcitol -)
42	Infected throat	Not 1-40	MR +, Indole +

Cont'd...../

Table 1 (Cont'd) - Appendix

BIOTYPES AND SEROTYPES OF KLEBSIELLA ISOLATED FROM THROAT SWABS

STRAIN NO.	SOURCE	SEROTYPE	BIOTYPE
43	Infected throat	Not 1-40	Typical (dulcitol -)
44	Infected throat	Not 1-40	Typical (dulcitol -)
45	Infected throat	Not 1-40	Lysine decarboxylase not produced
46	Normal throat	Acapsulate	Urea negative

Table 2 - Appendix

ANALYSIS OF NUMBER OF DIFFERENT STRAINS (BIOTYPES AND SERO-TYPES) IN 46 ISOLATES OF KLEBSIELLA FROM THROAT SWABS

(KA indicates typical Klebsiella Biochemistry)

SEROTYPE	NUMBER OF ISOLATES	ISOLATE REFERENCE NUMBERS	BIOCHEMISTRY	STRAIN DESIG-NATED
1	3	1,2,3	KA (dulcitol +)	T1
2	5	4	MR + Indole +	T2
		6	Malonate -	T3
		5,7,8	KA (dulcitol -)	T4
3	5	9,12	KA (dulcitol -)	T5
		10	Indole +	T6
		11,13	KA (dulcitol +)	T7
4	2	14	KA (dulcitol +)	T8
		15	KA (dulcitol -)	T9
8	1	16	KA (dulcitol -)	T10
9	2	17	KA (dulcitol -)	T11
		18	MR+,VP-,gluconate -	T12
11	4	19,21,22	KA (dulcitol +)	T13
		20	KA (dulcitol -)	T14
12	1	23	Malonate neg.	T15
13	1	24	KA (dulcitol -)	T16
15	2	25	KA (dulcitol +)	T17
		26	Acid and gas not produced from starch and glycerol	T18
16	1	27	KA (dulcitol +)	T19
23	1	28	KA (dulcitol -)	T20
24	1	29	KA (dulcitol -)	T21

Cont'd...../

Table 2 (Cont'd.) - Appendix

ANALYSIS OF NUMBER OF DIFFERENT STRAINS (BIOTYPES AND SERO-TYPES) IN 46 ISOLATES OF KLEBSIELLA FROM THROAT SWABS

(KA indicates typical Klebsiella Biochemistry)

SEROTYPE	NUMBER OF ISOLATES	STRAIN REFERENCE NUMBERS	BIOCHEMISTRY	STRAIN DESIG-NATED
29	4	30,31,33	KA (dulcitol -)	T22
		32	Acid and gas not produced from starch and glycerol	T23
35	7	34,39,40	KA (dulcitol -)	T24
		35,37	Acid and gas not produced from starch and glycerol.	T25
		36	Acid and gas not produced from starch, adonitol & glycerol.	T26
		38	Malonate negative.	T27
Not 1-40	5	41,42,43, 44,45	At least three different biotypes, and possibly more.	Not sero-logically classi-fied.
Acapsu-late	1	46	Urea negative	

Table 3 - Appendix

SEROTYPES AND BIOTYPES OF KLEBSIELLA ISOLATED FROM TRACHEOSTOMES

ISOLATE NUMBER	SOURCE	SEROTYPE	BIOTYPE	STRAIN DESIGNATED
47	Infected tracheotomy	24	KA (dulcitol -)	T21
48	Infected tracheotomy	21	KA (dulcitol +)	T29
49	Infected tracheotomy	3	KA (dulcitol -)	T5
50)	Normal tracheotomy	11	KA (dulcitol -)	T14
51)	Normal tracheotomy	1	KA (dulcitol -)	T30
52	Normal tracheotomy	9	KA (dulcitol -)	T11
53	Infected tracheotomy	8	KA (dulcitol -)	T10

Table 4 - Appendix

BIOTYPES AND SEROTYPES ISOLATED FROM "NORMAL" SPUTUM

Serotype	Number of Isolates	Isolate Ref. No.	Biotype	Strain Designated
1	1	54	KA dulcitol +	T1
2	1	55	MR +, VP -	S1
3	1	56	KA dulcitol +	T7
7	2	57	KA dulcitol +	S2
		58	Indole +	S3
9	3	59	KA dulcitol +	S4
		60 & 61	KA dulcitol -	T11
11	1	62	KA dulcitol +	T13
13	2	63	KA dulcitol +	S5
		64	KA dulcitol -	T16
16	1	65	MR +, VP -	S6
22	1	66	KA dulcitol +	S7
29	3	67	KA dulcitol +	S8
		68	MR +, VP - gluconate -	S9
		69	KA dulcitol -	T22
35	1	70	KA dulcitol -	T24
not 1-40	3	71, 72, 73	KA dulcitol - / KA dulcitol +	not classified

Table 5 - Appendix

CLINICAL DIAGNOSIS OF 20 CASES FROM WHICH COMMENSAL KLEBSIELLA WAS ISOLATED FROM SPUTUM

Series Number	Diagnosis	Sero-type	Hospital Acquired Infection
54	L.V. failure. Aortic and mitral incompetence.	1	No
55	Cardiomyopathy. Tricuspid incompetence.	2	Doubtful
56	Cor pulmonale. Emphysema.	3	No
57	Obstructive jaundice.	7	No
58	C.C.F. Diabetes mellitus.	7	Yes
59	Cor pulmonale. Bronchiectasis.	9	Yes
60	Carcinoma of lung.	9	No
61	Nephrotic syndrome Diabetes.	9	Yes
62	Asthma.	11	No
63	Infectious hepatitis.	13	No
64	Sequestration of lung. Post-thoracotomy sputum grew Klebsiella - no signs of infection.	13	Yes
65	Diabetes. D.V. thrombosis and cellulitis.	16	No
66	Asthma.	22	No
67	Pulmonary embolism. Pulmonary hypertension.	29	Yes
68	Carcinoma oesophagus. Post-operative Klebsiella isolation.	29	Yes

Continued...../

Table 5 - Appendix (Continued)

CLINICAL DIAGNOSIS OF 20 CASES FROM WHICH COMMENSAL KLEBSIELLA WAS ISOLATED FROM SPUTUM

Series Number	Diagnosis	Sero-type	Hospital Acquired Infection
69	Myeloid leukaemia.	29	Yes
70	Congestive cardiac failure.	35	Yes
71	Congestive cardiac failure.	not 1-40	No
72	Diabetes mellitus. Peripheral neuropathy.	not 1-40	No
73	Cervical spondylosis.	not 1-40	No

Table 6 - Appendix

BIOTYPES AND SEROTYPES OF 38 ISOLATES FROM SPUTUM IN ASSOCIATION WITH RESPIRATORY INFECTION

Sero-type	Total No. of Isolates	Reference Number	Biotype	Strain Designated
2	5	74	KA dulcitol +	S10
		75,76,77,78.	KA dulcitol -	T4
5	1	79	KA dulcitol +	S11
8	1	80	KA dulcitol -	T10
9	7	81	MR+, indole +	S12
		82,84,86.	KA dulcitol -	T11
		83	MR+, VP-	S13
		85,87.	KA dulcitol +	S4
10	1	88	KA dulcitol -	S14
11	2	89	KA dulcitol -	T14
		90	KA dulcitol +	T13
15	2	91	KA dulcitol -	S15
		92	KA dulcitol +	T17
16	2	93,94.	KA dulcitol +	T19
19	1	95	VP-, no ag. adonitol or glycerol.	S16
20	1	96	KA dulcitol +	S17
31	2	97	Gelatin +	S18
		98	No ag. adonitol or glycerol.	S19
35	1	99	No ag. adonitol or glycerol.	S20
not 1-40	12	102,103,104 106,108.	KA dulcitol -	Not classified. At least 4 different strains
		100,105,107 111.	KA dulcitol +	
		101	MR+, VP-	
		109,110	No ag. starch or glycerol.	

Table 7 - Appendix

WBC AND 8:00 AM TEMPERATURE IN CASES OF PULMONARY INFECTION

Diagnosis	Isolate Number	WBC	Temperature
Acute bronchitis	74	4.8	101°F
	75	-	98°F
	76	6.6	98°F
	81	7.6	98°F
	91	6.4	99°F
	93	7.2	100°F
	94	-	-
	95	5.3	99°F
	100	16.0	102°F
	101	19.6	100°F
	102	6.2	100°F
	103	3.5	99°F
	104	6.4	99°F
Bronchopneumonia	77	7.8	102°F
	79	3.2	98°F
	82	14.9	102°F
	89	10.1	98°F
	92	-	103°F
	96	-	103°F
	105	7.4	98°F
	106	14.5	102°F
	107	-	99°F
Lobar pneumonia	80	5.5	103°F
	83	19.5	100°F
	84	14.6	101°F
	85	9.9	98°F
	86	8.7	99°F

Continued...../

Table 7 - Appendix (Continued)

WBC AND 8:00 AM TEMPERATURE IN CASES OF PULMONARY INFECTION

Diagnosis	Isolate Number	WBC	Temperature
Lobar pneumonia (Continued)	87	15.7	102°F
	90	17.0	103°F
	98	11.5	103°F
	99	11.3	101°F
	108	19.7	101°F
	109	4.2	99°F
	110	12.0	102°F
	111	10.0	101°F
Lobar pneumonia and lung abscess	78	20.7	102°F
	88	7.6	98°F
	97	5.7	98°F

Table 8 - Appendix

BIOTYPES AND SEROTYPES FOUND IN 79 ISOLATES OF KLEBSIELLA AEROGENES FROM URINE

Sero-type	No. of Isolates	Strain Ref.No.	Biotype	Strain Designated	Hospital Acquired
2	2	112	KA dulcitol -	T4	Yes
		113	MR+ VP-	S1	Yes
3	1	114	KA dulcitol +	T7	Yes
7	3	115	KA dulcitol -	U1	No
		116	KA dulcitol -	U1	No
		117	MR+ VP- gluconate - malonate -	U2	Yes
8	1	118	KA dulcitol -	T10	No
9	3	119	KA dulcitol +	S4	No
		120	MR+ VP- indole +	S12	Yes
		121	MR+ VP-	S13	No
10	6	122	MR+ VP+ adonitol -	U3	No
		123	KA dulcitol +	U4	No
		124	KA dulcitol -	S14	No
		125	KA dulcitol -	S14	Yes
		126	KA dulcitol -	S14	No
		127	KA dulcitol +	U4	Unknown
14	2	128	KA dulcitol +	U5	Yes
		129	MR+ VP- gluconate -	U6	No
15	9	130	MR+ VP- gluconate - adonitol -	U7	No
		131	KA dulcitol +	T17	No
		132	KA dulcitol +	T17	No
		133	KA dulcitol -	S15	No
		134	MR+ VP- gluconate -	U8	Yes

Continued...../

Table 8 - Appendix (Continued)

BIOTYPES AND SEROTYPES FOUND IN 79 ISOLATES OF KLEBSIELLA AEROGENES FROM URINE

Sero-type	No. of Isolates	Strain Ref.No.	Biotype	Strain Designated	Hospital Acquired
15 (Cont.)		135	KA dulcitol -	S15	Yes
		136	(indole + (gelatin +	U9	No
		137	KA dulcitol +	T17	No
		138	(MR+ VP- (gluconate -	U10	Yes
16	1	139	malonate -	U11	No
17	2	140	KA dulcitol -	U12	No
		141	adonitol -	U13	No
19	3	142	malonate -	U14	No
		143	KA dulcitol -	U15	Yes
		144	lysine -	U16	No
20	1	145	KA dulcitol -	U17	No
23	2	146	KA dulcitol +	U18	No
		147	MR+ VP-	U19	No
24	1	148	KA dulcitol +	U20	Yes
26	3	149	KA dulcitol -	U21	No
		150	KA dulcitol +	U22	No
		151	KA dulcitol +	U23	Yes
29	2	152	KA dulcitol -	T22	Yes
		153	KA dulcitol +	S8	No
not 1-40	37	155,156) 159,160) 169,170) 172,173) 180,184) 187.)	KA dulcitol +	Not classified. At least 12 different biotypes,	No
		157,161) 165,168) 186.)	KA dulcitol +	unknown number of serotypes.	Yes

Continued...../

Table 8 - Appendix (Continued)

BIOTYPES AND SEROTYPES FOUND IN 79 ISOLATES OF KLEBSIELLA AEROGENES FROM URINE

Sero-type	No. of Isolates	Strain Ref.No.	Biotype	Strain Designated	Hospital Acquired
not 1-40 (cont.)	37	154,166 183,188 199.	KA dulcitol -	Not classified.	No
		158,175 179.	KA dulcitol -	At least 12 different biotypes, unknown number of serotypes.	Yes
		182	gluconate - malonate -		Yes
		176,185	MR+ VP-		No
		164	MR+ VP- lysine -		No
		190	MR+VP- malonate-		No
		162	adonitol - starch -		No
		167	adonitol -		Yes
		174	adonitol -		No
		178	indole +		Yes
		163	indole +		Yes
		171	starch - glycerol -		Unknown
		177	gluconate -		No
		181	urea -		No

Table 9 - Appendix

TEMPERATURE RANGE OF 30 FEBRILE PATIENTS WITH URINARY TRACT INFECTION

8:00 am oral temperature.	No. of cases	Isolates Number
99 - 99.9°F	1	130
100 - 100.9°F	15	114,115,117,128,150, 152,153,155,158,176, 178,182,186,190,160.
101 - 101.9°F	8	123,127,129,132,143, 148,167,272.
102 - 102.9°F	4	126,143,219,298.
103°F or more	2	144,138.

Table 10 - Appendix

BIOTYPES AND SEROTYPES FOUND IN 24 ISOLATES OF K. AEROGENES FROM THE FEMALE GENITAL TRACT

Sero-type	No. of Isolates	Strain Ref.No.	Biotype	Strain Designated	Hospital Acquired
3	2	191	KA dulcitol +	T7	No
		192	KA dulcitol +	T7	No
7	1	193	KA dulcitol +	S2	No
9	1	194	KA dulcitol +	S4	Unknown
15	1	195	KA dulcitol +	T17	No
17	1	196	adonitol - starch - glycerol -	G1	No
not 1-40	18	197-201, 203.	KA dulcitol +	Not classified. At least 3 biotypes.	No
		202,204) 205,206) 209-214)	KA dulcitol -		Only 204 and 205 hosp. ac
		207	glycerol -		
		208	glycerol -		

Table 11 - Appendix

CLINICAL SIGNS ASSOCIATED WITH ISOLATION OF VARIOUS SEROTYPES FROM VAGINAL TRACT

Clinical Group	Isolate Number	Local Signs	Other Relevant Clinical Features	Serotype	Temperature	WBC
Non-pregnant females of child-bearing age. (9 cases)	192	Discharge	T. vaginalis infection	3	98.8°	5.0
	201	Discharge	Fibroids	not 1-40	100.0°	11.5
	202	None	Vesico-vaginal fistula	not 1-40	98.0°	11.0
	203	Discharge	Endometrial hyperplasia	not 1-40	100.0°	7.1
	205	Discharge	Ca. uterus	not 1-40	102.0°	-
	210	Discharge	T. vaginalis infection	not 1-40	99.0°	-
	211	None	None	not 1-40	98.0°	9.3
	212	None	Torsian of ovarian cyst	not 1-40	98.0°	6.7
	213	Discharge	Metropathia haemorrhagiea	not 1-40	102.0°	-

Continued ...../

Table 11 - Appendix (Continued)

CLINICAL SIGNS ASSOCIATED WITH ISOLATION OF VARIOUS SEROTYPES FROM VAGINAL TRACT

Clinical Group	Isolate Number	Local Signs	Other Relevant Clinical Features	Serotype	Temperature	WBC
Puerperium (13 cases)	191	Normal lochia	Normal delivery two days previously.	3	99°	-
	193	Normal lochia	Respiratory infection causing fever.	7	102°	12.6
	194	Offensive lochia	Second day post-partum, normal delivery.	9	100°	14.8
	195	Normal lochia	Proteus urinary infection.	15	102°	-
	196	Offensive lochia	Diabetes. Thrombophlebitis.	not 1-40	103°	24.0
	198	Normal lochia	P.U.O. on third day.	not 1-40	102°	-
	199	Offensive lochia	Normal delivery.	not 1-40	100°	12.8
	200	Offensive lochia	Abortion with retained products of conception.	not 1-40	101°	9.8

Continued...../

Table 11 - Appendix (Continued)

CLINICAL SIGNS ASSOCIATED WITH ISOLATION OF VARIOUS SEROTYPES FROM VAGINAL TRACT

Clinical Group	Isolate Number	Local Signs	Other Relevant Clinical Features	Serotype	Temperature	WBC
Puerperium (Continued)	206	Offensive lochia	Normal delivery.	not 1-40	100°	5.4
	207	Normal lochia	P.U.O.	not 1-40	100°	-
	208	Offensive lochia	Normal delivery.	not 1-40	100°	6.3
	209	Normal lochia	A.R.M. normal delivery.	not 1-40	98°	-
	214	Normal lochia	Normal delivery.	not 1-40	98°	5.4
Pregnancy	197	Vaginal discharge	Cervical erosion.	not 1-40	99°	12.0
Post-menopausal	204	Vaginal discharge	Chronic cervicitis. Prolapse.	not 1-40	100°	-

Table 12 - Appendix

BIOTYPES AND SEROTYPES FOUND IN FIVE ISOLATES OF KLEBSIELLA FROM THE CENTRAL NERVOUS SYSTEM AND FROM EYE AND EAR SWABS

Source of Klebsiella	Sero-type	Isolate Ref.No.	Biotype	Strain Designated	Hospital Acquired
Cerebro spinal fluid	7	215	KA dulcitol -	U1	No
Eye swab	7	216	KA dulcitol +	S2	Yes
Eye swab	not 1-40	217	KA dulcitol +	not classified	Yes
Ear swab	9	218	KA dulcitol -	T11	No
Ear swab	not 1-40	219	KA dulcitol -	not classified	No

Table 13 - Appendix

Temperature RANGES ~~[illegible]~~ ASSOCIATED WITH KLEBSIELLA ISOLATED FROM THE CENTRAL NERVOUS SYSTEM AND FROM EYE AND EAR SWABS

8:00 am oral temperature	Number of Cases	Isolate Number	Source
98 - 99°F	3	216 217 218	Sticky eye. Sticky eye. Normal ear.
99 - 99.9°F	1	219	Mild otitis media.
100 - 100.9°F	1	215	Klebsiella meningitis.

Table 14 - Appendix

KLEBSIELLA FROM BLOOD CULTURES

Isolate Number and Diagnosis	Sero-type	Biotype	Strain Classified	Hospital Acquired
220 Prostatic abscess.	7	KA dulcitol -	U1	No
221 Acute glomerulo-nephritis.	2	KA dulcitol -	T4	No
222 Diabetes. Para-lytis ileus.	17	KA dulcitol +	B1	No
223 Obstructive jaundice. Respiratory infection.	9	MR+ indole +	B2	No
224	not 1-40	citrate -	not classified	No
225 224 and 225 from same patient. Subphrenic abscess.	3	MR+	B3	Yes

Table 15 - Appendix

WBC AND TEMPERATURE ON THE DAY OF TAKING EACH POSITIVE SPECIMEN FOR BLOOD CULTURE

Isolate Number	8:00 am Temperature	WBC
220	102°F	26.2
221	101°F	14.0
222	104°F	21.9
223	100°F	19.5
224 (same patient)	101°F	17.0
225 (same patient)	103°F	not done

Table 16 - Appendix

SEROTYPES AND BIOTYPES OF KLEBSIELLA FROM ASCITIC FLUID

Sero-type	Number of Isolates	Strain Ref.No.	Biotype	Strain Designated	Hosp. Acqu.
2	1	226	KA dulcitol -	T4	No
25	1	227	KA dulcitol -	A1	No
not 1-40	1	228	KA dulcitol -	not classified	No

Table 17 - Appendix

CLASSIFICATION OF KLEBSIELLA ISOLATES FROM THE UMBILICAL STUMP

Sero-type	No. of Isolates	Strain Ref.No.	Biotype	Strain Designated	Hospital Acquired
11	2	229 230	KA dulcitol + KA dulcitol +	T13 T13	Yes Yes
16	1	231	KA dulcitol -	W1	Yes
17	1	232	adonitol - starch - glycerol -	G1	Yes
19	1	233	KA dulcitol -	U14	Yes
23x29	1	234	KA dulcitol -	W2	Yes
not 1-40	2	235 236	adonitol - KA dulcitol -	not classified	Yes Yes

Table 18 - Appendix

KLEBSIELLA ASSOCIATED WITH FORMATION OF ABSCESS

Diagnosis and Age	Other Clinical Findings	Isolate Number	Sero-type
Ischiorectal abscess. Aged 56.	Multiple perianal sinuses. Epididymitis.	237	1
Scrotal abscess. Aged 45.	None.	238	2
Subphrenic abscess. Aged 49.	Diabetes. Klebsiella septicaemia.	239	3
Webspace infection with abscess. Aged 17.	Ulcer of index finger.	240	3
Multiple liver abscesses. Aged 5.	Previously healthy child.	241	7
Ischiorectal abscess. Aged 35.	None	242	not 1-40
Perinephric abscess. Aged 78.	Acute pyelonephritis. Diabetes.	243	not 1-40

Table 19 - Appendix

DETAILS OF TEMPERATURE AND PERIPHERAL WHITE COUNTS IN KLEBSIELLA ABSCESSES

Temperature range	Number of Cases	WBC
98 - 98.9°F	3	18.5
99 - 99.9°F	-	
100 - 100.9°F	2	12.0 17.0
101 - 101.9°F	-	
102 - 102.9°F	2	16.2 13.6
103°F +	-	

Table 20 - Appendix

CLASSIFICATION OF KLEBSIELLA ISOLATED FROM ABSCESSES

Sero-type	No. of Isolates	Strain Ref.No.	Biotype	Strain Designated	Hospita Acquire
1	1	237	KA dulcitol +	T1	No
2	1	238	KA dulcitol -	T4	No
3	2	239 240	MR+ KA dulcitol +	B3 T7	No No
7	1	241	KA dulcitol +	S2	No
not 1-40	2	242 243	KA dulcitol + KA dulcitol +	not classified	No No

Table 21 - Appendix

CLINICAL SIGNS ASSOCIATED WITH ISOLATION OF K. AEROGENES FROM WOUND SWABS

Isolate Number	Source of Specimen	Local Signs of Infection	Related Clinical Condition	WBC	Temperature
244	Post-operative wound swab.	Purulent wound.	Thoracotomy for Staphylococcal pneumonia.	11.6	100.1°F
245	Swab from post-operative wound.	Inflamed.	Carcinoma oesophagus. Oesophageal by pass and gastrostomy.	11.0	99°F
246	Swab from drainage tube.	Purulent.	Subphrenic abscess.	13.6	101°F
247	Swab from ulcer.	Inflamed.	Gangrenous ulcer of thigh.	9.8	99°F
248	Swab from pustule.	Purulent.	Pustules of lip and palate.	-	99°F
249	Swab from burn.	Normal.	First degree burn showing good healing.	15.0	98°F
250	Swab from area of plaster dermatitis.	Inflamed.	Osteotomy of right femur. Bilateral coxavara.	not done	100.2°F

Continued...../

Table 21 - Appendix (Continued)

CLINICAL SIGNS ASSOCIATED WITH ISOLATION OF K. AEROGENES FROM WOUND SWABS

Isolate Number	Source of Specimen	Local Signs of Infection	Related Clinical Condition	WBC	Temperature
251	Post-operative wound swab.	Normal wound.	Pharyngo-laryngectomy for post-cricoid carcinoma.	not done	100.4°F
252	Swab from post-operative wound.	Purulent.	Compound fracture.	not done	98.2°F
253	Swab from drainage tube.	Purulent.	Nephrectomy for renal calculus.	15.0	100°F
254	Post-operative wound swab.	Inflamed.	Laparotomy for haematemesis. No lesion found.	not done	101°F
255	Swab from ulcer.	Purulent.	Diabetic gangrene of toe.	6.8	101°F
256	Swab from ulcer of scrotum.	Inflamed.	Urethral stricture.	6.4	102°F
257	Swab from infected cut down site.	Purulent.	Gastroenteritis.	9.2	99.8°F

Continued..../

Table 21 - Appendix (Continued)

CLINICAL SIGNS ASSOCIATED WITH ISOLATION OF K. AEROGENES FROM WOUND SWABS

Isolate Number	Source of Specimen	Local Signs of Infection	Related Clinical Condition	WBC	Temperature
258	Post-operative wound swab.	Purulent.	Salpingo-oophorectomy for carcinoma ovary.	9.1	101°F
259	Swab from amputation stump.	Inflamed.	Diabetic gangrene of foot.	23.6	100°F
260	Post-operative wound swab.	Inflamed.	Hermaphrodite. Recession of clitoris.	12.5	99.4°F
261	Post-operative wound swab.	Purulent.	Urinary retention.	12.6	98.2°F
262	Post-operative wound swab.	Inflamed wound.	Mesenteric thrombosis.	12.0	101°F
263	Post-operative wound swab.	Purulent.	Pyosalpinx.	13.4	102°F
264	Post-operative wound swab.	Inflamed.	Carcinoma of penis.	9.6	98.2°F
265	Suprapubic cystostomy drain.	Purulent.	Urinary retention.	9.4	98°F

Continued...../

Table 21 - Appendix (Continued)

CLINICAL SIGNS ASSOCIATED WITH ISOLATION OF K. AEROGENES FROM WOUND SWABS

Isolate Number	Source of Specimen	Local Signs of Infection	Related Clinical Condition	WBC	Temperature
266	Swab from ulcer.	Inflamed base.	Gangrene.	9.6	99°F
267	Post-operative wound swab.	Normal.	Fracture neck of femur - insertion Smith Petersen pin.	7.3	98°F
268	Drainage fistula.	Purulent.	Vesico cutaneous fistula.	7.2	98.2°F
269	Swab from burn.	Inflamed.	Third degree burn.	not done	100°F
270	Swab from excoriation of groin.	Inflamed with pustule formation.	Congenital dislocation of hip.	not done	96°F
271	Swab from urinary sinus.	Purulent.	Urinary retention.	8.4	99.2°F
272	Swab from amputation stump.	Inflamed.	Gangrene of foot R. common iliac artery thrombosis.	not done	98.6°F
273	Swab from burn.	Purulent.	Second degree burns.	13.0	100.4°F

Continued...../

Table 21 - Appendix (Continued)

CLINICAL SIGNS ASSOCIATED WITH ISOLATION OF K. AEROGENES FROM WOUND SWABS

Isolate Number	Source of Specimen	Local Signs of Infection	Related Clinical Condition	WBC	Temperature
274	Swab from ureterostomy wound.	Inflamed.	Posterior urethral valve.	12.0	102°F
275	Swab from gangrenous ulcer.	Inflamed.	Diabetes. Gangrene of heel.	7.4	98.2°F
276	Swab from pustular rash of buttocks.	Purulent.	Rash in neonate - hospital delivery.	not done	98°F
277	Swab from ulcer.	Purulent.	Chronic leg ulcer.	not done	98.2°F
278	Swab from post-operative wound.	Purulent.	Carcinoma uterus.	25.6	101°F
279	Swab from post-operative wound.	Purulent.	Hysterectomy following septic abortion.	27.8	101-2°F
280	Swab from draining suprapubic fistula.	Purulent.	Carcinoma of bladder.	15.6	101°F

Table 22 - Appendix

TEMPERATURE RANGE IN 37 CASES OF WOUND INFECTION DUE TO KLEBSIELLA

Temperature	Number of Cases
Up to 98.9°F	12
99 - 99.9°F	7
100 - 100.9°F	6
101 - 101.9°F	9
102 - 102.9°F	3

Table 23 - Appendix

DETAILS OF BIOTYPES AND SEROTYPES OF KLEBSIELLA FOUND IN ASSOCIATION WITH WOUND AND SKIN INFECTION

Sero-type	No. of Isolates	Strain Ref.No.	Biotype	Strain Designated	Hospital Acquired
3	4	244	KA dulcitol -	T5	Yes
		245	KA dulcitol -	T5	Yes
		246	MR +	B3	No
		247	KA dulcitol +	T7	No
5	2	248	KA dulcitol -	W3	No
		249	KA dulcitol -	W3	No
7	1	250	KA dulcitol -	U1	Yes
9	2	251	KA dulcitol -	T11	Yes
		252	KA dulcitol -	T11	Yes
11 (x1)	2	253	KA dulcitol -	T14	Yes
		254	KA dulcitol +	T13	Yes
15(x17)	1	255	MR +	W4	No
16	1	256	KA dulcitol +	T19	Unknown
17	1	257	KA dulcitol -	U11	Yes
19	1	258	KA dulcitol -	U14	Yes
26	1	259	MR +, VP -	W5	Yes
35	3	260	MR+,VP-,indole +	W6	Yes
		261	KA dulcitol -	T24	No
		262	KA dulcitol +	W7	Yes
not 1-40	17	263,264, 267,268, 270,271.	KA dulcitol + (6 cases)	not classified, serotype	263,264, 267 only
		265,266, 273,274, 275,276, 277,278, 279,280.	KA dulcitol - (10 cases)		266,273, 274,276, 277 only
		269	glycerol -	unknown.	No
		272	MR+,VP-,adonitol -		Yes

Table 24 - Appendix

SPECIMENS OF KLEBSIELLA AEROGENES OBTAINED AT AUTOPSY

Isolate Number	Source of Specimen at Autopsy	Serotype	Biotype	Strain Classified
281	Swab of bladder wall.	13	MR +, VP +, indole +	W8
282	Swab meninges.	7	KA dulcitol -	U1
283	Swab lung.	not 1-40	indole +	not classified
284	Swab bronchus.	31	indole +	W9
285	Swab prostatic abscess.	7	KA dulcitol -	U1
286	Swab spleen.	7	KA dulcitol -	U1
287	Swab liver abscess.	7	KA dulcitol +	S2
288	Splenic abscess.	7	KA dulcitol +	S2
289	Kidney abscess.	7	KA dulcitol +	S2
290	Mesenteric lymph node.	7	KA dulcitol +	S2

Table 25 - Appendix

STOCK CULTURES OF KLEBSIELLA SPECIES USED IN ANTISERA PREPARATION, AND IN TESTING FOR CROSS REACTIONS

Catalogue Number	Batch	Name of Organism	Sero-type
5054	2	Klebsiella edwardsii var edwardsii	1
5055	1	K. aerogenes	2
5046	2	K. rhinoscleromatis	3
5050	4	K. ozaenae	4
5051	2	K. ozaenae	5
5052	2	K. ozaenae	6
9127	1	Klebsiella species	7
9128	1	" "	8
9129	1	" "	9
9130	1	" "	10
9131	1	" "	11
9132	1	" "	12
9133	2	" "	13
9134	2	" "	14
9135	2	" "	15
9136	2	" "	16
9137	2	" "	17
9138	2	" "	18
9139	1	" "	19
9140	2	" "	20

Continued...../

Table 25 - Appendix (Continued)

STOCK CULTURES OF KLEBSIELLA SPECIES USED IN ANTISERA PREPARATION, AND IN TESTING FOR CROSS REACTIONS

Catalogue Number	Batch	Name of Organism	Sero-type
9141	1	Klebsiella species	21
9142	1	" "	22
9143	1	" "	23
9144	1	" "	24
9145	1	" "	25
9146	2	" "	26
9147	1	" "	27
9148	1	" "	28
9149	1	" "	29
9150	1	" "	30
9151	1	" "	31
9152	1	" "	32
9153	1	" "	33
9154	2	" "	34
9155	1	" "	35
9156	1	" "	36
9157	1	" "	37
9158	1	" "	38
9159	1	" "	39
9160	1	" "	40
9161	2	" "	41

Continued...../

Table 25 - Appendix (Continued)

STOCK CULTURES OF KLEBSIELLA SPECIES USED IN ANTISERA PREPARATION, AND IN TESTING FOR CROSS REACTIONS

Catalogue Number	Batch	Name of Organism	Sero-type
9162	1	Klebsiella species	42
9163	3	" "	43
9164	2	" "	44
9165	2	" "	45
9166	2	" "	46
9167	2	" "	47
9168	2	" "	48
9169	2	" "	49
9170	2	" "	50
9171	1	" "	51
9172	2	" "	52
9173	1	" "	53
9174	1	" "	54
9175	1	" "	55
9176	1	" "	56
9177	1	" "	57
9178	1	" "	58
9179	1	" "	59
9180	1	" "	60
9181	1	" "	61
9182	1	" "	62

Continued...../

Table 25 - Appendix (Continued)

STOCK CULTURES OF KLEBSIELLA SPECIES USED IN ANTISERA PREPARATION, AND IN TESTING FOR CROSS REACTIONS

Catalogue Number	Batch	Name of Organism	Sero-type
9183	1	Klebsiella species	63
9184	1	" "	64
9185	1	" "	65
9186	1	" "	66
9187	1	" "	67
9188	1	" "	68
9189	2	" "	69
9190	1	" "	70
9191	1	" "	71
9192	1	" "	72

Table 26 - Appendix

VOLUMES OF ASCITIC FLUID OBTAINED FROM MICE IMMUNISED WITH KLEBSIELLA SPECIES INTRAPERITONEALLY

Sero-type	No. of mice Inoculated	Number Surviving	Total Volume Ascitic Fluid (ml)	Average volume per Mouse (ml)
1	6	5	40	8
2	6	6	46	7.66
3	6	4	54	13.5
4	6	6	70	11.66
5	6	5	30	6
6	6	6	48	8
7	6	3	35	11.66
8	6	5	20	4
9	6	6	52	8.66
10	6	6	58	9.33
11	6	6	48	8
12	6	5	60	12
13	6	6	85	16.16
14	6	4	20	5
15	6	6	70	11.66
16	6	5	53	10.6
17	6	6	48	8
18	6	6	54	9
19	6	6	40	6.66
20	6	6	25	6.16
21	6	6	40	6.66

Continued...../

Table 26 - Appendix (Continued)

VOLUMES OF ASCITIC FLUID OBTAINED FROM MICE IMMUNISED WITH KLEBSIELLA SPECIES INTRAPERITONEALLY

Sero-type	No.of mice Inoculated	Number Surviving	Total Volume Ascitic Fluid (ml)	Average Volume per Mouse (ml)
22	6	6	71	11.83
23	6	5	52	10.4
24	6	6	60	10
25	6	6	50	9.33
26	6	3	25	8.33
27	6	6	42	7
28	6	6	61	10.33
29	6	5	20	4
30	6	6	70	11.66
31	6	6	84	14
32	6	6	75	12.5
33	6	6	86	16.33
34	6	5	70	16
35	6	6	65	1.83
36	6	4	28	7
37	6	6	84	14
38	6	5	70	14
39	6	5	49	9.8
40	6	4	36	9

Total volume ascitic fluid = 2,094 ml

Number of mice tapped = 216

Average aspirate/mouse = 9.6 ml

Table 27 - Appendix

Z. med. Mikrobiol. u Immunol. 154 p 124-125. After Nimmich (1968)

QUALITATIVE ANALYSIS OF KLEBSIELLA POLYSACCARIDES

Klebsiella K-Typ	URONSAUREN		HEXOSEN			6-Desoxyhexosen	
	Galacturon-saure	Glucuron-saure	Galak-tose	Glu-cose	Man-nose	Fucose	Rham-nose
1		+		+		+	
2		+	(+)	+	(+)		
3	+		+		+		
4		+	(+)	+	+		
5		+	(+)	(+)	+		
6		+		+	+	+	
7		+	+	+	+		
8		+	+	+			
9		+	+				+
10		+	+	+	+		
11		+	+	+	(+)		
12		+	+	+			+
13		+	+	+	(+)		
14		+	(+)	+	+		+
15		+	+	+			
16		+	+	+		+	
17		+		+			+
18		+	+	+			+

Continued...../

Table 27 - Appendix (Continued)

QUALITATIVE ANALYSIS OF KLEBSIELLA POLYSACCHARIDES

Klebsiella K-Typ	URONSAUREN		HEXOSEN			6-Desoxyhexosen	
	Galacturon-saure	Glucuron-saure	Galak-tose	Glu-cose	Man-nose	Fucose	Rham-nose
19		+	+	+			+
20		+	+		+		
21		+	+		+		
22	u		+	+			
23		+	(+)	+			+
24		+		+	+		
25		+	(+)	+			
26		+	+	+	+		
27		+	+	+			
28		+	+	+	+		
29	+		+		+		
30		+	+	+	+		
31		+	+	+	+		
32			+				+
33		+	+	+	+		
34	+			+			+
35		+	+	+	+		
36		+	+	+			+
37	u		+	+			
38	u		+	+			

Continued...../

Table 27 - Appendix (Continued)

QUALITATIVE ANALYSIS OF KLEBSIELLA POLYSACCHARIDES

Klebsiella K-Typ	URONSAUREN		HEXOSEN			6-Desoxyhexosen	
	Galacturon-saure	Glucuron-saure	Galak-tose	Glu-cose	Man-nose	Fucose	Rham-nose
39		+	+	+	+		
40		+	+		+		+
41		+	+	+			(+)
42		+	+		+		
43		+	+		+		
44		+		+			+
45		+	(+)	+			+
46		+	+	+	+		
47		+	+				+
48	+			+			+
49	+		+		+		
50		+	+	+	+		
51		+	+	(+)			
52		+	+				+
53		+	+		+		+
54		+		+	(+)	+	
55		+	(+)	+			+
56	u		+	+			+
57	+		+		+		
58		+	+	+		+	

Continued...../

Table 27 - Appendix -(Continued)

QUALITATIVE ANALYSIS OF KLEBSIELLA POLYSACCHARIDES

Klebsiella K-Typ	URONSAUREN		HEXOSEN			6-Desoxyhexosen-	
	Galacturon-saure	Glucuron-saure	Galak-tose	Glu-cose	Man-nose	Fucose	Rham-nose
59		+	+	+	+		
60		+		+	+	+	
61		+	+	+	(+)		
62		+	+	+	+		
63	+		+			+	(+)
64		+		+	+		+
65		+		+	+		+
66		+	+		+		
67		+	+	+	+		+
68		+	+	+	+	+	
69		+	+	+	+		
70		+	+	+			+
71		+		+	(+)		+
72			(+)	+			+

(+) = small amounts

u = positive carbazol reaction, still not identified

ACUTE BRONCHITIS DUE TO KLEBSIELLA TYPE 9

R.S. Female. 75 years.

Isolate No. 81: Type 9. MR+, Indole + (Table 6-Appendix)

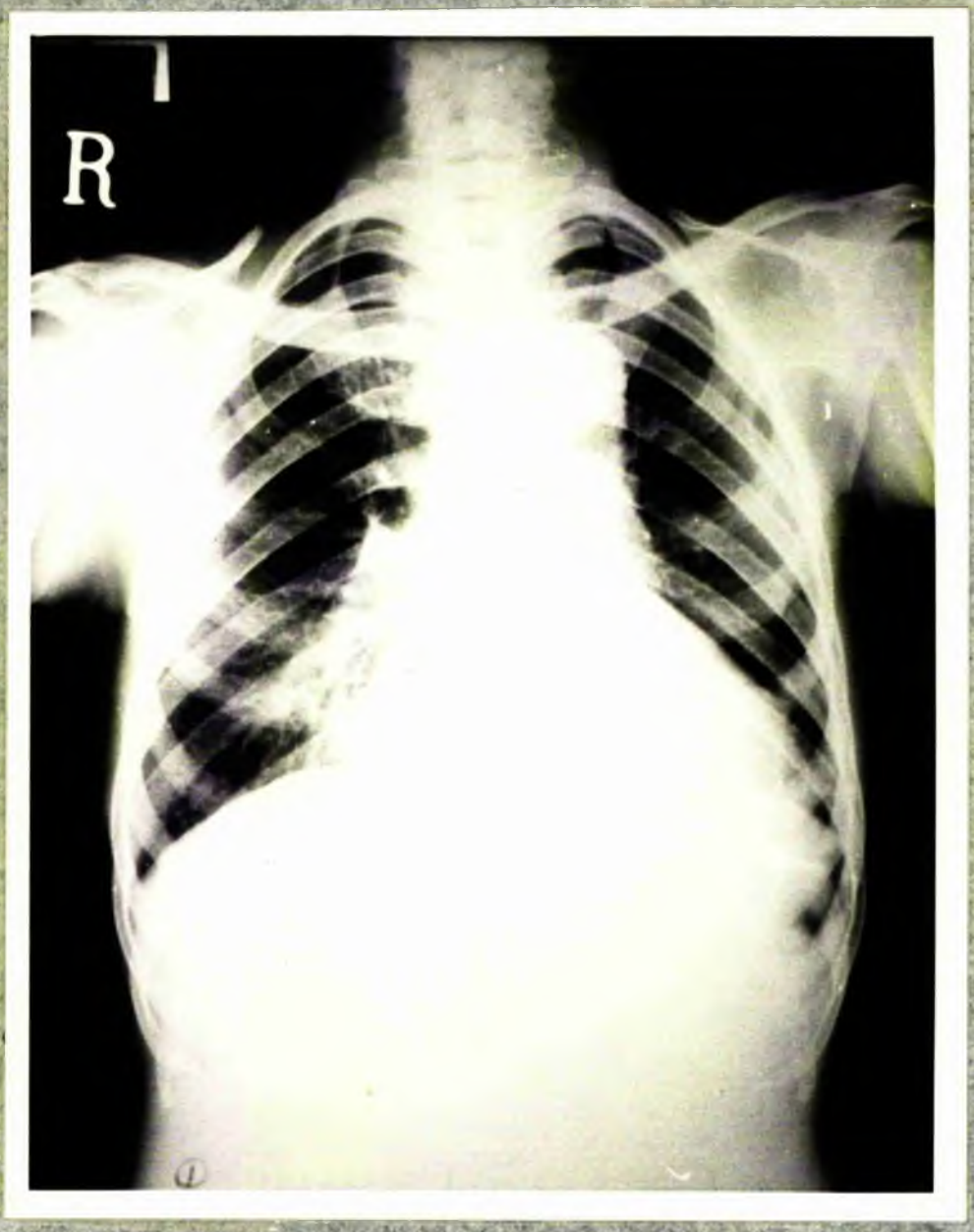
Antibiotics: No previous antibiotics given.

Clinical diagnosis: Acute bronchitis.
Obstructive jaundice and uraemia.

WBC: 7.6

Temperature: 98°F (Table 7 - Appendix)

X-ray chest: The heart is enlarged and aorta unfolded. There is a small adhesion to the pericardium on the left hand border. There are inflammatory changes in the left upper lobe.



ACUTE BRONCHITIS DUE TO KLEBSIELLA TYPE 15

H.F. Male. 73 years.

Isolate No. 91: Type 15. Typical biochemistry. (Table 6 - Appendix).

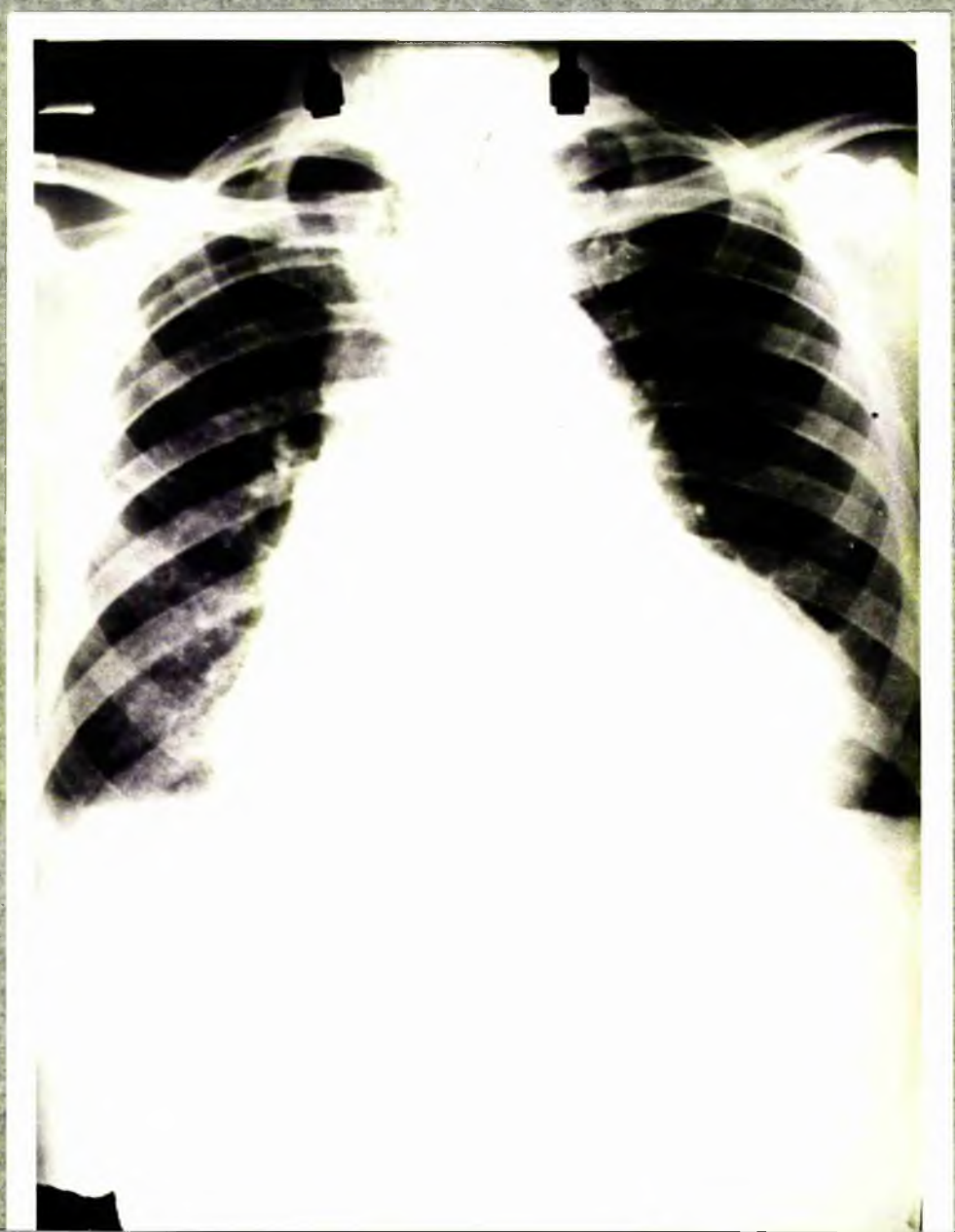
Antibiotics: No previous antibiotics given.

Clinical diagnosis: Acute bronchitis. Congestive cardiac failure. Hypertension.

WBC: 6.4

Temperature: 99°F (Table 7 - Appendix).

X-ray chest: The heart is grossly enlarged. The aorta is dilated and torturous. There is congestion of the lung fields, and a small pleural thickening at the right base.



ACUTE BRONCHITIS DUE TO KLEBSIELLA TYPE 16

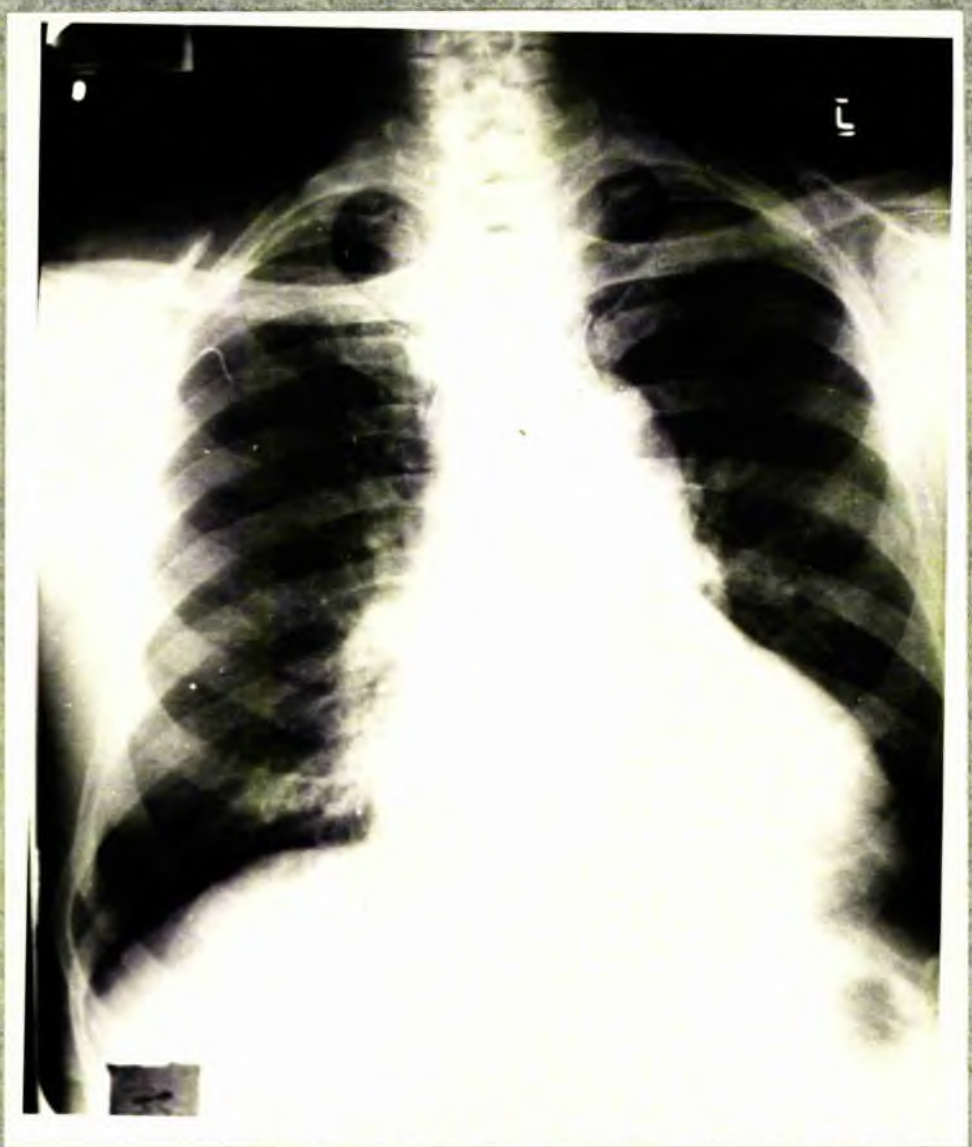
G.H. Male. 80 years.

Isolate No. 94: Type 16. Typical biochemistry. (Table 6 - Appendix).

Antibiotics: No previous antibiotics given.

Clinical diagnosis: Acute bronchitis.
Prostatic hypertrophy.

X-ray chest: The heart is enlarged and there is unfolding of the aorta. The right lower lobe shows some infiltration.



BRONCHOPNEUMONIA DUE TO KLEBSIELLA TYPE 15

C.F. Female. 22 years.

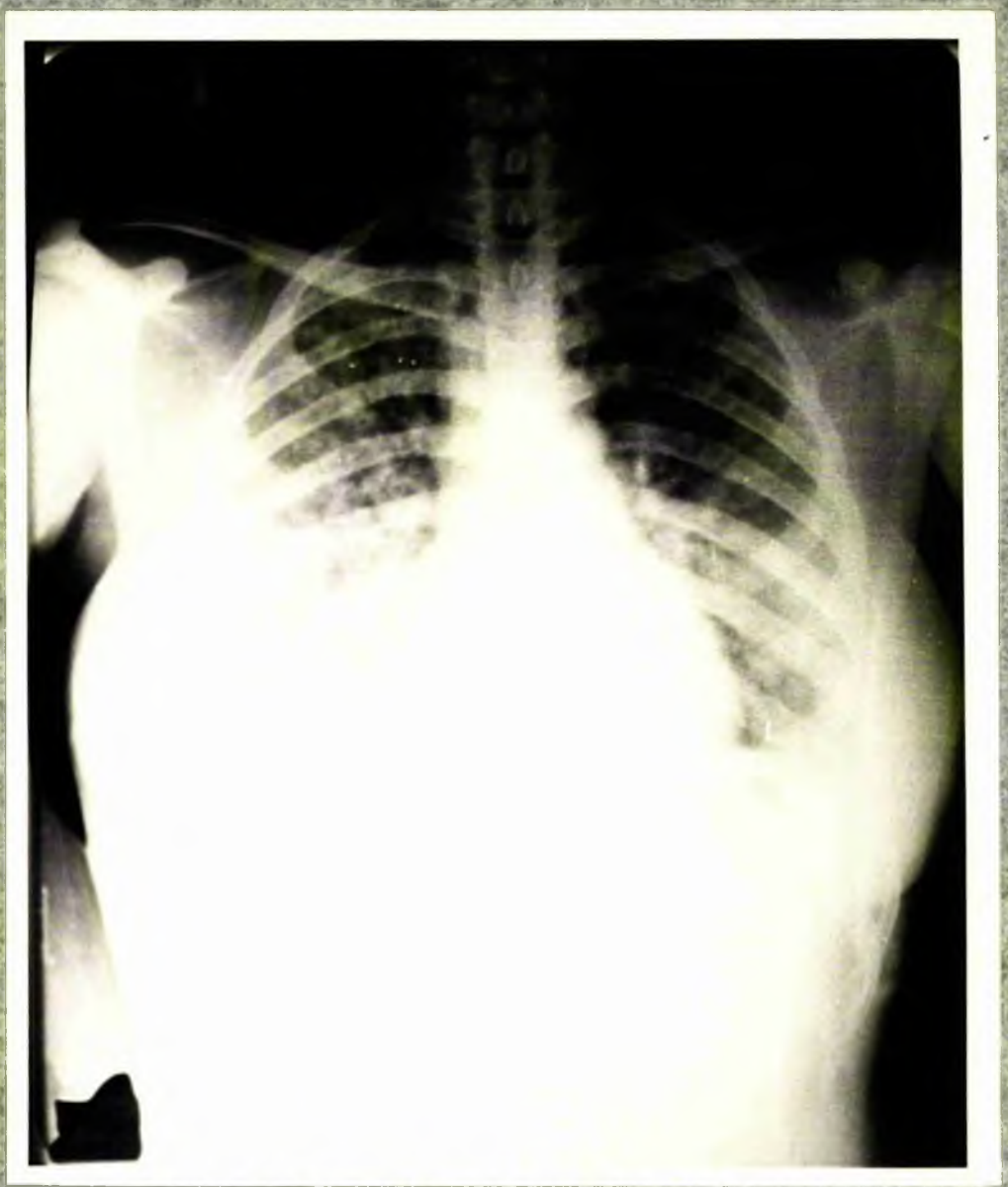
Isolate No. 92: Type 15. Typical Biochemistry. (See Table 6 - Appendix)

Antibiotics: Three days procaine penicillin.

Clinical diagnosis: Bronchopneumonia in a healthy adult.

Temperature: 103°F.

X-ray chest: (1) There are ill defined opacities in both lung fields. Appearances consistent with bronchopneumonia.



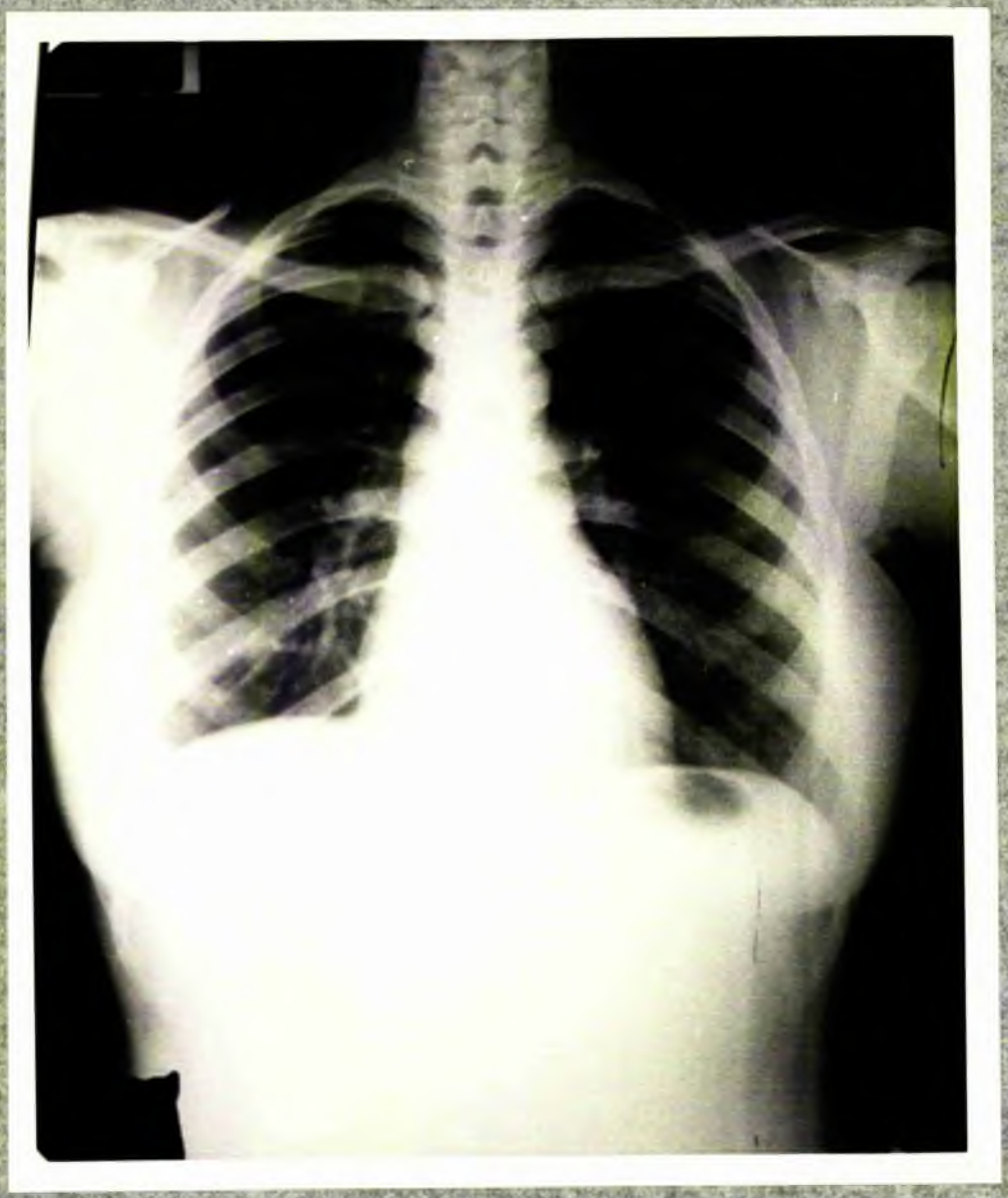
BRONCHOPNEUMONIA DUE TO KLEBSIELLA TYPE 15

C.F. Isolate No. 92. Type 15 bronchopneumonia (Cont.)

X-ray chest: (2) Ten days later after Tetracycline 250 mgms for nine days.

Sputum: Negative.

Report: Lungs are clear.



BRONCHOPNEUMONIA DUE TO KLEBSIELLA TYPE 9

C.H. Male. 43 years.

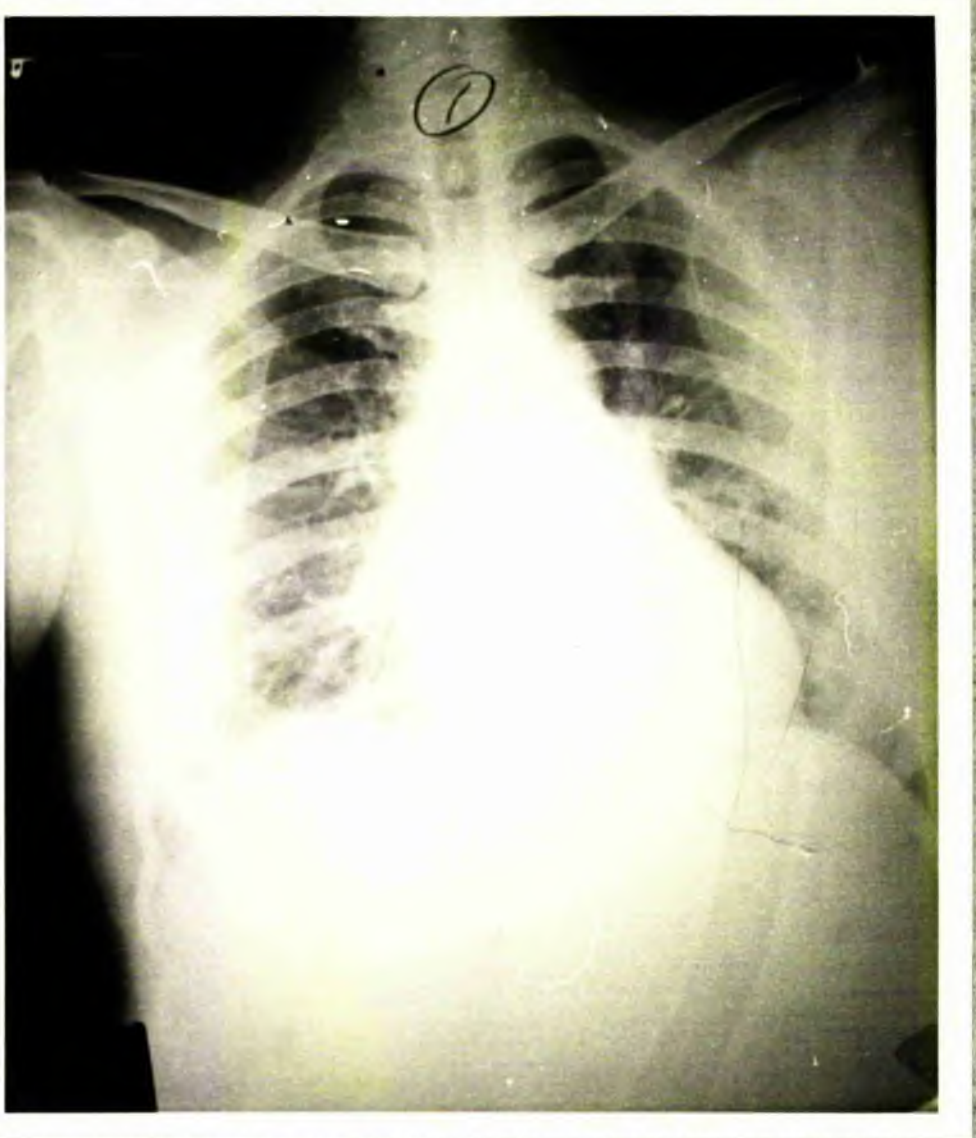
Isolate No. 82: Type 9. Biochemistry typical. (Table 6 - Appendix)

Antibiotics: None.

Clinical diagnosis: Bronchopneumonia. Diabetes mellitus.

WBC: 14,900. **Temperature:** 102°F (Table 7 - Appendix)

X-ray: The heart appears enlarged. There is patchy inflammatory change in both lower zones.



LOBAR PNEUMONIA DUE TO KLEBSIELLA TYPE 31

J.S. Male. 85 years.

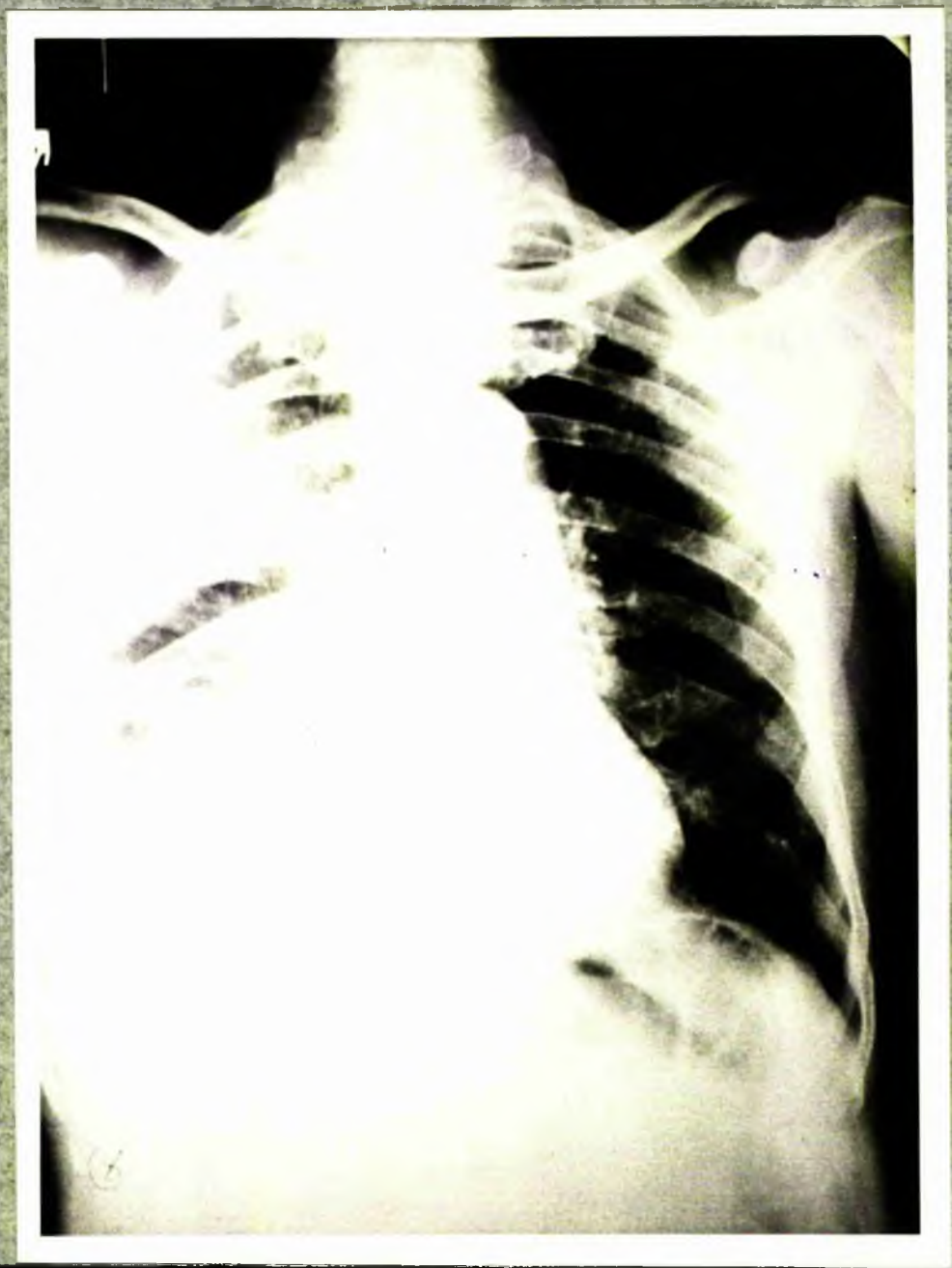
Isolate No. 98: Type 31. Biochemistry - no acid or gas from adonitol or glycerol. (Table 6 - Appendix)

Antibiotics: None.

Clinical diagnosis: Lobar pneumonia. Benign prostatic hypertrophy.

WBC: 11,500 Temperature: 103°F. (Table 7 - Appendix)

X-ray: (1) Antero-posterior view.

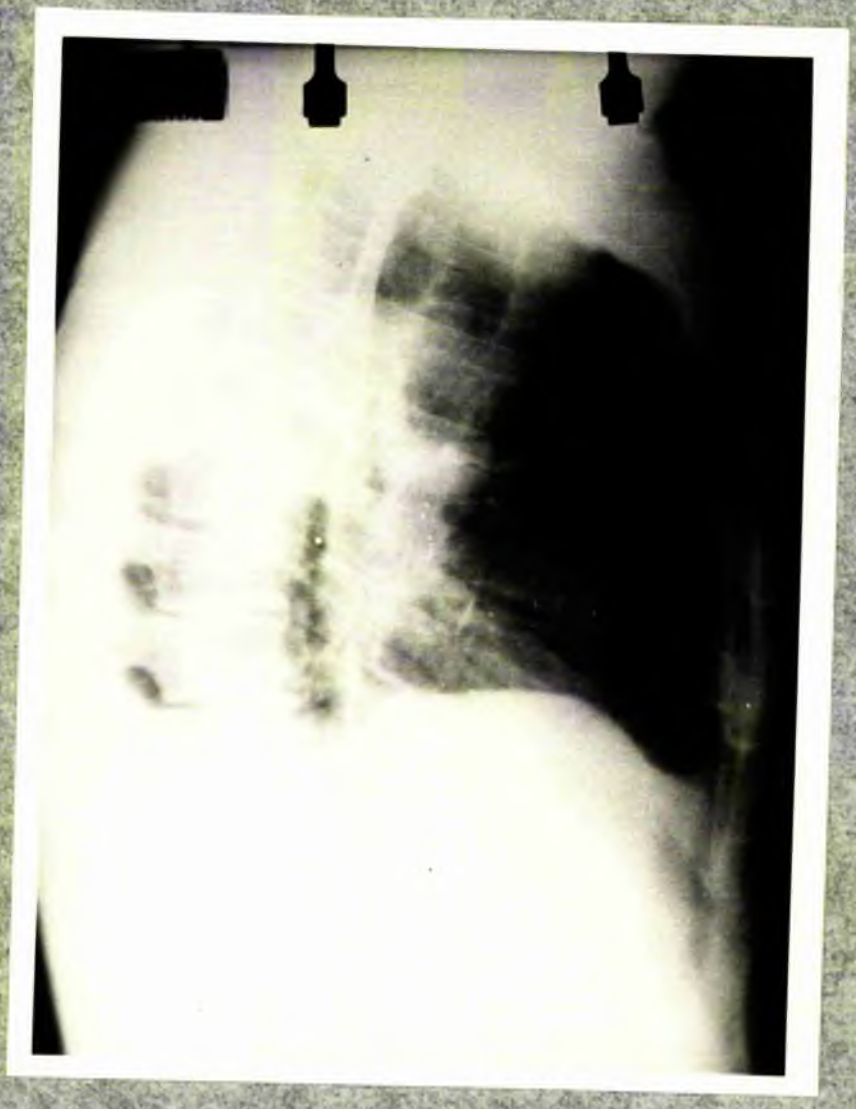


J.S. Isolate 98 (Continued)

X-ray: (2) Lateral view.

Report: There is consolidation in the right upper lobe, which is clearing.

There is elevation of the right side of the diaphragm, with fluid at the right base.



LOBAR PNEUMONIA DUE TO KLEBSIELLA TYPE 9

V.B. Female. 65 years.

Isolate No. 83: Type 9. MR +, VP -.
(Table 6 - Appendix).

Antibiotic history: Ten days penicillin and Streptomycin therapy for Streptococcus pyogenes acute bronchitis.

Developed Klebsiella aerogenes infection of sputum and signs of lobar pneumonia.

Sputum negative for S. pyogenes after four days therapy.

Clinical diagnosis: Klebsiella lobar pneumonia, secondary to Streptococcus pyogenes respiratory infection.

Diabetes mellitus.

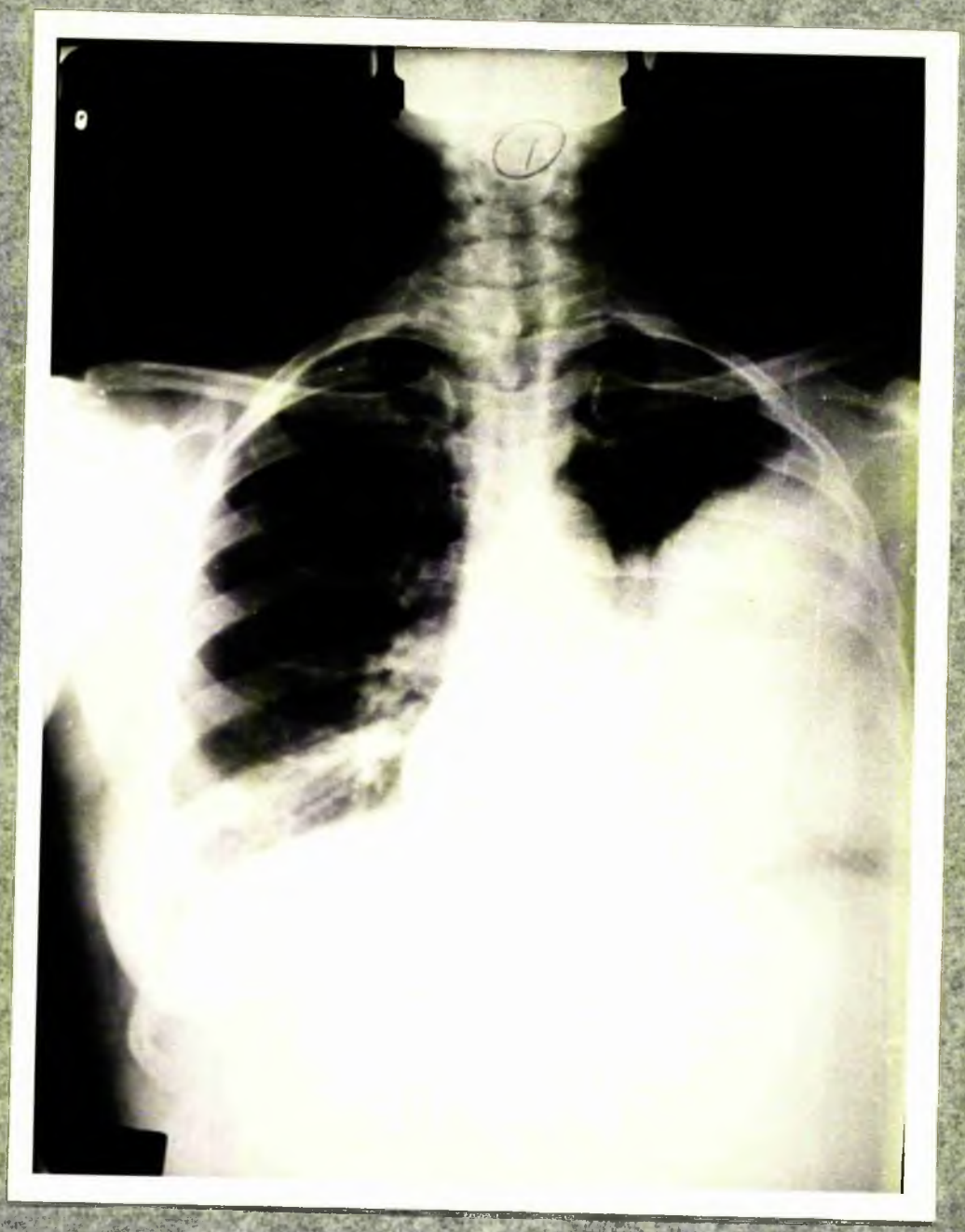
Temperature 100°F

WBC: 19,500 on day of isolating pure culture Klebsiella from sputum.

See following three pages for X-ray series.

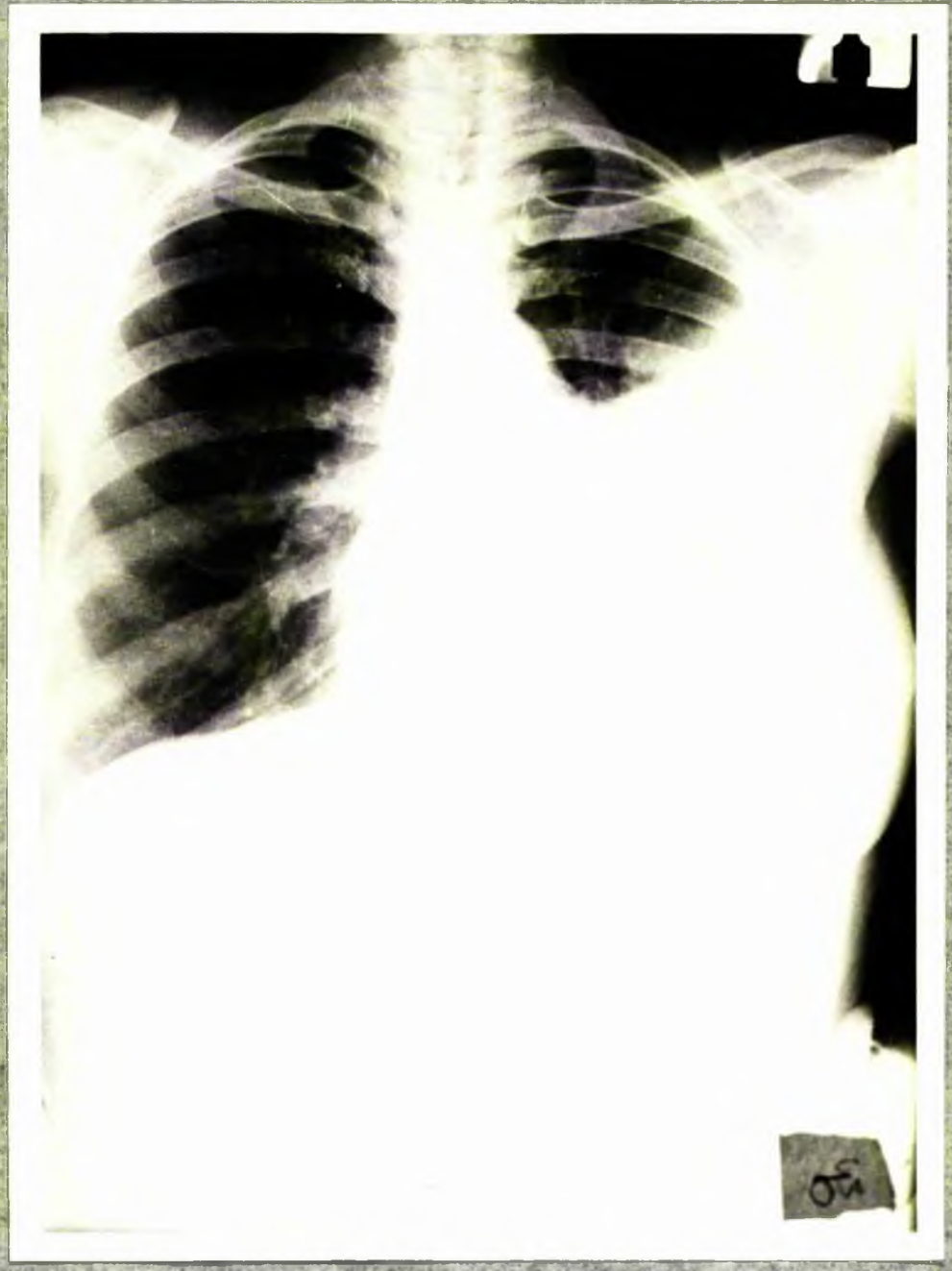
V.B. Isolate No. 83 (continued)

X-ray (1) There is a large area of consolidation on the left side mainly affecting the lower lobe.



V.B. Isolate No. 83 (continued)

X-ray
Ten days later (2) Cystic areas are now appearing within the consolidation, suggesting a Staphylococcal or Klebsiella infection.



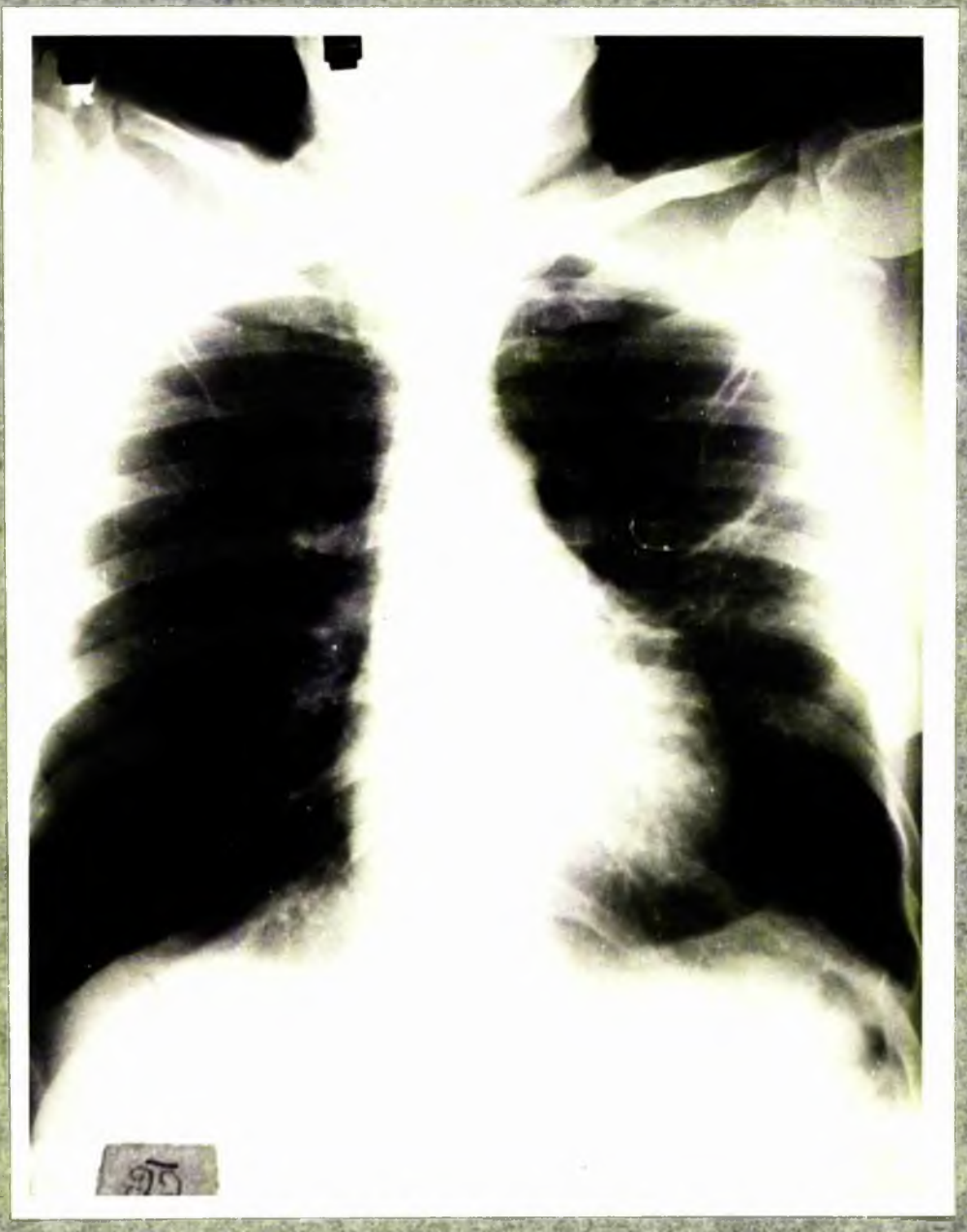
V.B. Isolate No. 83 (continued)

X-ray: (3) Twenty days after first X-ray.

Eighteen days ampicillin.

Sputum culture negative.

Report: Considerable improvement, with only minimal fibrosis remaining.



LOBAR PNEUMONIA DUE TO KLEBSIELLA TYPE 11

L.H. Male. 40 years.

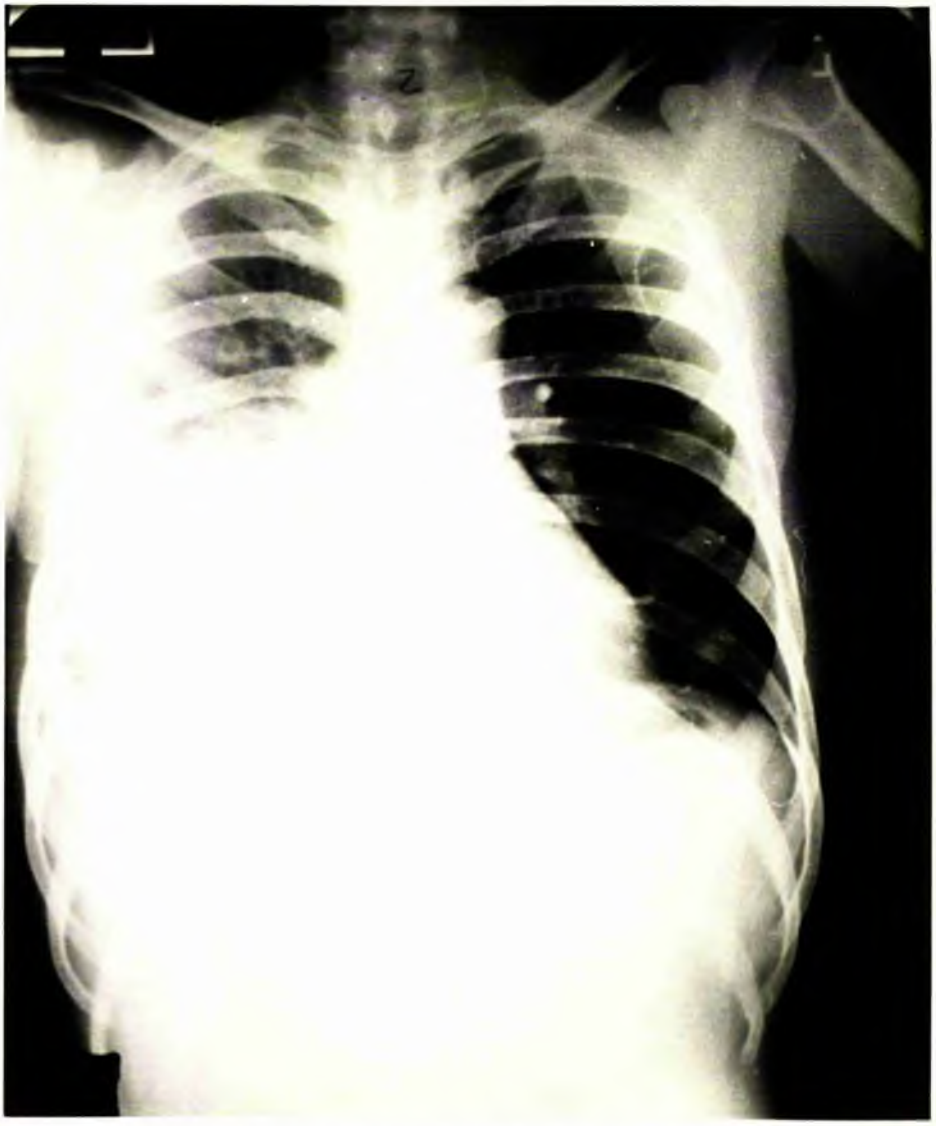
Isolate No. 90: Type 11. Biochemistry typical. (Table 6 - Appendix)

Antibiotics: No previous antibiotics given.

Clinical diagnosis: Lobar pneumonia in a previously healthy adult.

WBC: 17,000 **Temperature:** 104°F (Table 7 - Appendix)

X-ray report: There is right lower lobe consolidation.



LOBAR PNEUMONIA DUE TO KLEBSIELLA TYPE 8

L.K. Male. 48 years.

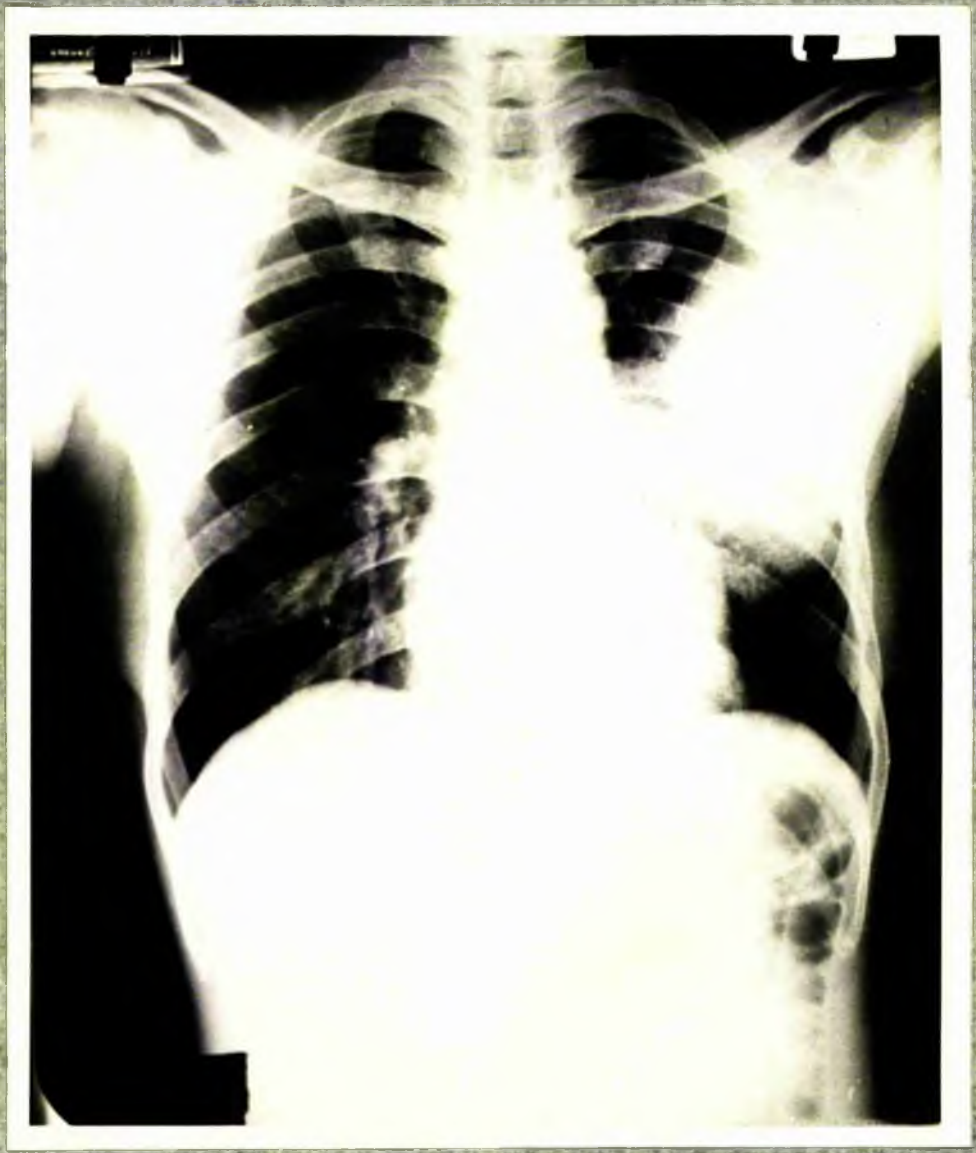
Isolate No. 80: Type 8. Typical biochemistry. (Table 6 - Appendix)

Antibiotics: No previous antibiotics given.

Clinical diagnosis: Left upper lobar pneumonia. Previously healthy adult.

WBC: 5,500 Temperature: 103°F (Table 7-Appendix)

X-ray: Shows an area of consolidation in the left upper lobe.



LUNG ABSCESS DUE TO KLEBSIELLA TYPE 31

L.C. Male. 12 years.

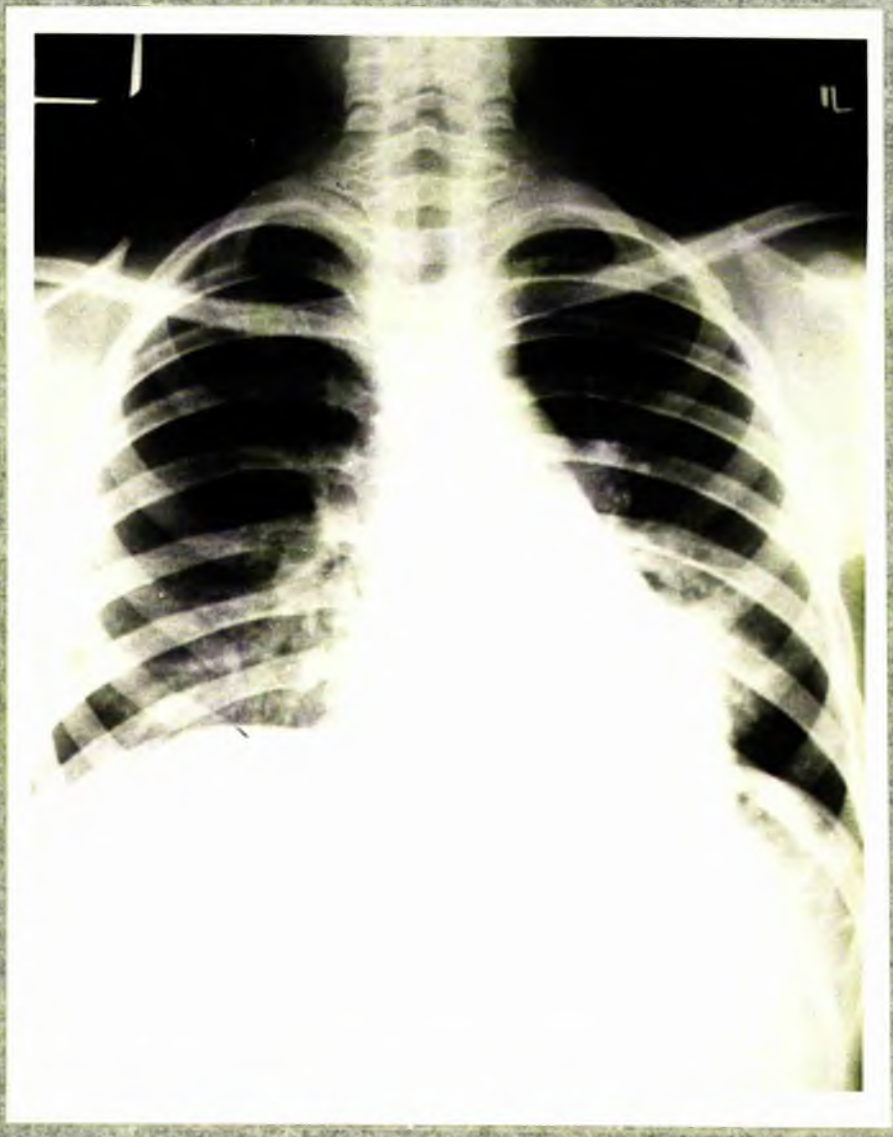
Isolate No. 97: Type 31. Biochemistry - gelatin liquefied. (Table 6 - Appendix)

Antibiotics: No previous antibiotics.

Clinical diagnosis: Lobar pneumonia and lung abscess.

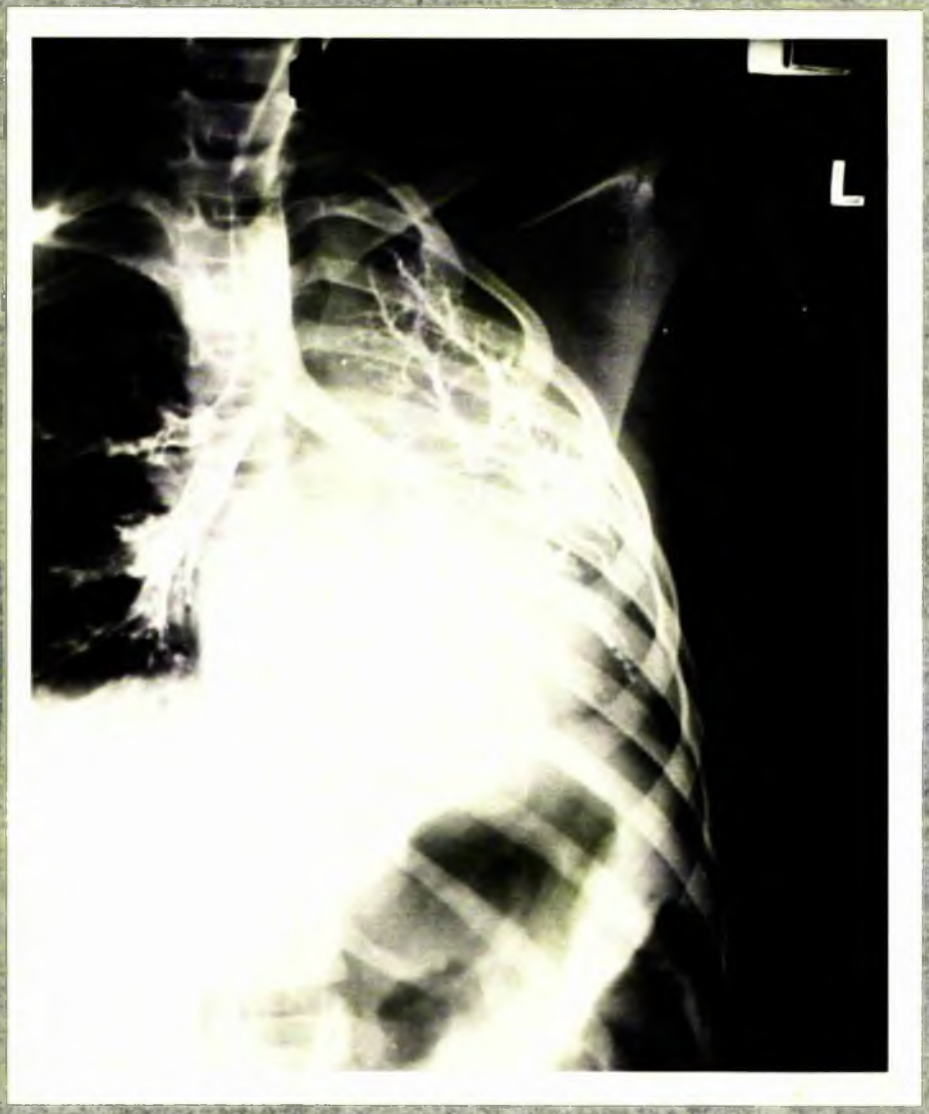
WBC: 5,700 Temperature: 98°F

X-ray chest: Area of consolidation at left base. May be lung abscess which is developing.



L.C. Isolate No. 97 (continued)

Bronchogram: The right lung and left upper lobe are normal. The bronchi of the left lower lobe are crowded and communicate with an abscess cavity.



LUNG ABSCESS DUE TO KLEBSIELLA TYPE 10

C.P. Male. 54 years.

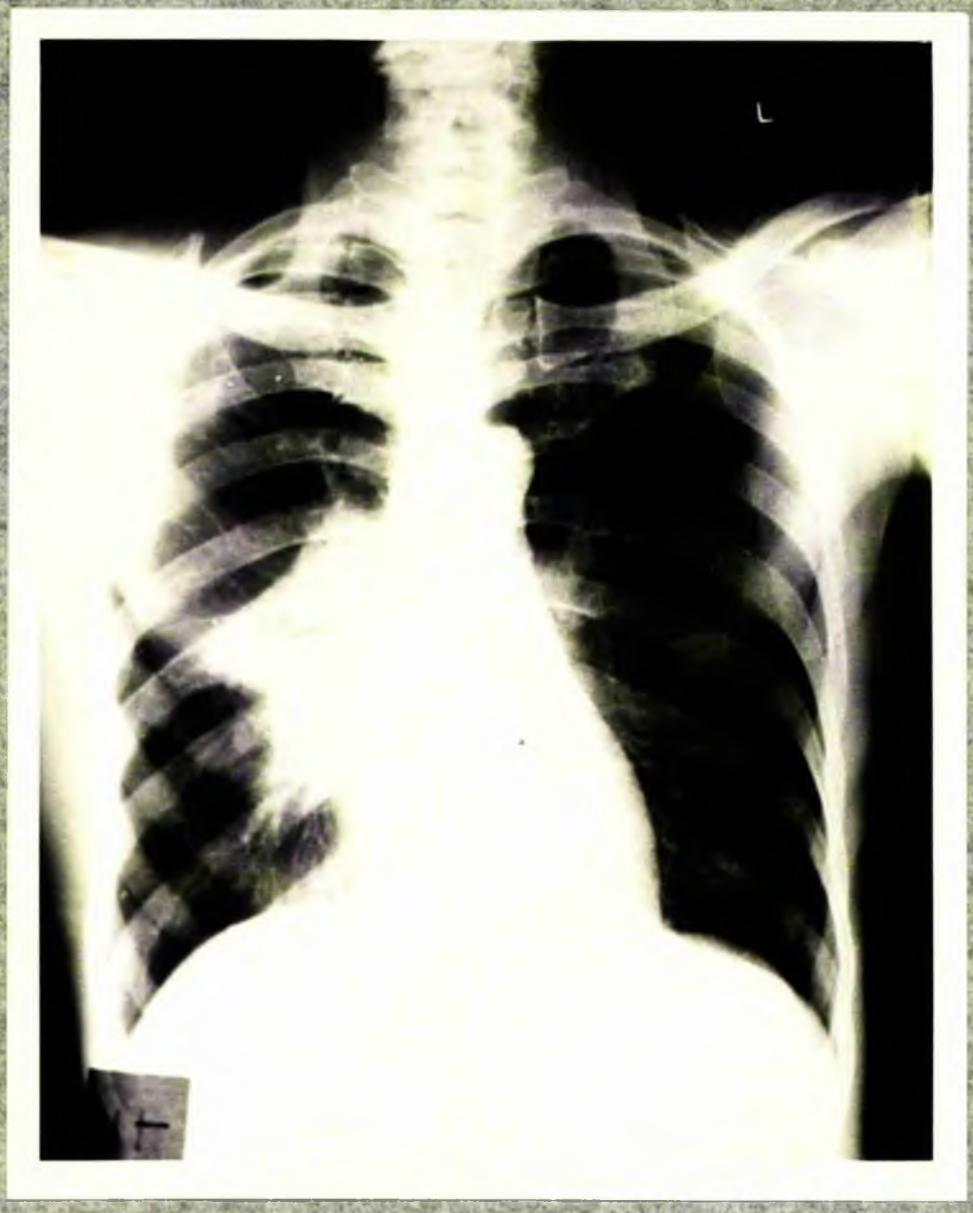
Isolate No. 88: Type 10. Biochemistry typical. (Table 6 - Appendix)

Antibiotics: No previous antibiotics given.

Clinical diagnosis: Lobar pneumonia with lung abscess.

WBC: 7,600 Temperature: 98°F (Table 7-Appendix)

X-ray chest: (1) There is a shadow in the region of the right hilum which may contain a cavity and fluid levels.

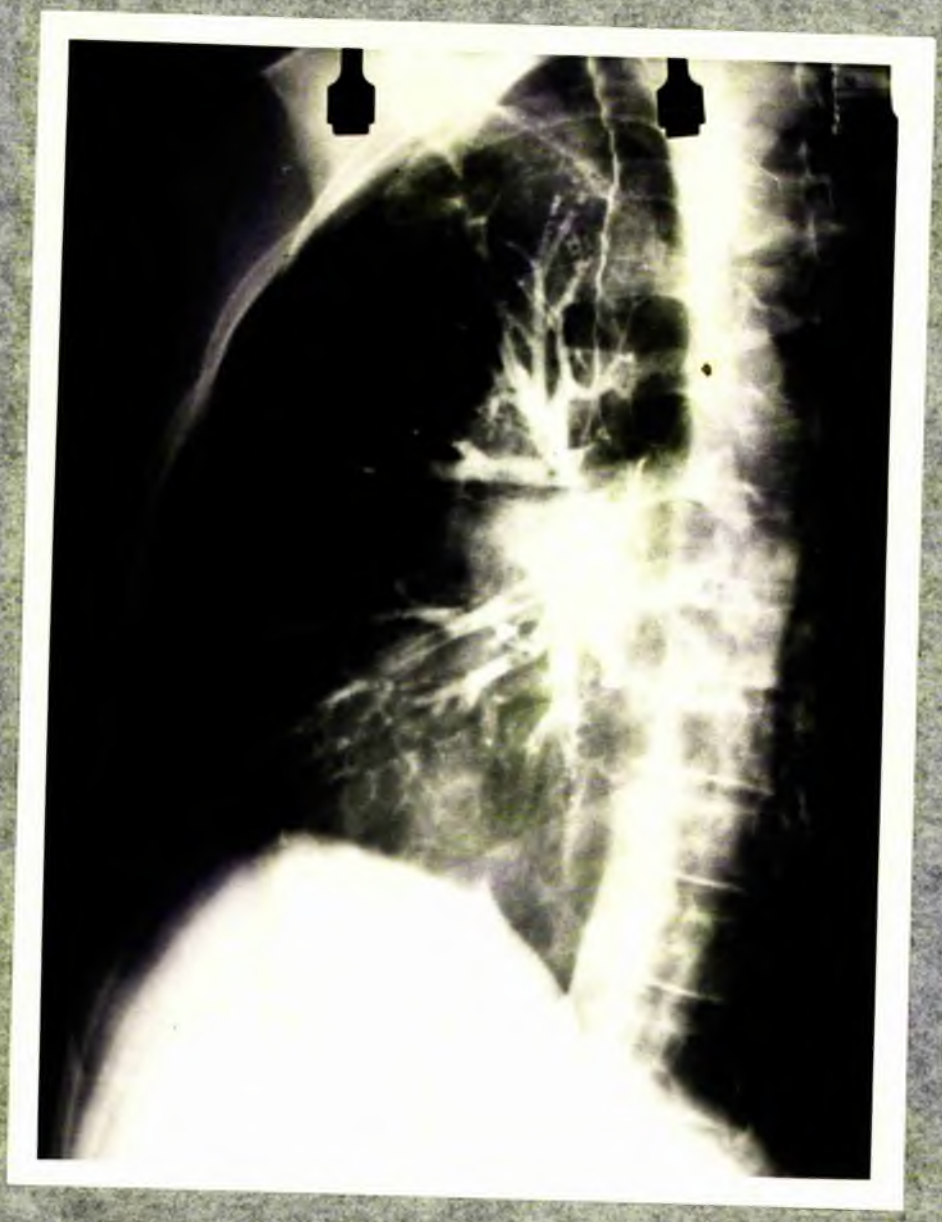


LUNG ABSCESS DUE TO KLEBSIELLA TYPE 10 (continued)

C.P. Isolate No. 88.

X-ray (2) Bronchogram

The right bronchial tree shows some mild cylindrical bronchiectasis in the neighbourhood of the abscess cavity in the apex of the lower lobe. The rest of the bronchi appear normal. Appearances are of inflammatory disease.



B.B. FATAL CASE OF KLEBSIELLA LIVER ABSCESS

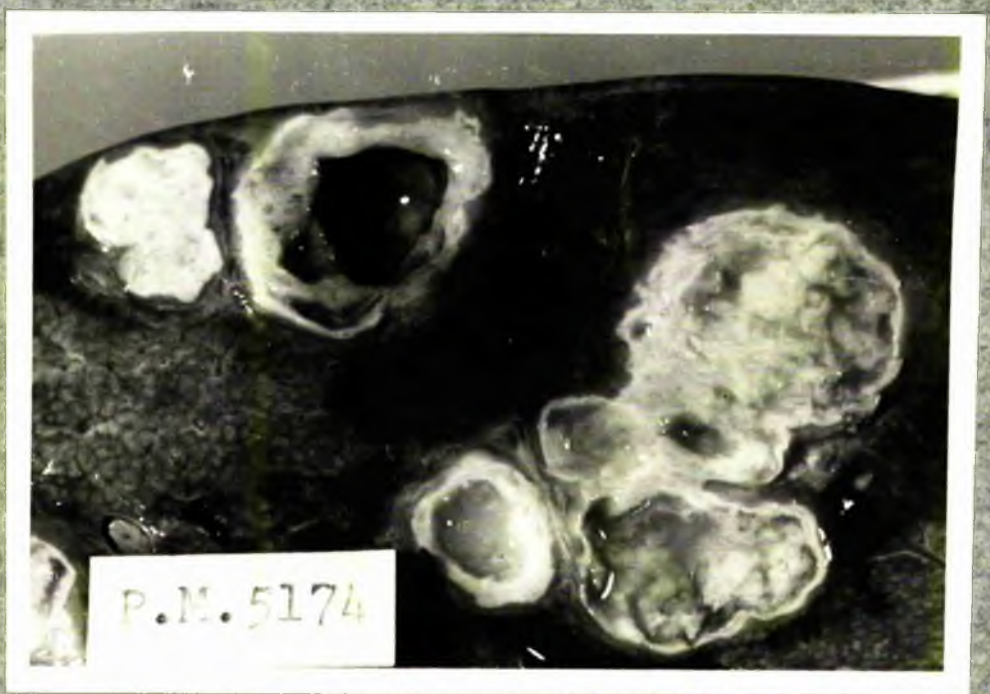
See results section page 147 - Case 1.

External view of enlarged liver (1360 g)

FATAL CASE OF KLEBSIELLA LIVER ABSCESS (Cont.)

Cut surface of liver containing numerous round abscesses containing greenish yellow pus.

Liver abscesses high power



R.J. (See page 152 - Case 9)

1. Septicaemia
2. Klebsiella prostatic abscess
3. Acute pyelonephritis with (a) papillitis necrotans
   (b) multiple abscesses
4. Purulent meningitis

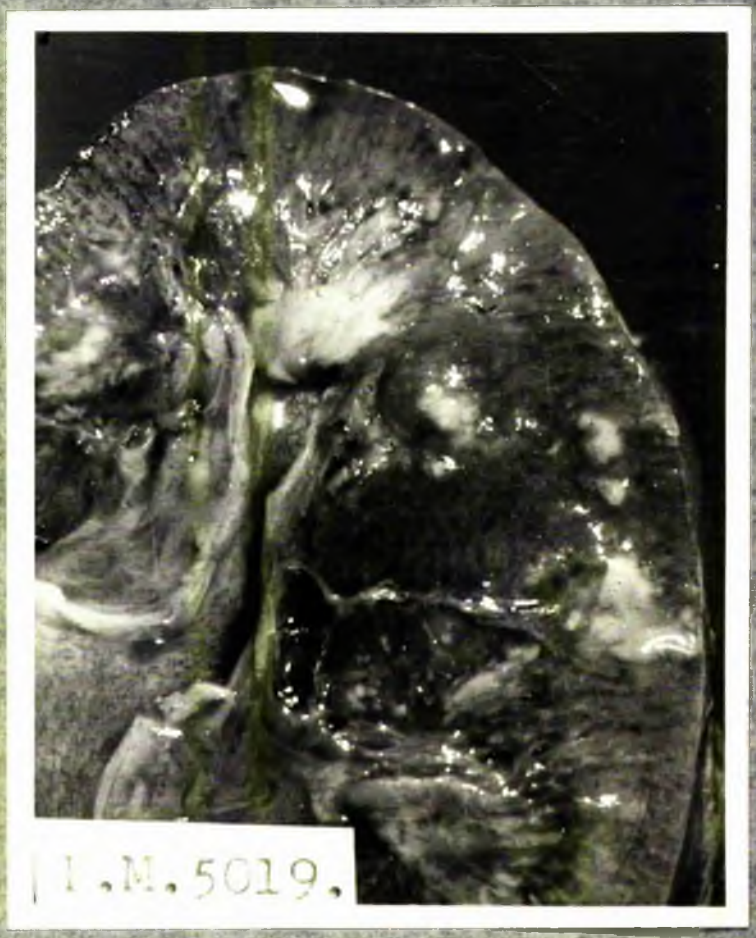
Kidney. R 315 gm. L 210 gm.

Both had perirenal fibrosis with densly adherent capsules. The surface showed a few old healed scars and also focal yellow abscesses, varying in size from 2 mm to 16 mm.

R.J. (Continued)

Kidneys (cut section)

The parenchyma was deep red with numerous yellow-green abscesses of variable size scattered throughout, but most prominently along the arcuate line. Most of the pyramids were either necrotic or converted to abscesses.



Lateral and A.P. views of mice before and after induction of ascitic response.

These photographs were taken 13 days after injection of Sarcoma 180 TC cells, when 14 mls of ascitic fluid was removed from the hyperimmunised mouse.

A. P. view of mice, before and after immunisation