THE BIOCHEMISTRY AND ANTIBIOTIC SENSITIVITY OF THE *KLEBSIELLAE*

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This paper presents a biochemical survey of a series of *Klebsiellae*. These findings are interpreted in the light of current taxonomical concepts. The antibiotic sensitivity of these organisms is also indicated.

Material

The material analysed here comprises a total of 82 strains. Thirty-two come from the National Collection of Type Cultures and are described in Table I. The remaining 50 strains were isolated at the Institute of Laryngology and Otology, mainly from infections of the upper respiratory tract. Data as to their source and serotype have already been presented (Epstein and Friedmann, 1958). In addition, the virulence of some of these strains for mice by oral and peritoneal routes of inoculation and the histopathology of pneumonic infections induced by them have been studied (Epstein and Payne, 1959; Epstein, 1959).

Techniques

Routine Biochemical Techniques.—All tests were performed at 37° C. and media inoculated by Pasteur pipettes from overnight peptone water growths. Cyanide and Koser citrate media were inoculated by loops and straight wires respectively. Unless otherwise stated, all tests were read daily for seven days.

Carbohydrate Fermentation.—Sixteen carbohydrates listed in Table II were tested with Andrade's indicator in media consisting of 1% carbohydrate (w/v) in peptone water.

Urease Test.—Christensen's (1946) weakly buffered urea agar medium was used.

Methyl-red and Voges-Proskauer Test.—The combined Ministry of Health (1939) oxoid media were used, and tests read at two days.

Koser Citrate Test.—Koser's (1923) medium was used, and positives confirmed by re-inoculation.

H₂S and Indole Production.—The combined SIM Difco (1953) medium was used and tested for indole at two and seven days.

Gelatin Liquefaction.—Liquefaction was observed in Difco gelatin agar slabs.

Nitrate Reduction.—Kauffmann's (1954) medium was used.

Cyanide Test.—The medium used was Taylor's (1956) modification of that described by Vagn Møller (1954b). This test is based on the finding by Braun

TABLE I
SOURCE AND SEROTYPE OF N.C.T.C. STRAINS ON
KLEBSIELLAE

Strain	Type	Source
<i>K. pneumoniae</i> (12)		
5054	1	Pneumonia
9494	1	Sputum
9502	2	Urine
9503	2	Unknown
5055	2	Unknown
9128	8	"
9129	9	"
9130	10	"
9131	11	"
9132	12	"
9133	13	"
3279	Untyped	Pneumonia coypu
<i>K. rhinoscleromatis</i> (6)		
5046	3	Nose
5047	3	"
5048	3	"
5049	3 (acapsulate form 5048)	"
1936	3	"
7799	3	"
<i>K. ozaenae</i> (7)		
5050	4	Nose
5051	5	Unknown
5053	5 (acapsulate form 5051)	"
5052	6	"
8883	Untyped	Sputum
9601	"	Nose
8895	"	Sputum
<i>K. "aerogenes"</i> (7)		
9527	I	Water
8844	II	Ice cream
8167	II	Water
8824	II	"
8851	II	"
8801	Untyped	Sputum
8808	"	Water

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(1938) that the inhibitory effect of potassium cyanide on *Klebsiellae* is less than on *Escherichiae*.

Base.—The composition is as follows:

Evans' peptone	..	..	..	..	..	3 g.
NaCl	..	..	..	..	..	5 g.
KH ₂ PO ₄	..	..	..	..	..	0.225 g.
Na ₂ HPO ₄ ·2H ₂ O	..	..	..	..	..	5.6 g.
Aq. dist.	..	..	..	..	..	100 ml.

The base is adjusted to pH 7.6 and then autoclaved in flasks. To 100 ml. of base, 1.5 ml. of a freshly prepared 0.5% potassium cyanide solution in sterile distilled water is added, giving a concentration of 1/13,300. The medium is distributed in 1 ml. amounts in sterile bijou bottles and at 4° C. is stable for one month. Readings are made at 24 and 48 hours. False positives may be obtained unless the caps of bottles are screwed tightly to achieve partial anaerobiosis.

Gluconate Test.—The medium is a modification by Shaw and Clarke (1955) of that originally described by Haynes (1951) for the identification of Pseudomonads. The test is based on the ability of some organisms to metabolize glucose by direct oxidation instead of by the usual oxidative phosphorylation. Gluconate is oxidized to a reducing compound, presumed to be potassium-2-ketogluconate. The medium is tubed in 3 ml. amounts and tested at two days with Benedict's qualitative reagent.

Malonate and Phenylpyruvic Acid Tests.—Shaw and Clarke (1955) combined Leifson's (1933) sodium malonate and Henriksen's (1950) phenylalanine oxidase test in a single medium by adding phenylalanine to Leifson's medium and supplementing with yeastrel. Both tests were read at 24 hours.

Amino-acid Decarboxylases.—The technique described here is based on Shaw and Clarke's (1955) method. It was, however, found impossible to obtain homogeneous suspensions from some mucoid strains of *Klebsiellae* by washing off agar slopes, and for this reason suspensions were harvested instead from fluid media.

Reagents.—All seven L. amino-acids listed in Table III were tested at pH 5.0 and 0.03 M, except glutamic acid, in which case the concentration was reduced to 0.01 M because of its strong buffer action at a low pH. A 0.0125 M phthalate buffer at pH 5.0 was used. The indicator was 0.04% bromcresol purple in distilled water at pH 5.0. All reagents were stored at 4° C., with a drop of toluene added to prevent the growth of moulds.

Tubes.—Non-sterile 3 in. by ½ in. tubes were used. Scrupulous chemical cleanliness was, however, essential, as traces of detergent produced false positive results.

Suspensions.—Strains under test were grown in peptone water at 37° C. for four hours.

As maximal decarboxylase activity develops at an acid pH, they were then subcultured to glucose broth and incubated for 16 hours at 37° C. The broths were then centrifuged at 3,000 r.p.m. for five minutes and supernatants discarded. The deposits were

suspended in saline, centrifuged, and the supernatants again discarded. Approximately 0.5 ml. of distilled water at pH 5.0 was added, and the deposits resuspended to give suspensions of concentrations greater than 10⁹ organisms/ml. (opacity tubes). As shown by Vagn Møller (1954a), the permeability of the bacterial cell membrane to amino-acids is increased by treatment with various lipid solvents. Consequently 0.05 ml. of toluene was added to the suspensions, which were then incubated at 37° C. for 10 minutes.

Test.—Apart from controls, seven tubes corresponding to the substrates were required for each strain under test. To each tube the following were added with a 50 dropper: (1) 2 drops of suspension, (2) 2 drops of substrate, (3) 2 drops of buffer, (4) 1 drop of indicator. The mixtures were then incubated at 37° C. for four hours.

Controls.—Two controls were employed with each set of tests, namely, (1) buffer, suspension, and indicator (no substrate); (2) buffer, heat-killed suspension, indicator, and substrate.

Readings.—The pH change was read at half, one, and four hours. Vagn Møller (1955) indicated that under these conditions of test pH change in an arginine substrate can be affected by arginine dihydrolase besides decarboxylase. For this reason the specificity of pH changes in arginine tubes was checked by Nesslerization, as ammonia formation precludes significant decarboxylase activity. Random tubes producing an indicator change in the lysine substrate were checked chromatographically. Butanol with 2N.NH₄OH was the solvent and an ascending technique with Whatman No. 1 paper was used. Spots were identified with 0.1% ninhydrin in chloroform.

Amino-acid Oxidases.—The technique is similar to that described for decarboxylase determinations. These tests, however, were carried out at a more alkaline pH, and the production of a keto-acid determined colorimetrically with ferric chloride rather than by indicator change.

Reagents.—L. amino-acids at pH 6.8 of the same molarity as previously were used. The buffer was 0.025 M phosphate at pH 6.8, and the keto-acid reagent 0.5 M ferric chloride.

Suspensions.—These were similarly prepared, except that strains were grown in nutrient rather than glucose broth and subsequently suspended in distilled water at pH 6.8.

Tests.—The mixtures were incubated at 37° C. and samples tested at one and 24 hours with a drop of ferric chloride.

Antibiotic Sensitivity.—Sensitivity to the eight antibiotics in Table II was tested by Kohn's (1953a) simple diffusion technique. Sensitivity limits of organisms inhibited by erythromycin, oleandomycin, and novobiocin were estimated by the usual tube technique. Titrations extended over a range of 40 to 0.15 units.

Results

Table II summarizes in percentage form the results of the above tests. For purposes of convenience, the 82 analysed strains are arranged in the seven groups indicated. Whereas all untyped and acapsulate strains from the Institute of Laryngology and Otology and the untyped N.C.T.C. strain 3279 are relegated to their appropriate group, the two acapsulate N.C.T.C. strains are classified with their corresponding capsular types. The captions *rhinoscleroma*, *ozaenae*, and *aerogenes* are used without prejudice at this stage.

The decarboxylase activity of all strains is analysed in Table III. Under the conditions of

TABLE II
SUMMARY OF BIOCHEMISTRY AND ANTIBIOTIC
SENSITIVITY OF 82 STRAINS OF *KLEBSIELLAE**

	<i>K. pneu- montiae</i> Type 1	<i>K. pneu- montiae</i> Type 2	<i>K. rhino- scleromatis</i> Type 3	<i>K. ozaenae</i> Types 4-6	<i>K. pneu- montiae</i> Types 8-13	Untypable and Acapsu- late Strains	<i>K. aerogenes</i>
No. of strains . .	23	5	7	9	6	25	7
Mucoid colonies	91	80	85	77	100	56	0
Tenacious colonies . .	78	60	71	66	16	4	0
Capsules . .	100	100	85	88	100	76	57
Motility . .	0	0	0	0	0	4	0
Adonitol . .	96	100	100	100	100	88	100
Inositol . .	74	80	42	100	100	52	100
Dulcitol . .	13	0	14	0	16	8	28
Glucose . .	100	100	100	100	100	100	100
" gas . .	91	100	14	33	83	96	85
Lactose . .	96	100	14	66	100	100	100
" gas . .	87	100	14	55	100	92	85
Saccharose . .	100	100	100	55	100	100	100
Mannitol . .	100	100	100	100	100	100	100
Maltose . .	100	100	100	88	100	100	100
Rhamnose . .	100	100	85	100	100	100	100
Xylose . .	100	100	100	100	100	100	100
Arabinose . .	100	100	100	100	100	100	100
Trehalose . .	100	100	100	100	100	100	100
Sorbitol . .	100	100	100	100	100	100	100
Salicin . .	96	100	100	100	100	96	100
Glycerol . .	61	100	85	100	100	52	100
Starch . .	100	100	100	100	100	100	100
Urea . .	87	100	14	44	100	52	85
" . .	65	100	14	44	100	100	85
Koser citrate . .	87	60	0	0	50	96	57
Voges Proskauer . .	13	40	100	100	50	4	42
Methyl red . .	96	100	100	88	100	100	100
Nitrate . .	0	0	0	0	0	0	0
Gelatin . .	0	0	0	0	0	8	71
Indole . .	0	0	0	0	0	0	0
H ₂ S . .	100	100	42	77	100	88	100
Cyanide . .	100	100	14	33	100	100	100
Gluconate . .	47	100	71	11	66	100	85
Malonate . .	4	0	0	0	0	0	0
Phenylpyruvic acid . .	100	100	14	33	100	24	100
Lysine decarboxylase . .	100	100	14	33	100	24	100

Percentage of strains sensitive to :							
Penicillin . .	0	0	0	0	0	0	0
Tetracycline . .	100	100	100	100	100	100	100
Streptomycin . .	100	100	100	100	100	96	100
Chloramphenicol . .	100	100	100	100	100	100	100
Polymyxin . .	78	60	71	88	66	60	71
Erythromycin . .	0	0	85	0	0	0	0
Oleandomycin . .	0	0	85	0	0	0	0
Novobiocin . .	0	0	85	0	0	0	0

*Expressed as percentages of strains giving positive reactions.

test, a shift of pH after half an hour is indicative of feeble enzyme activity. Feeble arginine decarboxylase activity was demonstrated in 40 strains. The specificity of these delayed pH changes was confirmed by Nesslerization, with negative results in all cases. As is apparent, the majority of typed strains, including aerogenes, but with the notable exception of all but one type 3 and more than half type 4-6 strains, possessed strong lysine in decarboxylase activity. These results are also listed in Table II for purposes of correlation with the other tests. The reaction products of randomly selected strains, exhibiting strong lysine decarboxylase activity, were determined chromatographically and spots with R_f values equivalent to cadaverine (decarboxylated lysine) were obtained (Fig. 1). By use of the combined malonate and phenylpyruvic acid medium, phenylalanine oxidase activity was detected in only one type 1 strain. Micro tests, however, proved uniformly negative.

Antibiotic sensitivity results determined by a diffusion technique are listed in Table II. Titrations demonstrated that all anaerogenic *rhino-*

FIG. 1.—Chromatographic identification of cadaverine produced by enzymatic decarboxylation of lysine. (1) Test: *Klebsiella* suspension, buffer, indicator, and lysine. (2) Control: Buffer, indicator, and cadaverine. (3) Control: Heated *Klebsiella* suspension, buffer, indicator, and lysine. (Solvent—*n*-butanol with 2N. NH₄OH; Whatman No. 1 paper; time of running—16 hr.)

TABLE III
AMINO-ACID DECARBOXYLASE ACTIVITY IN *KLEBSIELLAE*

	No.	Lysine				Arginine		Ornithine		Histidine		Tyrosine		Glutamic Acid		Phenylalanine	
		—	±	+	++	—	±	—	±	—	±	—	±	—	±	—	±
Type 1	23				23	10	13	18	5	23		23		23		23	
" 2	5				5	1	4	4	1	5		5		4	1	5	
" 3	7	5	1		1	7		7		7		7		7		7	
Types 4-6	9	4	2		3	9		9		9		9		9		9	
" 8-13	6				6	2	4	6		6		6		6		6	
Untypable and acapsulate ..	25	3	9		13	7	18	10	15	25		25		10	15	20	5
"Aerogenes"	7				7	6	1	7		7		7		7		7	
Total	82	12	12		58	42	40	61	21	82		82		66	16	77	5

++ = pH change less than ½ hour. + = pH change ½-1 hour. ± = pH change 1-4 hours. — = No change at 4 hours.

scleroma strains were sensitive to erythromycin, oleandomycin, and novobiocin over a range of 0.3 to 1.25 ml. with the exceptions of strain 1936 sensitive to 10 u./ml. oleandomycin and 5 u./ml. novobiocin, and strain 7799 sensitive to 5 u./ml. oleandomycin.

Discussion

Bergey's "Manual" (Breed, Murray, and Hitchens, 1948) divides the tribe *Escherichiae* into three genera, namely, *Escherichia*, *Aerobacter*, and *Klebsiella*. Edwards (1929) challenged the identity of *Aerobacter*, of which *aerogenes* was the type species, and concluded that there was no justification for its extragenetic differentiation from *Klebsiellae*. Kauffmann (1949), among other workers, proposed that *aerogenes* strains be classified in the *Klebsiella* genus. Nearly all strains originally submitted to the N.C.T.C. as *aerogenes* have recently been reclassified as *K. pneumoniae* (Medical Research Council, 1958). Cowan (1956) in a recent survey of problems of nomenclature suggested that *aerogenes* be accepted as a *Klebsiella* subgroup for the convenience of water bacteriologists, who would be loath to report on the isolation of *K. pneumoniae* from a potable water. By implication, however, he accepted that source of origin is not a valid taxonomic criterion and that the designation *aerogenes* has thus no true status. Furthermore, Clarke and Tracey (1956) recently ascertained that high yields of chitinase and cellulase, constitutive enzymes supposedly characterizing many species of soil and water bacteria including *aerogenes*, can also be isolated from *Klebsiellae* of human origin. Cowan (1954) also indicated the undesirability of the epithet *pneumoniae* for the *Klebsiella* type species. He suggested instead the name *friedlaenderi*, which does not suggest an obligate relationship with respiratory infections.

The *Klebsiella* genus was originally intended to include a heterogeneous group of non-motile

capsulated organisms, with an indole, methyl red, Voges-Proskauer and citrate formula (IMViC) — — + +. Both intragenetic and extragenetic biochemical and serological overlap have complicated the issue. Whether or not a particular organism be relegated to this genus may depend on the relative emphasis placed on any particular criterion. Serologically, for instance, an organism may belong to the *Escherichiae* and biochemically to the *Klebsiellae*—or vice versa. In such cases a decision as to which property represents the aberrance may prove impossible. In an attempt to discourage undue reliance on any given criterion as a basis for classification, Clarke (1955) suggested that the distribution of biochemical characters be best described on a statistical rather than on an "all or none" basis. Sneath (1957) has recently lent practical emphasis to this concept by demonstrating the application of mathematical computers to such taxonomical problems.

Kauffmann (1949, 1954) accepted only two biochemical species, *K. pneumoniae* and *rhinoscleroma*. Edwards and Fife (1955) further stressed that types 4-6, sometimes associated with *ozana*, represent a diffuse biochemical transition between these two species and not a discrete entity. Henriksen (1952) proposed that *ozanae* and *aerogenes* species be also recognized. Other workers, including Ørskov (1957), recommended the acceptance of further biochemical subtypes. The perpetuation of the name "*rhinoscleroma*" for type 3 organisms is based on tenuous evidence. Occasionally type 3 strains can be isolated from extranasal sites. Moreover, an aetiological relationship between the organism and disease is not proven. It is felt consequently that such strains should preferably be described numerically by their capsular antigen. The status of motile strains is still *sub judice*. Those liquefying gelatin are best classified as *Cloaca cloacae*, in accordance with Kauffmann (1954), or, as Cowan (1956) suggests, *Klebsiella cloacae*. Edwards and Fife

(1955) recommend the designation *Aerobacter cloacae*. Possibly non-liquefying strains represent biochemical variants, and, as Cowan (1956) further proposes, should also be included. The Institute of Laryngology and Otology strain 3502 of the present series was such a motile non-liquefying strain.

Typical *pneumoniae* strains are usually aerogenic, lactose, citrate, Voges-Proskauer, malonate, cyanide, gluconate, and lysine positive. Type 3 strains possess the reverse properties. Strains belonging to types 4-6 are sometimes aerogenic, citrate, cyanide, and lysine positive, and usually malonate and Voges-Proskauer negative. However, as indicated in Table II, biochemical aberrance is common in *pneumoniae*, especially in type 1 strains. It is less frequent in type 3 strains. One of the seven type 3 strains of the present series was aerogenic, lactose, and lysine positive. In fact, although serologically this strain (No. 615) belonged to type 3, it exhibited the biochemical features of a typical *pneumoniae*. Henriksen (1954) also described such strains and discussed this problem of mutually exclusive criteria.

Of the 82 strains of *Klebsiellae* studied in the present series, 15 were anaerogenic, of which six belonged to type 3 and five to types 4-6. As indicated previously, gas production in type 3 strains can be correlated with other biochemical features. The majority of anaerogenic strains were Voges-Proskauer negative and failed to ferment lactose. There were no other lactose-negative strains. Pickett and Cabelli (1953) stressed the low degree of biochemical reactivity of their lactose-negative strains, the majority of which, unlike those studied here, also failed to ferment adonitol and inositol. The fermentations of the former and to a lesser extent of the latter are fairly reliable differential tests between *Klebsiellae* and *Escherichiae*. Occasional strains failing to ferment these carbohydrates appeared otherwise biochemically typical. Dulcitol fermentation appears rare in *Klebsiellae*. Kauffmann (1954) pointed out that type 10 is characteristically dulcitol positive and this was in fact confirmed by the behaviour of the single type 10 strain studied. In addition, the aerogenic type 3 strain was also positive. In the few remaining dulcitol-positive strains, no other constant or significant biochemical deviation was observed.

Kligler (1914), on the basis of a biochemical comparison between *cloacae* and *aerogenes* strains, maintained that gelatin liquefaction was inversely related to gas production from glycerol. Edwards and Fife (1955) have shown, however, that

Klebsiellae occasionally fail to produce gas from glycerol, as did eight strains in the present series. No gelatin-liquefying strains were isolated, although, as Lautrop (1956) pointed out, extended incubation or the use of the Kohn (1953b) technique may possibly unmask some liquefiers. Delayed hydrolysis of urea and utilization of citrate is characteristic of most *pneumoniae* strains. Type 3 strains, with the exception of the aerogenic variant, were uniformly negative. Type 4-6 strains again behaved in an intermediate fashion, and those utilizing citrate did so only after four days. Occasional *pneumoniae* strains were also citrate and urease negative. Kauffmann (1954) pointed out that citrate-positive *Escherichiae* are very rare, and that no urease-positive strains have ever been described. Cowan (1957) has, however, encountered urease positive *Escherichiae*. Contrary to the findings of Kauffmann (1954), the single strains of *Klebsiellae* types 9 and 10 studied in this series were citrate positive. The methyl red and Voges-Proskauer are by no means clear-cut tests. Results depend on temperature, duration of incubation, method of testing, and, as indicated by Jennens (1954), the nature of the substrate. Of the 89 strains of *Klebsiella pneumoniae* recently studied by Vagn Møller (1954c), 89% were Voges-Proskauer positive at one day, but only 45% at four days. Variables such as these make it difficult to compare results under different conditions of test. However, in this study all type 3-6 strains were methyl red positive. Contrary to Leifson (1933), who found an absolute correlation between Voges-Proskauer production and malonate utilization, the strains studied here exhibited an approximate correlation only. Indole production, while rare in *Klebsiellae*, is usual in *Escherichiae*. Of 626 *Klebsiella* strains studied by Edwards and Fife (1955), only 26 were indole positive. "*Aerogenes*" type II strains are rare, but indole positive by definition. Apart from these, only two other strains from the Institute of Laryngology and Otology produced indole, namely, the motile strain 3502 referred to previously and also the acapsulate strain 3872 which was otherwise biochemically typical. Reduction of nitrates is a non-specific test and only one negative was encountered. It is generally accepted that failure to produce H_2S is a reliable exclusion test for *Klebsiellae*; all strains in this series were negative. Clarke (1953) has, however, demonstrated that by the use of N.C.T.C. methods of testing the reverse findings may be elicited.

Resistance to cyanide inhibition and the ability to form reducing end-products from gluconate

are differential tests of the utmost value. With the exception of some type 3-6 strains, the great majority of *Klebsiellae* tested were positive. There was, moreover, almost an absolute correlation between these tests.

The classical manometric studies of Gale (1946) on the decarboxylases demonstrated the varied and distinctive pattern of the enzymatic repertoire of different groups of bacteria. Subsequent workers, notably Woiwod (1949), Vagn Møller (1954c, 1955), and Shaw and Clarke (1955), introduced a variety of simplified techniques and stressed their practical application. Vagn Møller (1954c) examined a total of 671 *Enterobacteriaceae* and found that no strain, apart from *K. pneumoniae*, possessed powerful lysine and no other decarboxylase. The specificity of this test, as confirmed by the results in the present series, is thus high. Anaerogenic type 3 strains are consistently negative and *pneumoniae* strains almost invariably positive. Type 4-6 strains behaved in a variable manner, as did untyped and acapsulate strains. Unlike Shaw and Clarke (1955), who were able to demonstrate weak phenylalanine oxidase activity in some *Klebsiellae*, this was detected in one strain only by the combined medium and not by the micro test.

All strains examined were penicillin resistant, tetracycline, chloramphenicol, and streptomycin sensitive, with the exception of one, resistant to the last antibiotic. There is ample evidence, as reported by Finland, Murray, Harris, Kilham, and Meads (1946) and Ørskov (1952), that resistance to streptomycin often develops during treatment. Approximately two-thirds of strains were polymyxin sensitive. There appear to be scanty data on the sensitivity of *Klebsiellae* to some of the more recently introduced antibiotics. Coppo (1957) found that the minimum inhibitory concentration of novobiocin against 10 strains of "*aerogenes*" ranged from 250 to 500 u./ml. Erasmus (1956) reported on seven erythromycin-resistant untyped *Klebsiellae*. Apart from the aerogenic variant, all other type 3 strains exhibited surprising sensitivity to erythromycin, oleandomycin, and novobiocin. It is interesting that this previously unrecorded sensitivity pattern is restricted to the anaerogenic type 3 strains, which, as suggested previously, represent a homogeneous biochemical subtype.

Summary

A biochemical analysis of 82 *Klebsiella* strains is reported and some problems of nomenclature discussed. It is recommended that the name

friedlaenderi be substituted for *pneumoniae* as the *Klebsiella* type species and further that type 3 strains be no longer designated *rhinoscleroma*. No evidence for any other valid species was found.

It is suggested that the distribution of various properties, including biochemical, in the *Klebsiella* genus be best described on a statistical rather than on an "all or none" basis. It is further emphasized that the results of any biochemical test must be qualified by reference to the particular technique employed. Biochemical aberrance is common in *pneumoniae*, especially type 1 strains. *K. pneumoniae* is much more heterogeneous than the type 3 species, of which only two subtypes, the aerogenic and anaerogenic, appear to exist. Typical *pneumoniae* strains are usually aerogenic, lactose, citrate, Voges-Proskauer, malonate, cyanide, gluconate, and lysine positive, and exhibit delayed hydrolysis of urea. Type 3 strains, apart from the rare anaerogenic variety, possess the reverse properties. Type 4-6 strains on the whole behave in an intermediate fashion. The value of the cyanide, gluconate, and lysine decarboxylase tests is stressed, and there is a high degree of correlation between them. The majority of all *Klebsiella* strains ferment adonitol and inositol, but not dulcitol, fail to produce indole and H₂S, or to liquefy gelatin.

Attention is drawn to the sensitivity of anaerogenic type 3 strains to erythromycin, oleandomycin, and novobiocin. All other *Klebsiellae* are resistant to these antibiotics.

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