2. In the early stage of fat synthesis the fat is more acidic and contains a larger percentage of unsaponifiable substances than at any later period.

3. Until the stage of maximal activity of the mould is attained the actual amounts of both saturated and unsaturated component acids continue to increase, but in the subsequent phase of the life of the culture the weight of each of these components diminishes.

4. The results are not in accord with the hypothesis that, for the biosynthesis of fat, unsaturated acids are provided by dehydrogenation of saturated acids.

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The Composition of the Extracellular Polysaccharides of *Aerobacter–Klebsiella* Strains

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Since the earliest observations on coliform bacteria it has been known that organisms of the *Aerobacter–Klebsiella* group (i.e. *Aerobacter aerogenes*, *Aero. cloacae* and *Klebsiella* species) produce large amounts of slime and capsular material, and thus show mucoid growths. The polysaccharide nature of the slime and capsular material of these organisms was established by Emmerling (1900), Schardinger (1902), Toenniessen (1921) and Kramár (1922), and preliminary analyses of untyped strains showed galactose to be present in each. However, few detailed chemical studies have been made. The capsular polysaccharides of *Klebsiella pneumoniae* types A, B and C (corresponding to *Klebsiella* types 1, 2 and 3 of Edwards & Fife, 1952) have been examined more thoroughly and found to be glucose-containing polyuronides (Heidelberger, Goebel & Avery, 1925; Goebel & Avery, 1927; Goebel, 1927). Wilkinson, Dudman & Aspinall (1955) isolated the slime of *Klebsiella* type 54 (*Aero. aerogenes* A 3 (Sl)) from culture supernatants, and found it to be a polyuronide consisting of D-glucose (50%), L-fucose (10%) and an unidentified uronic acid (29%); a small amount of galactose (approx. 1%) detected in the slime was believed to be present only through contamination with an intracellular galactan. The composition of the slime was shown to be independent of the carbon source on which the organism had been grown. Recently, Levine, Stevenson, Bordner & Edwards (1955) examined the capsular polysaccharides of most known *Klebsiella* types by infrared spectrophotometry and found them all to be polyuronides, in some cases with esterified acetyl groups. No analysis has been reported of polysaccharides of *Aero. cloacae* strains.

Mucoid strains of *Aerobacter–Klebsiella* organisms have been differentiated into a very large number of immunologically distinct types (Edwards & Fife, 1952; Edmunds, 1954; Henriksen, 1954) whose specificities are determined by their capsular polysaccharide antigens (Julianelle, 1926; Edwards, 1929). These results indicate that, as with the pneumococcus, *Aerobacter–Klebsiella* strains of different serological type give rise to immunologically distinct extracellular polysaccharides. Levine *et al.* (1955) found the infrared spectrum of the capsular polysaccharide of each type to be different and distinct.

No attempts appear to have been made in previous studies of bacterial polysaccharides to isolate slime and capsular material separately in

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order to establish their relationship by comparative analysis. The view has been held that slime and capsule are of different origin, that slime is a secretion, whereas capsules arise through cell-wall swelling (Etinger-Tulczynska, 1933), but it is now generally believed that both slime and capsule are secretion products (see Dubos, 1945; Knaysi, 1951; Lamanna & Mallette, 1953). It has been shown with pneumococcus type III (Wood & Smith, 1949) and *Aerobacter–Klebsiella* strains (Edwards & Fife, 1952; Wilkinson, Duguid & Edmunds, 1954) that the slime and capsule of capsulate strains are identical antigenically. However, the chemical identity of slime and capsular polysaccharide has yet to be shown.

In the present investigation the composition of the extracellular polysaccharides of *Klebsiella* strains, representing five immunologically different types (types 8, 26, 29, 54 and 57 of the Edwards & Fife classification) was studied to find whether they differed from type to type. In addition, the slime and capsule of *Klebsiella* type 54 (strain A 3) were separately isolated and analysed. The extracellular polysaccharides of two strains of *Aero. cloacae* were also examined.

ORGANISMS AND METHODS

Organisms. *Klebsiella* strains A 1, A 3, A 4 and A 29 are capsulate strains isolated in this Department by Dr J. P. Duguid. Their biochemical and morphological characters are described by Edmunds (1954), who found them to be identical with known *Klebsiella* types of Edwards & Fife (1952). Strains A 1 and A 3 correspond with type 54, strain A 4 with type 8, and strain A 29 with type 57. Although strains A 1 and A 3 are identical antigenically, they differ in their characteristic capsule size, that of A 1 being much larger (Wilkinson *et al.* 1954). Strains E 26 (Sl) and E 29 (Sl) are non-capsulate slime-forming mutants isolated by Dr P. N. Edmunds from types 26 and 29 of Edwards & Fife. *Aero. cloacae* strains NCTC 5920 and NCTC 5936 were obtained from the National Collection of Type Cultures, Colindale, London; under most conditions they are non-capsulate organisms, producing small amounts of slime. The distribution of polysaccharide production in some of these strains has been studied by Duguid & Wilkinson (1953) and Wilkinson *et al.* (1954).

Cultural conditions. The organisms were grown in a simple synthetic medium of the following percentage composition: glucose, 1·0; $(NH_4)_2SO_4$, 0·03; Na_2HPO_4, 1·0; KH_2PO_4, 0·3; K_2SO_4, 0·1; NaCl, 0·1; $MgSO_4,7H_2O$, 0·02; $CaCl_2,6H_2O$, 0·002; $FeSO_4$, 0·0001 (all w/v). This medium contained the relatively high sugar and low nitrogen-source concentrations found to give maximal polysaccharide production (Duguid & Wilkinson, 1953; Wilkinson *et al.* 1954).

Initially, growth was carried out in Roux bottles in solid medium obtained by adding 2% of agar to the medium, but the use of solid medium was discontinued because of the risk of contaminating the polysaccharides with agar. This became especially important when small amounts of galactose were detected in some of the polysaccharide hydrolysates. Growth in aerated liquid medium, after inoculation with loopfuls of 24 hr. cultures, was carried out in 1 l. screw-capped bottles for 48 hr. at 35° in the manner described by Wilkinson *et al.* (1955).

Isolation and purification of the polysaccharides

Slime. The procedure for isolating slime polysaccharide was the same for capsulate and purely slime-forming strains. The liquid cultures were centrifuged at 13 000 rev./min. for 30–60 min. and the supernatants decanted and collected. Owing to the viscosity of the cultures it was not possible at this stage to remove completely all the cells, especially with strains producing large amounts of slime. The precipitation of the polysaccharide from the supernatant with acetone and the subsequent purification and final isolation of the purified material were carried out in the manner described by Wilkinson *et al.* (1955, method C).

Capsule. The capsular polysaccharide of capsulate strains was isolated from the cell residues collected from the initial centrifuging described above. Before decapsulation the cells were washed twice with equal volumes of water to remove any adherent slime. Unsuccessful attempts to remove the capsules of *Klebsiella* strain A 3 by shaking cells in the cold with water or NaCl solutions of up to 20% (w/v) concentration indicated that more drastic extraction conditions were necessary. Two different procedures were found to remove capsules successfully: (*a*) Boiling: cells were suspended in several vol. of water, the pH was checked and adjusted to 7·0 if necessary, and the suspension then boiled for 15 min. (*b*) Alkaline extraction: cells were suspended in 2–3 vol. of 1% (w/v) formaldehyde solution and set aside for 15 min. NaOH was added to a final concentration of 1% (w/v) and the suspension stirred at room temperature for 30 min. and then neutralized with N-HCl. The formaldehyde pretreatment was found to reduce cell disruption considerably, as shown by determining the amount of Kjeldahl N released by alkaline extraction from both pretreated and unpretreated cells. In the cells pretreated with formaldehyde, 25% of the total Kjeldahl N was in the supernatant fraction, but in the unpretreated cells the N value of the supernatant increased to 52%.

The boiled or NaOH-extracted suspension was then centrifuged and the sediment of decapsulated cells discarded. The capsular polysaccharide was precipitated from the supernatant by addition of acetone (2–3 vol.), the mixture being stirred with a spatula, when it was found that the polysaccharide adhered to the spatula as a stringy mass. This produced a partial purification from the intracellular polysaccharide which formed a flocculent precipitate which did not adhere to the spatula. The precipitate was then purified in the same way as the slime polysaccharides. The capsular polysaccharide of strain A 3 was isolated by both extraction procedures, and the boiled preparation found to be the purer product, as indicated by lower N and P values. All the other capsular polysaccharides were therefore isolated by the boiling method.

General analytical methods

The total N, total P, ash, reducing and anthrone values were determined by the methods described by Wilkinson *et al.* (1955). In addition to the 'anthrone equivalents' given previously, that of mannose was found to be 0·52.

Optical rotation. This was determined on 0·1% polysaccharide solutions in water, 1 dm. tubes being used. The turbidity of the solutions made it necessary to use this low polysaccharide concentration.

Identification and determination of component sugars

Glucose, galactose, fucose and mannose. These sugars were identified and estimated by the standard methods of paper chromatographic techniques applied to polysaccharide analysis. The polysaccharides were all hydrolysed under the same conditions, i.e. 24 hr. in 96% (w/v) formic acid at 100°, the formic acid was distilled off under reduced pressure, and the syrup further hydrolysed for 6 hr. in N-H_2SO_4 at 100°. The details are as described by Wilkinson *et al.* (1955).

For identification of the component sugars, the hydrolysates were neutralized with $BaCO_3$ and centrifuged and the supernatants evaporated to syrups. These were dissolved in small amounts of water and suitable small volumes applied to the starting lines of Whatman no. 1 chromatogram strips. The solvents used were *n*-butanol–ethanol–water mixture (5:1:4, by vol.) and *n*-butanol–acetic acid–water mixture (4:1:5, by vol.); irrigation with these solvents was carried out at room temp. for 96–120 hr. and 24–48 hr. respectively. Each hydrolysate was examined on replicate chromatograms, which were sprayed with a variety of reagents in order to detect different groups of sugars: (*a*) saturated aqueous aniline oxalate; (*b*) 1% (w/v) naphthoresorcinol in 0·25N-HCl; (*c*) the Morgan & Elson reagents (Partridge, 1948). Spots were identified by comparing their rate of movement, and colour, with those of monosaccharide standards run on the same chromatogram strips. In this way glucose, galactose, fucose and mannose were detected and identified.

For determination of the component sugars, the polysaccharides (15–30 mg. samples) were hydrolysed as described above, the uronic acid component was precipitated and removed as the Ba salt and the analysis carried out by the procedure used by Wilkinson *et al.* (1955). The reference sugar (15–30 mg.) used in these determinations depended on the composition of the polysaccharide; when mannose was present ribose was used, and when mannose was absent arabinose was used. These sugars were chosen because they could be easily separated from those in the hydrolysates.

Fucose. This was also determined separately on the unhydrolysed fucose-containing polysaccharides (75–100 μg. samples) by the colorimetric method of Dische & Shettles (1948) with the corrections described by Wilkinson *et al.* (1955).

Uronic acid. This was determined on the unhydrolysed polysaccharides by the decarboxylation method of McCready, Swenson & Maclay (1946). By trapping the CO_2 in 0·02N-NaOH and back-titrating with 0·02N-HCl, it was possible to carry out determinations on 20–30 mg. samples of polysaccharide. Control determinations with glucurone (20 mg.) gave reproducible recoveries of 104%.

Serological methods

Antiserum. Cultures of *Klebsiella* strain A3 were grown for 24 hr. at 35° on agar containing 0·5 g. of lactose and 0·5 g. of peptone/100 ml. Whole-culture suspensions (cells, capsule and slime) were made in 0·85% (w/v) NaCl solution to a density of about 10^9 cells/ml. Rabbits were injected with such suspensions to provide antiserum for *Klebsiella* strain A3. The antiserum, which was prepared and kindly provided by Dr P. N. Edmunds, was the same as that used by Wilkinson *et al.* (1954).

Precipitation tests. These were made to demonstrate the reaction of antiserum with solutions of some of the *Klebsiella* polysaccharides. Doubling dilutions of the antiserum in 0·85% (w/v) NaCl solution, from 1/2 to 1/32, were placed in narrow tubes and a polysaccharide solution (0·1% (w/v) in 0·85% (w/v) NaCl) superimposed. The tubes were incubated for 15 min. at 35° and examined, when a positive reaction was indicated by the appearance of a white ring at the interface.

Precipitin-absorption tests. The absorbing reagent (0·1%, w/v, polysaccharide solution in 0·85%, w/v, NaCl solution) was added dropwise with constant agitation to an equal volume of antiserum, and the mixture incubated at 35° for 30 min. After removal of the precipitate by centrifuging, the supernatant was tested with the appropriate polysaccharide solution to find whether precipitating activity had been lost as a result of absorption. If activity remained, more of the polysaccharide solution was added until absorption was complete.

RESULTS

The following polysaccharides were isolated, purified and examined; the slime of *Klebsiella* type 54 (A3), type 26 and type 29, and *Aero. cloacae* strains NCTC 5920 and NCTC 5936, and the capsular polysaccharide of *Klebsiella* type 54 (A1 and A3), type 8 (A4) and type 57 (A29). The yields of polysaccharide varied with strain and batch. For slime, the yields were of the order of 0·05–0·1 g./l. for *Klebsiella* type 54 (A3) and *Aero. cloacae* strains NCTC 5920 and NCTC 5936, and 0·2–0·3 g./l. for *Klebsiella* types 26 and 29. The yields of capsular polysaccharide varied between 0·2 and 0·4 g./l.

The polysaccharides were isolated as grey–white stringy masses. They were highly hygroscopic, absorbing moisture rapidly to a maximum of 15% in 4 hr., and when moistened swelled into gels. They dissolved in water with difficulty, in concentrations up to 1–2%, to give viscous solutions.

The homogeneity of the polysaccharide preparations was established by examination on the ultracentrifuge, 0·25% (w/v) solutions in 0·2M-NaCl being used. Their sedimentation diagrams indicated single molecular species in each preparation, with narrow molecular-weight distributions (Dudman & Greenwood, unpublished results).

Chemical composition

All the polysaccharides were found to contain uronic acid. With the exception of the slime of *Aero. cloacae* NCTC 5936, they were analysed quantitatively, and the results are given in Table 1.

At least two samples of each polysaccharide were hydrolysed, and the component sugars in each hydrolysate estimated in duplicate or triplicate. The results in Table 1 are expressed to the nearest whole number; the accuracy of the data relating to the component sugars is indicated by the closeness of the theoretical and experimental anthrone values. The components of the slime of *Aero.* Rao, 1951) and ethyl acetate–pyridine–water mixture (5:2:5, by vol.), but the results were unsuccessful. The specific colorimetric tests devised by Dische (1947, 1948) for distinguishing between glucuronic and galacturonic acids were carried out on hydrolysates of *Klebsiella* type 54 polysaccharides, with ambiguous results. The uronic acid components remain unidentified.

Table 1. *Analytical results on the polysaccharides of* Klebsiella (*and* Aerobacter cloacae) *strains*

Klebsiella type	54				8	57	26	29	
Strain	A3			A1	A4	A29	E26 (Sl)	E29 (Sl)	NCTC 5920
Nature of polysaccharide	Slime	Capsule		Capsule	Capsule	Slime	Slime	Slime	Slime
Method of extraction	—	Boiling	NaOH	Boiling	Boiling	—	—	—	—
Glucose (%)	47	43	37	48	22	0	35	0	17
Galactose (%)	1	2	4	1	51	21	26	31	26
Fucose (%)									
(*a*) Chromatographically	8	9	6	10	0	0	0	0	25
(*b*) Colorimetrically	9	9	7	11	—	—	—	—	28
Mannose (%)	0	0	0	0	3	44	23	13	0
Uronic acid (%)	30	27	26	29	25	28	17	25	22
Total percentage of sugar components	87	81	74	89	101	93	101	69	93
Reducing value of unhydrolysed polysaccharide (%, as glucose)	1·2	0·9	0·6	0·7	0·7	0·7	0·8	1·0	1·6
Anthrone value (%, as glucose)	59	59	54	60	52	40	55	40	58
Anthrone value theoretical (%, as glucose)	57	54	47	60	55	37	60	44	57
Ash (%)	7·8	6·2	9·2	5·5	2·3	3·2	5·0	8·9	4·3
N (%)	0·08	0·53	0·88	0·46	0·65	0·59	0·12	0·30	0·30
P (%)	0·46	0·11	0·38	0·02	0·25	0·02	0·59	1·08	0·19
$[\alpha]_D^{15}$	−59°	*	−82°	−77°	+95°	+220°	+138°	+128°	+110°

* Solution too turbid to read.

Table 2. *Highest dilution of antiserum giving precipitation reactions with various polysaccharides of* Klebsiella *type* 54

Antigen (polysaccharide)	A3 antiserum absorbed with					
	Nil	A1	A3 slime	A3* capsule	A3 (Sl)*	A3 (0) intracellular
A1	16	0	0	0	0	16
A3 slime	16	0	0	0	0	16
A3 capsule†	16	0	0	0	0	16
A3 (Sl)*	16	0	0	2	0	16
A3 (0) intracellular	8	8	8	4	8	0

* Freeze-dried preparation (Wilkinson *et al.* 1955). † Alkali-extracted.

aerogenes NCTC 5936 were identified as glucose, galactose, fucose and a uronic acid (21 %, estimated by the colorimetric method of Jarrige, 1947).

Attempts were made to identify the uronic acids of all the polysaccharides, by running the qualitative hydrolysates on chromatograms, together with standards of glucuronic, galacturonic and mannuronic acids, using solvents reported to be suitable for this purpose, e.g. ethyl acetate–acetic acid–water mixture (3:1:3, by vol.) (Rao, Beri &

Serological results

The immunological relationship of the following polysaccharides of *Klebsiella* type 54 strains were examined: capsule of A1, slime of A3, capsule of A3, slime of the non-capsulate mutant A3 (Sl) (described by Wilkinson *et al.* 1955), and an intracellular polysaccharide isolated from the non-slime-forming, non-capsulate mutant, A3 (0). (The origin and characteristics of this mutant have

been described by Wilkinson *et al.* 1954; the isolation and composition of the polysaccharide will be described in a later communication.) The results of the precipitation and precipitin-absorption tests, given in Table 2, show that the slime and capsule of A 3, the slime of A 3 (Sl) and the capsule of A 1 are antigenically similar or identical, confirming the findings of Wilkinson *et al.* (1954). They also show that the capsular polysaccharide of A 3 contained serologically detectable amounts of the A 3 (0) intracellular polysaccharide.

The high degree of serological activity of these purified polysaccharide preparations was demonstrated by titrating the A 3 antiserum (diluted 1/4) against increasing dilutions of A 3 (Sl) slime polysaccharide in 0·85% (w/v) NaCl, when positive results were obtained in the precipitation ring test with slime dilutions up to $1:10^6$.

DISCUSSION

The extracellular polysaccharides of the six strains of *Klebsiella* and two strains of *Aerobacter cloacae* examined were all found to be polyuronides, and they thus conform to the general pattern of bacterial extracellular heteropolysaccharides, most of which are known to possess acidic or basic component sugars. The results support the findings of Levine *et al.* (1955), based on infrared spectrophotometric evidence, that the capsular polysaccharides of all *Klebsiella* types contain uronic acids. All the polysaccharide samples examined here contained small amounts of nitrogen-containing material, which was shown not to be hexosamine, and was most probably the result of contamination with protein and nucleic acids.

Galactose was present in small amounts in the polysaccharides of *Klebsiella* type 54 strains (A 3 slime, A 3 capsule and A 1 capsule), but was most likely due to contamination with some intracellular polysaccharide. This view is supported by the results of the precipitin-absorption tests, which show that the A 3 capsular preparation containing the highest amount (4%) of contaminating galactose, when used to absorb anti-A 3 antiserum, slightly reduced the titre against the A 3 (0) intracellular polysaccharide. Preliminary analysis of this latter polysaccharide has shown it to be a galactan (Dudman & Wilkinson, unpublished results).

Fucose was unknown in bacteria until recently found in the polysaccharides of *Bacillus polymyxa* (Misaki, Hori & Teramoto, 1954), *Lactobacillus bifidus* (Norris, de Sipin, Zilliken, Harvey & György, 1954), *Klebsiella* type 54 strain A 3 (Sl) (Wilkinson *et al.* 1955), *Salmonella poona*, *Salm. grumpensis* and *Chromobacterium violaceum* (Davies, 1955). In the present study fucose was found in the polysaccharides of other strains of *Klebsiella* type 54 and the two strains of *Aero. cloacae*. Good correlation was obtained in the fucose determinations by the colorimetric and chromatographic methods.

The present results show that the five *Klebsiella* types produce extracellular polysaccharides which differ markedly in composition from each other. It will be seen from Table 1 that the polysaccharide of each type is qualitatively or quantitatively different from those of the other types studied here. Thus, in these *Klebsiella* types, immunological differences are accompanied by considerable differences in composition of the polysaccharide antigens. In many cases of immunological cross-reactions between unrelated organisms it has been found that the polysaccharide antigens contain uronic acid components. It is therefore believed that uronic acids dominate the specificity of antigens in which they are present, and that, when their steric arrangement in different antigens is sufficiently similar, cross-reactions take place. The absence of cross-reactions between any of the *Klebsiella* types studied here (see Edwards & Fife, 1952), despite the similar amounts of uronic acid present in the polysaccharide antigen of each type, indicates that the steric arrangement of uronic acid in each polysaccharide must be different, most probably as a result of the different composition of each polysaccharide.

By separately isolating and analysing the slime and capsular polysaccharide of *Klebsiella* type 54 strain A 3, it was shown that they are identical in composition. This is readily seen from the relative proportions of the component sugars, presented in Table 3. From the same table it can be seen that the polysaccharides of strains A 3 (Sl) and A 1 are identical in composition with A 3 polysaccharides The differences in $[\alpha]_D$ values (see Table 1) are not regarded as significant because the actual polarimetric readings were sufficiently small for differences of this magnitude to arise as a result of experimental error. Thus it has been shown that

Table 3. *Molecular ratios of component sugars in the extracellular polysaccharides of* Klebsiella *type* 54 *strains, relative to glucose as* 100

	Component sugars		
Polysaccharide	Glucose	Uronic acid*	Fucose†
A 1 capsule	100	56	25
A 3 slime	100	59	21
A 3 capsule‡	100	58	23
A 3 (Sl) slime§	100	54	22

* Assumed to be hexuronic acid.
† Calculated from colorimetric values.
‡ Extracted by boiling.
§ Freeze-dried preparation (Wilkinson *et al.* 1955).

the slime and capsule of a capsulate strain, and the slime of the non-capsulate slime-forming mutant derived from it, are chemically identical. Further proof of chemical identity, however, must await structural investigations and molecular-weight determinations.

The compositional and immunological identity of the polysaccharides of A 1, A 3 and A 3 (Sl) is of particular interest, in view of the results of Wilkinson *et al.* (1954), who found large physical differences between the capsules of A 1 and A 3 and the slime of A 3 and A 3 (Sl). Similarly, Macpherson, Wilkinson & Swain (1953) found differences in the ability of these polysaccharides, after prior NaOH treatment, to inhibit haemagglutination by the influenza group of viruses. Thus the capsular and slime polysaccharides of strain A 3 behaved similarly in inhibiting haemagglutination of PR 8 influenza virus at a concentration of 2·5 μg./ml., and a concentration of 333 μg./ml. of A 1 polysaccharide was required. A 3 (Sl) slime polysaccharide was completely non-inhibitory. Although all these type 54 polysaccharides were absorbed on the red-cell surface, it was found that there was a relationship between the degree of inhibition and the lowering of the red-cell surface charge, presumably owing to ionization of the carboxyl groups of the uronic acids of the polysaccharides. Thus there again appears to be some physical difference in the various polysaccharides, according to the availability of carboxyl groups for ionization. The production of the same polysaccharide in different physical states by closely related strains is one of the many problems about the apparently simple process of capsule and slime formation for which there is at present no explanation. Despite the advances made in the study of the chemical composition and factors influencing the production of bacterial polysaccharides, little is known as to why extracellular polysaccharides are produced in some strains as slime and capsule and in others as slime alone. Any hypothesis must account for the facts that in a capsulate strain the slime and capsule are composed of the same polysaccharide, and that by an apparently single mutable step a capsulate strain may give rise to a non-capsulate slime-forming mutant producing the same polysaccharide. It is possible that the problem of capsule formation may be finally elucidated when observations are made to discover what happens when a slime-forming capsulate strain becomes a slime-forming non-capsulate mutant.

SUMMARY

1. The extracellular polysaccharides of six *Klebsiella* strains, corresponding to five immunological types (types 8, 26, 29, 54 and 57), and two strains of *Aerobacter cloacae* (NCTC 5920, NCTC 5936), were isolated and purified, and shown to be complex polyuronides.

2. The polysaccharide of each *Klebsiella* type was found to be qualitatively or quantitatively different in composition from those of other types.

3. The slime and capsular polysaccharides of *Klebsiella* type 54 strain A 3 were separately isolated and analysed, and found to be identical in composition and immunological specificity.

4. The chemical and immunological relationship of the extracellular polysaccharides of *Klebsiella* type 54, strains A 1, A 3 and A 3 (Sl), was examined and they were found to be identical.

5. The slime polysaccharide of strain A 3 (Sl) in dilutions of $1:10^6$ reacted with homologous immune serum.

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The Fate of ^{131}I-Labelled Homologous and Heterologous Thyroglobulins in the Rat, Dog, Monkey and Rabbit

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Iodine is readily fixed by the thyroid gland, where it is rapidly incorporated into thyroglobulin. If radioactive iodide is administered to an animal the gland may be damaged and total destruction can easily be accomplished. Thyroglobulin from the damaged tissue passes into the circulation, where its presence has been recognized by means of chromatographic and ultracentrifugal methods (Tong, Taurog & Chaikoff, 1952; Robbins & Rall, 1952; Brown & Jackson, 1954; Robbins, Peterman & Hall, 1954). Examination of the plasma of patients with thyroid carcinoma after treatment with large doses of ^{131}I (approx. 150–200 mc) has shown that a range of patterns of radioactive components occurs (Brown & Jackson, 1954). These appear to be related to a varying ability of different individuals to metabolize the thyroid protein liberated into the blood as a result of the destructive effects of the radioactive iodide. It seemed of interest therefore to study the metabolism of thyroglobulin in experimental animals. In the present paper, results are described from an examination of the fate of ^{131}I-labelled homologous and heterologous thyroglobulin in various species. A preliminary account of some of this work has already been published (Brown & Jackson, 1955).

EXPERIMENTAL

Preparation and administration of labelled thyroglobulin. At a suitable interval after the injection of carrier-free [^{131}I]iodide, animals were killed, the thyroid glands removed, ground with powdered glass with additions of ice-cold water and centrifuged. The precipitate, which invariably contained less than 5% of the total radioactivity of the gland, was discarded. The supernatant was stored at −20°. Over 99% of the radioactive iodine in these preparations was protein-bound, and paper-electrophoretic examination indicated the presence of a single radioactive component which, in the presence of plasma, was associated with the α-globulins.

Portions of the appropriate thyroglobulin solution containing 50–5000 μg. of protein/kg. body wt. were injected intravenously into animals and samples of venous blood obtained at suitable intervals. In rats, the tissue distribution of radioactivity after the administration of thyroglobulin was studied in groups of animals which were killed at various times. The tissues under investigation were rapidly removed and digested in LiOH solution (10 g. of LiOH, 1 g. of KI in 100 ml. of 20%, v/v, ethanol) and portions counted in a M6 liquid counter. Plasma and whole-blood samples (0·1 ml.) were dried on aluminium trays and their radioactivity was measured directly under a mica end-window counter.

Chromatography and fractionation procedures. Portions (10 μl.) of plasma, to which marker diiodotyrosine, triiodothyronine and thyroxine had been added, were analysed by ascending chromatography on Whatman no. 1 paper in butanol–dioxan–2N NH_3 (4:1:5, by vol.). The position of the marker substances was ascertained by developing with ninhydrin; the paper was then cut into small sections and the radioactivity measured under an end-window counter. In some instances, plasma samples were fractionated by a procedure which has already been described (Brown & Jackson, 1954). This involved the treatment of plasma with silver phosphate (hence estimation of iodide) and precipitation of the proteins with methanol (hence determination of the radioactivity present as thyroglobulin).

RESULTS

All the results described refer to ^{131}I-labelled thyroglobulin.

Metabolism of rat thyroglobulin

The clearance of this protein from the plasma of the rat, dog, monkey and rabbit that follows the intravenous administration of rat thyroglobulin is