THE ANTIBACTERIAL ACTIVITY OF NEW DERIVATIVES OF 4-AMINOQUINOLINE AND 4-AMINOQUINALDINE

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Two new series of *N*-alkyl derivatives of 4-aminoquinoline and 4-aminoquinaldine are described. Compounds in both series show antibacterial and protein precipitating properties and it has been possible to correlate bactericidal action and protein precipitation for homologous members of the series. The bacteriostatic activity of both series of compounds is characterised by maxima at C = 12 to 14, with a marked decrease in potency at longer chain lengths. The bactericidal activity differs from this in that activity is not greatly diminished with the higher homologues. The 4-aminoquinaldinium salts are slightly more potent bactericidal agents than the corresponding 4-aminoquinolinium compounds. It is concluded that the mechanism of the antibacterial action of these derivatives may be closely related to the means by which they interact with proteins.

THE antimicrobial activity of certain polymethylene heterocyclic bis-quaternary ammonium salts has been described on a number of occasions (Collier, Potter and Taylor, 1953, 1955; Babbs, Collier, Austin, Potter and Taylor, 1956; Austin, Potter and Taylor, 1958; Austin, Lunts, Potter and Taylor, 1959). These compounds possess marked antifungal and antibacterial activity; the former reaching its highest point in the polymethylene bis-isoquinolinium series at a chain length of sixteen methylene groups (hedaquinium, Teoquil), and the latter its maximum activity in the polymethylene bis-4-aminoquinaldinium series at a chain length of ten methylene groups (dequalinium, Dequadin).

Continued interest in this field has led to the present investigation of the allied 1-alkyl derivatives of 4-aminoquinoline and 4-aminoquinaldine. A preliminary note on the activity of the latter series has already appeared (Cox and D'Arcy, 1959). The formation of insoluble complexes between quaternary ammonium compounds and proteins has been long known and the relationship between this property and antibacterial activity has been sought. For example Kuhn and Bielig (1940), using benzyl dimethyl dodecyl ammonium bromide and various proteins, demonstrated that the concentrations of quaternary salt required to precipitate proteins and to kill bacteria were about the same. Larose and Fischer (1952) using wool as the test protein showed that a similar relation existed between the bactericidal activity of some antiseptics and their degree of adsorption to wool.

Since, during our present investigation, it became clear that a rapid screening test would be useful to eliminate those compounds likely to have little or no antibacterial activity, before proceeding with more rigorous bacteriological testing, we have investigated a test based on the ability of certain quaternary compounds to precipitate proteins from very dilute solution. Using this test an attempt has been made to correlate protein

precipitating activity and the antibacterial activity of the 4-aminoquinolinium and the 4-aminoquinaldinium series.

EXPERIMENTAL

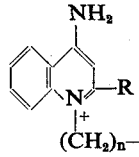
Materials and Methods

Chemical syntheses. 2-Methylcinchoninamide was prepared by a modification of a published method (German Patent 1913) and was converted into 4-aminoquinaldine by the Hofmann reaction using sodium hypochlorite; 4-aminoquinoline was prepared by the method of Royer (1949); all the alkyl halides were obtained using standard procedures.

The quaternary ammonium iodides were prepared by the reaction of a slight excess of the alkyl iodide with the heterocyclic amine in ethyl methyl ketone at 80° for from 6 to 90 hr. After recrystallisation and analysis the

TABLE I

ANALYTICAL RESULTS: 1-ALKYL 4-AMINOQUINOLINIUM AND 4-AMINOQUINALDINIUM IODIDES



R	n	Reaction time hr.	m.p. uncorrected	Crystn solvent*	Found per cent				Formula	Required per cent			
					C	H	I	N		C	H	I	N
H	9	6	139·5	E.M.K.	55·3	7·2	31·15	7·0	$C_{19}H_{29}IN_2$	55·3	7·1	30·8	6·8
,,	10	7	143	,,	56·2	7·3	30·2	6·9	$C_{20}H_{31}IN_2$	56·3	7·3	29·8	6·6
,,	11	6	136	,,	57·0	7·6	28·8	6·1	$C_{21}H_{33}IN_2$	57·3	7·6	28·8	6·4
,,	13	6	145·5	,,	58·7	8·1	27·5	6·0	$C_{23}H_{37}IN_2$	59·0	8·0	27·1	6·0
,,	15	16	157·5	,,	60·6	8·25	25·6	5·6	$C_{25}H_{41}IN_2$	60·5	8·3	25·6	5·6
,,	17	16	180·5	,,	61·9	8·6	24·05	5·4	$C_{27}H_{45}IN_2$	61·8	8·6	24·2	5·3
Me	6	68	228	EtOH	53·0	6·4	33·3	7·3	$C_{17}H_{25}IN_2$	53·2	6·6	33·0	7·3
,,	9	80	197	I.P.A.	56·1	7·4	29·7	6·8	$C_{20}H_{31}IN_2$	56·3	7·3	29·75	6·6
,,	10	90	192	E.M.K.	57·4	7·6	28·5	6·6	$C_{21}H_{33}IN_2$	57·25	7·55	28·8	6·4
,,	11	50	182	,,	58·5	7·7	27·6	6·4	$C_{22}H_{35}IN_2$	58·2	7·7	28·0	6·2
,,	13	80	180	,,	59·8	7·9	26·4	5·8	$C_{24}H_{39}IN_2$	59·8	8·1	26·3	5·8
,,	15	80	158	I.P.A./H_2O	61·4	8·35	24·8	5·5	$C_{26}H_{43}IN_2$	61·2	8·5	24·85	5·5
,,	17	100	165	E.M.K.	62·5	8·9	23·25	5·1	$C_{28}H_{47}IN_2$	62·4	8·8	23·6	5·2

*E.M.K. = ethylmethyl ketone, I.P.A. = isopropanol.

iodides were converted by the conventional silver salt method to the corresponding acetates. The latter were more soluble in water than the iodides and were therefore more convenient for the antibacterial testing.

Protein precipitation test. The proteins used for this test were pepsin B.P., casein (soluble and reprecipitated), gelatin (275-Duche), and egg albumin (Judex).

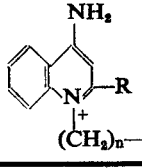
A solution of protein of concentration 0·125 per cent was prepared in 0·05 M sodium acetate and adjusted to pH 8·5 with sodium hydroxide. To 10 ml. portions of the solution specific amounts of a 0·125 per cent solution of a quaternary ammonium salt were added, and the final volume in each tube made up to 20 ml. with distilled water. This gave a protein final concentration of 62·5 mg./100 ml. The tubes were examined in a strong light, and after 1 hr. the weight of the quaternary salt required to cause turbidity was calculated.

Test for bactericidal activity. A bacterial cell suspension was prepared by washing the 20 hr. growth from an agar surface, centrifuging the bacterial cells (R.C.F. 600*g*/15 min.) and suspending them in sterile distilled water to a standard opacity (Wellcome opacity tube No. 7). The quaternary salts under examination were made up to a specified concentration in distilled water with a final volume of 9 ml. A control tube containing 9 ml. of sterile distilled water alone was included in each test.

At zero time, 1 ml. of culture suspension was added with shaking to each quaternary solution and also to the control tube. After time intervals of 2·5 and 15 min. 1 ml. samples of culture-quaternary mixture

TABLE II

ANALYTICAL RESULTS: 1-ALKYL 4-AMINOQUINOLINIUM AND 4-AMINOQUINALDINIUM ACETATES



R	n	Reaction time hr.	m.p. uncorrected	Crystn* solvent	Found per cent C	Found per cent H	Found per cent N	Formula	Required per cent C	Required per cent H	Required per cent N
H	9	18	120	I.P.A./I.P.AcO.	71·4	9·2	8·0	$C_{31}H_{53}N_3O_5\frac{1}{2}H_2O$	71·4	9·4	8·0
,,	10	18	125	,, ,,	73·7	9·8	7·9	$C_{32}H_{54}N_2O_2$	73·7	9·6	7·8
,,	11	18	122	,, ,,	74·0	9·8	7·8	$C_{33}H_{56}N_2O_2$	74·2	9·7	7·5
,,	13	18	128	,, ,,	75·3	9·8	7·2	$C_{35}H_{60}N_2O_2$	75·0	10·1	7·0
,,	15	17	133·5	,, ,,	75·3	10·45	6·7	$C_{37}H_{64}N_2O_2$	75·7	10·35	6·5
,,	17	17	130·5	,, ,,	76·5	10·6	6·2	$C_{39}H_{68}N_2O_2$	76·3	10·6	6·1
Me	6	16	207	,, ,,	70·4	8·7	8·4	$C_{19}H_{28}N_2O_2\frac{1}{2}H_2O$	70·15	9·0	8·6
,,	9	6	182	,, ,,	70·2	9·6	7·5	$C_{22}H_{34}N_2O_2H_2O$	70·2	9·6	7·4
,,	10	18	178	,, ,,	70·75	9·7	7·5	$C_{23}H_{36}N_2O_2H_2O$	70·7	9·8	7·2
,,	11	3	170	Acetone	71·0	10·0	7·1	$C_{24}H_{38}N_2O_2H_2O$	71·25	10·0	6·9
,,	13	14	161	I.P.A./I.P.AcO.	72·3	10·1	6·3	$C_{26}H_{42}N_2O_2H_2O$	72·2	10·25	6·5
,,	15	18	154	,, ,,	73·3	10·1	6·2	$C_{28}H_{46}N_2O_2H_2O$	73·0	10·5	6·1
,,	17	4	159	,, ,,	76·7	10·9	5·5	$C_{36}H_{56}N_2O_2$	76·6	10·7	5·9

* I.P.AcO. = isopropyl acetate.

were withdrawn and immediately mixed with 9 ml. of 2 per cent Bacto-oxgall to inactivate the excess quaternary ammonium compound in the sample. The inactivated suspensions were then shaken and, from these, 1 ml. quantities were serially diluted tenfold in distilled water and plated out on agar. The number of bacterial colonies formed after incubation for 24 hr. was counted, and the loss of viability determined by reference to the control count.

Test for bacteriostatic activity. The culture medium used was glucose-peptone water (0·5 per cent glucose, 1 per cent peptone (Bacto), 0·5 per cent sodium chloride; at pH 7·2). Five ml. quantities of this medium were distributed into test tubes and sterilised by autoclaving at 115° (10 lb. sq. in./10 min.). The quaternary compounds were dissolved in sterile distilled water to give double-strength solutions, and these were mixed aseptically with double-strength medium in the first tube of each series. Serial twofold dilutions were then made leaving 5 ml. of medium in each tube. The tubes were inoculated with 0·02 ml. of the appropriate

bacterial culture (*Staphylococcus aureus* C.N. 491: *Streptococcus viridans* Eden; *Escherichia coli*, Laboratory strain) containing approximately 1×10^6 organisms/ml. The tubes were incubated at 37° and the minimal inhibitory concentration (M.I.C.) of each quaternary was determined after 24 hr. and 5 days incubation.

TABLE III

KNOWN ANTIBACTERIAL AGENTS: CONCENTRATION OF CATION TO PRECIPITATE PROTEINS

Antibacterial agent	Cation μg./ml. to precipitate protein				Antibacterial activity against *Staph. aureus* C.N. 491 M.I.C. μg./ml.
	Casein	Pepsin	Gelatin	Egg albumin	
Chlorhexidine acetate	9·1	13	254	14	0·25
Benzalkonium chloride	51	27	250	40	0·31
Cetrimide	33	24	180	24	0·63
Dequalinium chloride	12	9	174	18	0·31
Domiphen bromide	33	5·1	76	15	0·31
Hedaquinium chloride	28	21	>625	26	0·31
5-Aminoacridine	37	>625	340	97	6·25
Polymyxin B	6	5	>625	12	0·63

TABLE IV

CONCENTRATION OF QUATERNARY CATION REQUIRED TO PRECIPITATE PROTEINS μg./ml. 4-AMINOQUINOLINIUM ACETATES

No. of carbon atoms in chain	Casein	Pepsin	Gelatin	Egg albumin
10	24	24	230	27
11	19	19	89	20
12	7·5	6·5	35	8
14	7	4·5	20	5
16	5	3	24	4·5
18	5	4·5	24	4

TABLE V

CONCENTRATION OF QUATERNARY CATION REQUIRED TO PRECIPITATE PROTEINS μg./ml. 4-AMINOQUINALDINIUM ACETATES

No. of carbon atoms in chain	Casein	Pepsin	Gelatin	Egg albumin
7	61	410	>525	127
10	23	26	156	21
11	10	13	58	24
12	11	10	32	5
14	11	5·5	19	4
16	11	5·5	19	3
18	8	5·5	25	4

RESULTS

Analytical. The results of analytical tests on the 1-alkyl-4-aminoquinolinium and the 1-alkyl-4-aminoquinaldinium iodides are shown in Table I and those of the corresponding acetates in Table II.

Protein precipitation test. Initial experiments with this technique were made with known quaternary ammonium compounds, and a wide difference in the behaviour of the compounds was noted. For example the following quaternary salts failed to precipitate any of the proteins tested: hexamethonium iodide, decamethonium iodide, decane-1,10-pyridinium bromide, benzyltrimethylammonium hydroxide, phenyltrimethylammonium-*p*-toluenesulphonate, decane-1,10-*N*-methylpyrrolidinium iodide, 3-phenylpropyl-4-aminoquinolinium acetate, and hexyl-3,5,5-trimethyl-4-aminoquinolinium acetate. However, it is known that

these compounds possess little or no antibacterial activity. In contrast to these results protein precipitation was caused by other quaternary ammonium compounds of known antibacterial activity, namely: benzalkonium chloride, cetrimide, dequalinium chloride, domiphen bromide, hedaquinium chloride; the antibacterials chlorhexidine acetate, 5-aminoacridine, and the antibiotic polymyxin B. The results with these latter compounds are summarised in Table III.

Figures for the protein precipitating activity of the acetates of the 4-aminoquinolinium series are shown in Table IV and those of the 4-aminoquinaldinium series in Table V.

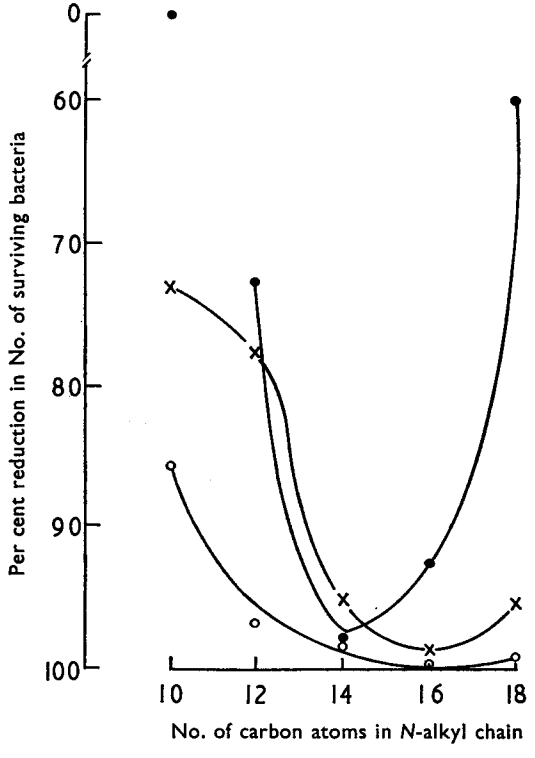


FIG. 1. Bactericidal activity of the 4-aminoquinolinium acetates against *Staph. aureus* (○—○), *Str. viridans* (●—●) and *E. coli* (×—×) at a drug concentration of 10 μg./ml., and a bacteria-drug contact time of 2·5 min.

Bactericidal activity. The results of bactericidal tests on members of the two series of compounds against the three organisms at a drug concentration of 10 μg./ml. and at a bacteria-drug contact time of 2·5 min. are shown graphically in Figs. 1 and 2. In a similar manner, Figs. 3 and 4 summarise their bactericidal activity at a contact time of 15 min. On these graphs the number of carbon atoms in the *N*-alkyl chain has been plotted against the percentage reduction in the survival of the exposed

bacteria. In each experiment the drug-bacteria ratio was 100 μg. drug: 0·7 mg. dry weight of bacteria.

From these results, it is apparent that both series of compounds have bactericidal activity against the organisms tested. In the 4-aminoquinolinium series, the hexadecamethylene member would seem to be the

TABLE VI

BACTERIOSTATIC ACTIVITY 4-AMINOQUINOLINIUM AND 4-AMINOQUINALDINIUM ACETATES

Micro-organism	Chemical series	Bacteriostatic activity (Minimal Inhibitory Concentration μg./ml. at 24 hr.)					
		Homologue: No. of carbon atoms in chain					
		7	10	12	14	16	18
Staphylococcus aureus C.N. 491	4-Aminoquinolinium	—	0·63	0·16	0·44	1·25	10
	4-Aminoquinaldinium	1·90	0·31	0·14	0·24	0·84	3·12
Streptococcus viridans	4-Aminoquinolinium	—	3·12	0·55	0·78	1·10	4·4
	4-Aminoquinaldinium	17·7	1·40	0·35	0·49	0·99	12·5
Escherichia coli	4-Aminoquinolinium	—	25	12·5	50	100	>100
	4-Aminoquinaldinium	100	12·5	8·8	17·7	100	>100

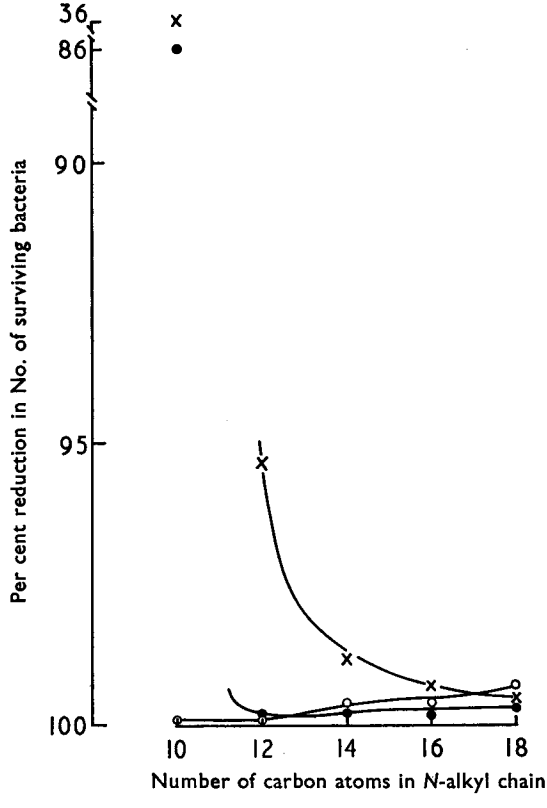


FIG. 2. Bactericidal activity of the 4-aminoquinaldinium acetates against *Staph. aureus* (○—○), *Str. viridans* (●—●), and *E. coli* (×—×), at a drug concentration of 10 μg./ml. and at a bacteria-drug contact time of 2·5 min.

most potent against the three species after 15 min. contact. In the 4-aminoquinaldinium series the hexadecyl and the octadecyl members are equally the most effective against *E. coli*, whilst against *Staph. aureus* and *Str. viridans* good activity is shown by the C_{12} and C_{14} members as well.

Bacteriostatic activity. The results of the bacteriostatic experiments with the 4-aminoquinolinium and the 4-aminoquinaldinium acetates are shown in Table VI; activity is shown against *Staph. aureus*, *Str. viridans* and *E. coli*.

The individual members of both series have high activity against the three bacterial species; in each series the maximal activity against *Staph.*

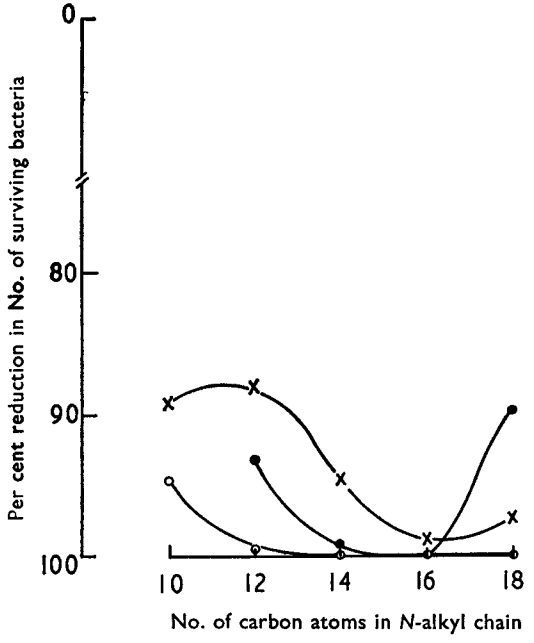


FIG. 3. Bactericidal activity of the 4-aminoquinolinium acetates against *Staph. aueus* (○—○), *Str. viridans* (●—●), and *E. coli* (×—×), at a drug concentration of 10 μg./ml. and at a bacteria-drug contact time of 15 min.

aureus and *Str. viridans* is shown by members with a polymethylene chain length of C = 12 to 14, whilst against *E. coli* activity is maximal at a chain length of C = 10 to 12.

Protein precipitating activity and bactericidal potency. The protein precipitating activities (μg. quaternary cation/ml.) of the two series against casein, pepsin, gelatin and egg albumin (Tables IV and V), have been plotted against chain length to give the results in Figs. 5 and 6, and it is interesting to note the similarities between these graphs and those obtained by plotting bactericidal activity against chain length, particularly in the 4-aminoquinaldine series (Figs. 2 and 4). For example: the chain length range over which the compounds display strong activity of both types

seems to be the same, and in addition both these activities seem to decrease at chain length C = 18 and beyond.

The correlation of protein precipitating activity and bacteriostatic activity is not so striking. Graphs drawn plotting bacteriostatic activity against chain length for the two series, reach maxima at C = 10 to 14 and then fall away rapidly at higher chain lengths.

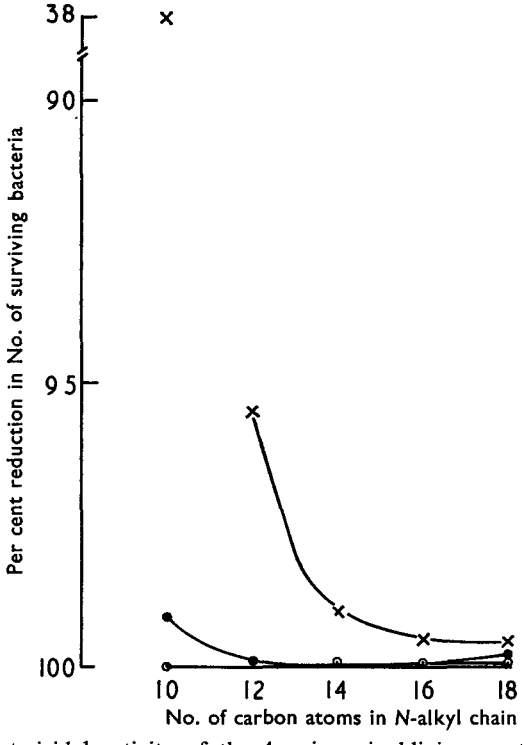


FIG. 4. Bactericidal activity of the 4-aminoquinaldinium acetates against *Staph. aureus* (○—○), *Str. viridans* (●—●), and *E. coli* (×—×), at a drug concentration of 10 μg./ml. and at a bacteria-drug contact time of 15 min.

DISCUSSION

Members of the 4-aminoquinolinium and 4-aminoquinaldinium series possess marked protein precipitating, bactericidal and bacteriostatic activity. Both series of compounds show maximal activity over the range of polymethylene chain length C = 12 to 16.

The results of the bactericidal tests show that members of the 4-aminoquinaldinium series are more potent than the homologous members of the 4-aminoquinolinium series. In addition the length of time that the organism is in contact with the drug has less effect upon bactericidal activity in the quinaldinium series than it has in the quinolinium series. In the latter it is much greater after 15 min. than after 2·5 min. contact time. In contrast, there is little difference in the bactericidal effects of the members of the quinaldinium series at the two times of contact.

Protein precipitation is caused by much the same concentration of each of the quaternary homologues in both series. However, higher concentrations of these compounds are needed to precipitate gelatin than to precipitate casein, pepsin and egg albumin. This is especially evident with the C_{10} homologues of both series, and there is an interesting analogy between the relatively poor activity of these members as bactericidal

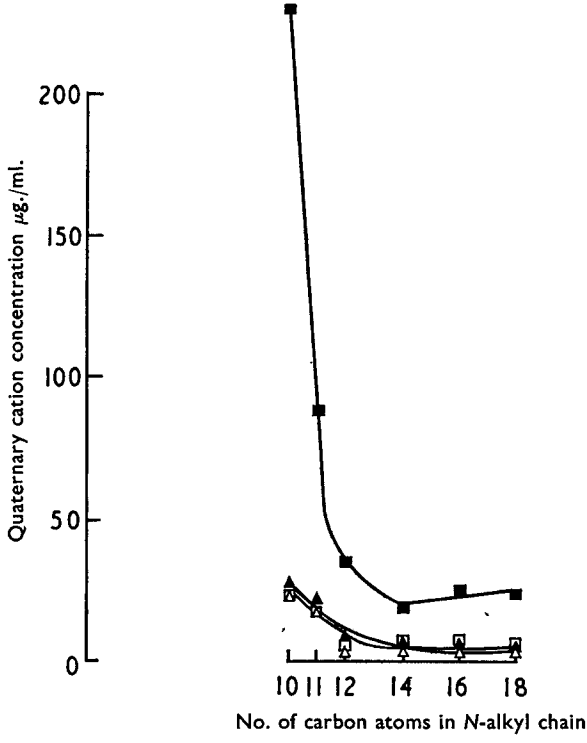


FIG. 5. Protein precipitating activity of the 4-aminoquinolinium acetates against casein (□—□), pepsin (△—△), gelatin (■—■), and egg albumin (▲—▲).

agents and their low activity as gelatin precipitants. The relation between protein precipitating and bactericidal activity can be put on a semi-quantitative basis by comparing the quaternary compound: protein ratio with the quaternary compound: bacteria ratio at the critical concentration of quaternary compound causing both protein precipitation and bactericidal effects.

A comparison of the relative amounts of protein and bacterial cells used in the experiments may be obtained from the formula, concentration of protein, divided by concentration of bacteria, both as mg. of dry weight per cent. This equals $62{\cdot}5 \div 7 \simeq 9$.

In this formula 62·5 is derived from the final concentration (mg. per cent) of protein in the quaternary compound-protein mixture used in the

protein precipitating experiments (0·625 mg./ml.), and where 7 is derived from the final strength (mg. per cent) of bacteria in the quaternary compound-bacteria mixture used in the bactericidal experiments (0·07 mg./ml.).

Therefore, since the concentration of quaternary compound used in all bactericidal experiments was 10 μg./ml., the equivalent concentration of quaternary compound required, in protein precipitating experiments, to give an equivalent quaternary compound: protein ratio to that in the

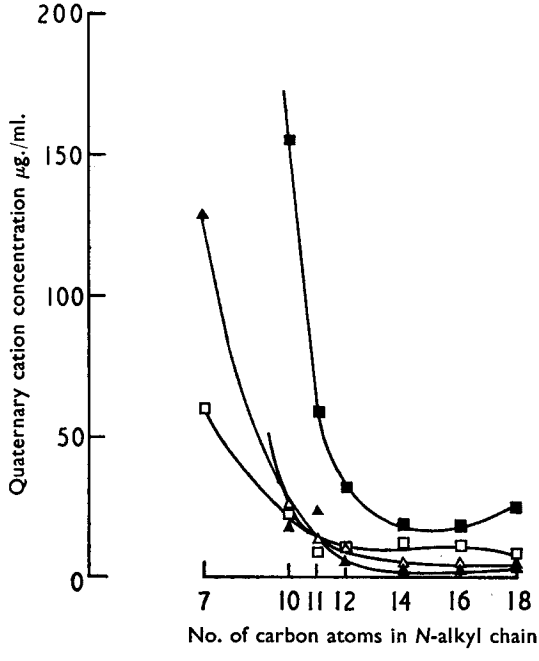


FIG. 6. Protein precipitating activity of the 4-aminoquinaldinium acetates against casein (□—□), pepsin (△—△), gelatin (■—■), and egg albumin (▲—▲).

bactericidal experiments should be 90 μg./ml. In the protein precipitation studies, it is found as expected that at a concentration of 90 μg./ml. the homologous salts of 4-aminoquinoline and 4-aminoquinaldine, in the range C = 10 to 18, precipitate albumin, casein and pepsin and, with the sole exception of the C_{10} quinolinium member, they also precipitate gelatin.

The results of the bacteriostatic experiments show maxima of activity in both series of compounds in the region of the C_{12}—C_{14} members, a range which also includes those compounds having good bactericidal activity. However, with polymethylene chain lengths greater than this range it is difficult to determine any correlation of bacteriostatic and bactericidal activity, since the bacteriostatic results are confused by precipitation of the salts in the culture media, and these are then not available to exert their antibacterial effect. There does not appear to be any close relation between the protein precipitating activity of the salts and

their bacteriostatic activity, but there is a good correlation of their protein precipitating and bactericidal activities which indicates that the mechanism by which these agents kill bacteria may well be related to their effects on protein.

The dodecyl acetate member of the 4-aminoquinaldine series (Laurodin) has been selected for fuller investigation.

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