# RESEARCH PAPERS

## SALTS OF DECAMETHYLENE-BIS-4-AMINOQUINALDINIUM ("DEQUADIN")*, A NEW ANTIMICROBIAL AGENT

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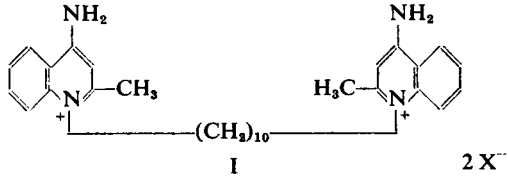
THE presence of high antimicrobial activity in polymethylene bis-*iso*-quinolinium (BIQ) salts[1] led to the synthesis and examination of a large number of related compounds, including the corresponding bis-quinolinium (BQ) salts[2] and many derivatives of both these series. Among these, bis-4-aminoquinaldinium (BAQD) salts showed broad activity and relatively low toxicity and the decamethylene member (I, BAQD 10, Dequadin) was selected for more extensive biological investigation




I

## MATERIALS AND METHODS

*Compounds.* The iodide, chloride, nitrate and acetate of Dequadin were used, their approximate solubilities in water at 25° C. being: iodide, 1 in 3500; chloride, 1 in 200; nitrate, 1 in 400; acetate, 3 in 4. Other members of the BAQD series (see Table I) were iodides. Methods of preparation and chemical properties of this series will be described elsewhere. The synthesis of the BIQ and BQ iodides (Table I) has already been described[1,2]. The following salts of other antibacterial agents were used: aureomycin hydrochloride, benzalkonium chloride, potassium benzylpenicillin, chlorhexidine acetate, domiphen bromide and streptomycin sulphate. Substances may be referred to below by the name of the active acid or base, but weights given are those of the salt. In most tests cetrimide was used for reference.

*Strains* in vitro. The microbial species used and the code numbers of some strains are shown in Table II. Both strains of *Proteus vulgaris* (L.H. 13 and 14) and the 12 penicillin-resistant strains of *Staphylococcus aureus* (L.H. 1 to 12) were recently isolated at the London Hospital. Strains not numbered in Table II had the following origins: staphylococci, streptococci and salmonellas were isolated from domestic animals at the Royal Veterinary College, London, or the Agricultural Research Council

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TABLE I

ACTIVITIES OF POLYMETHYLENE-BIS-*iso*QUINOLINIUM (BIQ), QUINOLINIUM (BQ) AND -4-AMINOQUINALDINIUM (BAQD) IODIDES AGAINST FOUR BACTERIAL SPECIES IN PEPTONE WATER. READINGS AFTER 5 DAYS AT 37° C.

| Series and number of carbons in chain | Geometric mean M.I.C. in μg./ml. | | | |
|---|---|---|---|---|
| | *Staph. aureus* (CN491) | *Myco. phlei* (NCTC.525) | *P. vulgaris* (L.H.14) | *Ps. pyocyanea* (NCTC.8203) |
| BIQ 10 | 50 | 100 | >100 | >100 |
| BIQ 11 | 50 | 20·4 | >100 | >100 |
| BIQ 12 | 12·5 | 3·13 | >100 | >100 |
| BIQ 13 | 5·00 | 2·50 | >100 | >100 |
| BIQ 14 | 0·77 | 0·70 | >100 | >100 |
| BIQ 15 | 0·44 | 0·63 | >100 | >100 |
| BIQ 16 | 0·12 | 0·88 | 100 – >100 | >100 |
| BIQ 17 | 0·20 | 0·99 | 50 | >100 |
| BIQ 18 | 0·22 | 1·10 | 25 | >100 |
| BIQ 19 | 0·16 | 1·40 | 8·89 | 100 – >100 |
| BIQ 20 | 0·49 | 3·13 | 6·25 | 100 – >100 |
| BQ 10 | 17·7 | 50 | >100 | >100 |
| BQ 11 | 12·5 | 25 | >100 | >100 |
| BQ 12 | 3·97 | 4·42 | >100 | >100 |
| BQ 13 | 2·50 | 3·54 | >100 | >100 |
| BQ 14 | 0·38 | 0·78 | >100 | >100 |
| BQ 16 | 0·18 | 0·88 | 100 | >100 |
| BQ 18 | 0·44 | 1·10 | 19·6 | 100 |
| BQ 20 | 0·63 | 3·13 | 12·5 | 70·7 |
| BAQD 8 | 0·67 | 1·97 | >100 | 100 – >100 |
| BAQD 10 | 0·35 | 1·66 | 63·0 | 67·3 |
| BAQD 12 | 0·35 | 0·88 | 35·4 | 70·7 |
| BAQD 14 | 0·39 | 0·79 | 35·4 | 84·1 |
| BAQD 16 | 0·67 | 1·69 | 70·7 | >100 |
| BAQD 18 | 0·99 | 3·95 | 70·7 | >100 |
| BAQD 20 | 2·48 | 70·7 | >100 | >100 |

Field Station, Compton; *Actinomyces dermatonomus* was isolated from sheep at the Animal Diseases Research Association, Edinburgh; fungi were from St. John's Hospital for Diseases of the Skin, except *Trichophyton verrucosum* which was isolated at the London School of Hygiene and Tropical Medicine; *Bacterium coli* and *Bacillus subtilis* were isolated at Ware.

*Growth inhibition* in vitro. Tube dilution tests were performed in a medium of 1 per cent. peptone (Difco) in water containing 0·5 per cent. dextrose and 0·5 per cent. sodium chloride, adjusted to pH 7·2. For *Actinomyces dermatonomus* 1 per cent. beef extract (Lab-Lemco) was added, for *Streptococcus pyogenes* CN10, 10 per cent. horse serum was added after autoclaving and *Mycobacterium tuberculosis* was grown in Dubos' medium with 4 per cent. bovine albumin. Drugs were serially diluted 1 to 2 and, after autoclaving, inoculated with suspensions of bacteria, adjusted to give approximately 50,000 organisms/ml. test medium. With the exception of *Myco. tuberculosis*, which was grown for 7 days, 18 hour cultures of bacteria were used for preparing inocula. After incubation at 37° C., growth was read by eye at 24 hours and 5 days, except for *Myco. tuberculosis*, which was read at 14 and 28 days. Results were expressed as the minimal inhibitory concentration (M.I.C.). For detailed studies of growth inhibition by Dequadin, *Staph. aureus* CN491 was used.

Plate tests were used for certain nutritionally exacting organisms. *Neisseria catarrhalis* and *Str. pneumoniæ* were grown on 10 per cent.

horse blood agar and *Hæmophilis influenzæ* on 10 per cent. boiled rabbit blood agar. Drugs were incorporated in the plates, whose surfaces were sown with approximately $10^8$ organisms per plate. Methods of anti-fungal test were similar to those previously described[2].

*Bactericidal action.* 5 ml. quantities of nutrient agar in 50 ml. conical flasks were seeded with 0·5 ml. of an 18 hour dextrose peptone water culture of *Staph. aureus* CN491 and incubated overnight at 37° C.

TABLE II

INHIBITORY ACTIVITY OF DEQUADIN IODIDE *in vitro*. IN BRACKETS, NUMBER OF STRAINS

| Microbial species | Strain No. | Geometric mean M.I.C. in μg./ml. at | | | |
|---|---|---|---|---|---|
| | | 24 hours | 5 days | 14 days | 28 days |
| *Actinomyces dermatonomus* | — | 0·63 | 0·63 | — | — |
| *Bacterium coli* | — | 6·87 | 11·9 | — | — |
| *Bacillus subtilis* | — | 2·50 | 2·50 | — | — |
| *Corynebacterium diphtheriæ* | NCTC 3989 | 0·31 | 0·35 | — | — |
| *Hæmophilus influenzæ* | NCTC 8468 | 17·7 | 25·0 | — | — |
| *Mycobacterium phlei* | NCTC 525 | 1·54 | 1·66 | — | — |
| *Myco. tuberculosis* | H37 | — | — | 2·14 | 2·69 |
| *Neisseria catarrhalis* | NCTC 4103 | 1·25 | 2·5 | — | — |
| *Pseudomonas pyocyanea* | NCTC 8203 | 41·0 | 67·3 | — | — |
| *Proteus vulgaris* (2) | L.H. 13 & 14 | 50·0–59·5 | 63·0–70·7 | — | — |
| *Salmonella dublin* | — | 25·0 | 50·0 | — | — |
| *Salm. typhi* | NCTC 5758 | 2·70 | 6·80 | — | — |
| *Salm. typhimurium* | — | 25·0 | 50·0 | — | — |
| *Staphylococcus aureus* | CN 491 | 0·32 | 0·35 | — | — |
| *Staph. aureus* (2) | — | 0·63 | 0·63–0·88 | — | — |
| *Staph. aureus*, penicillin-resistant (12) | L.H.1 to 12 | 0·16–0·63 | 0·31–0·63 | — | — |
| *Streptococcus agalactiæ* | — | 0·63 | 0·88 | — | — |
| *Str. dysgalactiæ* (2) | — | 0·44–1·25 | 0·63–1·25 | — | — |
| *Str. fæcalis* | 850 | 1·66 | 2·79 | — | — |
| *Str. pneumoniæ* | NCTC 7465 | 2·5 | 5·0 | — | — |
| *Str. pyogenes* | CN 10 | 1·10 | 1·10 | — | — |
| *Str. pyogenes* (3) | — | 0·31–0·88 | 0·44–1·25 | — | — |
| *Str. uberis* (2) | — | 0·79–1·25 | 0·99–2·50 | — | — |
| *Vibrio choleræ* | Madras 48210 | 4·26 | 5·36 | — | — |
| *Candida albicans* | — | — | — | 4·47 | — |
| *Microsporum canis* | — | — | — | 1·67 | — |
| *Trichophyton mentagrophytes* | — | — | — | 2·39 | — |
| *T. rubrum* | — | — | — | 0·59 | — |
| *T. verrucosum* | — | — | — | 1·67 | — |

Growth was washed off and shaken for 10 minutes with glass beads in 16 ml. water per culture flask. 5 ml. of this suspension, diluted as required, were thoroughly mixed with 5 ml. drug solution in buffered saline and maintained at 20° C. for 1 hour. The mixture was then diluted in 2 per cent. bovine bile ("Bacto-Oxgall") in water and 1 ml. samples plated out in 10 ml. nutrient agar to obtain viable counts.

*Local therapeutic action.* 0·1 ml. of an overnight broth culture of *Staph. aureus* 663, diluted 1 to 100 in 5 per cent. mucin in saline, was injected intraperitoneally (I.P.) into male white mice weighing 9 to 12 g. Half an hour later drugs were injected I.P. or subcutaneously (S.C.). The number of mice surviving was counted 10 days after inoculation and the ED50 and its standard error was estimated by the method of Miller and Tainter[3]. In experiments with *Str. pyogenes* CN10 a 6 hour culture diluted 1 to 1 million in blood broth was used.

*Toxicity.* In acute tests in mice, deaths were counted 7 days after treatment and the LD50 and its standard error estimated by the method of Miller and Tainter[3], with results obtained in 10 or more animals at

each dose level. In subacute tests, drugs were administered once daily, except on Saturdays and Sundays. In rabbits, saline solutions of Dequadin chloride were instilled in one of each pair of eyes, or creams containing Dequadin were applied to the shaved skin. Creams were applied also to the skin of hairless mice. In a chronic toxicity test, groups of 10 young female Wistar rats, maintained on M.R.C. diet No. 41, received Dequadin chloride at 0·05 and 0·01 per cent. of their drinking water. Rats were weighed weekly and at the end of the test period blood examinations were performed by conventional methods and tissues prepared for histological examination.

## RESULTS

### *Antimicrobial Action* in vitro

*Screening.* The results of comparative bacteriostatic tests of BIQ, BQ and BAQD iodides are expressed in Table I. High activity against *Staph. aureus* was present in all series, being particularly marked in BIQ 16, 17, 18 and 19, BQ 16 and BAQD 10, 12 and 14. Activity against *Myco. phlei* was also general, being highest in the neighbourhood of each tetradecamethylene member. *P. vulgaris* and *Pseudomonas pyocyanea* were most sensitive to the longest chains of the BIQ series and to BAQD 10, 12 and 14. For reasons discussed below, BAQD 10 was selected for further study.

TABLE III

SYNERGISM OF DEQUADIN IODIDE AND CHLORAMPHENICOL AGAINST *Staph. aureus* CN491 IN PEPTONE WATER AFTER 5 DAYS AT 37° C.

| Per cent. drug in mixture | | Geometric mean M.I.C. in μg./ml. |
|---|---|---|
| Dequadin | Chloramphenicol | |
| 100 | 0 | 0·3 |
| 28 | 72 | 1·2 |
| 13 | 87 | 2·0 |
| 9 | 91 | 3·0 |
| 6 | 94 | 4·0 |
| 0 | 100 | 13·2 |

*Antimicrobial spectrum.* All bacteria and fungi tested were sensitive to Dequadin iodide (Table II). It inhibited Gram-positive bacteria more readily that Gram-negative, and mycobacteria and fungi to an intermediate degree. The strains of penicillin-resistant staphylococci shown in Table II to be sensitive to Dequadin were also found to be sensitive to BIQ 16. As expected, the chloride, nitrate and acetate of Dequadin showed comparable activity to the iodide.

*Effect of inoculum size.* When the inoculum was increased a hundred-fold to

TABLE IV

EFFECT OF SERA ON THE INHIBITION OF *Staph. aureus* CN491 BY ANTIBACTERIAL AGENTS IN PEPTONE WATER AT 37° C.

| Antibacterial | Percentage Serum added to medium | M.I.C. in μg./ml. at 24 hours | M.I.C. in μg./ml. at 5 days |
|---|---|---|---|
| Dequadin chloride .. | None | 0·31 | 0·31 |
| | rabbit 10 | 0·31 | 0·63 |
| | bovine 10 | 0·63 | 0·63 |
| | horse 10 | 0·63 | 0·63 |
| | human 10 | 0·31 | 0·63 |
| | human 50 | 0·63 | 0·63 |
| Cetrimide .. .. | None | 0·63 | 0·63 |
| | human 10 | 6·25 | 12·5 |
| Benzalkonium chloride | None | 0·31 | 0·63 |
| | human 10 | 6·25 | 6·25 |
| Domiphen bromide .. | None | 0·31 | 0·31 |
| | human 10 | 1·56 | 3·13 |
| Chlorhexidine acetate .. | None | 0·25 | 0·25 |
| | human 10 | 2·5 | 5·0 |

about $5 \times 10^6$ organisms per ml. test medium, five times as high a concentration of Dequadin was needed to inhibit staphylococcal growth.

*Synergism.* In mixtures, Dequadin and chloramphenicol were roughly additive in action (Table III). A similar effect was also obtained with binary mixtures of Dequadin with aureomycin, penicillin, streptomycin and cetrimide.

TABLE V

ANTAGONISM OF BACTERIOSTATIC ACTION OF DEQUADIN AGAINST *Staph. aureus* CN491 IN PEPTONE WATER AT 37° C.

| Material added to medium Per cent. | | M.I.C. in μg./ml. at 24 hours | M.I.C. in μg./ml. at 5 days |
|---|---|---|---|
| None | | 0·31 | 0·31 |
| Egg yolk | 10 | 0·31 | 1·25 |
| Evaporated milk | 10 | 25 | 25 |
| Vegetable lecithin | 0·1 | 25 | 50 |
| Egg lecithin | 0·1 | 6·25 | 6·25 |
| Bovine bile | 0·2 | 2·5 | 2·5 |
| Bovine bile | 1 | 3·13 | 6·25 |
| Bovine bile | 2 | 6·25 | 12·5 |
| "Lubrol W" | 10 | 1·56 | 3·13 |

*Antagonism.* The effects of sera on the potencies of Dequadin and some other antibacterial agents in current use are expressed in Table IV. Among these, Dequadin alone was not appreciably antagonised.

The results of exploring possible antagonists of Dequadin are expressed in Table V. For bactericidal tests, bovine bile was chosen as antagonist, because it combined activity with ease of handling. The degree of antagonism of Dequadin by evaporated milk shown in Table V was observed also with two bovine strains of staphylococci.

*Bactericidal.* The bactericidal action of Dequadin nitrate was examined in 7 experiments, summarised in Table VI. In the conditions of these, 400 μg./ml., Dequadin and 200 μg./ml. cetrimide killed virtually all organisms. At 100 μg./ml., each drug killed more than 99·99 per cent. organisms. These experiments showed that the two drugs possessed bactericidal activity of a similar order.

TABLE VI

BACTERICIDAL ACTIONS OF DEQUADIN NITRATE AND CETRIMIDE ON *Staph. aureus* CN491 IN BUFFERED SALINE AT pH 7·2 FOR 1 HOUR AT 18° C.

| Concentration of drug in μg./ml. | Mean no. of survivors/ml. Dequadin | Mean no. of survivors/ml. Cetrimide |
|---|---|---|
| 500 | <10 | — |
| 400 | <10 | — |
| 250 | $2 \times 10^3$ | <10 |
| 200 | $4 \times 10^3$ | — |
| 100 | $10^3$ | $8 \times 10^3$ |
| 50 | $10^7$ | $6 \times 10^5$ |
| 25 | — | $2 \times 10^7$ |
| 5 | — | $10^{13}$ |
| None | $10^{14}$ | $10^{14}$ |

*Local Therapeutic Action*

*Staph. aureus.* Using an intraperitoneal inoculum adjusted to kill all control mice, the therapeutic action of Dequadin given intraperitoneally was compared with those of BAQD 12 iodide and cetrimide by the same route, and with Dequadin subcutaneously. The results of these experiments, involving 500 mice, are expressed in Figure 1. 4 mg./kg. Dequadin chloride, I.P., prevented death in all of 30 animals. Higher doses showed some toxicity. A similar picture was seen with BAQD 12. On the other hand, tolerated doses of Dequadin, S.C., or cetrimide, I.P., failed to protect more than one or two individuals.

*Str. pyogenes.* In similar experiments with streptococci a total of 388 mice was used. 0·9 mg./kg. Dequadin chloride, I.P., prevented death in all of 16 animals treated. Its ED50 was 0·26 ± 0·04 mg./kg. and its

LD50 in these infected animals was 11·0 ± 0·1 mg./kg. Dequadin S.C., and cetrimide I.P., were both ineffective.

*Toxicity*

*Acute.* During screening of the iodides in Table I, the acute I.P. toxicities of BAQD 10, 12 and 14, BIQ 20 and BQ 20 were compared in mice. The LD50 values (mg./kg.) were: BAQD 10, 20·9 ± 2·9; BAQD 12, 15·1 ± 1·1, BAQD 14, 18·9 ± 2·1; BIQ 20, 2·8 ± 0·2; BQ 20, 1·8 ± 0·2.

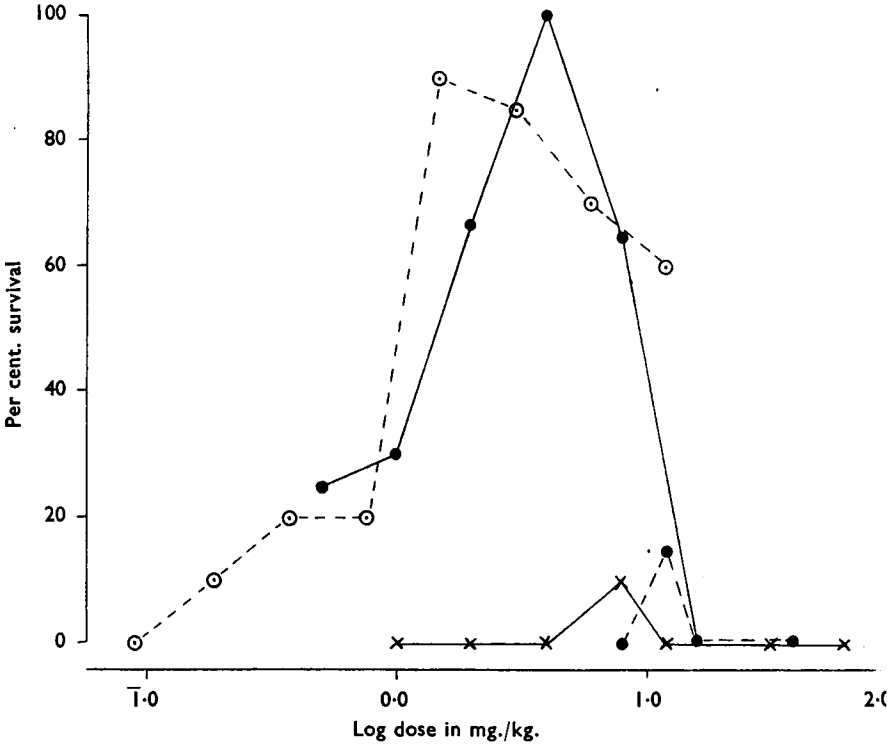


FIG. 1. Protection of mice by polymethylene-bis-4-aminoquinaldinium salts (BAQD 10 and 12). Drugs administered intraperitoneally (I.P.) or subcutaneously (S.C.) 30 minutes after I.P. inoculation of staphylococci.

⊙- - -⊙ BAQD 12 $I_2$,I.P. ●- - -● BAQD 10$Cl_2$,S.C.
●——● BAQD 10 $Cl_2$,I.P. ×——× Cetrimide,I.P.

In mice, the acute LD50 values (mg./kg.) of Dequadin chloride were: S.C., 70 ± 6·6; intravenous, 1·9 ± 0·2. After intravenous administration of Dequadin, mice appeared to die of respiratory paralysis.

Administered orally in 5 per cent. suspension in water, 2 g./kg. Dequadin chloride failed to kill any of 20 mice; while the same dose of the very soluble Dequadin acetate killed 6 of 20 animals.

In a series of intradermal injections in rabbits a solution containing 40 μg. Dequadin chloride/ml. saline caused erythema and induration in

5 of 8 injection sites and 20 $\mu$g. evoked the same reaction in 4 of 14 sites. 320 $\mu$g. cetrimide/ml. saline caused reactions in 5 of 8 sites and 160 $\mu$g. in 2 of 7 sites.

*Subacute.* Solutions containing 2 mg. Dequadin chloride/ml. saline appeared to be without effect on the eyes of rabbits when instilled daily over a period of 2 weeks. 0·4 per cent. Dequadin chloride or iodide in

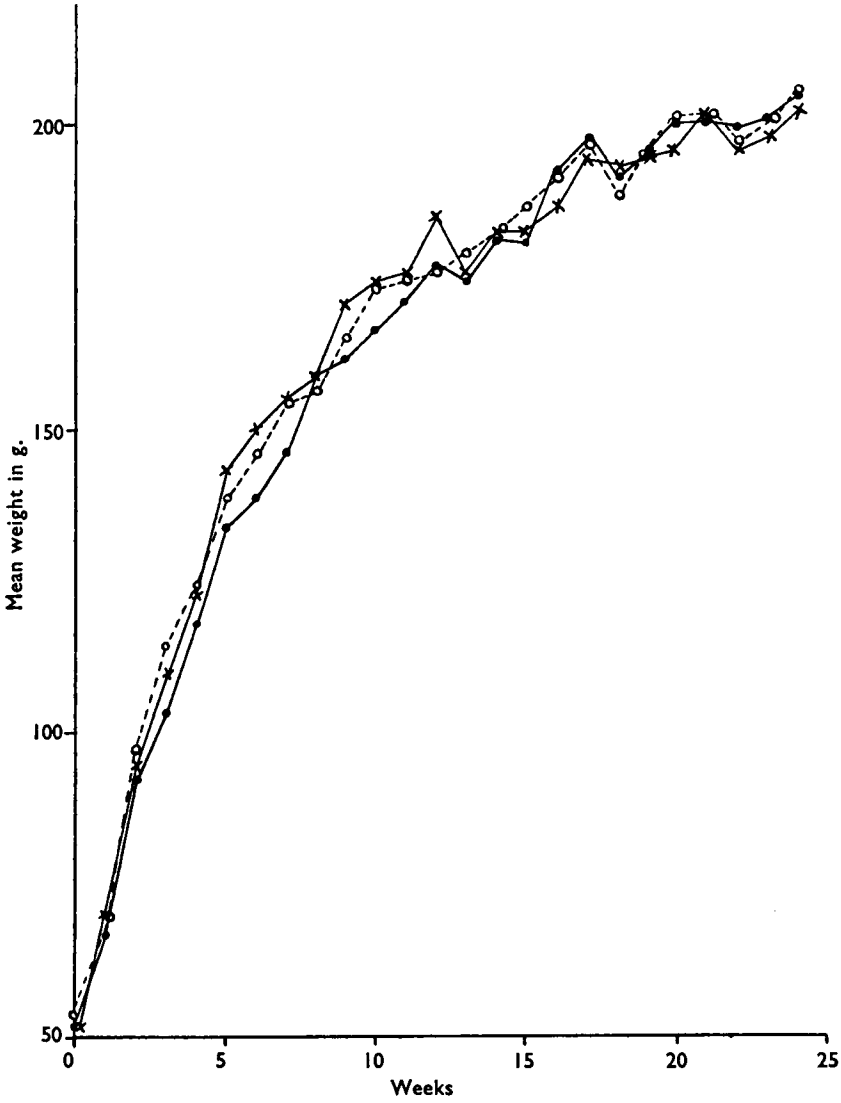


FIG. 2. Absence of growth inhibition in young rats by Dequadin administered in drinking water.

○- - -○ 0·05 per cent. Dequadin chloride
×——× 0·01 per cent. ,, ,,
●——● No addition

creams were applied daily over a period of 4 weeks to the shaved skins of rabbits without evoking any reaction. Similar experiments with the chloride were performed in hairless mice with the same result. As the skin reaction of hairless mice to toxic agents was unknown, chloroform was applied daily to an area of skin. Within 24 hours after the second application a sharp reaction, consisting of erythema, induration, and brownish pigmentation appeared. Some necrosis followed.

TABLE VII

MEAN VALUES OBTAINED FROM BLOOD EXAMINATION OF RATS RECEIVING DEQUADIN CHLORIDE IN THEIR DRINKING WATER FOR 26 WEEKS. L = LYMPHOCYTES; M = MONOCYTES; N = NEUTROPHILS; E = EOSINOPHILS; B = BASOPHILS.

| Per cent. drug in water | Per cent. hæmoglobin | Erythrocytes per $mm^3 \times 10^6$ | Leucocytes per $mm^3 \times 10^3$ | Differential counts per cent. | | | |
|---|---|---|---|---|---|---|---|
| | | | | L & M | N | E | B |
| 0·05 | 103 | 8·0 | 9·8 | 75·05 | 21·90 | 3·00 | 0·05 |
| 0·01 | 101 | 7·7 | 9·3 | 70·15 | 25·40 | 4·40 | 0·05 |
| Untreated controls | 100 | 7·8 | 10·5 | 75·45 | 20·30 | 4·25 | 0·00 |

*Chronic.* All rats receiving 0·05 per cent. Dequadin in their drinking water for 26 weeks survived; but one rat in the group receiving 0·01 per cent. drug died during the 5th week for unknown reasons. Compared with control animals, rats receiving Dequadin in their drinking water showed no depression of growth (Fig. 2). Blood examinations (Table VII) showed no substantial difference between treated and control rats. Histological examination of sections of brain, stomach, small and large intestines, liver, thyroid, spleen, kidney, heart, lung and ovary of all rats showed no pathological effects attributable to Dequadin.

## DISCUSSION

The activities of BIQ 10, 11, 12, 13, 14, 16, 18 and 20 against *Staph. aureus*, *Myco. phlei* and *Ps. pyocyanea* have been examined previously[1]. The present results with these compounds and species, given in Table I, confirm previous findings.

The peaks of activity against the bacteria in Table I occurred at similar positions in the BIQ and BQ series. On the other hand, in the BAQD series, the peaks against *Staph. aureus*, *P. vulgaris* and *Ps. pyocyanea* occurred at shorter chain lengths. This might be because the amino groups in the 4-position on the BAQD rings take over the role of the quaternary nitrogen atoms in the BQ and BIQ series as points of attachment to certain receptors in the bacterium.

In Table I, BAQD 10, 12 and 14 were roughly equivalent in activity to BIQ 20 and BQ 20; but the BAQD compounds were relatively less toxic and more easily prepared. In the BAQD series the decamethylene member was chosen for further study, because it could be most readily supplied. In view of comparative tests and of their structural similarity, it seems unlikely that the general properties of BAQD 12 or 14 differ greatly from those of BAQD 10.

One of the most striking features of Dequadin *in vitro* was the absence of appreciable antagonism by sera, even by 50 per cent. human serum in peptone water (Table IV). Ten per cent. of human serum antagonised the other antibacterials tested by a factor of about 10. For chlorhexidine, this factor was somewhat higher than that reported for rabbit serum[4], although our end-point with human serum was comparable to that obtained with rabbit serum by Davies, Francis, Martin, Rose and Swain. The difference between the two results lies in the relatively greater activity of chlorhexidine in peptone water than in the brain-heart infusion used by these authors.

In mice infected I.P. with virulent cocci, Dequadin was effective by the I.P., but not by the S.C. route. We concluded that its therapeutic action was essentially local. This and other observations described above led to the view that clinical trial of Dequadin as a local chemotherapeutic agent was justified. Results in limited trials of the chloride have so far been encouraging.

It is well known that many heterocyclic polymethylene bis-quaternary ammonium salts possess neuromuscular blocking activity. The paralysis of mice receiving Dequadin intravenously suggested that this drug was no exception, and this suggestion was borne out in an experiment on the tibialis preparation of the cat.

## Summary

1. The inhibitory activities of polymethylene-bis-*iso*quinolinium (BIQ), -quinolinium (BQ) and -4-aminoquinaldinium (BAQD) iodides, in which chain-length ranged between 8 and 20 methylene groups, were compared against *Staph. aureus*, *Myco. phlei*, *P. vulgaris* and *Ps. pyocyanea*.

2. Decamethylene-bis-4-aminoquinaldinium (BAQD 10, Dequadin) salts inhibited growth of all microbial species used, which included Gram-positive, Gram-negative and acid-fast bacteria, and fungi.

3. In binary mixtures, Dequadin was additive in bacteriostatic activity with aureomycin, chloramphenicol, penicillin, streptomycin and cetrimide.

4. Dequadin was not antagonised by serum, but was antagonised to varying degrees by bile, milk and lecithin.

5. When suspensions of *Staph. aureus* were exposed to 100 $\mu$g./ml. Dequadin for 1 hour at 20° C., more than 99·99 per cent. organisms were destroyed.

6. Dequadin protected all mice against death when administered intraperitoneally 30 minutes after they had been inoculated intraperitoneally with lethal bacterial suspensions. With streptococci, 0·9 mg./kg. and, with staphylococci, 4 mg./kg. were fully effective. Subcutaneously, Dequadin was only feebly active.

7. Iodides of BAQD 10, 12 and 14 showed approximately equal intraperitoneal toxicities in mice, which were lower than those of BIQ 20 and BQ 20.

8. In mice, the acute subcutaneous and intravenous and in rabbits the acute intradermal toxicities of Dequadin were determined.

9. Dequadin failed to exhibit toxic effects when administered subacutely to the eyes and skin of rabbits or to the skin of hairless mice, and chronically to rats in their drinking water.

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