- Haddow, A. (1935). *Nature, Lond.*, **136**, 868.
 Haddow, A. & Robinson, A. M. (1937). *Proc. Roy. Soc. B*, **122**, 442.
 Haddow, A., Scott, C. M. & Scott, J. D. (1937). *Proc. Roy. Soc. B*, **122**, 477.
 Lesnik, M. (1888). *Arch. exp. Path. Pharmacol.* **24**, 167.
 Maughan, G. B., Evelyn, K. A. & Browne, J. S. L. (1938). *J. biol. Chem.* **126**, 567.
 Peters, J. P. & Van Slyke, D. D. (1932). *Quantitative Clinical Chemistry*, **2**, 894. Baltimore: Williams and Wilkins.
 Stekol, J. A. (1937). *J. biol. Chem.* **121**, 87.
 Stekol, J. A. (1943). *Arch. Biochem.* **2**, 151.
 Stevenson, E. S. & White, A. (1940). *J. biol. Chem.* **134**, 709.
 White, A. & White, J. (1940). *Yale J. Biol. Med.* **12**, 427.
 White, J. (1940). *J. nat. Cancer Inst.* **1**, 337.
 White, J. & White, A. (1939). *J. biol. Chem.* **131**, 149.
 Zbarsky, S. H. & Young, L. (1943). *J. biol. Chem.* **151**, 487.
 Zondek, B. (1941). *Clinical and Experimental Investigations on the Genital Functions and their Hormonal Regulation*, Chap. 3. Baltimore: Williams and Wilkins.

The Antibacterial Action of 4:6-Dimethoxytoluquinone and its Fate in the Animal Body

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(Received 30 May 1945)

During the examination of a series of derivatives of fumigatin, 3-hydroxy-4-methoxytoluquinone, Oxford (1942) noted that while toluquinone was much less bacteriostatic, certain other quinones, particularly 4:6-dimethoxytoluquinone (synthesized: Anslow, Ashley & Raistrick, 1938; Aulin & Erdtman, 1938), were more bacteriostatic than fumigatin.

We have investigated the biological properties of the latter quinone for its possible use as a therapeutic agent by local or systemic administration. It was found at an early stage that the compound was inactivated in the presence of blood and it did not give any protection to mice against certain pathogenic organisms. Since we were, however, engaged in research on similar compounds we studied the mode of its deactivation and detoxication in the animal body, as a possible guide for further chemical work in the preparation of more active compounds.

After the completion of the work described in this paper a publication by Barber (1944) appeared on the antibacterial action of 4:6-dimethoxytoluquinone. She found that it had a powerful bacteriostatic action against *Staphylococcus aureus* and *Streptococcus pyogenes* in broth, but owing to its greatly decreased activity in the presence of blood or serum, and to the markedly antileucocytic properties of the compound, it was contra-indicated as a therapeutic agent. Our results agree with these findings.

EXPERIMENTAL

ACTION AGAINST BACTERIA

In vitro experiments

The following organisms were used: *Streptococcus pyogenes*, *Staphylococcus aureus*, *Diplococcus pneumoniae* (Type 1), *Ps. aeruginosa*, *Proteus vulgaris*, *Esch. coli*, *Cl. perfringens*.

Different types of culture media were used:

(a) *Synthetic media*. The synthetic media used were evolved from work described by McIlwain, Fildes, Gladstone & Knight (1939) on the nutrients essential for the growth of bacteria.

For staphylococci a medium was used consisting of: NaCl, 5.0 g.; K_2HPO_4 , 1.0 g.; KH_2PO_4 , 1.0 g.; $MgSO_4$, 0.2 g.; $CaCl_2$, 0.1 g.; ammonium lactate, 5.0 g.; acid-hydrolyzed casein, 1.0 g.; distilled water to 1 l.

For streptococci, the following medium was used: K_2HPO_4 , 0.5 g.; KH_2PO_4 , 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.2 g.; NaCl, 0.1 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; $MnSO_4 \cdot 4H_2O$, 0.01 g.; glucose, 20.0 g.; sodium acetate, 6.0 g.; adenine, 20 mg.; riboflavin, 0.2 mg.; calcium *DL*-pantothenate, 0.2 mg.; nicotinic acid, 0.2 mg.; pyridoxine, 0.2 mg.; biotin, 0.0001 mg.; distilled water to 1 l. This medium was Seitz-filtered before use, and 1% of acid-hydrolyzed casein was then added. For the other organisms the medium used was as follows: glycerol, 10.0 g.; NaCl, 5.0 g.; $CaCl_2$, 0.1 g.; $MgSO_4$, 0.5 g.; K_2HPO_4 , 2.0 g.; ammonium lactate, 6.0 g.; acid-hydrolyzed casein, 1.0 g.; distilled water to 1 l.

(b) *Papain digest broth*, containing 1% (w/v) glucose.

(c) *Papain digest broth*, with the addition of 10% (v/v) normal horse serum.

(d) *Normal human defibrinated blood*, used in Fleming's slide-cell technique.

Testing of anti-bacterial activity. Serial dilutions of 4:6-dimethoxytoluquinone or the corresponding quinol were added to the tubes containing the media. In making dilutions of 4:6-dimethoxytoluquinone, suitable precautions were taken to prevent oxidation.

The tubes of medium + 4:6-dimethoxyquinone dilutions were inoculated with one standard drop of a saline suspension of a 24 hr. agar culture, containing about 100×10^6 organisms/ml., and were incubated at 37°. The results were read after 24 and 48 hr. for bacteriostatic effects; the tubes

which showed no growth were subcultured on agar plates, incubated at 37°, and examined after 24 hr. for evidence of bactericidal action.

In Table 1, figures are given for bacteriostatic effects (a); the bactericidal effects (b) are shown for staphylococci only.

We found that the minimal antibacterial concentration was conditioned partly by the number of organisms in the inoculum (cf. Armstrong, Spink & Kahnke, 1943). Even when the inoculum was standardized, however, variation in the results occurred. An example of this is given by the results obtained with 4:6-dimethoxytoluquinone, the figures in six consecutive experiments being (1) 1:100,000, (2) 1:100,000, (3) 1:200,000, (4) 1:40,000, (5) 1:80,000 and (6) 1:80,000. The bactericidal results showed less variation. It is seen from Table 1 that the quinol was not less active than the quinone. Although serum decreased the activity of the quinone to some extent, there was presumably an interaction with the other constituents of defibrinated blood which rendered the quinone completely inactive.

Table 1. In vitro antibacterial activity of 4:6-dimethoxytoluquinone and 4:6-dimethoxytoluquinol

(The figures (the mean of several experiments) indicate the minimal effective concentrations: (a) bacteriostatic, (b) bactericidal (for *Staph. aureus* only).)

Media		Organisms						
		<i>Staph. aureus</i>	<i>Strept. pyogenes</i>	<i>Diplococcus pneumoniae</i> (Type 1)	<i>Esch. coli</i>	<i>Ps. aeruginosa</i>	<i>Proteus vulgaris</i>	<i>Cl. perfringens</i>
4:6-Dimethoxytoluquinone								
Glucose broth	(a)	1:100,000	±1:100,000	1:500,000	1:5,000	—	—	<1:50,000
	(b)	1:20,000	—	—	—	—	—	—
Serum broth	(a)	1:20,000	<1:50,000	1:100,000	<1:5,000	—	—	1:50,000
Synthetic medium	(a)	±1:500,000	±1:500,000	—	1:10,000	<1:1,000	<1:1,000	1:50,000
	(b)	<1:5,000	—	—	—	—	—	—
Defibrinated blood	(a)	<1:2,000	—	—	—	—	—	—
4:6-Dimethoxytoluquinol								
Glucose broth	(a)	1:100,000	1:500,000	—	<1:5,000	<1:5,000	<1:5,000	<1:50,000
	(b)	1:20,000	—	—	—	—	—	—
Serum broth	(a)	1:50,000	1:100,000	—	<1:5,000	<1:5,000	<1:5,000	—
Synthetic medium	(a)	1:500,000	±1:500,000	—	1:10,000	±1:5,000	<1:5,000	1:50,000

In vivo experiments

(a) *Systemic administration.* In mice infected with *Streptococcus pyogenes* or *Staph. aureus*, 4:6-dimethoxytoluquinone or the quinol had no protective effect when administered orally in doses of 0.1 or 0.05 mg./g., respectively, given twice daily, nor in the same doses given once daily by intraperitoneal injection. The treated and the control mice died within 48 hr. after infection.

(b) *Local administration.* In mice injected intramuscularly with a suspension of *Cl. perfringens* and 2.5% (w/v) CaCl₂, treatment by local intramuscular injections of 4:6-dimethoxytoluquinone at the site of infection failed to give any protection.

LOCAL TOLERANCE

Local toxicity on the conjunctiva. Two drops of 4:6-dimethoxytoluquinone solution in sterile distilled water at dilutions of 1:500 to 1:10,000, warmed to 37°, were applied twice daily under the left eyelids of rabbits, leaving the right eyes as controls. No irritation of the conjunctiva was observed after 2 days' treatment.

Action on tissue culture. This was tested on growing chick-heart embryo fibroblasts. Dilutions of 4:6-dimethoxytoluquinone were prepared in Tyrode's solution, and then added to a mixture of chick-plasma and embryonic extract on depression slides. Growth of fibroblasts was recorded after 24, 48 and 72 hr. No growth occurred with dilutions up to 1:40,000, whereas normal growth of fibroblasts took place in dilutions of 1:60,000. It can be inferred that 4:6-dimethoxytoluquinone is comparatively toxic to growing chick-embryo fibroblasts.

Action on leucocytes. Human leucocytes in contact for 1 hr. at 37° with dilutions of 4:6-dimethoxytoluquinone up to 1:20,000 showed greatly de-

creased phagocytic activity against a suspension of *Staph. aureus*.

Local tolerance by intramuscular injection. A 1:1000 solution (0.1 ml.) was injected intramuscularly into the thigh muscles of mice. Macroscopically there was no extensive damage; histological examination showed degeneration of some of the nuclei and swelling of the fibres, but on the whole the damage was small.

Local effect on wound healing. The effect of 4:6-dimethoxytoluquinone (5% (w/w) suspension in an ointment base) was tested on burn wounds produced in guinea-pigs by the method of Ungar & Robinson (1943). Enough ointment was applied daily on vaseline gauze to cover the wound area and the

treatment was continued for 8 days. There was no significant difference in the rate of healing of the treated animals as compared with control animals treated with the ointment base only.

TOXICITY

The acute toxicities were determined in mice by oral administration of the compounds suspended in arachis oil. The L.D.₅₀ (mean lethal dose) for 4:6-dimethoxytoluquinone and the quinol were respectively 0.6 and 0.3 mg./g. Absorption after oral administration was rapid, since toxic symptoms appeared within 5–10 min. The main symptom was general depression of the respiration, although the quinol produced an initial stimulation with moderately severe spasmodic convulsions of a mixed type.

A few experiments were made on the acute effects in larger animals. In a cat, anaesthetized with nembutal, 4:6-dimethoxytoluquinone (15 mg./kg.) injected intravenously in saline caused a brief rise in blood pressure and an increase in the depth of respiration. A further 20 mg./kg. caused a similar effect followed by respiratory irregularity. The blood at death was strikingly deoxygenated. Toluquinone itself, which is more toxic, showed certain differences. In a similar animal preparation toluquinone at 14.6 mg./kg. caused a transient rise in blood pressure with an increase in respiratory rate. A further 30 mg./kg. caused a rapid fall in blood pressure with rapid shallow respiration, and death resulted from pulmonary oedema. Toluquinone in its toxic effects resembles benzoquinone (Schulz, 1923), and these experiments extend the analogy to mammals, the earlier literature having dealt only with the effect on frogs. By oral administration to rabbits toluquinone is more toxic than 4:6-dimethoxytoluquinone, and death is always associated with pulmonary damage, which does not occur in animals fed 4:6-dimethoxytoluquinone.

METABOLISM EXPERIMENTS

These experiments were undertaken to ascertain the mode of inactivation, detoxication and fate in the animal body of 4:6-dimethoxytoluquinone; for comparison some experiments were also carried out with toluquinone.

Methods of estimation

Estimation of quinones. The colorimetric methods adopted for the estimation of the quinones were based on the reaction described by Craven (1931) between quinones with a labile hydrogen or halogen atom adjacent to the carbonyl group, and ethyl cyanoacetate. The method of Valeur (1899) was also used in some instances.

(1) *Estimation of 4:6-dimethoxytoluquinone.* By modifying Craven's reaction, a satisfactory pro-

cedure has been devised for estimating 4:6-dimethoxytoluquinone.

Total 4:6-dimethoxytoluquinone in urine is determined after boiling for 1 hr. under a reflux condenser in 2N-HCl containing 0.2% SnCl₂. After cooling the hydrolysate, ammonium hydroxide solution (50% (v/v) of 0.880 sp. gr. ammonia, in water) is added until all the Sn(OH)₂ is precipitated; the quinone is then extracted with chloroform and the mixture centrifuged to remove Sn(OH)₂ from the chloroform layer. 2 ml. of the chloroform extract are treated with 0.1 ml. redistilled ethyl cyanoacetate followed by 8 ml. ethanolic ammonia (2 parts of 95% ethanol to 1 part of 0.880 sp. gr. ammonia). The blue colour which develops at room temperature has a generalized absorption above 5900 Å; for photoelectric comparison an orange filter transmitting above 5400 Å was used. Using a 1 cm. cell, 0.01–0.25 mg. 4:6-dimethoxytoluquinone in 2 ml. chloroform can be estimated. The colour reaches its maximum intensity in 15 min. and is stable for a further 30 min.

4:6-Dimethoxytoluquinone added to urine could be recovered quantitatively using the above procedure. Free 4:6-dimethoxytoluquinone is determined in a chloroform extract of the unhydrolyzed material.

(2) *Estimation of toluquinone.* Total toluquinone is determined after acid hydrolysis as described above for 4:6-dimethoxytoluquinone. The hydrolysate is extracted twice with an equal volume of amyl alcohol and a sample of this extract shaken with an equal volume of saturated Na₂CO₃ solution. After centrifuging, the clear amyl alcohol extract is used for colour development. To 2 ml. extract is added 0.5 ml. saturated KClO₄ solution (to produce even colour development in the case of hydrolyzed rabbit urine extracts), followed immediately by 8 ml. ethanolic ammonia solution and 0.1 ml. ethyl cyanoacetate. The contents of the tube are mixed after each addition and the reading is made 35–45 min. later. When the amyl alcohol extract is coloured, a blank determination is carried out replacing the ethyl cyanoacetate by water. Toluquinone added to urine can be recovered quantitatively using the above procedure. Free toluquinone is estimated in an amyl alcohol extract of the unhydrolyzed material.

Inorganic and total sulphates were determined in urine by the method of Marenzi & Banfi (1939).

Glucuronic acid. Glucuronide excretion was usually estimated in the present work by the method of Maughan, Evelyn & Brown (1938), as used by Mozolowski (1940). After the completion of these experiments Hanson, Mills & Williams (1944) published a paper on the estimation of glucuronic acid. The glucuronic acid content of several samples of normal rabbits' urine, and of urine from rabbits fed 4:6-dimethoxytoluquinone, was then estimated, employing the method given by these authors. It was found necessary to increase the time of heating, in the case of the urine containing the conjugated

4:6-dimethoxytoluquinone derivative, to 4 hr. in order to obtain maximum recovery of glucuronic acid. The average recovery of glucuronic acid was 136 % of that obtained previously under the conditions of Maughan *et al.* (1938). The value for the glucuronic acid content of the urines after feeding 4:6-dimethoxytoluquinone given in this paper should therefore be increased by approximately 30–40 %. Corrected values are included in Table 2.

The standard glucurone was prepared by the method of Williams (1940) from ammonium glucuronide isolated from the urine of rabbits fed *dl*-menthol. The rabbits were only used for short periods (Martin & Rennebaum, 1943).

Conjugation of 4:6-dimethoxytoluquinone and toluquinone with amino-acids in vitro

It is known that quinones react with various ammonium salts and amino-acids. Fischer & Schrader (1910) have shown that strong ethanolic solutions of quinones (including toluquinone and benzoquinone) combine with esters of amino-acids to form deep red solutions from which crystals may separate. Under these conditions, two molecules of toluquinone combine with one molecule of amino-acid. Cooper & Nicholas (1927), and Cooper & Haines (1928, 1929) have further shown that dilute aqueous solutions of quinones combine with aqueous solutions of various amino-acids and proteins. The disappearance of free quinone was taken as a criterion of conjugation, using Valeur's method (1899) of estimation, which depends on the liberation of iodine from hydriodic acid. In experiments of this type the ethyl cyanoacetate method is not satisfactory, since the quinol liberated in the reaction also reacts with ethyl cyanoacetate, making the interpretation of the results obscure.

4:6-Dimethoxytoluquinone and the corresponding quinol were incubated at 37° at pH 7.3 for varying lengths of time up to 4 days with glycine, alanine, and albumen, altering the concentration of the reagents. On estimation of the free quinone by Valeur's method, no evidence of reaction was obtained.

In the case of toluquinone, equal volumes of 0.2 % toluquinone and glycine were allowed to react at room temperature in the dark and samples removed at intervals and analyzed for free quinone as above. The free COOH group of any conjugated amino-acid was also determined by titration with 0.01N-NaOH. The reaction mixture turned an intense red. In a typical experiment, after allowing the reaction to proceed for 43 hr., 34 % of the free toluquinone had disappeared. The results of the acidimetric titration indicated that glycine had combined with toluquinone in the proportion of 1.2 molecules of glycine to 2 molecules of tolu-

quinone. This is in approximate agreement with Fischer & Schrader (1910).

Disappearance of 4:6-dimethoxytoluquinone added to blood in vitro

The observations by Austin & Drabkin (1935) and Scudi (1942), that quinones in general cause methaemoglobin formation in laked blood, have been confirmed for 4:6-dimethoxytoluquinone, but this is of little significance *in vivo*. Even when large doses of the drug are fed, its low solubility and rapid excretion prevent detectable amounts of methaemoglobin from appearing in the systemic circulation. When citrated rabbit blood was incubated at 37° with one-tenth of its volume of a warm saturated solution of 4:6-dimethoxytoluquinone in saline, the proportions of free quinone recovered from the blood at 2, 75, 130 and 1320 min. were, respectively, 79, 53, 24 and 0 %. No quinone could be recovered after acid hydrolysis. The disappearance of free quinone was greater when incubated with whole blood or suspended washed red cells than with plasma, e.g. after 2 hr. incubation with one-tenth of the volume of saturated 4:6-dimethoxytoluquinone 29 % free quinone was recovered from a suspension of washed rabbit red cells and 63 % from the corresponding plasma. Again no quinone was recovered after acid hydrolysis. By modifying the hydrolysis procedure, a small proportion of the conjugated 4:6-dimethoxytoluquinone could be recovered from the blood previously incubated with 4:6-dimethoxytoluquinone until no free quinone was left. A maximum recovery of 20 % was obtained by increasing the concentration of SnCl₂ tenfold.

A mixture of citrated rabbit blood, hydrochloric acid containing SnCl₂ and saturated 4:6-dimethoxytoluquinone solution, extracted immediately with chloroform, gave only 50 % recovery of free quinone. This indicated that 4:6-dimethoxytoluquinone probably very readily forms an unhydrolyzable complex with acid haematin; for this reason further experiments in this direction were not attempted.

Feeding experiments on rabbits

Preliminary qualitative tests suggested that after feeding 4:6-dimethoxytoluquinone to rabbits a considerable amount of the compound was excreted conjugated with glucuronic acid. This is perhaps due, among other things, to the fact that in the organism the compound probably exists as the quinol because of its high oxidation reduction potential (Page & Robinson, 1943). The combined form could be detected in the urine within 30 min. of feeding the compound.

The rabbits were housed in metabolism cages and the urine and faeces collected separately. The diet consisted of 100 g. of washed sugar-beet pulp, bran and oats (4 : 1 : 1)

Table 2. *The effect of oral administration of 4:6-dimethoxytoluquinone on the urinary excretion of glucuronide and quinone by rabbits*

(C1-C4=pre-experimental period. TQ1-TQ4=experimental period. S1-S4=control period with food intake corresponding to TQ1-TQ4. A=values obtained using method of Maughan *et al.* (1938). B=corrected values using factor of 1.36 obtained after 4 hr. hydrolysis.)

24 hr. periods	Dose of 4:6-dimethoxytoluquinone 700 mg.				650 mg.				625 mg.			
	Urine volume (ml.)	Glucuronide* (mg.)		Quinone (mg.)	Urine volume (ml.)	Glucuronide* (mg.)		Quinone (mg.)	Urine volume (ml.)	Glucuronide* (mg.)		Quinone (mg.)
		A	B			A	B			A	B	
C1	250	165	—	—	350	235	—	—	280	—	—	—
C2	220	94	—	—	310	124	—	—	310	230	—	—
C3	250	162	—	—	330	202	—	—	310	205	—	—
C4	270	117	—	—	290	127	—	—	320	212	—	—
TQ1	60	261	355	325	70	268	365	375	190	320	436	277
TQ2	—	—	—	—	—	—	—	—	240	180	245	110
TQ3	200	205	279	29	275	242	329	50	330	270	—	0
TQ4	190	74	—	0	200	63	—	0	—	—	—	—
S1	75	63	—	0	118	82	—	0	120	117	—	0
S2	—	—	—	—	—	—	—	—	240	240	—	0
S3	160	109	—	0	175	110	—	0	200	200	—	0
S4	130	89	—	0	200	115	—	0	—	—	—	—

* Expressed as glucurone.

wetted with 300 ml. water, and 150 g. fresh cabbage daily. 4:6-Dimethoxytoluquinone (250 mg./kg. body wt.) suspended in warm arachis oil (in which it is partially soluble) or toluquinone (70 mg./kg.) suspended similarly, was fed by stomach-tube as the experiment required. Larger doses were often fatal. The urine was preserved with chloroform or toluene, introduced into the collecting vessel, to prevent bacterial hydrolysis of the conjugated excretion product.

Table 2 shows the total hydrolyzable quinone excreted in rabbits. No free 4:6-dimethoxytoluquinone or toluquinone was excreted. In the case of 4:6-dimethoxytoluquinone an average of 54 % of the drug was recovered in a conjugated acid hydrolyzable form, while with toluquinone the average was 46 %. We have not been able to account for the rest of the ingested quinones. No 4:6-dimethoxytoluquinone, either free or combined, detectable by our conditions of hydrolysis, is eliminated in the faeces.

In addition to the excretion of conjugated acid-hydrolyzable quinones, the excretion of glucuronic acid and inorganic and total sulphates has been followed. In the case of toluquinone, although it would have been desirable to follow the excretion of amino-acids, this was not possible, since even if all the toluquinone given had been excreted combined with amino-acid, no significant increase in the total excretion of amino-acid would have been observed.

The urine was collected for 24 hr. periods over a pre-experimental period of 4 days and for a further 4 days after the compound was given. Both compounds caused a marked decrease in the food intake, accompanied by a corresponding decrease in urinary volume. This in itself affected considerably the level of glucuronic acid excretion. In some experiments the urine was collected for a further control period

of 4 days, the food intake being adjusted to correspond with that over the experimental period. There was no increase in the excretion of ethereal sulphate after ingestion of 4:6-dimethoxytoluquinone or toluquinone; a slight increase would not have been significant. Table 2, however, shows the increased excretion of glucuronide and conjugated acid-hydrolyzable quinone after feeding 4:6-dimethoxytoluquinone. The absolute increase in glucuronide excretion cannot be given since the basal daily excretion varies within considerable limits. If we suppose, however, that 4:6-dimethoxytoluquinone combines with glucuronic acid in molecular proportions, it is highly probable that the whole of the 4:6-dimethoxytoluquinone appears in the urine as glucuronide.

Table 3. *The effect of oral administration of toluquinone on the urinary glucuronide and quinone excretion by a rabbit*

(The animal received a dose of 65 mg. toluquinone.)

(C1-C4, pre-experimental period; TQ1-TQ3, experimental period; S1-S3, control period with food intake corresponding to TQ1-TQ3.)

24 hr. periods	Urine volume (ml.)	Glucuronide* excreted (mg.)	Quinone excreted (mg.)
C1	230	208	—
C2	270	174	—
C3	270	194	—
C4	320	204	—
TQ1	150	150	50
TQ2	130	135	20
TQ3	170	125	0
S1	270	196	—
S2	150	140	—
S3	120	162	—

* Expressed as glucurone.

The corresponding experiment with toluquinone itself (Table 3) showed no increase in glucuronide excretion, so that, unlike 4:6-dimethoxytoluquinone, toluquinone is presumably not detoxicated by conjugation with glucuronic acid.

DISCUSSION

Although 4:6-dimethoxytoluquinone is highly bacteriostatic *in vitro*, its activity is decreased or abolished in the presence of serum and blood, and it is inactive *in vivo*. It is comparatively toxic to leucocytes and growing chick embryo fibroblasts and is unlikely to be of value as a therapeutic agent. One of our colleagues, Dr Harrison, has found it to be fungicidal against *Actinomyces praetorianus* and *Trichophyton laticolor* in concentrations of about 1 : 100,000, and it may possibly have some application in this field.

Since compounds of this type are common products of mould metabolism it is of interest to note that 4:6-dimethoxytoluquinone is very readily conjugated with glucuronic acid in the animal and that an adequate blood concentration cannot be obtained. Most of the ingested quinone can be recovered from the urine in a form mainly or entirely glucuronide. This has no antibacterial activity and is detectable in the urine within 30 min. of feeding the compound. Neither free nor acid-hydrolyzable conjugated quinone can be recovered from the faeces. The free quinone disappears fairly rapidly when it is incubated with whole blood *in vitro*, but the speed of disappearance is insufficient for this combination in the blood to assume significant proportions in the animal body.

SUMMARY

1. The *in vitro* antibacterial activities of 4:6-dimethoxytoluquinone and 4:6-dimethoxytoluquinol

against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Diplococcus pneumoniae* (Type 1), *Ps. aeruginosa*, *Proteus vulgaris*, *Esch. coli* and *Cl. perfringens* have been investigated. Both compounds were found to be highly bacteriostatic against Gram-positive organisms.

2. The bacteriostatic activity of 4:6-dimethoxytoluquinone was decreased in the presence of serum and abolished in the presence of blood.

3. Oral administration of the quinone gave no protection in mice infected with *Streptococcus pyogenes* or *Staphylococcus aureus*, and local injections were ineffective against experimental *Cl. perfringens* infections.

4. In tissue-culture experiments, the quinone, in a concentration of 1 : 40,000, inhibited the growth of chick-heart embryo fibroblasts. It also decreased the phagocytic action of leucocytes.

5. The quinone is rapidly conjugated with glucuronic acid when administered orally to rabbits, and appears in the urine in this form within 30 min. of feeding; the glucuronide has no antibacterial activity.

6. The fate of 4:6-dimethoxytoluquinone in the body is different from that of toluquinone itself, which is detoxicated by conjugation with amino-acids and not with glucuronic acid.

7. On account of its general toxic reactions, its inactivation in the presence of blood and rapid conjugation with glucuronic acid in the animal body, 4:6-dimethoxytoluquinone presumably has little therapeutic value.

The work described in this paper was carried out as part of a programme of the Therapeutic Research Corporation of Great Britain Ltd., to which acknowledgements are given. We are indebted to Dr Ashley for the supply of the quinones used.

REFERENCES

- Anslow, W. E., Ashley, J. N. & Raistrick, H. (1938). *J. chem. Soc.* p. 439.
- Armstrong, W. D., Spink, W. W. & Kahnke, J. (1943). *Proc. Soc. exp. Biol., N.Y.*, **53**, 230.
- Aulin, G. & Erdtman, H. (1938). *Svensk kem. Tidskr.* **50**, 42.
- Austin, J. H. & Drabkin, K. L. (1935). *J. biol. Chem.* **112**, 67.
- Barber, M. (1944). *J. Path. Bact.* **55**, 434.
- Cooper, E. A. & Haines, R. B. (1928). *Biochem. J.* **22**, 317.
- Cooper, E. A. & Haines, R. B. (1929). *Biochem. J.* **23**, 4.
- Cooper, E. A. & Nicholas, S. D. (1927). *J. Soc. Chem. Ind., Lond.*, **46**, 59T.
- Craven, R. (1931). *J. chem. Soc.* p. 1605.
- Fischer, E. & Schrader, H. (1910). *Ber. deutsch. chem. Ges.* **45**, 525.
- Hanson, S. W. F., Mills, G. T. & Williams, R. T. (1944). *Biochem. J.* **38**, 274.
- McIlwain, H., Fildes, P., Gladstone, G. P. & Knight, B. C. J. G. (1939). *Biochem. J.* **33**, 223.
- Marenzi, A. D. & Banfi, R. F. (1939). *Biochem. J.* **33**, 1879.
- Martin, G. J. & Rennebaum, E. (1943). *J. biol. Chem.* **151**, 417.
- Maughan, G. B., Evelyn, K. A. & Brown, J. S. L. (1938). *J. biol. Chem.* **128**, 567.
- Mozolowski, W. (1940). *Biochem. J.* **34**, 823.
- Oxford, A. E. (1942). *Chem. & Ind.* **61**, 189.
- Page, J. E. & Robinson, F. A. (1943). *Brit. J. exp. Path.* **24**, 89.
- Schulz, O., cit. Ellinger, A. (1923). *Heffter's Handbuch d. exp. Pharmacol.* **1**, 1026 (Berlin).
- Scudi, J. V. (1942). *Proc. Soc. exp. Biol., N.Y.*, **50**, 16.
- Ungar, J. & Robinson, F. A. (1943). *J. Pharmacol.* **80**, 217.
- Valeur, A. (1899). *C.R. Acad. Sci., Paris*, **129**, 552.
- Williams, R. T. (1940). *Biochem. J.* **34**, 272.