IV. THE SPERMICIDAL ACTIVITY OF QUINONES AND QUINOLS.

By JOHN MASSON GULLAND.

From the Biochemical Department, The Lister Institute, London.

(Received November 23rd, 1931.)

INTRODUCTION.

IN the course of his investigation [Baker, 1930, 1931] into the spermicidal effect of various substances Dr J. R. Baker invited me to collaborate with him in the examination of the action of pure chemical compounds on guinea-pig spermatozoa. By mutual agreement, many results of the biological tests of this joint work have been published elsewhere (Baker, 1932; in the press), but are unaccompanied by any comment on their significance from the chemical standpoint, although Dr Baker mentioned there that an article by me on this question was in preparation.

It is one purpose of this preliminary communication to mention the results of some further tests which Dr Baker has made and kindly permits me to quote. Its chief intention, however, is to discuss briefly possible modes of action of quinones and related compounds on the components of animal or vegetable tissues. An inspection of the relevant literature reveals that in the past only one of the many possibilities has been entertained—namely, the interaction of quinones with the primary amino-groups of proteins or their decomposition products, either as such, or as constituents of bacteria. Admittedly I have indulged in considerable speculation, but this is unavoidable in attempting to outline the scope of the problem in a preliminary communication. My thanks are due to Dr Baker for allowing me to include in this discussion results which he obtained before I joined him.

I take this opportunity to state that my main interest in this subject is not concerned with the contraceptive possibilities of the results obtained. It appears to me, however, that spermatozoa, by reason of their motility and of the spatial arrangement of their chemical components, are excellent material for the study of the effect and mode of action of chemical substances on the vitality of cells.

TECHNIQUE EMPLOYED.

The details of the technique have been amply elaborated by Baker in previous publications. The term "killing concentration" used in them and in this paper is the least concentration (weight/volume) in the series 1:50,

1 : 100, 1 : 200, 1 : 400, 1 : 800, *etc.*, which suffices to immobilise in 30 minutes in four consecutive experiments all guinea-pig sperms suspended in a glucose-saline fluid buffered at p_H 8.1 at 37°.

RESULTS OF BIOLOGICAL TESTS.

The results to be described deal with two main groups of compounds—namely, quinol derivatives and phenolic aldehydes—but others have been included on account of their chemical relationship to these groups. The figures in brackets indicate the killing concentrations, and a list is appended showing the compounds mentioned in order of their lethality.

Quinol derivatives.

In studying the toxicity of chemical substances to cells, it is natural to turn attention in the first place to the phenols on account of their germicidal properties. In the case of spermatozoa, phenol (1 : 200) is relatively inactive. From the few experiments performed, it would appear that the introduction of carbon side-chains into the molecule increases the lethality, since a mixture of cresols (tricresol) (1 : 400) is more active than phenol, but is surpassed by thymol (1 : 3200). In this respect there is a parallel with the increase in germicidal values of the homologous phenols as compared with that of the parent substance [Morgan and Cooper, 1912; Tilley and Schaffer, 1926, 1927].

The simple dihydroxybenzenes show striking variations depending on the orientation of the hydroxyl groups. Quinol (1 : 6400) is much more active than catechol (1 : 200), which in turn is more effective than resorcinol (1 : 100). It should be noted that hexylresorcinol (1 : 6400) shows enhanced efficiency as compared with resorcinol, presumably for the same reason that it is more germicidal [Johnson and Lane, 1921; Leonard, 1924, 1925; Elliot and Barbour, 1925]. The monomethyl ethers of dihydroxyphenols—quinol monomethyl ether (1 : 100) and guaiacol—are no more reactive than phenol, which suggested that a polyhydroxylic compound might have some specific action.

Attention was then directed to the isomeric trihydroxybenzenes. Phloroglucinol and pyrogallol (1 : 50) are practically inactive, whereas hydroxyquinol (1 : 6400) is rather more efficient than quinol. This superiority of quinol and hydroxyquinol at once focused interest on the group of *p*-dihydroxybenzenes, and noteworthy results were obtained—methoxyquinol (1 : 25,600), methylquinol (1 : 25,600), butylquinol (1 : 12,800). 2 : 6-Dimethoxyquinol, however, shows a decreased activity. This observation, coupled with the fact that the tests are carried out in an aerated solution at about p_H 8 in which oxidation of quinols might be expected to occur [*cf.* La Mer and Rideal, 1924; Euler and Brunius, 1928], led to the investigation of the *p*-quinones. These were all more active than the corresponding quinols, and out of nine tested, six—toluquinone, butylquinone, methoxyquinone,

ethylquinone, benzoquinone, *p*-xyloquinone (all 1 : 51,200)—were more spermicidal than mercuric chloride, hitherto the most active agent known.

The disubstituted quinones, 2-methoxy-5-methylquinone (methoxytoluquinone) (1 : 6400) and thymoquinone (1 : 12,800), were less lethal than those just mentioned, as also was duroquinone (no effect at 1 : 25,600), in which all four nuclear hydrogens are replaced by methyl groups. Unfortunately, the sparing solubility of duroquinone in water prevented its being tested at higher concentrations.

A few quinonoid dyestuffs—malachite green, crystal violet, pyronine G—and also nitrobenzene and dinitrobenzene were examined, but of these only malachite green (1 : 12,800) proved relatively efficient.

In view of the facile oxidation of *p*-phenylenediamine to benzoquinone which can be effected in the chemical laboratory, tests were carried out with this diamine (slight activity at 1 : 100) and with the *ortho*- (1 : 50) and *meta*- (fails at 1 : 50) isomerides. It must be concluded that no such oxidation occurs under the conditions of the biological test, and that whatever may be the explanation for the high spermicidal activity of the quinones and quinols it does not hold for the diaminobenzenes.

Table I. *Killing concentrations.*

Substances are arranged in order of activity, except where they are bracketed together. In the section "not fully tested," 3 indicates full activity of sperms, 0 indicates all immobile. Intermediate activity is graded 1, 2, *etc.*

1 : 51,200	1 : 400
Toluquinone	Tricresol
Butylquinone	1 : 200
{Methoxyquinone	Catechol
{Ethylquinone	Phenol
{Benzoquinone	
{ <i>p</i> -Xyloquinone	1 : 100
1 : 25,600	{Quinol methyl ether
Mercuric chloride	{Acetaldehyde
Methoxyquinol	{Chloral hydrate
{Formaldehyde	{Resorcinol
{Paraformaldehyde	1 : 50
Methylquinol	Pyrogallol
1 : 12,800	<i>o</i> -Phenylenediamine
Butylquinol	Fail to kill at 1 : 50
Malachite green	<i>m</i> -Phenylenediamine
Thymoquinone	
1 : 6,400	<i>Not fully tested.</i>
{2-Methoxy-5-methylquinone	Duroquinone. No effect at 1 : 25,600
{Hexylresorcinol	Nitrobenzene
{2 : 6-Dimethoxyquinol	Dinitrobenzene
{Hydroxyquinol	<i>p</i> -Nitrosodimethylaniline
{2-Hydroxy-3-ethoxybenzaldehyde (ethoxy-	Pyronine G
{ salicylaldehyde)	Guaiacol 3 at 1 : 1600
{2-Hydroxy-3-methoxybenzaldehyde	<i>iso</i> Vanillin 3 at 1 : 1600
{ (<i>o</i> -vanillin)	<i>p</i> -Dimethylaminobenzaldehyde 3 at
Quinol	1 : 1600
1 : 3,200	<i>p</i> -Hydroxybenzaldehyde 2 + at 1 : 1600
Salicylaldehyde	<i>p</i> -Phenylenediamine 1, 2, 2, at 1 : 100
Crystal violet	Phloroglucinol 1, 1, 1, at 1 : 400
Thymol	Not sufficiently soluble to try at 1 : 200
	Phloroglucinaldehyde 0, 0, 1 at 1 : 1600
	Vanillin 0 at 1 : 400, 3 at 1 : 1600

Phenolic aldehydes.

The high toxicity of formaldehyde (1 : 25,600) is striking when compared with that of acetaldehyde and chloral (both 1 : 100), and is doubtless analogous to its vigorous bactericidal action.

The aromatic aldehydes also exhibit marked divergences in activity. Salicylaldehyde, methoxysalicylaldehyde (*orthovanillin*), and ethoxysalicylaldehyde (all 1 : 6400) are about sixteen times as effective as vanillin, *iso*-vanillin, *p*-hydroxybenzaldehyde, and *p*-dimethylaminobenzaldehyde, all of which fail to kill at 1 : 1600, and are probably lethal at 1 : 400. It is interesting to note that the *o*-phenolic aldehyde phloroglucinaldehyde is intermediate in character, being somewhat more active than the last mentioned group, though inferior to the substituted salicylaldehydes.

DISCUSSION.

In the action of chemical substances on living cells it is evident that many factors may play a simultaneous part in producing the final result. For example, the effects due to alterations in surface tension, permeability of tissues, adsorbability and other similar physical causes may entirely outweigh those due to specific chemical groups. It would therefore be dangerous to contrast the behaviour of standard material under conditions which were changed only by the addition of different chemical substances in varying proportions, unless a close relationship existed not only between the radicals present, but also in the molecular size of each compound. The members of the group of quinones and quinols described above are probably free from any significant variations in these respects, and consequently a chemical explanation may be sought of their vigorous spermicidal properties as compared, for example, with those of the phenols and resorcinols. In order to do this, it will be necessary to consider briefly the modern conceptions of reactivity of such compounds.

In the terms of the electronic theory of the mechanism of reactions [Robinson, 1925; Rây and Robinson, 1925; Allan, Oxford, Robinson and Smith, 1926; Lapworth and Robinson, 1928], the reactivity of phenol is expressed by either of the formulae I or II. In these a definite polarisation of the molecule

has occurred owing to the tendency of the oxygen of the hydroxyl group to increase its covalency with the neighbouring carbon atom. This effect is carried through the conjugated system of the benzene ring, and results in the

production at the *o*- and *p*-positions of an excess of electrons as compared with the non-polarised state. The carbon atoms at these positions are termed anionoid centres, and are attacked by kationoid, or electron-seeking, reagents.

The case of resorcinol is merely an elaboration of that of phenol. The individual effects of the two hydroxyls are combined (III), and the intense sensitivity of resorcinol to kationoid reagents is well known.

As regards quinones, however, the situation is reversed. On the same theory the properties associated with doubly-linked oxygen are explained by its tendency to decrease its covalency by attracting electrons away from the adjacent carbon atom. The oxygen atoms of a quinone thus become anionoid centres, and the electron drift is carried on through the two conjugated systems of which benzoquinone, for example, is composed (IV and V). The carbon atoms *X* and *Y* in the β -positions to the carbonyl groups are thus the kationoid centres, and will be attacked by anionoid, or electron-donating, reagents.

The following, among many other possible illustrations, will serve to indicate the great differences between the two types of reactivity. Phenol is very readily substituted by bromine and by dilute nitric acid: benzoquinone takes up bromine additively [Sarauw, 1881; Nef, 1890] and is not attacked by cold, fuming nitric acid [Liebermann, 1877; Nietzki, 1882]. On the other hand, benzoquinone and resorcinol readily react when heated together [Pummerer and Huppmann, 1927], the anionoid centre of resorcinol combining with the kationoid centre of the quinone to yield tetrahydroxydiphenyl (VI). No such reaction takes place between phenol and resorcinol.

In virtue of this property of reacting with electron-donating reagents, the quinones combine chemically with many different types of compounds, the union in many cases being extremely facile [see Houben, 1923]. It is of interest to discuss here only their behaviour with substances or groups which may reasonably be expected to occur in living tissues. It should be noted, however, that the omission of a particular group, or type, does not exclude the possibility that it may, as a component of the spermatozoon, undergo reaction with a quinone.

Quinol derivatives.

The reactivity of quinols as a class is phenolic in character. Nevertheless, it will be observed that the quinols are, in general, only slightly less active than the corresponding quinones. It seems probable, therefore, that they exercise their spermicidal properties not by precipitation of cell colloids as do

phenols and allied germicides, but by either (i) undergoing oxidation to the quinones by oxygen in the alkaline fluid (*cf.* Harger [1922, 1924] on the action of primary and secondary aliphatic amines on quinol), or (ii) by interfering with the oxidising systems of the cell.

Reactions of p-quinones.

Primary amines. Primary, secondary and tertiary aromatic amines form highly coloured additive complexes with quinones [Jackson and Clarke, 1905; Fecht, 1908; Schlenk and Knorr, 1909; Siegmund, 1910; Brass, 1913; Suchanek, 1914; Kremann *et al.*, 1922; Hixon, 1923]. When those derived from primary amines are oxidised by aerial oxygen, by a molecule of a quinone or by other means, they are easily converted into nuclear condensation products, of which dianilinoquinone (VII) is typical. In the interaction of quinones with aliphatic primary amines, however, the first isolable product is the corresponding alkylaminoquinone [Fichter and Willmann, 1904; Fichter, 1908; Zincke, 1881].

In extending these foregoing results to animal tissues, Fischer and Schröder [1910] prepared the substance (VIII) from benzoquinone and glycine ester, and observed that toluquinone formed a similar compound. Hilpert [1925] selected glycine anilide to represent a peptide, and found that one molecule only entered the nucleus of benzoquinone (IX), toluquinone and α -naphthoquinone respectively.

No definite compounds have been isolated as the result of the action of quinones on proteins or their decomposition products, apart from amino-acids. Several investigators, however, have been attracted by the similarity between the red colours of the anilinoquinones and related compounds and those produced when quinones react with proteins, peptones or amino-acids, and have associated the production of colour with the formation of substituted aminoquinones from the primary amino-groups of these substances [Raciborski, 1906; Scharvin, 1913; Suida, 1913; Cooper, 1913; Morgan and Cooper, 1921]. The high bactericidal value of the quinones has been studied at some length on the basis of this type of action by the germicide on the primary amino-groups not only of the complex proteins, but also of simple water-soluble cell constituents [Morgan and Cooper, 1924; Hilpert, 1925; Cooper and Nicholas, 1927; Cooper and Haines, 1928, 1929].

Cooper [1913] and Morgan and Cooper [1921] have suggested that compounds analogous to quinonedianil (X) may result from interaction of primary amino

groups of cell constituents and quinones. This view is largely based on the fact that gelatin is not attacked by an aqueous solution of quinone dioxime, whereas after treatment with quinone it may be isolated in a coloured state having altered physical properties. The evidence for this type of reaction seems somewhat inadequate. A study of the literature shows that in general nuclear condensation is preferred to "anil" formation, and that quinone dioxime does not retain the high nuclear reactivity of the parent substance. This may be deduced on theoretical grounds from electronic considerations, and it is clearly unsatisfactory to compare the reactivity of the two substances.

As mentioned above, the possibility that groups, other than primary amino-groups, of cell constituents may enter into combination with quinones does not appear to have been entertained. It is noteworthy, however, that Cooper and Haines [1928] observed in a quantitative experiment that the amount of benzoquinone taken up by gelatin is considerably in excess of that required by the primary amino-groups. Since the nitrogen of peptide groups does not react with quinones, the additional amount of benzoquinone is regarded by them as being adsorbed and not chemically combined, but it seems not improbable that entry into chemical union may account for some at least of the "adsorbed" quinone in their experiment. Further, Suida [1913] found that 2:5- and 2:6-xyloquinone and thymoquinone do not dye wool, whereas benzoquinone, toluquinone and others do so, and concluded that these dialkylquinones lacked the power of forming aminoquinonoid derivatives. Reference to Table I shows that two of these are excellent spermicides and may therefore have the power of entering into other types of compounds than those which benzoquinone forms with wool. These facts suggest that reactions such as are described below may play as important a part in the action of quinones on cell substances as do those in which primary amino-groups participate.

Secondary amines. These are known to occur in animal tissues, and there is ample evidence that they react chemically with quinones to produce substituted aminoquinones, analogous in constitution to tetramethyldiaminobenzoquinone [Mylius, 1885; Harger, 1922]. In the case of reduced heterocyclic compounds containing imino-groups, Schmidt and Sigwart [1913] have recorded the production of aminoquinones and the examples quoted, *e.g.* piperidine, have analogies with the constituents of animal material. These authors and Möhlau and Redlich [1911] observed that pyrrole and α -methyl-indole readily react with quinone, whereas carbazole and tetrahydrocarbazole do not; the suggestion made by Möhlau and Redlich that carbon-to-carbon condensation has occurred here would seem to be correct and probably limits the possibility of this type of combination to pyrroles having a free α - or β -position, unless it can be shown that indoles occur naturally in which the β -position is unsubstituted.

Phenoquinones and related substances. The additive complexes which quinones form with aromatic primary, secondary and tertiary amines have already been mentioned, but it is very doubtful if this type of combination would play

a part in the action of quinones on spermatozoa. Additive complexes, however, are formed with great ease also when quinones react with phenols, two hydroxyl groups, in general, uniting with one molecule of the quinone. Very many cases of such phenoquinones (XI) are known [Beilstein, 1925], quinhydrone (XII) being a special example, and the occurrence of phenolic groups in living matter has long been established. Quinhydrone is stable up to p_H 8 [La Mer and Parsons, 1923], and complexes of this type would thus appear to be possible within the p_H limits of the living cell and the glucose-saline fluid.

The conversion in the cell of phenoquinones into diphenylquinones is possible, but improbable, since in the laboratory it involves the use of aluminium chloride as a condensing reagent [Pummerer and Prell, 1922]. Resorcylic groups, being highly efficient as anionoid reagents (see above), might conceivably react in this way, but their presence in animal, as opposed to vegetable, organisms has not been demonstrated.

Sulphydryl compounds. Thiophenols and mercaptans unite readily with benzoquinone and toluquinone, forming thiophenoquinones—additive complexes similar to the phenoquinones. When these are oxidised or undergo autoxidation they readily yield substituted thiolquinones, of which di-*iso*amylthiolquinone (XIII) is typical [Tarbouriech, 1901; Posner, 1904; Posner and Lipski, 1904].

The importance of the part played by sulphydryl groups in living processes is fully recognised, and it is probable that the thioliminazoles would react with quinones in a similar manner from their analogy with the thiophenols.

Aldehydes. When aqueous solutions of *p*-benzoquinone and acetaldehyde, isovaleraldehyde or benzaldehyde are exposed to light, a dihydroxyphenylketone (XIV) is produced [Klinger and Standke, 1891; Klinger and Kolvenbach, 1898]. With these results in view, it is noteworthy that the presence of aldehydic groups has been proved or suspected in the intermediates of animal

processes and in enzymes, *e.g.* erepsin [Josephson and Euler, 1927; Waldschmidt-Leitz and Rauchalles, 1928].

Alcohols. A few examples are known in which quinone reacts with alcohols to form ethers of 2 : 5-dihydroxyquinones [Möhlau and Klopfer, 1899; Knoevenagel and Büchel, 1901]. The reaction, however, is in general not facile and requires a condensing agent.

Oxidation-reduction. The ease with which quinones can enter into oxidation-reduction systems is fully established [Oppenheimer, 1926]. Wieland and Bertho [1928], Neuberg and Simon [1926] and Lüers and Mengele [1926] found that quinones can act as hydrogen acceptors in acetic or alcoholic fermentations, and Dixon [1926] observed that quinone played a similar part in the xanthine-oxidase system. By *in vitro* experiments Wieland and Bergel [1924], Bach [1914] and Traube [1911] showed that *p*-benzoquinone transformed α -amino-acids to aldehydes, and Ciamician and Silber [1901] used it to oxidise alcohols to aldehydes, polyhydric alcohols to aldoses (ketoses), and glucose to glucosone.

The effect of quinones on sulphydryl-disulphide systems does not appear to have been investigated, although it might be anticipated from a comparison of oxidation-reduction potentials [Michaelis, 1929] that they would maintain the systems in an oxidised state. Posner and Lipski [1904] have observed that xyloquinone, in which the nuclear activity is somewhat decreased by the substituents (see later), oxidises thiophenol to diphenyldisulphide.

It is well recognised that quinol is dehydrogenated by enzymes, as for example by muscular tissue [Wieland and Lawson, 1931; see also Wieland and Sutter, 1928; Battelli and Stern, 1912], and it has in addition remarkable anti-oxidant properties [Moureu and Dufraisse, 1922, 1, 2; 1928].

Enough has been indicated above to show that the high spermicidal values of *p*-quinones and quinols can receive abundant explanation, whether their action is regarded as chemical union with cell constituents, or as interference with vital enzymic processes, and it is hoped that further investigations will allow definite interpretations to be placed on their modes of action.

orthoQuinones and orthoquinols.

It may be asked why catechol is so much less active than quinol since much of what has been said undoubtedly would apply, and in many cases does apply, to it and to *o*-benzoquinone. The explanation of this unexpected variation is probably to be found in the greater reactivity of *o*-benzoquinone and catechol as compared with the *p*-isomerides. In the alkaline media in which the test is carried out, *o*-benzoquinone would be extremely rapidly polymerised to complex inactive substances on account of its instability [Willstätter and Pfannenstiel, 1904], whereas *p*-benzoquinone is comparatively much less labile [Meunier and Queroix, 1925].

Phenolic aldehydes.

It seems reasonable to assume that the phenolic aldehydes which have been tested form a group in which the physical effect of each member on the cell should not differ very greatly. Here again as with the quinones and related compounds, it is possible to search for a chemical explanation of activity.

The moderately high lethality of salicylaldehyde and its vicinal methoxy- and ethoxy-derivatives suggested a hypothesis which related the action of these aldehydes with that of the quinones. Dakin [1909] showed that the sodium salts of *o*- and *p*-hydroxyaldehydes of the benzene series are readily and often almost quantitatively oxidised to the corresponding *o*- and *p*-dihydroxybenzenes by hydrogen peroxide. The same author [1922] has drawn attention to the similarity between hydrogen peroxide oxidations *in vitro* and many oxidations *in vivo*, and it seemed possible that the *o*-hydroxyaldehydes were in themselves little reactive, and that the spermatozoa by this oxidation brought about their own extermination. This hypothesis became untenable, however, when it was found that vanillin is about sixteen times less active than *o*-vanillin. From the experimental results it seems possible to correlate spermicidal activity with the adjacent orientation of the hydroxyl and aldehydic groups, but further discussion is reserved for a future publication.

COMPARISON OF THE SPERMICIDAL AND GERMICIDAL POWERS OF *p*-QUINONES
AND THE CORRELATION OF SPERMICIDAL AND CHEMICAL ACTIVITY.

In conformity with previous work, the killing concentrations quoted in this paper are calculated on equal, and not equimolecular, weights of chemical substances. This is unfortunate from the point of view of an absolute experimental comparison of the spermicidal powers of different members of the quinone, and quinol, groups. From a consideration of molecular weights, however, and from the protocols of experiments, it is possible to arrive at the following series of *p*-quinones in order of spermicidal activity:

Toluquinone $\doteq$ butylquinone > methoxyquinone $\doteq$ ethylquinone > *p*-benzoquinone $\doteq$ *p*-xyloquinone > thymoquinone > 2-methoxy-5-methylquinone.

The experimental accuracy of the technique employed does not allow a definite decision to be reached between toluquinone and butylquinone. As an illustration of their superiority over *p*-benzoquinone, however, it may be mentioned that toluquinone is very nearly twice as spermicidal as benzoquinone. Similarly it is not possible to make a definite decision between methoxyquinone and ethylquinone, nor between benzoquinone and *p*-xyloquinone. The first six members of the series lie comparatively close together, and are separated by an interval from thymoquinone, which in turn is followed at some distance by methoxytoluquinone.

It is evident that monoalkylquinones are more spermicidal than benzoquinone, and that disubstituted quinones are less spermicidal. It should be

noted that the substituents have been selected so that they are themselves unlikely to exert any influence through chemical reaction with the cells. The case of methoxyquinone is a special one, and will be discussed below.

These results are strikingly different from those of Cooper and his collaborators on the germicidal powers of quinones; they find, in general, that bactericidal activity is diminished on ascending the homologous series.

Erdtman [1932]¹ has recently conducted a systematic investigation into the chemical reactivity of a number of *p*-benzoquinones. As a test of their reactivity to anionoid reagents, he has examined under standard conditions the ease with which they are converted into triacetoxy-derivatives by acetic anhydride and sulphuric acid [Thiele, 1898], and has also studied the readiness with which they yield the corresponding quinols with sulphur dioxide. He finds that the following series holds,

and considers that the superiority of the monosubstituted quinones over benzoquinone is due to the neutralisation by the substituent of the electronic attraction of the carbonyl group, whereas in the disubstituted quinones the electronic effects of both carbonyl groups are decreased. In both cases the magnitude of the neutralisation is dependent on the strength of electronic repulsion of the substituent.

The striking similarity between the spermicidal and chemical activities of the *p*-quinones lends great support to the views which have been expressed above as to the reasons for their lethality. The only anomalous result is that of methoxyquinone, which is the most reactive chemically, but is only slightly greater than benzoquinone in spermicidal effect. The explanation is simple; so reactive is this quinone chemically that during the course of the biological test it undergoes polymerisation to a considerable extent at the alkaline p_H of the glucose-saline buffer solution.

Again taking molecular weights into account, the group of quinols may be arranged in order of their lethality to spermatozoa—methoxyquinol > methylquinol $\doteq$ butylquinol > 2 : 6-dimethoxyquinol > hydroxyquinol > quinol. The similarity of the first part of the series to the corresponding portion of the quinone series is striking, and suggests that the quinols may exert their spermicidal action in the form of quinones by undergoing gradual oxidation in the alkaline fluid. Hydroxyquinol, which should in theory resemble methoxyquinol, probably takes a low place on account of its instability in alkaline solution, and, as compared with the position of benzoquinone in the quinone series, quinol is less active than might be expected. This result might conceivably receive an explanation in the rates of oxidation of the quinols, and the question is being investigated.

¹ I wish to express to Dr H. Erdtman my indebtedness to him not only for allowing me to quote these results, but also for giving me the opportunity on various occasions of discussing with him questions relating to the chemical reactivity of quinones.

PREPARATION OF CHEMICAL SUBSTANCES.

I am greatly indebted to Dr H. Erdtman, Dr Wilson Baker, Dr S. Sugasawa and Mr T. H. Mead for gifts of some of the compounds which have been tested. The following compounds have not previously been described.

2 : 5-Dihydroxyphenyl propyl ketone. Powdered aluminium chloride (30 g.) was added gradually to an ice-cold solution of quinol dimethyl ether (30 g.) and *n*-butyryl chloride (23 g.) in carbon disulphide (30 cc.). After 12 hours, ice was added, and the mixture was steam-distilled to remove solvent and traces of quinol dimethyl ether. The product of the reaction, 2 : 5-dimethoxyphenyl propyl ketone, was not readily volatile in steam, and was extracted from the residue with ether, dried, and obtained as a brown oil by evaporation of the solvent. Without further purification, this ketone (30 g.) was demethylated by being heated under reflux with a boiling mixture of concentrated hydrobromic acid (100 cc.) and glacial acetic acid (100 cc.) for 18 hours. The liquid was poured into water, and the product extracted with ether, freed from acids by being shaken with aqueous potassium bicarbonate solution, and obtained in the crystalline state when the solvent was evaporated. 2 : 5-Dihydroxyphenyl propyl ketone was purified by distillation, B.P. 193°/16 mm., followed by crystallisation from carbon tetrachloride. It formed yellow prismatic tablets, M.P. 87–89°, which changed to a powdery state shortly before melting, and dissolved readily in benzene and chloroform. (Found: C, 66.5; H, 6.6. $C_{10}H_{12}O_3$ requires C, 66.7; H, 6.7 %.) The preparation of this substance by heating together quinol, *n*-butyryl chloride and zinc chloride is less satisfactory. The method described has been used with equal success for the preparation of 2 : 5-dihydroxyacetophenone, but this was accompanied by a small amount of a deep-red by-product, which, on treatment with sodium acetate and hydroxide, exhibited the colour changes associated with an anthocyanidin.

2 : 5-Dihydroxybutylbenzene (butylquinol). The ketone described above (10 g.) was reduced according to Clemmensen's method with amalgamated zinc and equal volumes of concentrated hydrochloric acid and water. After 10 hours, the hot solution was filtered, cooled, and extracted with ether. The extract, when washed, dried and distilled, yielded a brown oil which was fractionated at reduced pressure. Butylquinol passed over at 180–185°/20 mm., and crystallised from carbon tetrachloride in small, colourless needles, M.P. 89°. (Found: C, 73.2; H, 8.6. $C_{10}H_{14}O_2$ requires C, 72.3; H, 8.4 %.)

Butyl-1 : 4-quinone. Butylquinol was dissolved in hot water, and the solution was quickly cooled so that no separation of solid occurred. A large excess of aqueous ferric chloride solution was added, and butylquinone separated immediately as a yellow oil which rapidly crystallised. It was collected, dried in a vacuum desiccator, and purified by sublimation at 100°, since it was readily soluble in all the usual organic solvents including light petroleum. It formed orange-yellow needles, M.P. 32°, which were very rapidly attacked by 2*N* sodium hydroxide. (Found: C, 72.9; H, 7.3. $C_{10}H_{12}O_2$ requires C, 73.1;

H, 7.3 %.) On being kept, the quinone was transformed into a dark-coloured oil in the course of 24 hours; from this, some unchanged quinone could be extracted by means of light petroleum.

SUMMARY.

- (1) *p*-Quinones and quinols are highly toxic to guinea-pig spermatozoa.
- (2) Possible reasons for their great toxicity, as compared with that of mono-hydroxyphenols and resorcinol derivatives, are discussed on the basis of chemical interaction of the spermicide with cell constituents, and an explanation is suggested for the relative lack of toxicity of *o*-benzoquinone and catechol.
- (3) A series of *p*-quinones arranged in order of spermicidal efficiency resembles very closely a series of the same, or similar, quinones placed in order of chemical activity.
- (4) Monoalkyl-*p*-quinones are more spermicidal than *p*-benzoquinone, which is more toxic than dialkyl-*p*-quinones.
- (5) Aromatic *o*-hydroxyaldehydes are much more spermicidal than *m*- or *p*-hydroxyaldehydes.

My thanks are due to Dr J. R. Baker for his invitation to collaborate with him in this work, and for carrying out the numerous biological tests.

REFERENCES.

- Allan, Oxford, Robinson and Smith (1926). *J. Chem. Soc.* 401.
 Bach (1914). *Biochem. Z.* **58**, 205.
 Baker (1930). *J. Hyg.* **29**, 323.
 — (1931). *J. Hyg.* **31**, 189, 309.
 Battelli and Stern (1912). *Ergebn. Physiol.* **12**, 96.
 Beilstein (1925). *Handbuch der organischen Chemie*, 4th ed. **7**, p. 165.
 Brass (1913). *Ber. deutsch. chem. Ges.* **46**, 2903.
 Ciamician and Silber (1901). *Ber. deutsch. chem. Ges.* **34**, 1530.
 Cooper (1913). *Biochem. J.* **7**, 186.
 — and Haines (1928). *Biochem. J.* **22**, 317.
 — (1929). *Biochem. J.* **23**, 4.
 — and Nicholas (1927). *J. Soc. Chem. Ind.* **46**, 59 T.
 Dakin (1909). *Amer. Chem. J.* **42**, 477.
 — (1922). *Oxidations and reductions in the animal body*, 2nd ed. (Longmans, Green and Co., London.)
 Dixon (1926). *Biochem. J.* **20**, 703.
 Elliot and Barbour (1925). *Canad. Med. Assoc. J.* 787.
 Erdtman (1932). *Svensk Kemisk Tidskrift*, in the press.
 Euler and Brunius (1928). *Z. physikal. Chem.* **139**, 615.
 Fecht (1908). *Ber. deutsch. chem. Ges.* **41**, 2986.
 Fichter (1908). *Liebig's Ann.* **361**, 397, 400.
 — and Willmann (1904). *Ber. deutsch. chem. Ges.* **37**, 2388.
 Fischer and Schröder (1910). *Ber. deutsch. chem. Ges.* **43**, 525.
 Harger (1922). *Proc. Nat. Acad. Sci.* **8**, 57.
 — (1924). *J. Amer. Chem. Soc.* **46**, 2540.
 Hilpert (1925). *Biochem. Z.* **166**, 71.

- Hixon (1923). *J. Amer. Chem. Soc.* **45**, 2333.
- Houben (1923). Die Methoden der organischen Chemie, 2nd ed., 3, 602 *et seq.* (Thieme, Leipzig.)
- Jackson and Clarke (1905). *Amer. Chem. J.* **34**, 441.
- Johnson and Lane (1921). *J. Amer. Chem. Soc.* **43**, 348.
- Josephson and Euler (1927). *Z. physiol. Chem.* **162**, 85.
- Klinger and Kolvenbach (1898). *Ber. deutsch. chem. Ges.* **31**, 1214.
- and Standke (1891). *Ber. deutsch. chem. Ges.* **24**, 1340.
- Knoevenagel and Bückel (1901). *Ber. deutsch. chem. Ges.* **34**, 3993.
- Kremann, Sutter, Sitte, Strzelba and Dobotzky (1922). *Monatsh.* **43**, 269.
- La Mer and Parsons (1923). *J. Biol. Chem.* **57**, 613.
- and Rideal (1924). *J. Amer. Chem. Soc.* **46**, 223.
- Lapworth and Robinson (1928). *Mem. Manchester Lit. Phil. Soc.* **72**, 43.
- Leonard (1924). *J. Amer. Med. Assoc.* **83**, 2005.
- (1925). *Lancet*, i, 448.
- Liebermann (1877). *Ber. deutsch. chem. Ges.* **10**, 2179.
- Lüers and Mengele (1926). *Biochem. Z.* **179**, 238.
- Meunier and Queroix (1925). *J. Soc. Leather Trades Chem.* **9**, 26.
- Michaelis (1929). Oxydation-Reduktions Potentiale. (Springer, Berlin.)
- Möhlau and Klopfer (1899). *Ber. deutsch. chem. Ges.* **32**, 2147.
- and Redlich (1911). *Ber. deutsch. chem. Ges.* **44**, 3605.
- Morgan and Cooper (1912). Rep. 8th Int. Congress App. Chem. **19**, 243.
- — (1921). *Biochem. J.* **15**, 587.
- — (1924). *J. Soc. Chem. Ind.* **43**, 352 T.
- Moureu and Dufraisse (1922, 1). *Compt. Rend. Soc. Biol.* **86**, 321.
- — (1922, 2). *Compt. Rend. Acad. Sci.* **174**, 258.
- — (1928). *Chem. Ind.* **47**, 819, 848.
- Mylius (1885). *Ber. deutsch. chem. Ges.* **18**, 468.
- Nef (1890). *J. prakt. Chem.* [2], **42**, 182.
- Neuberg and Simon (1926). *Biochem. Z.* **171**, 256.
- Nietzki (1882). *Liebig's Ann.* **215**, 137.
- Oppenheimer (1926). Die Fermente und ihre Wirkungen, II. (Thieme, Leipzig.)
- Posner (1904). *Liebig's Ann.* **336**, 85.
- and Lipski (1904). *Liebig's Ann.* **336**, 149.
- Pummerer and Huppmann (1927). *Ber. deutsch. chem. Ges.* **60**, 1442.
- and Prell (1922). *Ber. deutsch. chem. Ges.* **55**, 3105.
- Raciborski (1906). *Anzeiger Akad. Wiss. Krakau.* 553.
- Rây and Robinson (1925). *J. Chem. Soc.* **127**, 1618.
- Robinson (1925). *Chem. Ind.* **44**, 18, 456.
- Sarauw (1881). *Liebig's Ann.* **209**, 111.
- Scharvin (1913). *Z. angew. Chem.* **26**, 254.
- Schlenk and Knorr (1909). *Liebig's Ann.* **368**, 277.
- Schmidt and Sigwart (1913). *Ber. deutsch. chem. Ges.* **46**, 1491.
- Siegmund (1910). *J. prakt. Chem.* [2], **82**, 409.
- Suchanek (1914). *J. prakt. Chem.* [2], **90**, 467.
- Suida (1913). *Z. physiol. Chem.* **85**, 308.
- Tarbouriech (1901). *Bull. Soc. Chim.* (iii), **25**, 313.
- Thiele (1898). *Ber. deutsch. chem. Ges.* **31**, 1247.
- Tilley and Schaffer (1926). *J. Bact.* **12**, 303.
- — (1927). *J. Bact.* **14**, 259.
- Traube (1911). *Ber. deutsch. chem. Ges.* **44**, 3145.
- Waldschmidt-Leitz and Rauchalles (1928). *Ber. deutsch. chem. Ges.* **61**, 645.
- Wieland and Bergel (1924). *Liebig's Ann.* **439**, 196.
- and Bertho (1928). *Liebig's Ann.* **487**, 95.
- and Lawson (1931). *Liebig's Ann.* **485**, 193.
- and Sutter (1928). *Ber. deutsch. chem. Ges.* **61**, 1060.
- Willstätter and Pfannenstiel (1904). *Ber. deutsch. chem. Ges.* **37**, 4744.
- Zincke (1881). *Ber. deutsch. chem. Ges.* **14**, 94.