THE SPERMICIDAL POWERS OF CHEMICAL CONTRACEPTIVES.

IV. MORE PURE SUBSTANCES.

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CONTENTS.

Introduction.

In an earlier paper of this series (Baker, 1931 *a*), a method was described whereby substances may be graded according to their powers of immobilising guinea-pig sperms. It was thought best at first to test a wide variety of substances, and thirty-six were graded and reported upon in the paper referred to above. In order to discover general principles underlying the immobilisation of sperms by chemical means, it was felt that the most profitable way of continuing the research would be to test a number of allied substances, quite irrespective of whether they were likely themselves to prove of practical value. For this purpose I chose two groups, the phenols and the aldehydes. I had not gone far with the investigation of them, when it became apparent that the work could only be carried out with the help of an organic chemist. I was very fortunate in securing the co-operation of Dr J. M. Gulland, who not only suggested various substances for trial, but also prepared those which were not available, including two extremely spermicidal substances (butylquinone and butylhydroquinone) which had not been prepared before. Dr Gulland proposes later to publish a paper on the chemical interpretation of this work. I wish to make it clear that the work on the phenols, quinones and aldehydes, described in this paper, could not have been carried out without his co-operation, for which I am very grateful.

A variety of substances hitherto untested are also reported on in this paper.

I wish to thank Mr A. F. Walden, Dr H. Erdtmann, and Mr C. W. Carter for chemical advice. The work has been carried out under the auspices of the Birth Control Investigation Committee in the Department of Zoology and Comparative Anatomy at Oxford, by kind permission of Prof. E. S. Goodrich, F.R.S.

Recapitulation.

This section is included for the benefit of those who have not read the earlier paper referred to above.

The technique which is described in the earlier paper aims at finding what is the least concentration of any substance, in the series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, etc., per cent., which suffices to immobilise every guinea-pig sperm in half an hour at 37° C. in four consecutive experiments, the control sperms showing normal activity. The least concentration sufficing for this is called the killing concentration. Each substance is also tried three times at one-half of the killing concentration, and the activity of the sperms is graded. The highest activity is 3 +. Complete absence of any movement is 0. 3, 2 +, 2, 1 + and 1 are intermediate grades of activity. The grading of the activity of sperms at one-half of the killing concentration enables one to place substances, whose killing concentration is the same, in the order of their spermicidal powers.

Improvements in technique.

It has not been found necessary to make any material alteration in the general technique.

I now make up B.G.S. with less acid potassium phosphate. The increased alkalinity (about *p*H 8·1) favours the activity of the sperms, and hinders the growth of moulds to some extent. The fluid is as follows:

Buffered glucose-saline.

Glucose	3·0 grm.
Sodium hydrogen phosphate ($Na_2HPO_4 . 12H_2O$)	0·6 „
Sodium chloride	0·2 „
Acid potassium phosphate (KH_2PO_4)	0·01 „
Distilled water	100 c.c.

While the general technique is satisfactory, it does not give reliable results with the salts of metals whose phosphates are insoluble, for they are partly precipitated by the phosphates of the B.G.S. I have therefore elaborated a new fluid for sperm suspensions when such substances are to be tested. It is not necessary to describe in detail the research which led up to the final formula of the fluid which I call acetate glucose-saline (A.G.S.) which takes the place of B.G.S. in these cases. Both citrates and acetates were tried as buffers, but sodium acetate was finally decided upon, since a few metals have insoluble citrates, while all acetates are soluble. It is necessary to make up the solution shortly before the experiment, for if one adds sufficient acetate to buffer against the increasing acidity of dissolved glucose, then the amount of acetate is so great as to be toxic to sperms. For the same reason the amount of glucose is reduced, and it is alkalinised shortly before the experiment. The amount of sodium chloride is increased so as to compensate in tonicity for the reduction in the amount of glucose. The final fluid is prepared as follows:

Acetate glucose-saline (A.G.S.).

Solution A:

Sodium chloride	1·2 grm.
Sodium acetate	0·26 ,,
Distilled water	100 c.c.

Solution B:
2·4 % glucose in distilled water

Shortly before the fluid is required, the *p*H of the glucose solution is raised to about 8·5 by the addition of 0·1 per cent. NaOH drop by drop until a sample of it gives a bluish green reaction with the "Universal" indicator of the British Drug Houses. 1·5 c.c. of solution A is then mixed with 1·5 c.c. of solution B to make the 3 c.c. of A.G.S. required for making the sperm suspensions.

A.G.S. does not result in sperms showing such extreme activity as they often do in B.G.S., but it serves its purpose well.

Acids were formerly graded by a modification of the usual technique, but it is not a good test for them, and I therefore omit them in the list of substances showing the order of their spermicidal powers (p. 175). I have shown that the effect of the hydrogen ion in immobilising sperms altogether outweighs the effect of the anion, and therefore the effect of acids on sperms simply depends on the extent to which they ionise. One might compare the spermicidal powers of acids with those of other substances by suspending the sperms in a fluid buffered against acidity to the same extent as the contents of the vagina after coition. Even so, there would be the difficulty that acids immobilise sperms without killing them, except at a low *p*H.

A small addition should be made to section 15 of the description of the technique, given in the previous paper. Section 15 describes the addition of the sperm suspension to the solution of the substance under investigation. The beginning of this section should be as follows: "The sperm suspension in the other tube is transferred to the experimental tube with a pipette. The point of the pipette is held below the surface of the fluid in the experimental tube while the bulb is being squeezed, to ensure immediate mixing of the fluids. The experimental tube now contains...." etc.

The following should be added at the end of section 23: "When a coloured substance is being tested, it is not possible to forget the identity of the experimental slide."

General results.

The results of the investigation are shown in Table I. The preliminary experiments, at concentrations higher than what was subsequently found to be the killing concentration, are omitted from the table, in order to simplify it. The table includes three substances which were included in the similar table in my previous paper. These are formaldehyde, mercuric chloride, and mag-

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Table I.

	2 %	1 %	$\frac{1}{2}$ %	$\frac{1}{4}$ %	$\frac{1}{8}$ %	$\frac{1}{16}$ %	$\frac{1}{32}$ %	$\frac{1}{64}$ %	$\frac{1}{128}$ %	$\frac{1}{256}$ %	$\frac{1}{512}$ %	$\frac{1}{1024}$ %	$\frac{1}{2048}$ %
Toluquinone	.	.	.	.	.	.	.	.	.	.	0 0 0 0	0 0 0 1+	3+ 3+
Butylquinone	.	.	.	.	.	.	.	.	.	.	0 0 0 0	0 1 3	3
Methoxyquinone	.	.	.	.	.	.	.	.	.	.	0 0 0 0	2 3+	.
Parabenzoquinone	.	.	.	.	.	.	.	.	.	.	0 0 0 0	2+ 3 3+	3+
Mercuric chloride (2nd test)	.	.	.	.	.	.	.	.	.	0 0 0 0	0 0 0 1	0 0 0 1+ 2	1+ 1+ 2+
Methoxyhydroquinone	.	.	.	.	.	.	.	.	.	0 0 0 0	0 0 1 2	0 0 1+ 3 3	2+ 3 3+
Formaldehyde (2nd test)	.	.	.	.	.	.	.	.	.	0 0 0 0	1 1 1+	2	.
Paraformaldehyde	.	.	.	.	.	.	.	.	.	0 0 0 0	1+ 1+ 1+	.	.
Methylhydroquinone	.	.	.	.	.	.	.	.	.	0 0 0 0	0 3 3	.	.
Malachite green	.	.	.	.	.	.	.	.	0 0 0 0	0 0 0 1	1+ 2	.	.
Butylhydroquinone	.	.	.	.	.	.	.	.	0 0 0 0	0 0 2	0 2 3	.	2+ 3
Methoxytoluquinone	.	.	.	.	.	.	.	0 0 0 0	0 0 1	1 2	2+ 3	.	.
Dimethoxyhydroquinone	.	.	.	.	.	.	.	0 0 0 0	0 1 1	1 1+	.	.	.
Oxyhydroquinone	.	.	.	.	.	.	.	0 0 0 0	0 0 0 1 1	2 2+	3		
Orthoethoxysalicylaldehyde	.	.	.	.	.	.	.	0 0 0 0	1 1 1+	.	.	.	.
Orthovanillin	.	.	.	.	.	.	.	0 0 0 0	1+ 1+ 1+	.	.	.	.
Hydroquinone	.	.	.	.	.	.	.	0 0 0 0	0 3 3+	3+	.	.	.
Salicylaldehyde	.	.	.	.	.	.	0 0 0 0	1 1+ 1+	1+	.	.	.	.
Crystal violet	.	.	.	.	.	.	0 0 0 0	0 0 2	3 3 3	.	.	.	.
Thymol	.	.	.	.	.	.	0 0 0 0	1+ 2 2	.	.	.	.	.
Mercurochrome	.	.	.	.	0 0 0 0	0 0 1+	2	.	.	.	.	.	.
Carmine (with four times as much borax)	.	.	.	.	0 0 0 0	0 1+ 2+	1+ 3	3	.	.	.	.	
Zinc sulphate (with seven molecules of water)	.	.	.	0 0 0 0	0 0 1	1 1 1+	2	.	.	.	.	.	
Catechol	.	.	0 0 0 0	0 0 2	2+ 3 3	3	.	.	.	.	.	.	.
Phenol	.	.	0 0 0 0	0 1+ 2+	3	.	.	.	.	.	.	.	.
Hydroquinone methyl ether	.	0 0 0 0	0 0 0 1	2 2+	.	3	.	.	.	.	.	.	.
Acetaldehyde	.	0 0 0 0	0 0 1	2 2+	.	3+	.	.	.	.	.	.	.
Chloral hydrate	.	0 0 0 0	0 1 1	3	3+	.	.	.	.	.	.	.	.
Resorcinol	.	0 0 0 0	1 1 1	1 1+	.	.	.	.	.	.	.	.	.
Pyrogallol	0 0 0 0	0 0 1	2	.	.	.	.	.	.	.	.	.	.
Orthophenylene diamine	0 0 0 0	0 1 3+	2	.	.	.	.	.	.	.	.	.	.
Zinc sulphocarbolate	1 1 1	1 1	1 1	1	1+	2	1+	2	2	3	.	.	.
Magnesium sulphate (2nd test)	2 2 3	.	.	.	.	.	.	.	.	.	.	.	.
Metaphenylene diamine	3 3 3	.	.	.	.	.	.	.	.	.	.	.	.
Glycerol	3 3 3+	.	.	.	.	.	.	.	.	.	.	.	.

nesium sulphate. They have been retested because of errors in their original testing. In the original test, solutions of formaldehyde were made up on the assumption that "formalin" is 40 per cent. formaldehyde. It has now been retested on the assumption that "formalin" is 37 per cent. formaldehyde by weight. Mercuric chloride and magnesium sulphate have been retested, using A.G.S. instead of B.G.S. for the sperm suspensions. All three substances have been shown to be slightly more spermicidal than appeared in the first test, but not sufficiently to raise them to a higher grade.

Several of the substances (*e.g.* malachite green and crystal violet) are obviously unsuited for practical use. They have been tested so as to make possible a general comparison of the germicidal and spermicidal powers of substances.

The list below shows all the substances which have been tested, including those reported on in the previous paper, with the omission of acids. Substances whose spermicidal powers are not significantly different are bracketed together.

It will be noticed that toluquinone is the most spermicidal substance yet tested. The first four substances are all quinones. It was not anticipated that mercuric chloride would be surpassed.

Killing concentration $\frac{1}{512}$ per cent:
- Toluquinone.
- Butylquinone.
- {Methoxyquinone.
- Parabenzoquinone.}

Killing concentration $\frac{1}{256}$ per cent.:
- Mercuric chloride.
- Methoxyhydroquinone.
- {Formaldehyde.
- Paraformaldehyde.}
- Methylhydroquinone.

Killing concentration $\frac{1}{128}$ per cent.:
- Malachite green.
- Butylhydroquinone.

Killing concentration $\frac{1}{64}$ per cent.:
- {Saponine.
- Methoxytoluquinone.
- Hexylresorcin.
- Dimethoxyhydroquinone.
- Oxyhydroquinone.}
- {Iodine (with four times as much potassium iodide).
- Orthoethoxysalicylaldehyde.
- Orthovanillin.}
- Hydroquinone.

Killing concentration $\frac{1}{32}$ per cent.:
- Salicylaldehyde.
- Crystal violet.
- {Thymol.
- Sodium oleate.}
- Sodium palmitate.

Killing concentration $\frac{1}{16}$ per cent.:
- Potassium permanganate.

Killing concentration $\frac{1}{8}$ per cent.:
- Mercurochrome.
- Carmine (with four times as much borax).

Killing concentration $\frac{1}{4}$ per cent.:
- {Sodium taurocholeate.
- Zinc sulphate (with seven molecules of water).}
- {Potassium aluminium sulphate (anhydrous).
- Sodium glycocholeate.}
- {Oxyquinolin sulphate (chinosol).
- Quinine urea hydrochloride.}
- {Tricresol.
- Potassium borotartrate.}

Killing concentration $\frac{1}{2}$ per cent.:
- {Quinine hydrochloride.
- Potassium oxyquinolin sulphonate.
- Potassium cyanide.
- Quinine bisulphate.}
- Catechol.
- Phenol.

Killing concentration 1 per cent.:
- {Hydroquinone methyl ether.
- Acetaldehyde.}
- {Chloral hydrate.
- Resorcinol.}

Killing concentration 2 per cent.:

- Pyrogallol.
- Potassium capronate.
- Orthophenylene diamine.

These fail to kill at 2 per cent.:

- Ammonium chloride.
- Zinc sulphocarbolate.
- Potassium butyrate.
- Sodium citrate.
- Hexamine.
- Sodium tartrate.
- Magnesium sulphate.
- Sodium diborate.
- Ethyl alcohol.

- Metaphenylene diamine.
- Glycerol.
- Sodium dichlorylsulphamidbenzoate.

An incomplete investigation was made of the substances which are listed below in alphabetical order. Insolubility prevented a full test of some of them.

	Activity of sperms
Acetone	3 at $\frac{1}{32}$ %
Acriflavine	3 at $\frac{1}{16}$ %
Auramine	3 at $\frac{1}{32}$ %. Not soluble enough to try at $\frac{1}{16}$ %
Caffeine	3 at $\frac{1}{16}$ %
Camphor	3 at $\frac{1}{16}$ %
Chloroform	3 at $\frac{1}{16}$ %
Guaiacol	3 at $\frac{1}{16}$ %
Haematein (dissolved in $\frac{1}{64}$ % potassium alum in saline)	3 at half-saturated solution
Isovanillin	3 at $\frac{1}{16}$ %
Menthol	1 + at $\frac{1}{16}$ %
Mercury potassium iodide	0, 0 at $\frac{1}{32}$ %, 2 + at $\frac{1}{64}$ %
Musk (synthetic)	3 at half-saturated solution
Paradimethylaminobenzaldehyde	3 at $\frac{1}{16}$ %
Parahydroxybenzaldehyde	2 + at $\frac{1}{16}$ %
Paraphenylene diamine	1, 2, 2 + at 1 %
Phloroglucinol	1, 1, 1 at $\frac{1}{4}$ %: 1 +, 2 at $\frac{1}{8}$ %: not soluble enough to try at $\frac{1}{2}$ %
Phloroglucinaldehyde	0, 0, 1 at $\frac{1}{16}$ %; 2 +, 2 +, 2 + at $\frac{1}{32}$ %
Potassium bichromate	3 at $\frac{1}{32}$ %
Potassium chlorate	3 at $\frac{1}{16}$ %
Sodium nitrite	2 at $\frac{1}{16}$ %
Sodium veronal	3 + at $\frac{1}{16}$ %
Tannin	3 at $\frac{1}{32}$ %
Urea	3 at $\frac{1}{4}$ %
Vanillin	0 at $\frac{1}{4}$ %, 3 at $\frac{1}{16}$ %

Most substances are without effect upon sperms at one-eighth of the concentration at which they kill every time, and many are without effect at one-quarter. Most have considerable effect at one-half. There are some substances, however, to which these generalisations do not apply. At one end of the scale of these exceptional substances comes hydroquinone, at the other zinc sulphocarbolate. With hydroquinone the killing of sperms may almost be regarded as an "all or none" reaction. Hydroquinone always kills at $\frac{1}{64}$ per cent. At half this concentration sperms showed the following grades of activity in three experiments: 0, 3, 3 +. Whether it kills every sperm, or has no effect whatever, probably depends on the number of sperms present. If there were many substances like hydroquinone, it might be necessary to devise some means of standardising the number of sperms more accurately than is done in the usual technique. Methylhydroquinone behaves in the same way as hydroquinone. Zinc sulphocarbolate has precisely the opposite effect. It fails to kill every sperm even at 2 per cent. (though at this concentration a trained eye is required to detect the minute movements of a very small number of the sperms). Nevertheless it still has effect at $\frac{1}{128}$ per cent. There is no other substance which approaches zinc sulphocarbolate in this respect. Perhaps this property renders it somewhat unsuitable for practical use, for it might result in an injured sperm achieving fertilisation.

Several substances definitely stimulate sperms at certain concentrations. I have proved this for strychnine hydrochloride, brucine hydrochloride, and chloral hydrate (Baker, 1931 *b*). Chloral hydrate stimulates at one-sixteenth of the concentration at which it kills every sperm every time. I have evidence, but not yet proof, that chinosol and metaphenylene diamine also stimulate at certain concentrations. Carter (1930) has shown that at certain concentrations thyroxin prevents the rapid decrease in metabolic activity which sea-urchin sperms otherwise undergo soon after they have been suspended in sea water. Perhaps this is the same phenomenon as mine, but Carter thinks that the egg secretions of the sea-urchin may contain a substance allied to thyroxin, and that the reaction of sperms to it may thus be adaptive. The possibility that this is the case with guinea-pig sperms is precluded by the diversity of the substances which stimulate them.

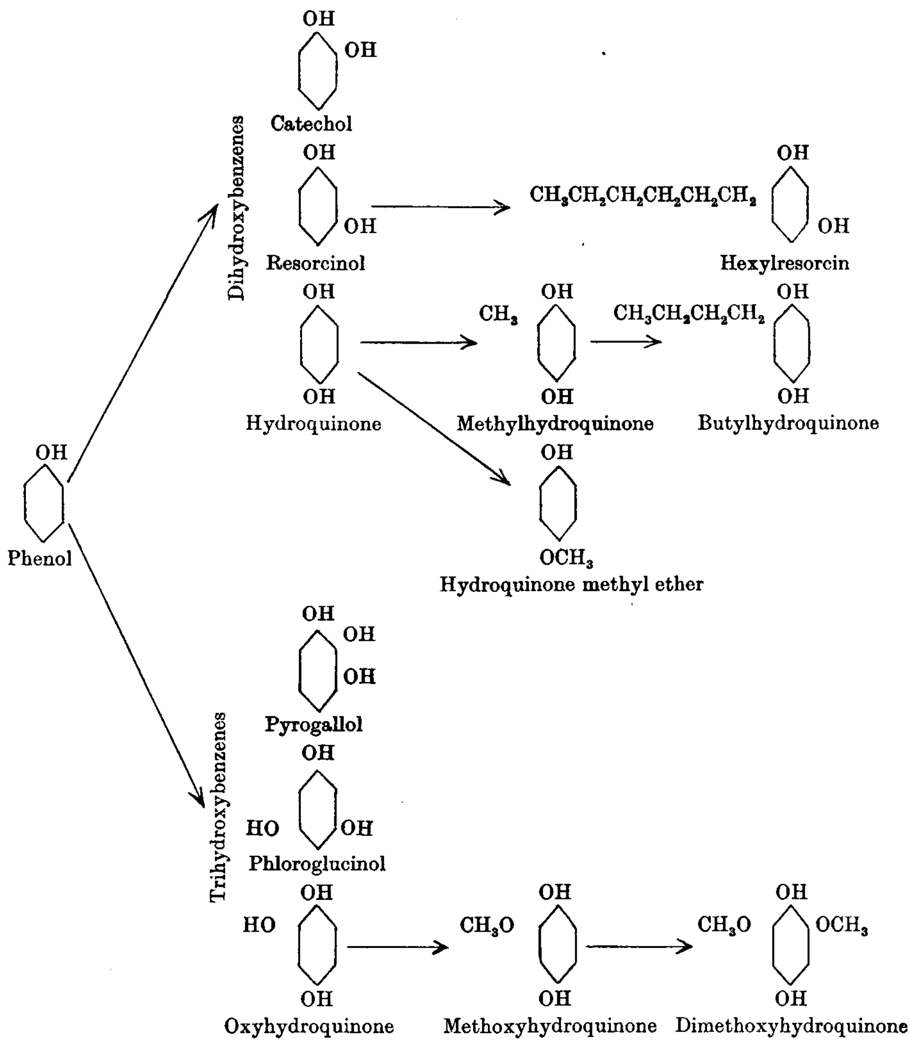
The phenols.

The chemical constitution of the phenols investigated is shown below. In this diagram, and also in the ones illustrating the quinones and aldehydes, the arrows indicate chemical relationships only, and not methods of preparation.

Phenol itself is a weak spermicide, only killing at $\frac{1}{2}$ per cent. The first step in this investigation was to find the effect of adding another hydroxyl group in each of the three possible positions, forming catechol (pyrocatechin), resorcinol and hydroquinone. Catechol has almost exactly the same spermicidal power as phenol. Resorcinol only kills at 1 per cent. However, when the hydroxyl groups are placed in the para-position, an enormous increase in spermicidal power occurs, for hydroquinone kills at $\frac{1}{64}$ per cent. I could at first scarcely believe the results, when it began to become apparent that one position isomer was sixty-four times as spermicidal as another. Actually, however, Günther had discovered the high spermicidal power of hydroquinone nearly a quarter of a century before, in the course of his most important study of spermicides (Günther, 1907).

Now the addition of alkyl groups to resorcinol is known to increase the germicidal power (Dohme and others, 1926), and I had already shown that hexylresorcin kills at $\frac{1}{64}$ per cent., as compared with 1 per cent. for resorcinol. The idea of adding alkyl groups to hydroquinone, to produce substances of even greater spermicidal power, naturally suggested itself. This was impossible without the co-operation of an organic chemist, and it was at this point that my collaboration with Dr Gulland started, which has resulted in the discovery of some extremely spermicidal substances. The addition of a methyl group multiplied the spermicidal power by four, for methyl hydroquinone kills at

$\frac{1}{256}$ per cent., the same concentration as mercuric chloride. It was anticipated that a longer side chain would result in still higher spermicidal power, and Dr Gulland therefore undertook to prepare butylhydroquinone. This substance, however, was found to be less spermicidal than methylhydroquinone, since it only kills at $\frac{1}{128}$ per cent. There is evidently an optimum length of side chain,



containing less than four carbon atoms. In a parallel way, the germicidal power of the resorcins falls off when more than six carbon atoms are included in the side chain (Dohme and others, 1926).

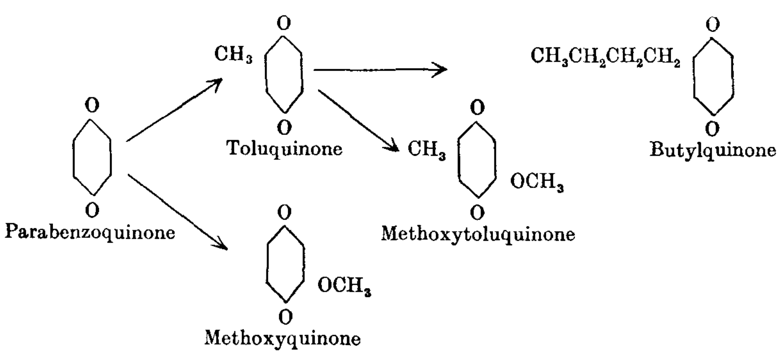
We next tried substituting an alkyl group for the hydrogen of one of the hydroxyl groups of hydroquinone. Hydroquinone methyl ether was found only to kill at 1 per cent. It is evidently essential that the hydroxyl groups should remain intact, if the spermicidal power of hydroquinone is to be retained.

That concluded our work on phenols with two hydroxyl groups. When three hydroxyl groups co-exist in the molecule there are three isomeric phenols possible, namely pyrogallol, phloroglucinol and oxyhydroquinone. Pyrogallol is scarcely spermicidal, for it only kills at 2 per cent. This seems to argue strongly against Günther's suggestion, that the high spermicidal power of hydroquinone is due to its reducing power; for pyrogallol is also an extremely powerful reducer. Phloroglucinol fails to kill at $\frac{1}{4}$ per cent. It is not soluble in saline at 1 per cent., and therefore it cannot be tried at $\frac{1}{2}$ per cent. In contrast to the small spermicidal powers of its two isomers, oxyhydroquinone kills at $\frac{1}{64}$ per cent., the same concentration as hydroquinone. Two hydroxyl groups in the para-position seem to confer high spermicidal powers, irrespective of whether a third hydroxyl group co-exists. The substitution of a methyl group for the hydrogen of this third hydroxyl group multiplies the spermicidal power four times, for methoxyhydroquinone kills at $\frac{1}{256}$ per cent. It comes next to mercuric chloride in order of spermicidal power. Dimethoxyhydroquinone, on the contrary, has the same spermicidal power as oxyhydroquinone.

Those phenols which are easily oxidised were dissolved shortly before each test.

The quinones.

The high spermicidal power of hydroquinone and its derivatives led us to try the quinones. The chemical constitution of those tested is shown below.

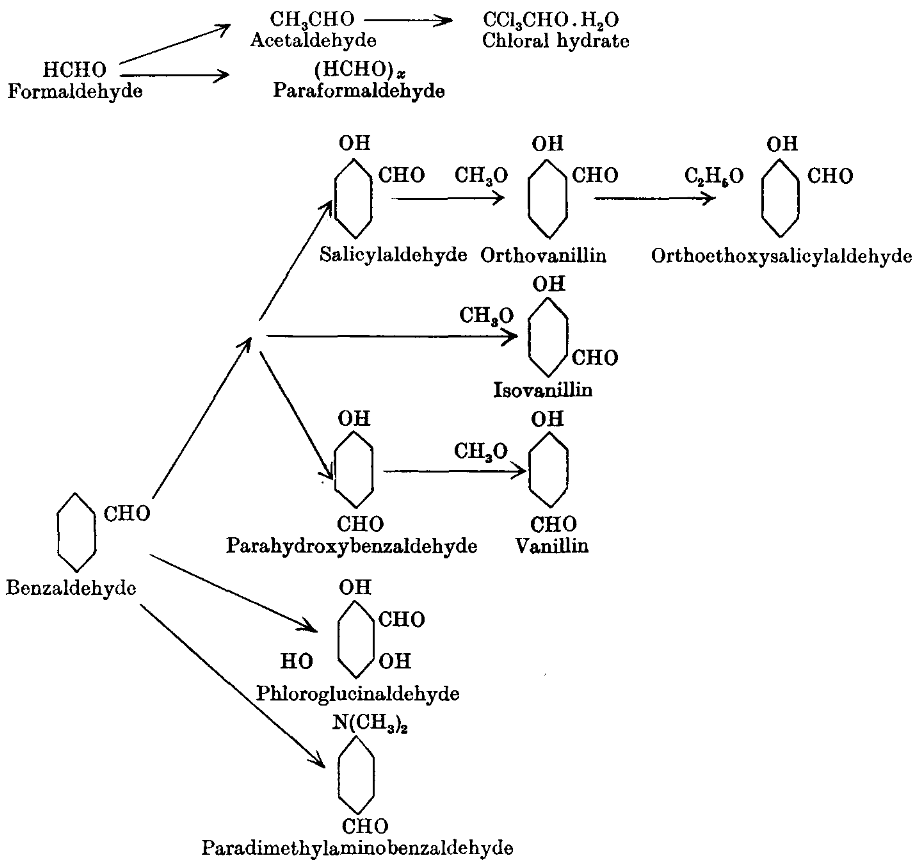


We at once found a substance more spermicidal than any that had been tested before, for quinone itself (parabenzoquinone) kills at $\frac{1}{512}$ per cent. The introduction of a methyl group increases the spermicidal power still further, although not sufficiently to put toluquinone in a higher grade. Toluquinone is the most spermicidal substance yet tested. Thinking that a side chain of four carbon atoms might result in even higher spermicidal power, Dr Gulland prepared butylquinone. This was found to be slightly less spermicidal than toluquinone. Evidently the optimum number of carbon atoms is less than four. This is exactly analogous to what we found with methyl and butylhydroquinone.

Dr Erdtmann most kindly presented us with a few milligrams of methoxyquinone, which was found to have the same spermicidal power as parabenzoquinone. Methoxytoluquinone, on the contrary, has only one-eighth of the spermicidal power of the other quinones, for its killing concentration is $\frac{1}{64}$ per cent.

The aldehydes.

The chemical constitution of the aldehydes tested is shown below. Benzaldehyde was not tested, but it is shown because the chemical constitution of the vanillins and related substances must be referred to it.



Formaldehyde kills at $\frac{1}{256}$ per cent., the same concentration as mercuric chloride. The condensation of several molecules into one has no effect on spermicidal power, for paraformaldehyde is almost exactly equally effective. The next member of the series, acetaldehyde, has inexplicably little spermicidal power, for it only kills at 1 per cent. If three chlorine atoms are substituted for three hydrogen atoms in acetaldehyde, the spermicidal power is unaffected, for chloral hydrate also kills at 1 per cent.

Dr Gulland suggested trying the vanillins. The remarkable result was obtained that while orthovanillin kills at $\frac{1}{64}$ per cent., its two isomers, vanillin and isovanillin, are without effect at $\frac{1}{16}$ per cent. (It is vanillin that is the essential substance in the vanilla of commerce.) Salicylaldehyde was next tried, and found to kill at $\frac{1}{32}$ per cent. Salicylaldehyde and orthovanillin agree with one another, and differ from vanillin and isovanillin, in that the hydroxyl and aldehyde groups are in the ortho-position in the former two substances. I therefore asked Dr Gulland to suggest other related substances of sufficient solubility, to provide a test of the idea that high spermicidal power may be conferred by the ortho-position of hydroxyl and aldehyde. Altogether the following substances were tried:

Ortho.

Salicylaldehyde	(killing	concentration	$\frac{1}{32}$	per cent.)
Orthovanillin	,,	,,	$\frac{1}{64}$	,,
Orthoethoxysalicylaldehyde	,,	,,	$\frac{1}{64}$	,,
Phloroglucinaldehyde	(0, 0, 1 at $\frac{1}{16}$ per cent.)			

Meta and para.

Isovanillin	(no effect at $\frac{1}{16}$ per cent.)
Parahydroxybenzaldehyde	(scarcely any effect at $\frac{1}{16}$ per cent.)
Vanillin	(no effect at $\frac{1}{16}$ per cent.)

It seems allowable to presume a causal connection between the ortho-position of the hydroxyl and aldehyde groups and a moderately high spermicidal power.

Paradimethylaminobenzaldehyde is without effect at $\frac{1}{16}$ per cent.

PRACTICAL CONSIDERATIONS.

The object of the work on the phenols, quinones and aldehydes was not to discover substances suitable for practical use, but to find general principles underlying the killing of sperms, whose application might later result in the discovery of such substances. The comparatively small spermicidal powers of quinine bisulphate is forced upon one by the discovery of four substances which are 256 times as spermicidal. (N.B. In such statements as these it must of course not be taken that the last figure is significant. The exact killing concentration is not determined, but only the least concentration, in the series 1, $\frac{1}{2}$, $\frac{1}{4}$, etc. per cent., that suffices to kill.) Manufacturers seem to attach an almost mystical significance to chinosol, which I am at a loss to understand. Not only is its killing concentration relatively small ($\frac{1}{4}$ per cent.), but at certain concentrations it seems actually to stimulate sperms.

It goes without saying that the position of a substance in the order of spermicidal powers is by no means in itself an index of its suitability for practical use. It may be injurious, or unstable, or volatile, or liable to stain tissues or fabrics, or insufficiently soluble. It is of little practical significance that a substance has a killing concentration of *e.g.* $\frac{1}{256}$ per cent., if it is so insoluble that this is close to its saturation concentration. Sperms lying in mucus in folds in the vagina may be acted upon at a concentration far below saturation, though plenty of the spermicide is present in the cavity of the vagina. Such sperms might later achieve fertilisation, especially if the bulk of the spermicide is washed out by a douche. A very soluble substance coming much lower in order of spermicidal power would be preferable to such a substance as this.

A substance whose saturated solution is harmless to the vagina, yet which kills sperms at a concentration much below saturation, would be a useful contraceptive, because any desired amount of it could safely be placed within the vagina. Probably only fairly insoluble substances would be likely to fulfil these conditions, as very soluble spermicidal substances would be likely to be harmful to the vagina in saturated solution. Paraformaldehyde is relatively insoluble, yet spermicidal much below saturation. It seems worth while to test it for harmful effects, especially as Dr Carleton has shown formaldehyde to be harmless far below the killing concentration.

Hydroquinone and oxyhydroquinone and their derivatives seem unlikely to be of practical use, on account of their instability and the dark stain which they make on tissues and fabrics. Methylhydroquinone seems to have these disadvantages less than the others, and it is one of the most spermicidal. The quinones tested, except methoxyquinone, are free from these disadvantages, and may prove to have practical applications in suppositories or douches. However, Dr Carleton kindly allows me to state that he has found 0·2 per cent. parabenzoquinone harmful to the vagina of the rabbit. Methoxytoluquinone, though less spermicidal than the others, especially commends itself on account of its solubility and stability. It has not yet been tested for harmful effects.

Orthovanillin and the closely related orthoethoxysalicylaldehyde may be found to be useful. Dr Carleton is now testing the former for harmful effects. Both have the disadvantage of staining skin. Although any smell is probably an aesthetic disadvantage, yet the smell of these substances is not unpleasant. Salicylaldehyde is not so stable, and has the disadvantages of being a liquid and having a very pungent smell.

It would appear worth while to try orthovanillin and its allies by the Rideal-Walker test. They might find application as germicides.

Dr Gulland is now suggesting further substances for trial, other than phenols, quinones and aldehydes. Certain hypotheses about the relation between chemical constitution and spermicidal power, which have occurred to him as a result of the work described in this paper, will be tested. If these hypotheses are substantiated, they should serve as a guide to yet further research, which may result in the discovery of an ideal chemical contraceptive.

In such work as this, it is important not to consider the present cost of production of the various substances tested. If one were to restrict oneself to cheap substances, one's choice would be much limited. If an ideal chemical contraceptive were discovered, it seems probable that the great demand for it would result in the discovery of inexpensive methods of large-scale production.

Perhaps the best method of contraception to-day for general use is a rubber occlusive pessary used in conjunction with a chemical contraceptive. This combination has been recommended for many years by Dr Stopes. The best occlusive pessaries seem well designed, but when one remembers the minute size of sperms, one can easily imagine them swimming past. If there are lacerations of the cervix, or if the occlusive pessary becomes dislodged, the necessity for a chemical contraceptive in addition is especially evident. It remains for the chemical contraceptive to be improved.

SUMMARY.

1. In the list showing all substances yet tested in the order of their spermicidal powers, the first four substances are all quinones.

2. In the dihydroxybenzenes and their derivatives, the para-position of the two hydroxyl groups confers high spermicidal powers.

3. In the trihydroxybenzenes and their derivatives, the para-position of two of the three hydroxyl groups confers high spermicidal powers.

4. The introduction of a methyl group into quinones and into the di- and trihydroxybenzenes in certain positions increases the spermicidal power.

5. In the hydroxybenzaldehydes and their derivatives, the ortho-position of the hydroxyl and aldehyde groups confers high spermicidal powers.

6. Among the substances so far tested which may be found to be of practical use are orthovanillin and methoxytoluquinone. These are highly spermicidal, sufficiently soluble and stable in solution. It remains to be seen whether they are harmless to the vagina.

POSTSCRIPT.

Some more substances have been tested since this paper was written. Ethylquinone and paraxyloquinone both kill at $\frac{1}{512}$ per cent., and must be bracketed with parabenzoquinone and methoxyquinone. The first six substances in order of spermicidal power are thus all quinones.

Thymoquinone kills at a little less than $\frac{1}{128}$ per cent. It is not soluble enough to try at exactly $\frac{1}{128}$ per cent. It must be bracketed with butylhydroquinone. Duroquinone is without effect at $\frac{1}{256}$ per cent., and insolubility prevents test at a higher concentration.

The following are without effect at $\frac{1}{64}$ per cent.: nitrobenzene, metadinitrobenzene, paranitrosodimethylaniline and pyronin G.

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