THE SPERMICIDAL POWERS OF CHEMICAL CONTRACEPTIVES.

II. PURE SUBSTANCES.

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(With 3 Text-figures.)

CONTENTS.

Introduction.

This is the second of a series of papers on an extensive investigation of chemical contraceptives, undertaken at the suggestion of the Birth Control Investigation Committee and financed by it. I wish to thank the Committee for its continued support, and in particular Dr C. P. Blacker and Prof. J. S. Huxley, who have helped me in many ways. Prof. E. S. Goodrich, F.R.S., has kindly allowed the whole of the work to be done in the Department of Zoology and Comparative Anatomy at Oxford. That the work has resulted in the discovery of some very spermicidal substances must be attributed largely to the fertile suggestion of Prof. J. B. S. Haldane that I should investigate substances which reduce surface tension. Dr H. M. Carleton has always been generous with valuable advice. Dr F. H. A. Marshall, F.R.S., has been kind enough to read the proofs. The makers of the various commercial pessaries have most kindly given me quantitative analyses of them, but do not allow me to publish them. The qualitative analyses are not secret. I wish to thank the manufacturers for their co-operation.

The first paper in this series (Baker, 1930) dealt with the effects of commercial pessaries on guinea-pig sperms. An investigation of their effects on human sperms is almost complete, and will form the third paper in this series. The present paper is concerned not with commercial pessaries, but with chemically pure substances, free from any vehicle, diluent or foam-producing

https://doi.org/10.1017/S0022172400010767 Published online by Cambridge University Press

mixture. It is only by an investigation of this sort that one can properly interpret the effects of commercial pessaries, and find new substances for commercial use. The investigation was conducted with guinea-pig sperms, on account of the impossibility of procuring sufficient human semen for this investigation as well as for the investigation of pessaries. On the whole guinea-pig sperms seem to react to spermicides in much the same way as human ones. I hope in the future to undertake an investigation of this subject. A coefficient might be found, whereby one could calculate directly at what concentration a given substance would kill human sperms, if the concentration at which it kills guinea-pig sperms were known. This would be a very great convenience. The guinea-pig was selected because it is easily kept and bred in the laboratory, matures quickly, remains fecund throughout the year, has a conveniently large epididymis, and produces very active sperms.

It appears that chemical contraceptives are coming to be regarded as unreliable, and only suitable for use in conjunction with other methods. That this is so must be due largely to the lack of physiological investigation of the subject. There are two ways of studying chemical contraceptives. Firstly there is the clinical method of asking women for their experience with various pessaries and tabulating the results. This has been done on a large scale by Dr Marie Stopes and others, and no one will deny the importance of their work. The objection to this type of investigation is that it is of necessity an experiment without controls. The second method of studying chemical contraceptives is the physiological one which I have adopted. The objection to it is that the experiments are performed in glass tubes and not in the vagina. Whilst admitting that this objection has weight, I must protest against the idea, quite unfounded on observation, that the vagina is a sort of chemical laboratory, equipped to change the chemical constitution of every substance placed within it. The great advantage of the physiological method is that every experiment has a control. Further, new substances may be tested without unwarrantable risks on women. The truth surely is that both the clinical and the physiological method should be used, and workers in neither field should disparage the research of those in the other.

Throughout this paper sperms will be spoken of as "killed" if they are completely immobilised by any substance at the temperature of the body. This is done because it has not been shown that any substance, with the possible exception of hydrogen ions, can immobilise Mammalian sperms at the temperature of the body without preventing them from becoming capable later of achieving fertilisation. The words "spermicide" and "spermicidal" are preferred, on grounds of euphony, to "spermaticide" and "spermaticidal."

The substances tested.

Substances already used as chemical contraceptives, substances thought likely to be useful, and certain very poisonous substances were tested. The latter were tried for purposes of comparison only. Many friends were kind

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enough to suggest substances for trial. Prof. Haldane's suggestions are discussed below. Thirty-six substances were investigated in such a manner that it is possible to place them all in the order of their spermicidal powers. They are arranged alphabetically below. So that there may be no doubt whatever about what substances are meant, the chemical formula of each is given.

Acetic acid	CH_3COOH
Ammonium chloride	NH_4Cl
Boric acid	H_3BO_3
Citric acid	$C_3H_4OH(COOH)_3 . H_2O$
Cresol	$C_6H_4 . OH . CH_3$
Dioxyquinolin sulphate	$(C_9H_6NOH)_2 . H_2SO_4$
Ethyl alcohol	C_2H_5OH
Formaldehyde	$HCHO$
Hexamine	$(CH_2)_6N_4$
Hexyl resorcin (Caprokol)	$C_6H_3(OH)_2(CH_2)_5CH_3$
Hydrocyanic acid	HCN
Iodine	I
Lactic acid	$CH_3CHOH . COOH$
Magnesium sulphate	$MgSO_4 . 7H_2O$
Mercuric chloride	$HgCl_2$
Phenol	C_6H_5OH
Potassium aluminium sulphate (anhydrous)	$K_2SO_4 . Al_2(SO_4)_3$
Potassium borotartrate	$KBO . C_4H_4O_6$
Potassium butyrate	$KC_4H_7O_2$
Potassium capronate	$KC_6H_{11}O_2$
Potassium cyanide	KCN
Potassium oxyquinolin sulphate (Chinosol)	$K_2SO_4 . (C_9H_6NOH)_2H_2SO_4$
Potassium permanganate	$K_2Mn_2O_8$
Quinine bisulphate	$C_{20}H_{24}N_2O_2 . H_2SO_4 . 7H_2O$
Quinine hydrochloride	$C_{20}H_{24}N_2O_2 . HCl . 2H_2O$
Quinine urea hydrochloride	$C_{20}H_{24}N_2O_2 . HCl . CO(NH_2)_2 . HCl . 5H_2O$
Saponine	$C_nH_{2n-8}O_{10}$
Sodium citrate	$2C_3H_4OH(COONa)_3 . 11H_2O$
Sodium diborate	$Na_2Br_4O_7$
Sodium dichlorylsulphamidbenzoate	$C_6H_4 . COONa . SO_2NCl_2$
Sodium glycocholeate	$NaC_{26}H_{42}NO_6$
Sodium oleate	$NaC_{18}H_{33}O_2$
Sodium palmitate	$NaC_{16}H_{31}O_2$
Sodium taurocholeate	$NaC_{26}H_{44}NSO_7$
Sodium tartrate	$(CHOH . COONa)_2 . 2H_2O$
Succinic acid	$C_2H_4(COOH)_2$

A less complete investigation was made of the following:

Acetone	$(CH_3)_2CO$
Aconitine nitrate	$C_{34}H_{45}NO_{11} . HNO_3$
Copper acetate	$Cu(CH_3COO)_2$
Pilocarpine nitrate	$C_{11}H_{16}O_2N_2 . HNO_3$
Potassium bichromate	$K_2Cr_2O_7$
Sodium hydroxide	$NaOH$
Strychnine hydrochloride	$C_{21}H_{22}N_2O_2 . HCl . \frac{1}{2}H_2O$
Sulphuric acid	H_2SO_4
Tannic acid	$C_{14}H_{10}O_9 . 2H_2O$

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TECHNIQUE.

The technique employed is described here in great detail, because it is hoped that it may be accepted as a standard technique for grading the spermicidal powers of pure substances. The technique enables one to place all substances in "grades" of spermicidal power. The substances in each grade are, on the average, twice as spermicidal as the substances in the next lower grade.

It would have been very convenient if it had been possible to test the effect of each substance dissolved in distilled water; but since distilled water kills sperms by osmosis, it was necessary to dissolve each substance in a fluid isotonic with the sperms. 0·9 per cent. sodium chloride solution is not suitable by itself, for sperms are not very active in it. I made a special investigation (Baker, 1930*a*) to find in what fluid guinea-pig sperms are most active, and elaborated a fluid called Buffered Glucose-Saline, which will be called B.G.S. for short throughout this paper. This fluid has the following composition:

Acid potassium phosphate (KH_2PO_4) ...	0·03 grm.	(Dissolve this first)
Sodium hydrogen phosphate ($Na_2HPO_4 . 12H_2O$)	0·6 ,,	
Sodium chloride	0·2 ,,	
Glucose	3·0 ,,	
Distilled water	100 c.c.	

The sperm suspensions were made in B.G.S. throughout this investigation (except when acids were being studied), and the various substances tested for spermicidal powers were dissolved in 0·9 per cent. sodium chloride solution. The sperm suspension in B.G.S. was added to an equal quantity of 0·9 per cent. sodium chloride solution containing a definite amount of the substance under investigation. The sperms were thus finally suspended in a solution containing equal parts of B.G.S. and 0·9 sodium chloride solution, together with a known amount of the substance investigated. The control sperms were in a solution of equal parts of B.G.S. and 0·9 per cent. sodium chloride, which is so effective a fluid that the control sperms showed a high degree of activity in nearly every experiment. Those who have only used ordinary saline or Ringer's or Locke's solution would be surprised at the extreme activity of guinea-pig sperms when suspended at 37° C. in B.G.S. or B.G.S. mixed in equal quantities with saline. This laborious investigation, which has occupied a large proportion of my time over a period of nearly two years, could never have been carried through if I could not have relied upon having active control sperms as a matter of course. Controls were invariably examined in every experiment.

The substances themselves were not dissolved directly in B.G.S. for two reasons. Firstly, prolonged contact might cause a reaction between the substance and the phosphates or the glucose of the fluid. Secondly, moulds tend to grow after a time in B.G.S., and a great waste of time would have been involved in making up all solutions afresh from this cause.

Although this technique is effective for the great majority of substances, there are some which present difficulties. Silver salts cannot be tested by it, since silver chloride is at once precipitated. Salts of metals other than potas-

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sium and sodium present the difficulty that their phosphates are insoluble, and therefore they are partly precipitated when their solution is added to B.G.S. It is possible to omit the phosphates in these cases, but one must be careful not to enter the results of the experiment if the control sperms are inactive as a result of the omission. The amount of phosphates in B.G.S. is not great enough, however, to render the usual technique inapplicable to these salts. A different fluid takes the place of B.G.S. when acids are being tested, as will be explained later.

The substances are dissolved in 0·9 per cent. saline at various concentrations in the series 4, 2, 1, $\frac{1}{2}$, $\frac{1}{4}$ per cent., etc. To test a substance at 1 per cent., for instance, a 2 per cent. solution must be used, since it will be mixed with an equal volume of B.G.S. The lowest concentration in this series which kills all sperms in half an hour in four consecutive experiments is determined for each substance.

The glass pipettes, bottles, capsules, specimen tubes, and microscopical slides and coverslips used in the experiments are kept clean but not sterile. Sterility is unnecessary, since sperms are adapted to the non-sterile conditions of the vagina. Pipettes and bottles are cleaned with distilled water. The rest of the glassware is thoroughly washed in tap-water and dried with clean cloths.

The following is a detailed description of the technique.

(1) A thermostat is maintained at 37° C. It contains coverslips, pipettes, and a damp chamber (Fig. 1). The latter is of glass, with a glass lid sealed

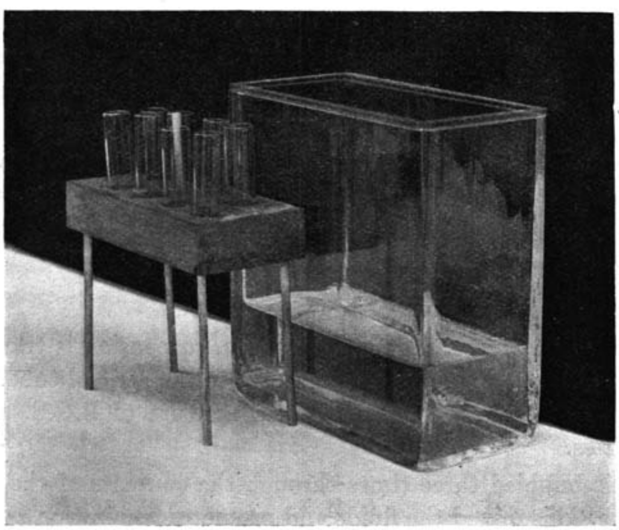

Fig. 1. The damp chamber, with rack containing specimen tubes which fits inside it.

with vaseline to prevent evaporation. There is an inch or two of water in it, containing a crystal of thymol to prevent the growth of moulds. The damp chamber contains small specimen tubes in a wooden rack, which is held out

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of the water on four legs. Sperm suspensions will be introduced into the tubes later in the experiment. Since no evaporation can take place in the saturated atmosphere of the damp chamber, the tubes are not corked, and therefore the sperm suspensions do not lack oxygen nor accumulate carbon dioxide. Specimen tubes are preferred to test tubes, since their flat bottoms prevent the sperms from becoming too much aggregated together by gravity.

(2) A thermostatic heater is regulated to deliver a constant stream of water at about 37° C., which passes through a hollow microscope stage. This will prevent the sperms from becoming torpid through cold during examination under the microscope.

(3) The substance to be investigated is dissolved in 0·9 per cent. sodium chloride solution, generally at a concentration of 2 per cent. for the first trial, if the substance is sufficiently soluble.

(4) 0·5 c.c. of 0·9 per cent. sodium chloride solution is introduced into one of the specimen tubes in the damp chamber by means of a graduated pipette. This is the control tube.

(5) 0·5 c.c. of the solution made up in section 3 is introduced into another specimen tube. This is the experimental tube.

(6) An adult male guinea-pig is killed by a blow on the head, and the fur damped in the pelvic region to prevent hairs from getting in the way.

(7) 3 c.c. of B.G.S. are placed in a glass capsule.

(8) The tails of both epididymes of the guinea-pig are cut out and placed in the B.G.S.

(9) Each is cut across once with scissors. Each fragment is pressed several times with flat-pointed forceps to cause the sperms to come out. The fragments are then removed and thrown away.

(10) The fluid is sucked into and out of a pipette to break up any clumps of sperms and form an even suspension.

(11) 0·5 c.c. of the sperm suspension is transferred to each of two empty specimen tubes in the damp chamber in the thermostat.

(12) The fluids are left to warm up.

(13) After a quarter of an hour the sperm suspension is transferred with a pipette from one of the tubes containing it to the control tube. The control tube now contains sperms suspended in a mixture of B.G.S. and saline in equal quantities.

(14) Air is bubbled three times through the contents of the control tube with a pipette. This mixed the fluids and promotes respiration by the sperms.

(15) The sperm suspension in the other tube is transferred to the experimental tube. The latter now contains sperms in a mixture of B.G.S. and saline in equal quantities, together with the substance under investigation at a concentration of 1 per cent., if it has been made up, according to section 3, at 2 per cent.

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(16) Air is bubbled three times through the contents of the experimental tube.

(17) The time is recorded at the start of the experiment.

(18) Two hollowed microscopical slides are labelled with a grease-pencil. One is labelled C (control), the other with letters denoting the name of the substance under investigation.

(19) Over the grease-pencil lettering on each slide is fixed a blank gummed-paper label, stuck down at one side in such a way that it may be turned aside to disclose the grease-pencil lettering. The object of this arrangement is explained in section 23.

(20) The labelled slides are placed on the floor of the thermostat.

(21) Twenty-five minutes after the start of the experiment, air is bubbled through the control tube, and with the same pipette three drops of the fluid are transferred to the hollow of the slide marked C. A warm coverslip is applied. Three drops of fluid do not fill the hollow of the slide. A large bubble of air is included below the coverslip, which prevents the sperms from becoming inactive quickly owing to inability to respire. The slide is left on the floor of the thermostat.

(22) The process described in the last section is applied to the contents of the experimental tube and to the slide labelled with letters denoting the name of the substance under investigation.

(23) The slides are shuffled together till the observer does not know which is which. Bias, conscious or unconscious, is thus avoided. The shuffling is particularly valuable when two or three substances are being tested at the same time. (See section 38.)

(24) As exactly as possible half an hour after the start of the experiment (section 17), the slides are removed one by one from the thermostat and examined under the microscope with a $\frac{1}{6}$ in. objective and No. 2 eyepiece, on the hot stage mentioned in section 2.

(25) The activity of the sperms is recorded on the blank labels on the slides, as follows:

III indicates that the majority of the sperms are moderately or very active.

II indicates that 10 per cent. of the sperms are moderately active, *or* that the majority are feebly active, *or* that there is any greater amount of activity that is less than III.

I indicates any activity less than II, including the slightest movement in a single sperm.

0 indicates that examination of ten microscopical fields fails to reveal the slightest movement in a single sperm. If the observer has seen no movement with certainty, but thinks it possible that a single sperm has moved, he starts again and begins to examine ten more microscopical fields.

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Currents in the suspension, caused by movement of the coverslip, sometimes give a deceptive appearance against which the observer must guard.

The following sign is occasionally useful:

III + indicates extreme activity.

The following signs are used to prevent the delay which would arise from prolonged indecision:

II + indicates that it cannot be quickly decided whether the sperms should be graded as II or III.

I + indicates that it cannot be quickly decided whether the sperms should be graded as I or II.

It is usually possible to place sperms in grades III or II in a few seconds. Grade 0 takes up to a minute, since ten fields must be examined, and grade I may take as long, if the first active sperm is seen in the tenth field.

At first sight this method of grading seems complicated and arbitrary, but experience proves it to be quick and reliable. It is very important that the grading should be done quickly, in order that the slides may be examined as exactly as possible half-an-hour after the start of the experiment. Quickness is of course particularly important when several substances are being examined at the same time.

(26) The blank labels are turned aside to disclose the identity of the slides.

(27) If the control sperms show an activity of III or III +, the result of the experiment is recorded.

(28) If the control sperms show an activity of less than III, the result is not recorded. This only happens when an immature or diseased guinea-pig is used.

(29) If the activity of experimental sperms is reduced to 0, the experiment is repeated with the substance at half the concentration used before. It is repeated again and again if necessary, at lower and lower concentrations in the series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$ per cent., etc., until active sperms are found.

(30) If the activity of the experimental sperms is not reduced to 0, the experiment is repeated at double the concentration used before. It is repeated again and again, if necessary, at higher concentrations in the series $\frac{1}{2}$, 1 and 2 per cent. 2 per cent. is not exceeded.

(31) The lowest concentration which suffices to kill all sperms is thus ascertained, and the experiment is performed again at this concentration.

(32) If all sperms are killed, the experiment is performed a third time at this concentration.

(33) If all sperms are killed, the experiment is performed a fourth time at this concentration.

(34) If all sperms are killed, this concentration is recorded as the "killing concentration." The killing concentration is the lowest concentration in the

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series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$ per cent., etc., which reduces the activity of the sperms to 0 in four consecutive experiments.

(35) If in any of the experiments of sections 31, 32 and 33 all the sperms are not killed, the next higher concentration must be tried until the killing concentration, defined in section 34, is found.

(36) All substances whose killing concentration is the same are placed in the same "grade" of spermicidal power. If the killing concentration is $\frac{1}{2^x}$, then the substance is said to be in "grade x." Thus sodium oleate always kills sperms at $\frac{1}{32}$ per cent., but not at $\frac{1}{64}$ per cent. Its killing concentration is $\frac{1}{32}$ per cent. or $\frac{1}{2^5}$. Sodium oleate therefore falls into grade 5. It is obvious that substances in each grade have, on the average, twice the spermicidal power of the substances in the next lower grade.

(37) The experiment is performed three times with each substance at half the killing concentration. This enables the observer to compare the spermicidal powers of substances which fall into the same grade.

(38) From one to three different substances may be tested at the same time against the same control. A single substance must never be tested more than once at the same concentration against the same control.

(39) The technique described above is suitable for most substances except acids. Hydrogen-ions themselves immobilise sperms, irrespective of what the acid may be. (See below, under "Acidity.") If the experiments with acids were carried out in the way described above, one might simply be measuring what concentration of each acid is sufficient to overcome the sodium hydrogen phosphate buffer in the B.G.S. A fluid containing so much sodium hydrogen phosphate is clearly unsuitable for an investigation of the spermicidal powers of acids.

A neutral fluid is therefore used while acids are being tested, instead of the alkaline B.G.S. This neutral fluid is made as follows. 6 grm. of $Na_2HPO_4 . 12H_2O$ are dissolved in 100 c.c. of water, and this solution is added to 5·2 per cent. glucose solution until a sample of it gives a yellowish-green reaction with the "Universal" Indicator of British Drug Houses. A definite formula cannot be given for this fluid, since the acidity of glucose solutions varies.

RESULTS OF THE MAIN INVESTIGATION.

Table I gives the whole of the results of this investigation, except a few inconsistent results which must have been caused by mistakes in diluting the substances to the various concentrations. In such an extensive investigation as this it was perhaps to be expected that an occasional slip would be made. When an apparently inconsistent result was obtained, experiments were performed to find whether the inconsistency was apparent or real. If the result was shown to have been due to a mistake, neither the inconsistent result

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Table I.

For signs used in this table refer to text, p. 195, *et. seq.*

	2 %	1 %	$\frac{1}{2}$ %	$\frac{1}{4}$ %	$\frac{1}{8}$ %	$\frac{1}{16}$ %	$\frac{1}{32}$ %	$\frac{1}{64}$ %	$\frac{1}{128}$ %	$\frac{1}{256}$ %	$\frac{1}{512}$ %	$\frac{1}{1024}$ %
Mercuric chloride	.	.	.	0	0	0	0	0	0 0 0	0 0 0 0	0 0 III	II
Formaldehyde	.	.	.	0	0	0	0	0	0	0 0 0 0	I I II	.
Saponine	.	.	0	0	0	0	0	0 0 0 0	0 0 I	I+	II+	.
Hexyl resorcin	.	.	.	.	.	.	0	0 0 0 0	0 0 I	I+	III	.
Iodine	.	.	.	.	0	0	0	0 0 0 0	0 I II	.	.	.
Acetic acid	.	.	.	.	.	0	0 0 0 0	I I+ II	.	.	.	.
Succinic acid	.	.	.	.	.	0	0 0 0 0	I II II	.	.	.	.
Sodium oleate	.	.	0	0	0	0	0 0 0 0	II II II+	.	.	.	.
Sodium palmitate	.	.	.	.	.	.	0 0 0 0	II III III	.	.	.	.
Lactic acid	.	.	.	.	.	0 0 0 0	0 I I	I	.	.	.	.
Potassium permanganate	.	.	0	0	0	0 0 0 0	0 I I	0 I+ II+	.	.	.	.
Citric acid	.	.	.	.	.	0 0 0 0	0 I I	II	.	.	.	.
Sodium taurocholeate	.	.	.	0 0 0 0	0 0 0 I	I+	III	.	.	.	.	.
Potassium aluminium sulphate	.	.	.	0 0 0 0	0 0 I	II II+	.	.	.	.	.	.
Sodium glycocholeate	.	.	0	0 0 0 0	0 0 I	II III III	.	.	.	.	.	.
Dioxyquinolin sulphate	.	.	.	0 0 0 0	0 I I	.	.	.	.	.	.	.
Quinine urea hydrochloride	.	.	.	0 0 0 0	0 I I+	.	.	.	.	.	.	.
Cresol	.	.	0	0 0 0 0	I II II	.	.	.	.	.	.	.
Potassium borotartrate	.	.	0	0 0 0 0	I II II+	.	.	.	.	.	.	.
Quinine hydrochloride	.	0	0 0 0 0	0 0 0 I	I+ I+	II+	.	.	.	.	.	.
Potassium oxyquinolin sulphate	.	.	0 0 0 0	0 0 0 I	I+ II II	.	.	.	.	.	.	.
Potassium cyanide	.	.	0 0 0 0	0 0 I	I	II	.	.	.	.	.	.
Quinine bisulphate	.	0	0 0 0 0	0 0 I I	I I	III	.	.	.	.	.	.
Phenol	.	0	0 0 0 0	0 I+ II+	III	.	.	.	.	.	.	.
Hydrocyanic acid	.	0	0 0 0 0	I+ I+ II	II+	.	.	.	.	.	.	.
Potassium capronate	0 0 0 0	I I I	II	.	.	.	.	.	.	.	.	.
Boric acid	0 I I	I+	.	.	.	.	.	.	.	.	.	.
Ammonium chloride	0 I I	II	III	.	.	.	.	.	.	.	.	.
Potassium butyrate	I I I	.	III	.	.	.	.	.	.	.	.	.
Sodium citrate	I+ II II	.	.	.	.	.	.	.	.	.	.	.
Hexamine	II+ II+ II+	II+	.	.	.	.	.	.	.	.	.	.
Sodium tartrate	II II III	.	.	.	.	.	.	.	.	.	.	.
Magnesium sulphate	II+ II+ III	.	.	.	.	.	.	.	.	.	.	.
Sodium diborate	II III III	.	.	.	.	.	.	.	.	.	.	.
Ethyl alcohol	II+ III III	.	.	.	.	.	.	.	.	.	.	.
Sodium dichlorylsulphamid-benzoate	III III III+	.	.	.	.	.	.	.	.	.	.	.

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nor the experiments performed to test the inconsistency are recorded in the table. Thirteen inconsistent results were obtained, mostly towards the beginning of the investigation. Twenty extra experiments were performed, which showed these results to be due to mistakes. Table I shows the 292 consistent results obtained in the investigation.

The following is an abstract of Table I. In each grade the substances are arranged in the order of their spermicidal powers, as determined by the results of the experiments at half the killing concentration, or in some cases at one-quarter the killing concentration. The order within each grade cannot be regarded as final.

Grade 8. (Killing concentration $\frac{1}{256}$ per cent.)

Mercuric chloride   Formaldehyde

Grade 6. (Killing concentration $\frac{1}{64}$ per cent.)

Saponine   Hexyl resorcin   Iodine

Grade 5. (Killing concentration $\frac{1}{32}$ per cent.)

Acetic acid   Sodium oleate
Succinic acid   Sodium palmitate

Grade 4. (Killing concentration $\frac{1}{16}$ per cent.)

Lactic acid   Potassium permanganate   Citric acid

Grade 2. (Killing concentration $\frac{1}{4}$ per cent.)

Sodium taurocholeate   Quinine urea hydrochloride
Potassium aluminium sulphate   Cresol
Sodium glycocholeate   Potassium borotartrate
Dioxyquinolin sulphate

Grade 1. (Killing concentration $\frac{1}{2}$ per cent.)

Quinine hydrochloride   Quinine bisulphate
Potassium oxyquinolin sulphate (Chinosol)   Phenol
Potassium cyanide   Hydrocyanic acid

Grade − 1. (Killing concentration 2 per cent.)

Potassium capronate

Ungraded. (These fail to kill at 2 per cent.)

Boric acid   Sodium tartrate
Ammonium chloride   Magnesium sulphate
Potassium butyrate   Sodium diborate
Sodium citrate   Ethyl alcohol
Hexamine   Sodium dichlorylsulphamidbenzoate

These substances will now be considered in the order of their spermicidal powers.

Mercuric chloride. This appears to be the most spermicidal substance that exists. In an attempt to find whether any other substance surpassed it, four

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substances which were not included in the main investigation were tried at $\frac{1}{512}$ per cent., to find whether they had any effect at all at this concentration. Table I shows that mercuric chloride has considerable effect at $\frac{1}{512}$ per cent. These substances were aconitine nitrate, pilocarpine nitrate, sulphuric acid and sodium hydroxide. The two former seemed to have a slightly stimulating effect on the sperms at this concentration, when compared with the controls, though this cannot be regarded as certain. The two latter substances were without effect. The extreme spermicidal power of mercuric chloride is of academic rather than practical interest, for no one would include such a poisonous substance in a pessary. It presumably kills by forming mercury albuminates with the proteins of the sperms.

Formaldehyde. The solutions were made up on the assumption that the "formalin" used was actually 40 per cent. formaldehyde. Probably the concentration was slightly less, so that formaldehyde may be slightly more spermicidal than the experiments show. Anyhow, it runs mercuric chloride very close. Dr H. M. Carleton has made some experiments with it, and finds that it is without effect upon the vagina or uterus of the rabbit when used at concentrations far greater than the killing concentration. I wish to thank Dr Carleton for allowing me to mention this unpublished result. A weak solution of formalin would probably be an effective contraceptive if used as a vaginal douche. One cannot detect formaldehyde if a solution at the killing concentration is taken into the mouth and swallowed. The preparation of a solid pessary containing or producing formaldehyde presents difficulties, which are referred to under the heading "Hexamine."

Saponine. This poisonous glucoside was suggested by Prof. J. B. S. Haldane on account of its power of reducing surface tension. Unless experiments prove the contrary, it may be suspected of being too irritant for use as a contraceptive. Powdered quillaia bark is contained in the Monsol pessary.

Hexyl resorcin. This substance, sometimes known as Caprokol, was tried on account of its very great power of reducing surface tension. Of all the substances tried it seems the most likely to be useful as a contraceptive, though no experiments have yet been made to find whether it is harmless. It is thirty-two times as spermicidal as quinine bisulphate or chinosol. It is not very soluble, but this may in a sense be an advantage, for with very soluble substances there is always the possibility of dangerously high concentrations occurring in the vagina if there is very little vaginal fluid.

Iodine. This was dissolved with four times its weight of potassium iodide. It would probably be too irritant for practical use.

Acetic acid. Diluted vinegar is said by Stopes (1925) to be widely used as a contraceptive. It has not, to my knowledge, been incorporated in a solid pessary (as lactic acid has). It suffers from the same disadvantage as other acids, namely that semen is alkaline, and that this alkalinity must be neutralised before the sperms are affected. The vaginal fluid is also said to be alkaline during sexual excitement. It is interesting to notice that a monobasic acid

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is the most spermicidal. It had been suggested to me that dibasic acids would be more effective than monobasic, and tribasic than dibasic. This is not so.

Succinic acid is dibasic. It has almost exactly the same spermicidal powers as acetic acid. It belongs to the poisonous oxalic acid series, and is only significant for comparative purposes.

Sodium oleate. Prof. J. B. S. Haldane suggested soaps, on account of their power of reducing surface tension. His expectations were fully realised, for sodium oleate kills at $\frac{1}{32}$ per cent. It is therefore sixteen times as spermicidal as quinine bisulphate or chinosol, which are so commonly used in pessaries. Its effect upon sperms is not due to the alkalinity of the solution, for neutralised solutions kill at the same concentration. This was proved by a series of experiments in which the $\frac{1}{16}$ per cent. sodium oleate solution was neutralised with lactic acid before it was mixed with an equal quantity of sperm suspension in B.G.S. The 0·9 per cent. sodium chloride solution in the control tube was acidified by the same amount of lactic acid, lest it might be thought that the inactivity of the experimental sperms was due to the lactic acid itself, and not to the sodium oleate. In all other respects the experiments were carried out as usual. The following results were obtained in four consecutive experiments:

Experimental sperms (Sodium oleate $\frac{1}{32}$ %, previously neutralised)				Corresponding controls			
0	0	0	0	III +	III	III	III

It is clear that it is not the alkalinity of sodium oleate solutions that causes them to be so spermicidal.

I found that sodium oleate is also very spermicidal with human sperms, though less so than with guinea-pig ones. (See Potassium capronate.) I therefore prepared a number of pessaries with various soaps in gelatine gels, with and without the addition of glycerine, and these seemed likely to be far more efficient than any existing pessary. Dr Carleton kindly allows me to say that he finds that these pessaries are harmful to the vagina and uterus of the rabbit. Weak soap solutions could probably be used with advantage as contraceptive douches.

The great spermicidal power of soap raises some interesting points. No woman who was desperately anxious to have a baby would soak herself (vagina included) in a weak solution of quinine or chinosol shortly before coition; yet she would have no hesitation in soaking herself in a soap solution in a hot bath, although soap is very much more spermicidal than quinine or chinosol. Bath water certainly enters the vagina in the case of women who have borne children. It seems to be conceivable that the fall in the birth rate among the wealthier classes during the latter half of the nineteenth century may be correlated with the installation of proper hot water systems in the houses of the rich at that time. Another interesting point is that occlusive pessaries made of rubber are often placed in soapy water before introduction into the vagina, so that they may slip in easily. Their efficacy no doubt depends largely on the soap.

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Sodium oleate and palmitate are slightly more spermicidal than appears from the table, for the solutions were made up on the assumption that the samples used did not contain water.

Dr M. Stopes and Mrs Margaret Sanger inform me that soap-suds have been used as a contraceptive by prostitutes and others.

Sodium palmitate has almost exactly the same spermicidal power as the oleate. It is less soluble.

Lactic acid has the advantage that it is normally present in the vaginal fluid. The solutions were made up in such a way that 100 c.c. of a $\frac{1}{8}$ per cent. solution contained $\frac{1}{8}$ grm. of actual lactic acid, not $\frac{1}{8}$ grm. of the 75 per cent. solution sold as lactic acid. This monobasic acid has almost exactly the same spermicidal power as the tribasic citric acid. It is open to the same objection as other acids. (See Acetic acid.) It is sold in the form of a cocoa-butter pessary. It is said by Stopes (1925) to be used also as a douche.

Potassium permanganate is said by Stopes (1925) to be used as a douche at $\frac{1}{20}$ per cent. Its killing concentration with guinea-pig sperms is $\frac{1}{16}$ per cent., but very few sperms remain alive at $\frac{1}{32}$ per cent.

Citric acid is said by Stopes (1925) to be used in douches. It is tribasic, but less spermicidal than the monobasic acetic acid. It is open to the same objection as other acids. (See Acetic acid.)

Sodium taurocholeate. The bile salts were suggested by Prof. Haldane on account of their power of reducing surface tension. They are not very powerful spermicides. Possibly they will be found useful in conjunction with other more spermicidal substances.

Potassium aluminium sulphate. This was made up in such a way that 100 c.c. of a 1 per cent. solution contained 1 grm. of the anhydrous salt. Alum is said by Stopes (1925) to be mentioned as a contraceptive in Sanskrit writings. It is a constituent of the foaming pessary finil. Perhaps its astringent effect on mucous membranes may render it rather unsuitable for continued use, but this has not been proved.

Sodium glycocholeate. See Sodium taurocholeate, above.

Dioxyquinolin sulphate is a constituent of the foaming pessaries, semori and finil.

Quinine urea hydrochloride is a constituent of a very small pessary made by Messrs Parke, Davis and Co. The only other constituent is sodium chloride. It is not known whether it has the disadvantage of having an appreciable anaesthetizing effect on the vagina.

Cresol. Monsol fluid, which enters into the composition of the monsol pessary, contains substances allied to cresol.

Potassium borotartrate is a constituent of semori.

Quinine hydrochloride. This was dissolved in such a way that 100 c.c. of a 1 per cent. solution contained 1 grm. of the salt as one buys it, with two molecules of water.

Potassium oxyquinolin sulphate. There is some doubt whether chinosol can

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be regarded as a pure substance. Martindale (1928) regards it not as a double sulphate of potassium and oxyquinolin, but as a mixture, in varying proportions, of potassium sulphate and dioxyquinolin sulphate. It is sold in the form of a cocoa-butter pessary by Messrs Lambert.

Potassium cyanide. This was tested merely for comparison with other substances. It is remarkable that such a poisonous substance should only have one-sixteenth the spermicidal power of soap. It was dissolved in such a way that 100 c.c. of a 1 per cent. solution contained 1 grm. of the actual salt, not of the 95 per cent. pure substance as one buys it.

Quinine bisulphate has probably been as much used as any substance as a chemical contraceptive. It is sold in the form of a cocoa-butter pessary by Messrs Lambert. In this investigation it was dissolved in such a way that 100 c.c. of a 1 per cent. solution contained 1 grm. of the salt as one buys it, with seven molecules of water. Only 59 per cent. of this salt is alkaloid. In quinine hydrochloride 82 per cent. is alkaloid. It is therefore remarkable that the bisulphate is nearly as spermicidal as the hydrochloride. In view of the widespread distrust of chemical contraceptives used by themselves, the low spermicidal power of the substance most commonly used is significant.

Phenol is less spermicidal than one would expect from its germicidal power. It was tested by the ordinary technique, not by the modification for acids.

Hydrocyanic acid. The same remarks apply to prussic acid as to potassium cyanide. The solution was brought at 2 per cent. in water, and 0·9 per cent. of sodium chloride added.

Potassium capronate. When the high spermicidal power of sodium oleate on guinea-pig sperms was discovered, it was tried on human semen. Although effective, it was less so than on guinea-pig sperms. It appeared probable that its reduced power was due to the large calcium content of human semen, which caused the precipitation of the insoluble calcium oleate. For this reason lower members of the acetic acid series were chosen instead of the oleic, on account of the solubility of their calcium soaps. Pessaries containing the potassium and sodium soaps of coconut oil were prepared for the same reason. Unfortunately the capronate has slight spermicidal powers, and the butyrate still less.

Boric acid. This antiseptic has surprisingly little spermicidal power. It is a constituent of finil.

Ammonium chloride was tried on account of the ease with which it penetrates cell membranes.

Potassium butyrate. See Potassium capronate.

Sodium citrate. It was thought that the salts of tribasic acids might be effective.

Hexamine. It was thought that hexamine might be a convenient producer of formaldehyde. Unfortunately it produces formaldehyde very slowly, especially in alkaline solutions, and is scarcely spermicidal.

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Sodium tartrate is a product of the interaction of the foam-producers in effervescing pessaries.

Magnesium sulphate is the chief ingredient of the very large pessary called "contraps."

Sodium diborate. Borax has scarcely any effect on sperms.

Ethyl alcohol is almost without effect at 2 per cent.

Sodium dichlorylsulphamidbenzoate is the least spermicidal of all the substances tried. It is supposed by the makers to be the essential constituent of speton, but actually the spermicidal powers of that pessary must be due to the carbon dioxide produced by the tartaric acid and sodium bicarbonate. Perhaps sodium dichlorylsulphamidbenzoate would be found to be more spermicidal if it were dissolved directly before the experiment. In a paper distributed by the manufacturers, Prof. Kionka concludes that it must be a good spermicide because he has found it to be germicidal.

Notes on other substances.

A few experiments were made on other substances, but not sufficient for grading purposes.

Copper acetate reduces the activity of sperms, but not to 0, at $\frac{1}{32}$ per cent. At this concentration potassium bichromate, acetone and tannin acid have no effect. Strychnine hydrochloride has no effect even at 1 per cent. It cannot be tested at 2 per cent., because it will not dissolve at 4 per cent. I have never seen sperms so active as with strychnine hydrochloride at $\frac{1}{8}$ per cent., but I have not made sufficient experiments to prove that strychnine actually activates them at this concentration. If so, practical use might be made of this knowledge in cases where infertility results from the inactivity of the sperms. A very small quantity of strychnine hydrochloride, dissolved in B.G.S., might be added to semen caught in a sheath. The semen could then be inserted in the vagina or uterus.

Aconitine nitrate, pilocarpine nitrate, sulphuric acid and sodium hydroxide are mentioned above under the heading "Mercuric chloride."

Foaming mixtures.

Effervescing pessaries (semori, finil and speton) contain tartaric acid and sodium bicarbonate, which react in the vagina to form a foam of bubbles of carbon dioxide, whose purpose it is to spread the spermicidal substance or substances to all parts. Now carbon dioxide has itself the power of immobilising sperms, and I therefore decided to test foaming mixtures by the usual technique. The mixtures were dissolved in such a way that 100 c.c. of a 1 per cent. solution contain 1 grm. of a mixture of tartaric (or citric) acid and sodium bicarbonate in the appropriate proportions. The sodium tartrate (or citrate) formed is probably partly responsible for the results obtained.

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The only differences from the usual technique were the following:

(1) 0·5 c.c. of sperm suspension in B.G.S. is placed in each of two specimen tubes in the damp chamber in the thermostat and 0·5 c.c. of 0·9 per cent. sodium chloride solution is added to it.

(2) The experimental tube, containing a weighed amount of dry foaming mixture is placed in the thermostat, not in the damp chamber.

(3) It is transferred to the damp chamber immediately before the start of the experiment.

(4) At the start of the experiment, 1 c.c. of sperm suspension is transferred with a pipette from one of the tubes containing it to the experimental tube. The other tube containing sperm suspension is the control tube.

The following results were obtained. The control sperms showed an activity of III + or III in each experiment.

	8 %				4 %			2 %		1 %
Tartaric foaming mixture	0	0	0	0	I	I	I	II +	II +	II +
Citric foaming mixture	0	0	0	0	I	I	I	I	I +	I +

Each mixture therefore kills at 8 per cent. every time. Now supposing that 7·5 c.c. of fluid are present in the human vagina after coition, and that human sperms behave in the same way as those of the guinea-pig to the products of foaming mixtures, it is clear that 0·6 grm. of foaming mixture would suffice to immobilise all the sperms present. One of the commercial foaming pessaries contains more than this amount.

Since the foaming mixtures only kill at 8 per cent., their spermicidal powers are really very low; but one can safely introduce very much more of them into the vagina than one can of most spermicidal substances.

I shall undertake experiments to find whether the products of these foaming mixtures actually kill or only temporarily immobilise sperms. It seems probable that a pessary consisting simply of about a gram of foaming mixture would be much more spermicidal than many non-foaming pessaries on the market. The presence of an excess of acid would probably increase the spermicidal power.

THE EFFECTS OF VARIOUS SUBSTANCES ON THE BEHAVIOUR AND STRUCTURE OF SPERMS.

Experiments were performed in order to find out in what way some of the substances investigated kill sperms. Six substances were investigated from this point of view, namely formaldehyde, hexyl resorcin, sodium oleate, lactic acid, dioxyquinolin sulphate and quinine bisulphate. It was thought unnecessary to extend this study to all the substances whose spermicidal powers had been investigated.

Unfortunately I could find in the literature no accurate description of the structure of the head of the guinea-pig sperm. The best is that of Meves (1899),

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but he gives figures of transverse sections of the head which do not correspond with his surface views. I had therefore first to study the structure of the normal guinea-pig sperm. (See Fig. 2.)

The nucleus is shaped like the bowl of a spoon. Meves calls the convexity of the bowl "dorsal" and the concavity "ventral," and indeed sperms seem usually to come to rest with the convexity uppermost, though in swimming the progression is spiral. The acrosome is also somewhat spoon-shaped, but with the convexity "ventral." Its lip is recurved. It is attached to the front end of the nucleus, which it is said by Meves and all other workers to ensheath. So far as I can make out, this is inaccurate. The acrosome seems to me only to cover the nucleus to any extent *ventrally*. Dorsally it only covers the extreme anterior end of the nucleus. The line of the posterior end of the acrosome is quite easily seen crossing the nucleus, and it has been supposed that the acrosome extends to this line both above and below the nucleus. Very careful focussing has made me almost certain that this line is only seen by transparency when the sperm is viewed from "above."

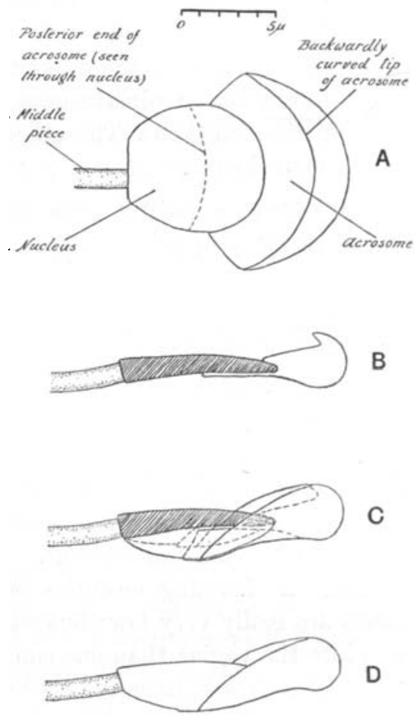

Fig. 2. Head of normal guinea-pig sperm. A. "Dorsal" view. B. Longitudinal section. C. Viewed from the side as a transparent object. D. Viewed from the side as an opaque object.

Fig. 2 was drawn from numerous observations upon fresh sperms. The section B is an optical section. B and C are to be regarded as expressions of my opinion as to the structure of the acrosome, and not as pictures of what can be seen in a single sperm viewed from the side. The backward extent of the acrosome below the nucleus can only be determined in ventral or dorsal view.

In Figs. 2 and 3 the nucleus, where seen in optical section, is marked with parallel straight lines. The middle piece is dotted.

Two series of experiments were performed to find the effects of the six substances mentioned on the behaviour and structure of sperms.

In one series the experiments were carried out as usual up to section 16, the substance being present at *half* the concentration which always kills in half an hour. Directly the sperm suspension had been added to the solution of the substance investigated, a slide was prepared and examined forthwith under the $\frac{1}{6}$ in. objective on the hot stage, and at intervals up to a quarter of

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an hour later. The movements of those sperms which were active were carefully observed, to find whether special types of movement characterised the various substances. Controls were always examined.

In the other series the experiments were carried out as usual up to section 17, the substance being present at *twice* the concentration which always kills in half an hour. A flat microscopical slide was prepared half an hour after the addition of the sperm suspension to the solution of the substance investigated.

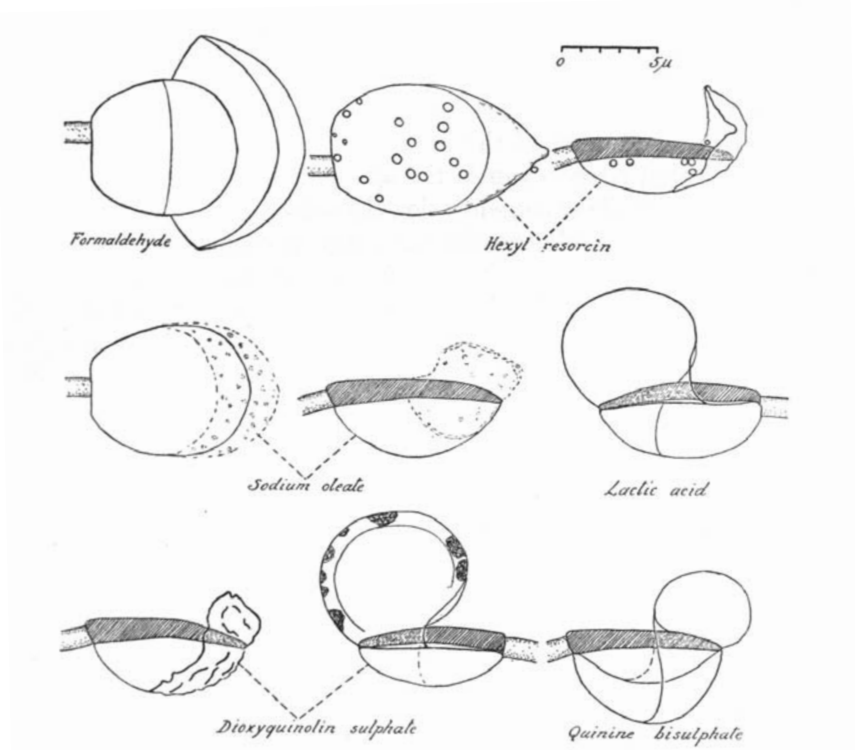

Fig. 3. Heads of guinea-pig sperms killed by various substances at twice the killing concentration.

The sperms were then subjected to close examination under an oil-immersion objective, to find what structural alterations, if any, had taken place in them. Controls were always examined. Some of the sperms were also fixed in a chromosium fixative after the substance had acted for half an hour, and permanent preparations were made and studied.

In what follows each of the six substances is dealt with separately. Under each heading the results of both series of experiments are recorded. The reader is referred to Fig. 3 for representations of sperms acted upon by the six substances studied at twice the killing concentration.

Formaldehyde. At half the killing concentration some of the sperms show spiral progression, probably on account of the shape of the acrosome not being

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affected by formaldehyde. Other sperms oscillate rapidly. By oscillation is meant repeated small to-and-fro movements of the head and tail without progression. At twice the killing concentration there is no visible effect upon the sperm, which resembles a living one. This is the only substance among the six which does not distort the acrosome.

Hexyl resorcin. Since very few sperms remain active at half the killing concentration, hexyl resorcin was tried at one-quarter the concentration instead. No characteristic behaviour on the part of the sperms could be detected, some behaving in one way and some in another. At twice the killing concentration the acrosome is shrunk. Its outline is distinct, but often contorted. Large refringent granules are scattered over the nucleus, possibly derived from the substance of the internal part of the acrosome. It seems probable that hexyl resorcin causes the acrosome to swell up and burst, for at one-quarter of the killing concentration the acrosome of those sperms which are dead is commonly swollen. The shrunken acrosome seen at twice the killing concentration is probably the collapsed remnant.

Sodium oleate. Rapid oscillation through a small distance is characteristic of those sperms which are not killed outright by sodium oleate at half the killing concentration. At twice the killing concentration the acrosome is very much shrunk and transparent, and lacks a clear outline. It appears to contain small, indistinct granules. As with hexyl resorcin, it has apparently swollen up and burst, for swollen acrosomes are seen at half the killing concentration.

Lactic acid. At half the killing concentration, the tails of the active sperms tend to curl up slowly and then straighten out quickly, resulting in slow, non-spiral movement in the arc of a small circle. The concavity of the curved tail is directed towards the centre of the circle. At twice the killing concentration the acrosome is swollen into a rounded structure situated on the dorsal surface of the nucleus, roughly circular in optical section when viewed from the side.

Dioxyquinolin sulphate. At half the killing concentration quite active lashing movements of the tail occur, but very few sperms progress spirally. There is no oscillation. At twice the killing concentration the acrosome is commonly swollen up into a bladder situated dorsally. The bladder is thick-walled and the walls have opaque patches on them. In many sperms it appears that the acrosome has swollen up into a bladder which has then burst. In these the acrosome is shrunk and distorted. Its outline, which remains very distinct, is puckered.

Quinine bisulphate. At half the killing concentration those sperms which are active tend to oscillate rapidly through a very short distance without any progression. There are no large bending movements of the tail. At twice the killing concentration the acrosome is swollen up into a rounded structure on the dorsal surface of the front end of the nucleus, similar to that produced by lactic acid. Sometimes it appears to have burst, for the acrosome is reduced to a small granular mass capping the anterior end of the nucleus.

Careful measurements fail to reveal any swelling or shrinkage of the nucleus

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as a result of the action of any of these substances, nor is the middle-piece visibly affected. The tail is often rather strongly curved just behind the middle-piece in sperms subjected to the action of dioxyquinolin sulphate and quinine bisulphate, but this is not to be regarded with certainty as a characteristic reaction. It is the acrosome which is the most vulnerable part of the sperm, so far as structural changes are concerned. Prof. G. T. Popa, of the Histological Laboratory at Jassy, Rumania, has arrived at this conclusion quite independently in a large investigation of the biology of the sperms of several Mammals. It was my fortune to see a demonstration of his work at the Second International Congress for Sex Research.

OSMOSIS.

A series of experiments was performed to find to what extent guinea-pig sperms were susceptible to changes in the osmotic pressure of a substance which is not chemically destructive to them. For this purpose sperms were suspended in various concentrations of sodium chloride.

The experiments were carried out as follows. Solutions of sodium chloride were made up at 5, 2, 1·5, 0·9 (control) and 0·5 per cent. 1 c.c. of each was placed in a specimen tube in the usual damp chamber in the thermostat. 1 c.c. of distilled water was placed in another tube in the damp chamber. The fluids were left to warm up. An adult male guinea-pig was killed by a blow on the head, and the tail of one epididymis removed. The tail was cut into four approximately equal portions, of which one was placed in the tube containing 0·9 per cent. sodium chloride, and one or more in one or more of the other tubes. The portions of epididymis were squeezed with forceps and removed. Air was bubbled through the fluids with pipettes. Twenty-five minutes after the introduction of the sperms into the fluids, air was bubbled through again and hollowed slides prepared from the contents of each tube. As exactly as possible half an hour after the introduction of the sperms into the fluids, the slides were examined under the microscope on the hot stage. The experiment was repeated until it had been performed three times at each experimental concentration, and seven times at the control concentration (0·9 per cent.). The control sperms were not very active, on account of the acidity of sodium chloride solutions. The following results were obtained:

0%	0·5%	0·9%	1·5%	2%	5%
0	.	III	.	.	.
0	.	II+	.	I	0
.	.	II+	.	I	0
0	II	II+	I+	.	.
.	.	II+	I	I	0
.	III	III	I	.	.
.	I+	III	.	.	.

The results show that sperms are very susceptible to changes in osmotic pressure. The increase from 0·9 to 1·5 per cent. results in the death of the great majority of the sperms. Acidity cannot be the cause of the inactivity of sperms

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at increased concentrations, for 2 per cent. sodium chloride has the same *p*H (about 6·2) as 0·9 per cent. Distilled water always kills every sperm.

No doubt many of those substances tested in the main investigation which only kill at high concentrations do so largely by virtue of their osmotic pressure.

Acidity.

The reader is referred to the section upon acetic acid, above, for some remarks on acids.

A series of experiments was performed to find the effects of fluids at various *p*H on sperms. Acetic, succinic, citric and lactic acids were investigated. Each was dissolved at various concentrations in a glucose-saline solution containing phosphates, and the *p*H of each solution was determined. The technique was almost precisely the standard one. The *p*H given is that of the fluid without the sperms. The activity of the sperms was determined as usual after half an hour at the temperature of the body. The control sperms showed an activity of III or III + in every case. The following results were obtained:

*p*H 4·0–4·9	Acetic	0	0	0	0
	Succinic	0	0	0	0
	Citric	0	0	0	0
	Lactic	0	0	0	0
*p*H 5·0–5·9	Acetic	0	0	I	II
	Succinic	I	I	I +	II +
*p*H 6·0–6·9	Acetic	III			
	Citric	I	II	III	
	Lactic	II	II	III	

This may be summarised as follows: Sperms are all killed below *p*H 5. At *p*H 5·0–5·9 their activity is usually reduced to about I (less than 10 per cent. moderately active). At *p*H 6·0–6·9 their activity is usually reduced to about II (less than half moderately active).

It is important to note that it is the concentration of hydrogen-ions, and not the nature of the anions, that determines the inactivity of the sperms. It makes little or no difference what acid is used; all have the same effect at the same *p*H. Again, citric acid always kills at $\frac{1}{16}$ per cent. when tested by the standard technique; but sodium citrate fails to kill at 2 per cent.

An apparent exception to the rule that the anion does not matter is afforded by boric acid. A solution of boric acid at *p*H 6·3 reduced the activity of sperms to I in two experiments. This is not to be interpreted, however, as indicating any special spermicidal power of the boric anion. It is simply that boric acid dissociates very slightly, and therefore the solution at *p*H 6·3 was a very concentrated one, thirty-two times as concentrated as a solution of citric acid at approximately the same *p*H. No doubt the boric acid exerted its effects largely by osmosis.

Surface tension.

The importance of Prof. Haldane's suggestion that substances which lower surface tension would be likely to be good spermicides has already been

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stressed. It was this conception which resulted in the discovery of the great spermicidal powers of hexyl resorcin.

I was anxious to find whether there was any direct correlation between the power to reduce surface tension and the power to kill sperms. In order to arrange these substances in the order of their power to reduce surface tension, I dissolved each of them at a concentration of $\frac{1}{32}$ per cent. in 0·9 per cent. sodium chloride solution. I then transferred each solution drop by drop with a pipette to a measuring cylinder, and counted the number of drops required to make 10 c.c. The same pipette was used for each substance, and it was held vertically throughout.

The observations were made at room temperature, which did not vary greatly. The number of drops is shown below. The last figure is probably insignificant, owing to the lack of precision in the method employed. The substances are arranged in the order of their capacity to reduce surface tension.

	Number of drops	Killing concentration (%)
Hexyl resorcin	741	$\frac{1}{64}$
Sodium taurocholeate	542	$\frac{1}{4}$
Sodium glycocholeate	493	$\frac{1}{4}$
Sodium oleate	476	$\frac{1}{32}$
Potassium capronate	438	2
Saponine	410	$\frac{1}{64}$
Sodium palmitate	403	$\frac{1}{32}$
Sodium chloride 0·9 %	385	

It will be observed that there is no direct relation between capacity to reduce surface tension and spermicidal power. The bile salts are very surface-active, but only kill at $\frac{1}{4}$ per cent.

Substances which reduce surface tension should be included in pessaries, not only because such substances are usually spermicidal, but also because their presence renders it more probable that the smallest crevices in the folds of the vagina will be reached. Perhaps the bile salts will find an application here, used in conjunction with a more spermicidal substance.

THE IDEAL CHEMICAL CONTRACEPTIVE.

It appears to me that the ideal chemical contraceptive should have the following characters:

(1) It should be inexpensive ($\frac{1}{2}d.$ or less).

(2) It should be solid, and therefore require no special appliance for introduction into the vagina.

(3) It should be small (less than 1·5 grm.).

(4) It should be unaffected by the ordinary range of climate.

(5) It should leave no trace on the skin when handled.

(6) It should contain no volatile or odorous substance.

(7) It should be absolutely non-irritant and harmless to the vagina, uterus and penis. It is possible that chemical contraceptives may sometimes find

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their way to the uterus, so the absence of any action on the uterine mucosa must be assured.

(8) It should be without physiological effect if absorbed into the blood stream.

(9) It should form a dense foam of small bubbles within the vagina, to carry the spermicidal substance to all parts.

(10) It should contain a substance which will give strength and permanence to the foam.

(11) It should contain a substance reducing surface tension, not only because such substances are usually good spermicides, but also to ensure the smallest crevices in the folds of the vagina being reached.

(12) It should dissolve completely in 7·5 c.c. of water at the temperature of the body, leaving no insoluble vehicle or sediment behind.

(13) One-twentieth of it should suffice to kill every sperm in 5 c.c. of human semen at the temperature of the body under laboratory conditions in half an hour.

(14) Its spermicidal power should not be affected by acidity or alkalinity within the range to which it is likely to be subjected.

(15) It should contain no substance which could induce any genetic modification in those sperms which were not killed outright by it. This is a subject upon which nothing is known, beyond the fact that there are no statistics showing an increase in the birth-rate of abnormal individuals since the use of chemical contraceptives became widespread.

No pessary on the market approaches this ideal. Douches and pastes may be used by those who have no aesthetic objection to the use of special appliances for insertion, though douches must be less certain in action from being used after coition.

Methods of research in chemical contraception.

It cannot be denied that research in chemical contraception has been haphazard and almost entirely chemical and clinical. The chemical manufacturers seem to have assumed that substances which are known to be good germicides must necessarily be good spermicides. This research has shown this assumption to be erroneous. It suffices to mention the high spermicidal and low germicidal power of sodium oleate.

It seems to me that the subject should be treated in an orderly way, with co-operation between physiologist, chemist, histologist, and clinical worker. Research could be carried out as follows:

(1) The physiologist should test substances for spermicidal powers with guinea-pig sperms.

(2) The chemist should suggest substances for use as vehicles, foam-producers, foam-stiffeners, and reducers of surface tension.

(3) The histologist should test the substances recommended by the physiologist and chemist for effects upon vagina and uterus.

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(4) The chemist should decide in what ways the substances passed by the histologist as harmless may be mixed together to form pessaries, in such a way that no substance interferes with the action of another substance.

(5) The physiologist should test the pessary for spermicidal powers with human sperms. I have elaborated a complicated technique for this purpose, which will be described in detail in the next paper of this series.

(6) Those pessaries which are passed by the physiologist as efficient should be tested on women in a birth-control clinic.

SUMMARY.

1. A technique for comparing the spermicidal powers of pure substances is described in detail. It is hoped that this may be accepted as the standard technique for the purpose.

2. The killing concentration of each substance is determined by this technique. The killing concentration is defined as the lowest concentration, in the series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$ per cent., etc., which suffices to kill every guinea-pig sperm suspended in glucose-saline solution in half an hour at the temperature of the body in four consecutive experiments, the majority of the control sperms being moderately or very active.

3. 36 substances have been graded by this technique.

4. Mercuric chloride and formaldehyde were found to be the most spermicidal substances. The killing concentration of each is $\frac{1}{256}$ per cent.

5. Hexyl resorcin kills at $\frac{1}{64}$ per cent., soaps at $\frac{1}{32}$ per cent.

6. Formaldehyde and hexyl resorcin, among other substances, seem likely to be useful as contraceptives.

7. The significance of the high spermicidal power of soaps is discussed.

8. Quinine bisulphate and chinosol, which are perhaps more commonly used as contraceptives than any other substances, only kill at $\frac{1}{2}$ per cent.

9. Certain very poisonous substances have very slight spermicidal powers. This applies to potassium cyanide, prussic acid and strychnine hydrochloride.

10. Foaming mixtures, consisting of acids and sodium bicarbonate, could probably be used alone as contraceptives.

11. The acrosome is the part of the sperm most vulnerable to spermicides. It tends to swell up and burst.

12. Sperms are very susceptible to changes in osmotic pressure.

13. It is the hydrogen ions and not the anions of acids that kill sperms.

14. The suggestion that tribasic acids would be found to be more spermicidal than dibasic, and dibasic than monobasic, is not substantiated.

15. Substances which reduce surface tension are often effective spermicides.

16. The characters of the ideal chemical contraceptive are discussed.

17. The need for co-operation in research in chemical contraception is stressed.

https://doi.org/10.1017/S0022172400010767 Published online by Cambridge University Press

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(*MS. received for publication* 2. IX. 1930.—Ed.)

https://doi.org/10.1017/S0022172400010767 Published online by Cambridge University Press