

**THE CHEMICAL CONTROL,
OF CONCEPTION**

THE CHEMICAL CONTROL OF CONCEPTION

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TO
JULIAN HUXLEY
FROM HIS FORMER PUPIL

PREFACE

THIS book is the result of many years' work, resulting from a suggestion by Dr. C. P. Blacker that a scientific study of chemical contraception should be made. I wish to thank the Birth Control Investigation Committee for its long-continued support, and in particular to express my gratitude to Dr. Blacker, who, as its Secretary, has always been generous with advice and encouragement. Professor E. S. Goodrich, F.R.S., has kindly allowed the work to be done in the Department of Zoology and Comparative Anatomy at Oxford. Since the spring of 1932 Mr. R. M. Ranson has worked with me on this subject, and has not only performed a large number of tests of spermicidal power, but also has made many valuable suggestions for the improvement of the technique. Dr. J. M. Gulland gave me the great benefit of his knowledge of organic chemistry during the course of the work on the mode of action of the aldehydes, phenols and quinones, and synthesized many substances for trial, some of which had not been prepared before. This part of the work could not have been done without his collaboration. Dr. H. M. Carleton has always worked in close collaboration with me, and has kindly written a chapter on the morbid histology

of chemical contraception, of which he has made a special study under the auspices of the Birth Control Investigation Committee.

A summary is given at the end of each chapter.

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THE CHEMICAL CONTROL OF CONCEPTION

CHAPTER I

HISTORICAL INTRODUCTION

THERE formerly existed an antithesis between pure and applied science. Workers in pure science were sometimes even pleased by the thought that their discoveries could not possibly find practical application. The applied scientist often found difficulty in appreciating the value of research which appeared to have no application in human affairs. With the advance of science, however, the antithesis is disappearing, for a very practical reason. Research is getting more and more expensive. As each science develops, so its equipment tends to cost more and more. The funds available for pure research are strictly limited. When a proposed research has an obvious practical application, it is usually not difficult to find funds for it. Now it is not difficult to find many fields of research, of the greatest academic interest, that have practical bearings. If those who command the funds take a generous and long-sighted view, a real advance may be made in pure science, while the work pays for itself in practical results.

To some extent these remarks might be regarded almost as truisms by medical men, for in medicine

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the line between pure and applied science has never been so clearly drawn as elsewhere. The great majority of physiologists have at least considered the possibility of a medical career. The subject of this book is, in its applications, a medical one, and it would have been an advantage if the subject had been taken seriously in hand by a medical man, and after due investigation had been discussed without any regard to current opinion and well-meaning bias on a subject formerly taboo. Until recently, however, the medical profession has for one reason or another adopted an attitude of aloofness. It avoided any expression of opinion when a storm was raised around Charles Bradlaugh and Mrs. Besant in the Victorian atmosphere of 1877-8 by the public discussion of the restriction of population. No doubt opinion was influenced by a mystical hankering after the "natural," which also impeded medical progress by causing people to regard it as proper, because natural, that women should suffer great pain in childbirth. People who pretend to desire the natural life should have the courage of their pretended convictions and go and live it to the full among savage communities, instead of trying to prevent the improvement of the lot of civilized beings.

Medical men, then, and physiologists in general, have avoided the scientific study of contraception. Surely, one would say, biologists would be attracted. The subject is bound up with eugenics, which is far more a biological than a medical subject. Yet biologists have shirked the subject too, for a different reason. The reason is the antithesis between pure and applied science, which is probably as strong in zoology as in any other science. If it were not so,

zoologists could not fail to have been attracted by the study of contraception, for population problems are problems of zoology, and it is zoologists, not medical physiologists, who have made the most comprehensive studies of the sperm.

The result of the facts so far discussed is that there has been very little scientific research on contraception, despite the vast amount of contraceptives sold and their enormous influence on civilization.

The history of contraception has been the history of customs rather than the history of science. The history of scientific chemical contraception can be told in a few pages. The first man attracted to the subject was the great Swiss zoologist, Kölliker, who had been the first to prove, in 1841, that sperms are not parasites, but, true sexual elements, or, as we should now say, germ cells. He was directed to the subject of the action of substances on sperms by the chance discovery, in 1854, that sodium hydroxide activated immobile dog sperms (Kölliker, 1855). He worked chiefly with sperms from the vas deferens and epididymis of the bull, horse, dog and rabbit. His human material, from corpses, was unsatisfactory. The experiments were conducted at room temperature. He found that many substances immobilized sperms in high and low, but not in intermediate, concentrations. It is obvious to us now that he was here observing simply the effects of osmotic pressure. Had he realized the necessity of maintaining a suitable concentration of salts in his suspension fluids, his work would have had far more value. It appears that he often simply dissolved the substance under investigation in water. He says of some substances that they act on sperms

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“like water,” a result which cannot surprise us. After noting the effect on the sperms, he sometimes made the solution what we should now call isotonic, to see whether the sperms had been permanently or only temporarily immobilized. In some cases he did dissolve the substance in isotonic sugar solution. He did not elaborate a standard test for comparing the spermicidal powers of substances. He found that the movement of sperms may be slowed down by acids and restored by alkalis. He showed that alkaloids have low spermicidal powers.

A comprehensive investigation of spermicides was made in 1905 and 1906 by Professor Gustav Günther at the Veterinary High School in Vienna (Günther, 1907). He worked with the ejaculated semen of horses, bulls and dogs. He also tried human semen, but unfortunately found that the specimens at his disposal were usually useless (presumably deficient in active sperms). He added a drop of semen to nine drops of 0.9 per cent. sodium chloride solution, in which the substance under investigation had previously been dissolved, usually at $\frac{1}{10}$ or $\frac{1}{100}$ per cent. After quickly mixing the fluids he at once prepared a microscopical slide (leaving no bubble of air below the coverglass), and examined the sperms under a low magnification. The experiment was performed at the temperature of the laboratory. Günther's criterion of spermicidal power was the quickness with which the sperms died. He noted the number of minutes elapsing before the time when no movement at all could be detected in any of the sperms. It appears that in the great majority of cases he only made one experiment at each concentration. He tested 101 different

substances, mostly alkalis, acids, inorganic salts, alcohols, organic antiseptics, organic reducing agents, and alkaloids, together with a few miscellaneous substances, including urea, dextrin and blood-sera. He confirmed the fact that sperms which have been rendered less active by acids can be reactivated by alkalis, but showed that the converse does not hold true. He showed also that alkalis could reactivate sperms immobilized by other substances than acids. He discovered the high spermicidal power of hydroquinone. He was thus led to the investigation of other organic reducers, and found that they tend to be highly spermicidal, adurol (monochlorhydroquinone) being the most spermicidal of all the substances which he tested. He confirmed the low spermicidal power of alkaloids.

In 1931 Professor E. Gellhorn, of the University of Oregon, U.S.A., published the results of his study of the effects of salts on sperms. He suspended cavy sperms in isotonic solutions of various salts and noticed how long they took to die. Using the same anion throughout, he found lithium the most spermicidal cation, then cæsium, then ammonium, then sodium, then rubidium, and finally, least toxic of all, potassium. With frog sperms, curiously enough, the cation order was almost exactly reversed. Using the same cation throughout, Gellhorn was able to arrange the anions in order of toxicity. With cavy sperms sulphocyanide and fluoride were the most toxic, then citrate and iodide, then chloride, nitrate and bromide, and finally sulphate, phosphate, acetate and tartrate. The anion order, which resembles the Hofmeister series, is almost the same with frog

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sperms. From the practical point of view these interesting experiments have the disadvantage of not measuring the toxicity of cations and anions in the presence of those which are essential to the active life of sperms, and which are always present in normal cases. Gellhorn made another series of tests, which to some extent meets this criticism.

He suspended frog sperms in $\frac{N}{40}$ sodium chloride solution, to which he added the salts of various metals at one-twentieth of the concentration of the sodium chloride. Lead acetate and ferric chloride were the most spermicidal of the salts he tested in this way, then zinc chloride, and lastly cadmium chloride. The other salts investigated (cobalt nitrate, barium chloride, strontium chloride, and particularly magnesium and calcium chloride) caused the sperms to live longer than in sodium chloride solution alone.

In the third decade of this century two separate organizations, the Birth Control Investigation Committee in England and the Committee on Maternal Health in the United States, began to organize laboratory research on the subject, with practical objects in view. Owing to certain restrictions imposed by the laws of the United States, the Committee on Maternal Health arranged to have the work done for them at the Animal Breeding Research Department (now the Institute of Animal Genetics) of the University of Edinburgh. It was carried out between 1929 and 1932 by Dr. C. I. B. Voge, under the general direction of Professor F. A. E. Crew. Dr. Voge examined sperms after varying periods of subjection to various concentrations of spermicides.

Some of his results are given in his book, "The Chemistry and Physics of Contraceptives," in which he discusses also the composition of many commercial products.

My own laboratory work on chemical contraception, under the Birth Control Investigation Committee, started in 1928 and still continues. From the spring of 1932 I have had the great advantage of the assistance of Mr. R. M. Ranson. The work has been done in the Department of Zoology and Comparative Anatomy at Oxford, by permission of Professor E. S. Goodrich. (Dickinson and Bryant (1932, p. 222) state that my work started later than Dr. Voge's, and is being done at Cambridge. These statements are erroneous.) Working in the Department of Physiology at Oxford, Dr. H. M. Carleton has made a study of the pathology of chemical contraception, also under the auspices of the Birth Control Investigation Committee. My chief objects have been to devise methods of grading the spermicidal powers of pure substances and suppositories, to find the principles underlying the killing of sperms by chemical means, and to discover new powerful spermicides which are harmless to the vagina. Mr. Ranson's and my contributions to these subjects form the material of the rest of this book, except Chapter VII., which Dr. Carleton contributes. Much of it has already been published in the *Journal of Hygiene* and the *Quarterly Journal of Experimental Physiology*, and is reproduced here by kind permission of the editors and publishers. It has been thought advisable to collect the material into a book, not only because it is thus made more readily available, but also

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because an opportunity is given for the correction of errors in the earlier work, and for the omission of discarded techniques. A considerable amount of hitherto unpublished matter is also given, large parts have been entirely rewritten, and the whole has been arranged in a consistent order. There is nothing in any of the six papers in the *Journal of Hygiene* (Baker, 1930, 1931, 1931a, 1932, 1932a, Baker and Ranson, 1935) or in the two papers in the *Quarterly Journal of Experimental Physiology* (Baker, 1930a and 1931b) that is not also given in this book, except matter that was subsequently discovered to be erroneous or capable of being expressed in a better way.

It is no part of the purpose of this book to press the advantages of chemical over other methods of contraception. It has the obvious advantage of simplicity in use, and will probably often be used as a second line of defence by those who place more reliance on other methods. That any reliance at all has been placed on it is remarkable, since there has been, till recently, little objective evidence of the capacity of supposed chemical contraceptives to kill sperms. It is hoped that the research recorded in this book will be regarded as a contribution to pure as well as applied science. The sperm gives a much more obvious sign of vitality than most cells, and requires no special methods of separation from other cells. These are great advantages for any study of the chemical killing of cells. Such a highly specialized cell as the sperm cannot, of course, be expected to give reactions typical of all cells, but its advantages are sufficiently great to have made it worth while to elaborate a standard technique

for testing the spermicidal powers of substances, irrespectively of whether there were any likelihood of the practical application of the results.

Throughout this book the word sperm is used instead of spermatozoon, which is clumsy and was given under a false conception of the significance of the object named. The words spermicide and spermicidal are preferred to spermaticide and spermatocidal.

SUMMARY. Medical men have avoided the subject of contraception, and there has been little laboratory research on it. In 1854 Kölliker made the first investigation of the action of substances upon sperms, but the lack of knowledge of osmotic pressure at that time prevented him from obtaining very important results. Günther made a comprehensive investigation in 1905-6. He discovered the low spermicidal powers of alkaloids and the high powers of many organic reducers. Recently Gellhorn studied the effects of ions on sperms. Voge has worked on the subject under the Committee on Maternal Health, and Dr. H. M. Carleton and the author, with the able assistance of Mr. R. M. Ranson, under the Birth Control Investigation Committee. The rest of this book is an account of the work done under the auspices of this Committee. It is hoped that it will be regarded as a contribution to pure as well as applied science.

CHAPTER II

FLUIDS FOR SPERM SUSPENSIONS

THE title of this chapter will probably make many readers decide to skip directly to the summary on p. 25, and then pass on to the rest of the book. Nevertheless, the high activity of one's control sperms is absolutely essential in an investigation of this sort, and suitable suspension fluids are the very basis on which success depends. One may, of course, use human sperms in human semen; but if one does this, one is confronted with the impossibility of getting adequate supplies for a large investigation. Further, one cannot study the real effects of substances on the sperms themselves, owing to the reaction of the substances under test with the proteins of the semen. To investigate the principles of chemical contraception it is therefore necessary first to study sperms taken from the epididymis and suspended in a fluid which is favourable to their activity but unlikely to react with the substances under test. Before this could be done, it was necessary to elaborate a suitable suspension fluid.

The cavy or guinea-pig, *Cavia cobaya*, was chosen as the animal whose sperms were to be investigated in detail, because it is easy and cheap to keep in the laboratory, is pleasant to handle, breeds readily throughout the year, matures quickly, and provides an abundance of large and very active sperms (see

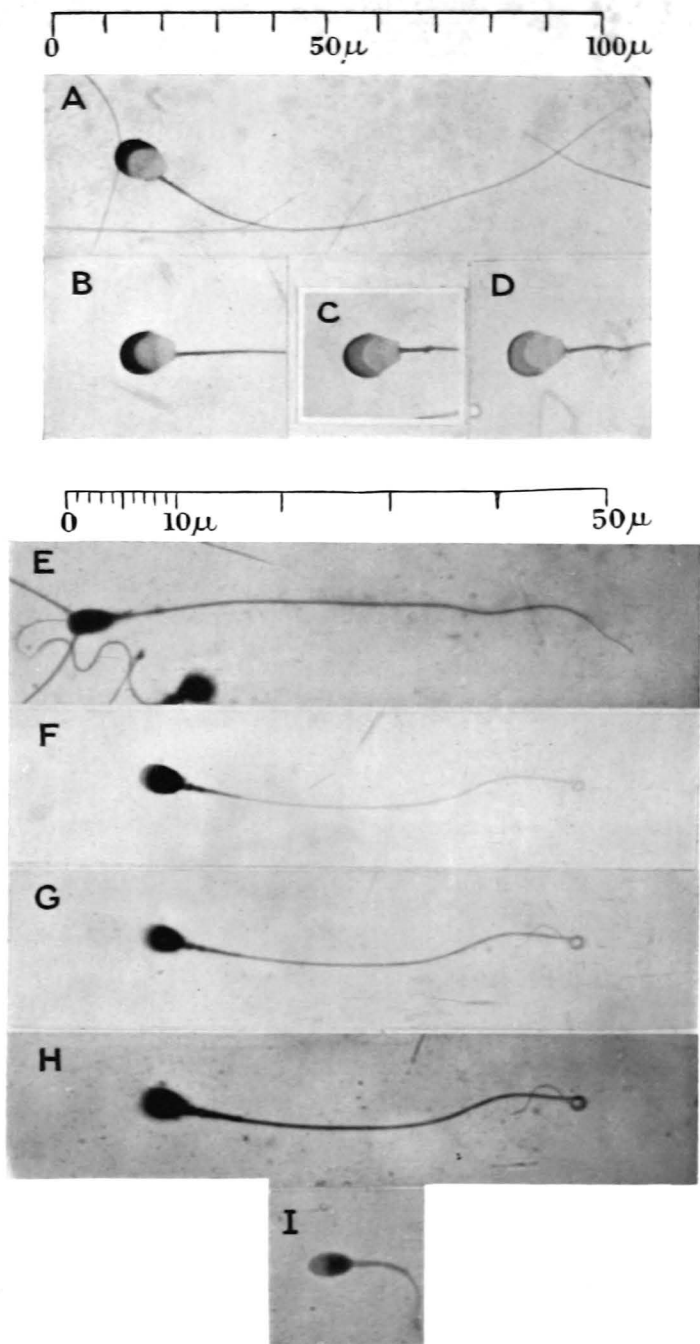


FIG. 1. (Untouched Photomicrographs by the Author.)
See opposite page.

FIG. 1.

The sperms of the cavy (above) and of man (below).

- A. Cavy sperm, $\times 680$. (Formaldehyde : Heidenhain's hæmatoxylin.)
- B. Front end of another cavy sperm. The acrosome is darkly stained. (Same treatment.)
- C. Another cavy sperm, lightly stained to show the turned-back front edge of the acrosome. (Same treatment.)
- D. Ditto.
- E. Human sperm, $\times 1330$, to show its full length. (Formaldehyde followed by mercuric chloride : hot Regaud's hæmatoxylin.)
- F. Another human sperm, $\times 1330$, focussed to show the acrosome (pale). (Same treatment.)
- G. The same sperm, $\times 1330$, focussed to show the middle-piece.
- H. The same sperm, $\times 1330$, focussed to show the end-piece (free axial filament).
- I. Front end of another human sperm, $\times 1330$, to show the acidophil part of the nucleus (dark). (Formaldehyde followed by potassium dichromate : acid fuchsin.)

On the scale at which the human sperms are shown, a slit only $\frac{1}{100}$ inch wide between a rubber occlusive pessary and the living tissue would appear over 13 inches wide. This discourages one from the use of occlusive pessaries in the absence of a chemical contraceptive.

Fig. 1). As we shall see in Chapter V., its sperms resemble human ones surprisingly closely in their reactions to the most diverse substances.

It is well known that mammalian sperms, after removal from the body, live much longer at low temperatures than at body-temperature. Those who have tried to devise methods for keeping sperms alive as long as possible have naturally used low temperatures, and they have not needed to find out whether their fluids were suitable for keeping sperms active at body-temperature. My object, on the contrary, has been to elaborate a fluid in which to keep mammalian sperms, taken from the tail of the epididymis, at maximum activity at the temperature of the body for a maximum length of time.

The best way to make a sperm suspension is not to snip up the tail of the epididymis into small pieces, as is usually done, but to divide it in two lengthwise by a single cut with scissors, and then to squeeze each half with forceps. The halves are then removed from the suspension. Nearly as many sperms are obtained as by the other method, and fragments of epididymis are almost entirely absent from the suspension.

A fairly concentrated suspension is advisable. Six c.c. of fluid may be allowed to the tails of two epididymides of the cavy. Sperms die more quickly in dilute suspensions, and, indeed, it seems probable that this is why, as Walton (1927) found, the fertility of rabbits is less when they are artificially inseminated with much diluted sperm suspensions. Presumably the secretion of the epididymis contains a substance which prolongs the vitality of the sperms, and this substance is less effective when diluted.

After a considerable amount of experiment, I have found that for the best results the sperm suspension should be placed in small, uncorked specimen tubes in a rack inside a glass damp-chamber with a glass lid (Fig. 2), the damp-chamber itself being placed inside a thermostat maintained at 37° C. Since the specimen tubes are uncorked, the sperms are not prevented from respiring; and there is no evaporation, as the tubes are in an atmosphere saturated with water. Occasionally the end of an empty pipette may be introduced into the suspensions and the bulb squeezed. The bubbles of air not only ensure thorough oxygenation, but also prevent the sperms from becoming too much aggregated together at the bottom of the tubes. Too great aggregation is also prevented by using flat-bottomed specimen tubes instead of test tubes.

In his very important work (1921) on the preservation of vitality in sperms at low temperatures, Wolf showed not only that plenty of oxygen is necessary, but also that glucose should be present in the suspension fluid, and that the fluid should be buffered at slight alkalinity. His fluid consists essentially of a mixture of Tyrode saline with isotonic glucose solution, to which is added a buffering solution, made separately, and oxygen.

The objections to Wolf's fluid are:—

- (1) That it is excessively complicated to make up;
- (2) That no experiments were performed to show that all the ingredients of Tyrode saline are beneficial to the life of sperms;
- (3) That no experiments were performed to show in what proportions the Tyrode solution should be

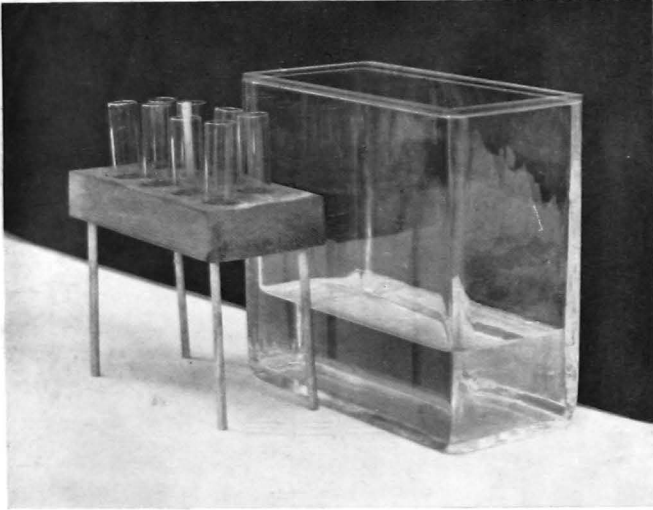


FIG. 2.—The damp chamber and the specimen-tube rack which fits inside it.

Reproduced by permission from "The Journal of Hygiene."

[To face p. 12.]

mixed with the isotonic glucose solution for the best results ;

(4) That no experiments were performed to show in what proportions the buffer salt solution should be mixed with the glucose-saline.

In addition, of course, Wolf was not trying to make a fluid for maximum sperm activity at 37° C.

To find a suitable suspension fluid for my purposes, solutions of sodium chloride, potassium chloride, disodium hydrogen phosphate, acid potassium phosphate, glucose and cane-sugar were made up, all isotonic with mammalian blood-plasma. Günther (1907) had previously shown that sperms live best in solutions nearly isotonic with blood. The sperm suspensions were kept in the damp-chamber in the thermostat, and the activity of the sperms was determined by examination under the microscope, on a warm stage, at one hour, six hours, and eleven hours after the preparation of the suspensions.

In the first series of experiments the best proportion in which to mix the glucose and sodium chloride solutions was investigated. The necessary alkalinity was got by the addition of disodium hydrogen phosphate solution. It was found that the exact proportion of glucose and sodium chloride is not important, but glucose without sodium chloride, and particularly sodium chloride without glucose, are distinctly worse than mixtures of the two. Yamane and Kato (1928) used glucose without sodium chloride, buffered at very slight alkalinity.

A second series of experiments was designed to find whether sodium acetate or sodium citrate can

advantageously take the place of disodium hydrogen phosphate as an alkaline buffer. The results were negative. The proportion in which the phosphate should be mixed with the glucose-saline for the best results was ascertained.

A third series of experiments was directed towards finding out whether there is any advantage in substituting potassium chloride, in whole or in part, for sodium chloride. Although potassium has been shown by Mârza (1930) to occur in the sperm, its presence is not necessary in a suspension fluid designed to give maximum activity. A small quantity of potassium, in the form of acid potassium phosphate, is included in the fluid as a convenient and easily obtained acid buffer; but there is no merit in potassium as such. Sperms do not require the same substances in their environment as the heart. Many workers with sperms have used such fluids as Ringer and Locke-Ringer, but these do not give high sperm activity over a long period, and they contain quite unnecessary substances. Wolf added calcium chloride to his fluid, presumably on the assumption that sperms must require it because the heart does; but the calcium must have been precipitated by the phosphates.

A fourth series of experiments was designed to find out whether there is any advantage in substituting cane-sugar for glucose. A negative result was obtained, but the difference was not marked.

In a fifth series of experiments various dilutions were tried. Slight dilution proved beneficial.

A comparison was instituted between the fluid as it now stood and a closely similar one, differing only in its components being present in round

figures, when expressed as grams and tenths of a gram per 100 c.c. of water. No disadvantage was found in the simpler method of expression, so the round figures were adhered to in the final fluid.

In order to buffer the fluid slightly against increase in alkalinity, 0.01 per cent. of acid potassium phosphate was added. At first 0.03 per cent. was used, but the greater alkalinity of the solution when the amount is reduced to 0.01 per cent. hinders the rapid growth of moulds, and sperms are at least as active in the more alkaline solution.

The pH of the final solution is about 8.1. Its composition is as follows :—

Buffered Glucose-saline (B.G.S.)

Glucose	3.0 gram.
Na ₂ HPO ₄ .12H ₂ O	0.6 „
Sodium chloride	0.2 „
KH ₂ PO ₄	0.01 „
Distilled water	100 c.c.

This solution will be called B.G.S. for short throughout the rest of this book. It is a convenient suspension fluid not only for experiments on spermicides, but also for artificial insemination, when sperms from the epididymis are used for this purpose. Mr. Ranson and I have used it with success in the artificial insemination of cavies (see p. 140). It is also being used to wash out the vagina of women when sterility is thought to be due to the vaginal contents being unfavourable to the active life of sperms.

The moulds which grow slowly in B.G.S. may be removed from time to time by filtering.

Ringer's solution is not nearly so well adapted to the active life of sperms as B.G.S. Cavy sperms suspended in Ringer's solution at 37° C. are usually nearly all immobile within six hours, a few only showing slight movements, while exact controls in B.G.S. usually show active movement in the majority of the sperms after the same period.

B.G.S. may be mixed with 0.9 per cent. sodium chloride solution in a fairly wide range of proportions without interfering seriously with its power of maintaining the active life of sperms. As will be seen later, equal volumes of the two solutions are mixed together in the standard test of the spermicidal powers of pure substances.

B.G.S. is a suitable fluid in which to test the powers of substances to immobilize sperms, because it contains nothing likely to interact with most of them. There are, however, two classes of substances to which this does not apply, namely, acids (see p. 22) and the salts of metals whose phosphates are insoluble. These metals are liable to be precipitated by the phosphates in B.G.S. A special fluid is therefore required when such substances are to be tested. It is not necessary to describe in detail the work which led up to the final formula of the fluid called 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 alkalinized shortly before the experiment. The amount of sodium chloride is increased so as to compensate in osmotic pressure for the reduction in the amount of glucose. The fluid is prepared as follows :—

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

Solution A.

Sodium chloride	1.2 gram.
Sodium acetate	0.26 „
Distilled water	100 c.c.

Solution B.

2.4 per cent. glucose in distilled water.

Shortly before the fluid is required, the pH of solution B is raised to about pH 8.5 by the addition of 0.1 per cent. sodium hydroxide solution drop by drop until a sample of it gives a bluish-green reaction with the "Universal" indicator of the British Drug Houses Ltd. 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 suspension.

Sperms do not show such extreme activity in A.G.S. as they usually do in B.G.S., but the fluid serves its purpose well.

Although B.G.S. and A.G.S. are useful suspension fluids for use in the investigation of the direct effects of substances other than acids on sperms, yet they have one great disadvantage from the practical point of view, namely, that there is no reason to suppose that substances would act similarly if the sperms were suspended in semen, for the latter might protect the sperms against various substances in varying degree. It was therefore decided to try suspending cavy sperms in human semen. (For a consideration of the differences in the reactions of the sperms of different species to spermicides, the reader is referred to Chapter V. It must suffice to say here that the differences between cavy sperms and human sperms in their reactions to the most diverse substances are very small.)

To make the rest of this chapter intelligible it is

necessary to explain very shortly the nature of the standard test for pure substances, which is very fully described in Chapter III. In this test cavy sperms from the epididymis are suspended in B.G.S. The purpose of the test is to find the least concentration of any substance, in the series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, etc., per cent., that suffices to immobilize every 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 immobilizing concentration. Each substance is also tried three times at one-half of the immobilizing 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. (Each of these figures has a meaning, which is explained in Chapter III.)

To find whether human semen provides any special protection to sperms against spermicides, seven substances were chosen for test on cavy sperms suspended both in B.G.S. and in human semen. These seven varied in spermicidal power from the most spermicidal substance known (toluquinone) to one which has such slight effect as not to immobilize all sperms at 2 per cent. (borax). The seven substances were of the most varied chemical composition. A complete test was made with cavy sperms suspended in B.G.S., and then another complete test was made, exactly similar to the first in every way except that the cavy sperms were suspended in fresh human semen instead of B.G.S. The results are given on the next page. Full details are shown in Appendices 1 and 8.

	Immobilizing Concentrations, per cent.	
	Cavy sperms in B.G.S.	Cavy sperms in human semen.
Toluquinone	$\frac{1}{512}$	$\frac{1}{256}$
Hexylresorcinol	$\frac{1}{64}$	$\frac{1}{64}$
Orthovanillin	$\frac{1}{64}$	$\frac{1}{8}$
Sodium oleate	$\frac{1}{32}$	$\frac{1}{2}$
Chinosol	$\frac{1}{4}$	1
Potassium borotartrate	$\frac{1}{4}$	2
Quinine bisulphate	$\frac{1}{2}$	$\frac{1}{2}$
Borax	Fails at 2	Fails at 2

In the tests in which human semen was used, no attention was paid to the human sperms, which are far smaller than the cavy sperms and of quite different shape, so that there was no possibility of confusing them.

The differences between B.G.S. and semen as suspension fluids are most striking. The immobilizing concentration is the same only with hexylresorcinol and quinine bisulphate, if we disregard borax, which fails to immobilize in either fluid. The difference, when present, is always in the same direction; the various substances are less spermicidal when the suspension fluid is human semen. Toluquinone is one-half as spermicidal in human semen as in B.G.S.; chinosol one-quarter; orthovanillin and potassium borotartrate one-eighth; and sodium oleate one-sixteenth.

If B.G.S. is used in tests, one learns the direct effect of substances on sperms, which is essential for an understanding of the connexion between chemical composition and spermicidal power. One

completely neglects, however, the protective action of semen, which is not only very important from a practical point of view, but also of considerable theoretical interest. It might therefore appear necessary to do one series of tests in B.G.S. and another in human semen. The impossibility of obtaining an adequate supply of fresh human semen for an extensive series of tests made it necessary to try to find a substitute for human semen, which would protect sperms against spermicides in the same way.

It was decided to use sodium oleate for the first experiments, because it is more sensitive to the protective action of human semen than any of the other substances tried. The addition of egg-white to B.G.S. in various proportions was tried, and it was found that it provided a protective action. It was only necessary to find in what proportion the egg-white should be mixed with the B.G.S. in order to give the closest approximation to the results given by human semen. Mixtures were made in the proportion of one volume of B.G.S. to 0.2, 2, 3, 3.5 and 4 volumes of beaten egg-white, and sperms were also suspended in undiluted egg-white.

The results are given in detail in Appendix 10. A study of them will show that the mixture which gives the closest resemblance to human semen is one volume of B.G.S. to 3.5 volumes of egg-white. This may be better expressed as 0.7 c.c. of B.G.S. to 2.3 c.c. of egg-white, since 3.0 c.c. is the amount of suspension fluid used in each test. A closer approximation to the ratio 1 : 3.5 cannot be obtained without using measurements below tenths

of 1 c.c., which would be both troublesome and unnecessary.

It was soon found that it made no difference to the results if 0.9 per cent. sodium chloride solution was substituted for B.G.S. in the mixture with egg-white, and this substitution was therefore made for the sake of simplicity. It is convenient to call this suspension fluid albumen-saline.

Albumen-saline

0.9 per cent. sodium chloride	
solution	0.7 c.c.
Beaten egg-white	2.3 c.c.

To prepare the egg-white, break an egg, separate the white, remove the chalaza from it, and beat it for a minute. It is preferable (though not necessary) to leave the beaten egg-white for one or two days in a covered glass vessel before using it. Sperms are then more active in the mixture made with it, probably because it becomes less viscous. It is presumably because of its high viscosity that sperms are not so active in albumen-saline as in B.G.S. Albumen-saline has a higher pH than human semen, but this does not matter, as sperms are not damaged by a wide variation on the alkaline side.

So far it had only been shown that albumen-saline was a suitable substitute for human semen when sodium oleate was being tested. It was necessary to make a test with other substances whose spermicidal powers in human semen were known. The result of this test is shown in detail in Appendix 2, and shortly on the next page.

	Immobilizing Concentrations, per cent.	
	Cavy sperms in human semen.	Cavy sperms in albumen-saline.
Toluquinone	$\frac{1}{350}$	$\frac{1}{350}$
Hexylresorcinol	$\frac{1}{84}$	$\frac{1}{84}$
Orthovanillin	$\frac{1}{8}$	$\frac{1}{8}$
Sodium oleate	$\frac{1}{2}$	$\frac{1}{2}$
Chinosol	1	$\frac{1}{2}$
Potassium borotartrate	2	2
Borax	Fails at 2	Fails at 2

The close resemblance between albumen-saline and human semen is most remarkable. Only chinosol differs when tested in the two different suspension fluids, and the difference is small. The results show that albumen-saline is a reliable substitute for human semen in finding the immobilizing concentrations of substances differing widely in chemical composition and spermicidal power. It seems very probable that the protective agent in human semen is a protein. There is no point in trying to reproduce the proteins of human semen as closely as possible, when egg-white fulfils the requirements of the test. It may be mentioned that gelatine and saliva also give some protection against sodium oleate.

Albumen-saline is a suitable fluid in which to suspend cavy sperms for tests of the spermicidal powers of all substances except acids. Acids present a special problem. Their spermicidal powers depend mostly on their capacity to overcome the buffer of the suspension fluid. There is therefore only one rational way of testing them, namely, with a sus-

pension fluid that has the same alkaline reserve as human semen. Neither B.G.S. nor albumen-saline fulfils this condition. Ten samples of human semen were taken (varying in amount from 2.0 to 6.7 c.c.). These were diluted with saline and an indicator added. The amount of 1 per cent. tartaric acid required to neutralize each was determined by titration. It was found that on the average it takes 1.7 c.c. of 1 per cent. tartaric acid to neutralize 5 c.c. of human semen. Experiments with the whites of ten hens' eggs showed that egg-white has far less alkaline reserve (though its pH is higher), for it only takes 0.8 c.c. of 1 per cent. tartaric acid, on the average, to neutralize 5 c.c. of egg-white. To make a substitute for human semen, with the same alkaline reserve, it is therefore necessary to mix egg-white with a stronger alkaline buffer. Various mixtures of egg-white and isotonic disodium hydrogen phosphate solution were made and titrated with 1 per cent. tartaric acid, until one was found that required approximately the same amount of acid for neutralization as human semen. This has the following composition :—

Phosphate-albumen

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 6 per cent. in	
water	1.4 c.c.
Beaten egg-white	1.6 c.c.

Since different eggs differ slightly in the alkaline reserve of their whites, it is a good plan to mix the whites of two or three eggs when making up this fluid.

Phosphate-albumen is used as suspension fluid in the tests of acids.

In the next chapter it will be shown that in the standard test for pure substances a certain volume of sperm suspension is mixed with the same volume of 0.9 per cent. sodium chloride solution holding the spermicide in solution. In the event of the spermicide being neutral or alkaline, the fluid after mixture is alkaline. Now there exist certain spermicides which are active in alkaline solution, but much less so in acid solution. It is necessary to have a faintly acid solution in which to test such substances. The acidity must, of course, be very faint, for otherwise it would itself immobilize the sperms. For the acid test of pure substances cavy sperms are suspended in ordinary albumen-saline, and the spermicide to be tested is dissolved in a special fluid, called "lactic-saline A," instead of 0.9 per cent. sodium chloride solution. Lactic-saline A is acidified with lactic acid, because lactic acid is a normal constituent of the vaginal fluid. In actual practice there may be sufficient lactic acid in the vagina to acidify the semen.

Strong lactic acid consists not only of acid, but also of (neutral) lactide and water. When it is diluted the lactide is gradually converted into lactic acid. To make a stable solution of precisely the right concentration for use in the tests, proceed as follows. It will save trouble if five times the quantities specified are used.

Lactic-saline A

Add exactly 3 c.c. of strong lactic acid of specific gravity 1.21 to 100 c.c. of distilled water. Add 11 c.c. of this solution to 89 c.c. of 0.9 per cent. sodium chloride solution. Boil for twenty-four hours with reflux condenser.

At the end of twenty-four hours the acidity has at last

reached a stable figure, and the solution may be kept indefinitely at room temperature. If the solution is not boiled, but simply made up and kept at 37° C., it will be found at the end of a month still to be getting more and more acid by gradual conversion of lactide to acid.

Lactic-saline A contains exactly the right amount of lactic acid for use in the standard test; that is, it is sufficiently acid to acidify the sperm suspension in albumen-saline, but not sufficiently acid to kill the sperms. To fulfil these conditions in the somewhat different test for suppositories, a stronger fluid called lactic-saline B is prepared as follows:—

Lactic-saline B

Add exactly 3 c.c. of strong lactic acid of specific gravity 1.21 to 100 c.c. of distilled water. Add 18 c.c. of this solution to 82 c.c. of 0.9 per cent. sodium chloride solution. Boil for twenty-four hours with reflux condenser. The solution may be kept indefinitely at room temperature or in the thermostat at 37° C.

I wish to acknowledge a great deal of help from Mr. Ranson in the elaboration of the lactic-saline fluids. A considerable amount of experiment was necessary before the exact proportions fulfilling the requirements were discovered. Both fluids have the appropriate osmotic pressure for the active life of sperms, and, as used in the tests, they reduce sperm activity simply by acidity without killing.

SUMMARY. The cavy (guinea-pig) is used as the experimental animal.

When it is desired to test the direct action of substances on sperms, the sperms are suspended in buffered glucose-saline (B.G.S.), a fluid in which they are very active. Phosphates are used as buffers in this fluid. When the salts of metals whose

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phosphates are insoluble are tested for spermicidal power, acetate glucose-saline (A.G.S.) is substituted for B.G.S.

When it is desired to imitate the protective action of semen on sperms, the sperms are suspended in albumen-saline. When acids are being tested, B.G.S., A.G.S. and albumen-saline are all unsuitable suspension fluids, and phosphate-albumen is used, which has the same alkaline reserve as human semen.

When it is desired to test the spermicidal powers of a substance in slightly acid environment, the substance is dissolved in lactic-saline instead of saline.

CHAPTER III

THE STANDARD TESTS FOR PURE SUBSTANCES

LITTLE progress can be made with a study of the principles underlying chemical contraception until some means have been found of attaching a figure to each substance proportional to its spermicidal power. Two ways present themselves: (1) Every substance may be tested at the same concentration, and the length of time required for immobilization of the sperms measured. (2) A standard time of action may be selected, and the lowest concentration required to kill the sperms discovered.

In general, the relation between the concentration (C) of a drug and the time (t) required for its action on cells may be expressed by the formula $C^n t = \text{constant}$ (Clark, 1933). Unfortunately the value of n varies according to the nature of the drug. In the disinfectant action of iodine and formaldehyde the value of n is 1.0, that is, the concentration varies inversely as the time. If the value of n were 1.0 with many substances, it would not matter much for what length of time one decided to allow the substances to act when finding the minimum concentration necessary to kill; for all substances would grade themselves in the same order, whatever length of time one were to choose as the standard. It is known, however, that in disinfection the value of n varies greatly. The reader is referred to Pro-

fessor Clarke's book for a full treatment of this subject. With phenol n is about 5, with thymol 4, and with the salts of the heavy metals between about 0.5 and 1.0.

If, as seems probable, the formula $C^n t = \text{constant}$ applies to the chemical killing of sperms, and if the value on n varies, then clearly the various substances would fall into different orders of spermicidal power, according to the special time or special concentration arbitrarily selected for the test. This is an unavoidable evil in any attempt to make a standard test whereby a number may be assigned to every substance, proportional to its spermicidal power.

Admitting this evil, we must make the best test we can. Which of the alternative methods mentioned in the first paragraph of this chapter shall we choose?

If we select a standard concentration, and measure the time, we are faced with two difficulties. Firstly, the life of sperms at the temperature of the body in the absence of any spermicide is strictly limited. After a few hours they may already be noticeably less active, and after twelve hours there may be no movement at all. Clearly, then, one is introducing a complication if one decides to measure the time. Secondly, it is not possible to choose a single concentration which will work with all substances. If one chooses a concentration sufficiently high to enable weak spermicides to be graded, then strong spermicides will all kill instantaneously, and it will not be possible to separate their spermicidal powers. One may, of course, choose more than one concentration, as Günther did; but if one does so, one

cannot give a figure to each substance proportional to its spermicidal power. That could only be done if time always varied inversely as concentration, *i.e.*, if n were always 1.0.

These disadvantages in the time-measuring method force us to choose a standard time, and to find the least concentration which suffices for the killing (or immobilization) of the sperms. In choosing a standard time, one must avoid such a long one that the sperms begin to become less active irrespective of the presence of the substance under test. One must also avoid choosing such a short time that weak spermicides have not time in which to act. The accurate grading of the spermicidal powers of weak spermicides is most important in a scientific study of the principles underlying the chemical killing of sperms. It is very convenient, in a standard test, always to mix a saline solution of the substance under test with an equal quantity of sperm suspension. That means that no substances can be tested above half-saturation. Now many rather insoluble substances will not kill at once even at half-saturation. If one allows a fairly long time for the substance to act on the sperms, it will be possible to grade its spermicidal powers, and thus investigate the effect of the particular grouping of the atoms in the molecule. It would be wrong to omit the study of rather insoluble substances on the ground that they are unlikely to be useful spermicides in practice. The discovery of useful spermicides is likely to follow on a real comprehension of the underlying principles, not upon the restriction of the test to those substances that are thought likely to be useful. For the sake, then, of

relatively weak spermicides and of rather insoluble substances, too short a time must be avoided.

The length of time which I have chosen for the standard test of pure substances is half an hour. This period avoids the disadvantages of too short and too long times. In the testing of suppositories a much shorter time is given, for the reasons stated in Chapter VI.

It is necessary to choose a series of concentrations at which all substances will be tested. In choosing the series one must not make the intervals too large, for then one would not be able to separate the spermicidal powers of substances unless they differed greatly. It would also be unjustifiable to make the intervals very small, because differences between the sperms of one cavy and another in their reaction to spermicides would then be large in comparison with the differences between their reactions to one concentration of the spermicide and to the next concentration in the series. A convenient series, which avoids both of these disadvantages, is the series 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, etc., per cent., and this is the series used in the standard test. If one states that a certain substance always kills at $\frac{1}{256}$ per cent., one must, of course, not attach any significance to the last figure, for the next concentration in the series is $\frac{1}{128}$ per cent. For this reason there is an advantage in calling the series $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, etc., per cent.; but on the whole the other notation is perhaps to be preferred.

It would be an advantage if the concentration of the sperms could be kept the same in all experiments. This could be done by making a sperm suspension, estimating the number of sperms by a

count of the contents of a small volume, and diluting the suspension to a standard concentration in terms of the number of sperms per cubic centimetre. Unfortunately this would take a long time, and would seriously limit the number of tests that could be carried out in a given length of time. This practical consideration has led me to decide not to control the number of sperms accurately in the standard test, but there is no reason why it should not be done in special cases. A rough approximation to a standard concentration is obtained by always suspending the contents of the tails of two epididymides in 3 c.c. of fluid. When part of this sperm suspension is subsequently diluted with an equal volume of saline containing the substance under test, the sperms are at a concentration which is favourable to high activity.

The standard temperature for the test is 37° C. It seemed best to take the body-temperature of man, although the cavy's temperature may be slightly higher. The experiment is performed inside the damp-chamber (Fig. 2), described in Chapter II., which is itself inside a thermostat maintained at 37° C.

For the examination of the sperms under the microscope it is quite essential to have a warm stage, since sperms are quickly inactivated by cold, and to a different degree on different days.

The enclosure of the whole microscope except the top of the tube in a thermostat is a clumsy and unnecessary arrangement for this purpose. The type of electric warm stage that is heated by a resistance in liquid paraffin has proved useless in my hands, firstly because the paraffin always leaks out, and secondly because the glass top of the

stage is too bad a conductor to keep the slide warm. The following is an effective arrangement. A small open tank is placed near the ceiling of the laboratory. It receives a supply of running water, and is provided with an overflow pipe running to waste. A tube runs down from the bottom of the tank to the bench, carrying water at constant pressure. This leads into a coiled metal tube passing through a water bath maintained by an electric thermostat a degree or two above 37° C.

This thermostatic water-bath was specially made by Messrs. Charles Hearson. It is supplied from the power mains. The water is heated in the coil and passes through a rubber tube to a hollow metal stage, which has two prongs below fitting into holes in the ordinary stage of the microscope. Hot water stages of this type may be obtained from microscope manufacturers. The hot stage has a small hole in the middle, through which light is admitted from a low power condenser. The stage is thin enough to allow the focussing of the light on the sperms. A blind tube running to near the middle of the stage allows the insertion of a small thermometer. The water is guided round the interior of the stage by partitions, and passes out through a rubber tube to a sink. A variable clip on this tube controls the rate of flow of the water through the heating coil, and thus enables one to keep the stage at the right temperature. The stage may be kept at about 37.5° C. to allow for the cooling at the surface of the coverslip.

The tank with overflow is a necessary part of the apparatus. If the water is led directly from the tap to the coil in the water-bath, variations in the pressure in the water mains cause the water to flow at varying speeds, and thus to be warmed to varying temperatures.

The criterion of the effectiveness of any substance as a spermicide is its capacity to kill every sperm outright. Great stress must be laid on this point. There is no other dependable criterion. If one finds only the lowest concentration that suffices to immobilize *nearly* every sperm, one's results are at once subjective and dependent on one's own personal

view of the meaning of "nearly." The grading of substances into those that always kill at 1 per cent., but sometimes fail at $\frac{1}{2}$ per cent., those that always kill at $\frac{1}{2}$ per cent., but sometimes fail at $\frac{1}{4}$ per cent., and so on, should be a completely objective matter, independent of personal views.

It is convenient to have some means of comparing the spermicidal powers of those substances which fall into the same grade. This may be done by testing substances several times at one-half of the lowest concentration that kills every time, and recording the activity of the sperms. The most accurate way of recording their activity would be by measuring their oxygen consumption or carbon dioxide production, but that would clearly be impossible in a routine test. The system of assessing the activity of sperms described below (p. 37) is intended to give results as independent as possible of personal whim, so that results obtained by different observers may be comparable.

It is very difficult to make a dependable assessment of the activity of sperms if one knows what activity one may expect. There is a strong, scarcely conscious prejudice in favour of making the same assessment as one made the time before when testing the same substance at the same concentration. For this reason it is best to test three different substances at the same time against the same control, and to cover the identity of all four slides with blank labels until the activity of the sperms on each has been assessed.

The standard test for pure substances (as opposed to suppositories, which are mixtures of two or more substances) is described in detail below.

THE TECHNIQUE OF THE STANDARD TEST

The glass pipettes, bottles, capsules, test tubes, specimen tubes and microscopical slides and coverslips used in the test are kept clean but not sterile. Sterility is not necessary, 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. One requires a bottle (say 2,000 c.c.) of 0.9 per cent. sodium chloride solution, a bottle (say 500 c.c.) of B.G.S., and a bottle (say 150 c.c.) of alkaline diluting fluid. The formula of B.G.S. is given on p. 15. Alkaline diluting fluid is this :—

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 6 per cent. in water	. 1 vol.
B.G.S. 1 „

1. Regulate a thermostat to 37° C., and place in it coverslips, graduated and ordinary pipettes, a bottle of alkaline diluting fluid, and a damp-chamber. The damp-chamber is of glass, with a glass lid (Fig. 2, p. 12). Put some water in the bottom of it. Any non-volatile disinfectant may be added to prevent the growth of moulds. Place in the damp-chamber a wooden tube-rack, provided with long legs to hold it above the water. In the rack place four small test tubes and four small specimen tubes, each about 12 mm. in internal diameter and about 5.5 cm. long.

2. Regulate a thermostatic heater to deliver a constant stream of water, which must pass through a hollow microscope stage, and maintain it at approximately 37.5° C.

3. Dissolve the substance to be investigated in 0.9 per cent. sodium chloride solution at some concentration in the series 4, 2, 1, $\frac{1}{2}$, etc., per cent. (When liquid substances are to be tested, either they must be measured by weight, as solids are, or else their specific gravity must be taken into consideration when they are measured by volume. Thus, e.g., a 1 per cent. solution is one that contains 1 gm. (not 1 c.c.) of the substance in 100 c.c. of fluid.) A concentration of 1 per cent. is often convenient for the first trial. When the sperm suspension is added (section 16), the substance will be at $\frac{1}{2}$ per cent.

4. Introduce 0.5 c.c. of 0.9 per cent. sodium chloride

solution into one of the test tubes in the damp-chamber by means of a graduated pipette.

5. Introduce 0.5 c.c. of the solution made up in section 3 into another test tube in the damp-chamber.

6. Kill an adult male cavy by a blow on the head, and damp the fur in the pelvic region to prevent hairs from getting in the way. Leave the animal for a few minutes to prevent post-mortem reflexes from interfering with dissection.

7. Meanwhile place 3 c.c. of B.G.S. in a glass capsule. (Substitute A.G.S. for B.G.S. in this section and in sections 8, 15 and 16, when the salts of metals whose phosphates are insoluble are being tested.)

8. Cut out the tails of both epididymides of the cavy and place them in the 3 c.c. of B.G.S.:

9. Cut each across once with scissors. Press each fragment several times with flat-pointed forceps to cause the sperms to come out. Remove the fragments and throw them away.

10. Suck the fluid into a pipette and press it out again three times to break up any clumps of sperms and form an even suspension.

11. Transfer 0.5 c.c. of the sperm suspension to each of two empty specimen tubes in the damp-chamber in the thermostat. These tubes are called the control tube and the experimental tube.

12. Set a timer at 0 minutes. The fluids are left to warm up.

13. Fourteen minutes later transfer the contents of the test tube mentioned in section 4 to the control tube with a pipette. (A test tube is used rather than a specimen tube because it is easier to remove the last drops from a test tube than from a specimen tube.)

14. Put the point of the empty pipette at the bottom of the control tube and squeeze the bulb. Do this three times. The bubbling of air through the suspension mixes the fluids and aids the respiration of the sperms.

15. Suck part of the mixed fluid into the pipette and then distribute it all round the inside of the control tube near its top. In falling to the bottom of the tube the fluid catches any sperm suspension which may have stuck to the side of the tube when it was introduced. The control tube now

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contains sperms suspended in a mixture of B.G.S. and saline in equal volumes.

16. When the timer records fifteen minutes, transfer the contents of the test tube mentioned in section 5 to the experimental tube, which now contains sperms in a mixture of B.G.S. and saline, together with the substance under investigation at half the concentration at which it was made up in section 3. (If two or three substances are being tested at the same time, in accordance with section 50, the process described in this section should be started when the timer records about fourteen and a half minutes.)

17. Treat the contents of the experimental tube exactly as those of the control tube were treated in sections 14 and 15. (The performance of the process described in section 15 is particularly important with the experimental tube. If it is not carried out, sperms sticking to the side of the tube may avoid contact with the substance under test until section 23, and thus vitiate the results.) This section must be complete when the timer records sixteen minutes.

18. Four hollowed microscopical slides are distinguished by diamond or grease-pencil marks. It is convenient to label two of them "C" (control) and the other two "1." (Six or eight slides will be required if two or three substances are being tested at the same time in accordance with section 50. They should be labelled in two sets, "C," "1" and "2," or "C," "1," "2" and "3.")

19. Fix a blank gummed-paper label over the distinguishing mark on each slide, sticking it down at one side only in such a way that it may be turned aside to disclose the mark. The object of this arrangement is explained in section 24.

20. Place the labelled slides on the floor of the thermostat.

21. When the timer records forty minutes, bubble air three times through the control tube with a pipette.

22. With the same pipette transfer two drops of the fluid in the control tube to the hollow of one of the slides marked C. Apply a warm coverslip. Two 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. Leave the slide on the floor of the thermostat.

23. Apply the process described in sections 21 and 22 to

the contents of the experimental tube and to one of the slides marked 1.

24. Shuffle the slides together until you do not know which is which (unless a coloured substance is being tested, in which case it is not possible to forget the identity of the slides). Bias, conscious or unconscious, is thus avoided. (The shuffling is particularly valuable when two or three substances are being tested at the same time, in accordance with section 50.)

25. When the timer records forty-four minutes, remove one of the slides containing sperms from the thermostat and examine it under the microscope with a 4-mm. objective and low-power eyepiece. (With a low-power objective it is not possible to detect minute movements of the sperms.) (If two or three substances are being tested at the same time, the examination of the slides may begin when the timer records forty-three minutes, but it must be complete when it records forty-seven minutes. The object is to examine the slides as exactly as possible half an hour after the introduction of the sperm suspension into the tubes containing the substances under investigation.)

26. Record the activity of the sperms on the blank labels on the slides, as follows :—

- 3 indicates that the majority of the sperms are moderately or very active.
- 2 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 3.
- 1 indicates any activity less than 2, including the slightest movement in a single sperm.
- 0 indicates that the examination of ten microscopical fields fails to reveal the slightest movement in a single sperm. If you have seen no movement with certainty, but think it possible that a sperm has moved, start again to examine ten more microscopical fields. Currents in the suspension occasionally give a deceptive appearance.

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

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- 2+ indicates that it cannot be quickly decided whether the sperms should be graded as 3 or 2.
- 1+ indicates that it cannot be quickly decided whether the sperms should be graded as 2 or 1.

It is usually possible to place sperms in grade 3 or 2 in a few seconds. Grade 0 takes up to a minute, since ten fields must be examined, and grade 1 may take as long, if the first active sperm is seen in the tenth field.

It is occasionally useful to use the sign 3+ to indicate extreme activity.

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 tested at the same time.

27. Repeat sections 25 and 26 with the other slide containing sperms.

28. Turn the blank labels aside to disclose the identity of the slides.

29. If the control sperms show an activity of 3 or 3+, record the activity of the experimental sperms. If the control sperms show an activity of less than 3, do not record the activity of the experimental sperms, but discard the experiment altogether. This only happens when an immature or diseased cavy is used.

30. If the experimental sperms show any activity whatever (from 1 to 3+), pass on directly to section 41.

31. If the experimental sperms show no activity whatever (0), test the reaction of the contents of the experimental tube with red litmus paper, and then proceed to find out whether they are really killed or only temporarily immobilized, as follows (sections 32-39):—

32. Add 3 c.c. of (warm) alkaline diluting fluid to both the control and the experimental tubes, and draw part of the mixtures into and out of the pipettes three times to mix thoroughly.

33. Test the contents of the experimental tube again with red litmus paper. If it does not go blue, add more

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alkaline diluting fluid from a graduated pipette until it does. Add the same amount of alkaline diluting fluid to the control tube.

34. When the timer records fifty-five minutes, prepare a slide from the contents of the control tube, exactly as in sections 21 and 22.

35. Prepare a slide from the contents of the experimental tube in the same way.

36. While the timer records between approximately fifty-nine and sixty-one minutes, determine the activity of the sperms on the control and experimental slides as in sections 25 and 26.

37. If the control sperms show an activity of 2+, 3 or 3+, proceed to section 38. After dilution an activity 3 or 3+ is not insisted upon. If the activity of the control sperms is less than 2+, discard the experiment. (This scarcely ever happens.)

38. Record the activity of the experimental sperms. If the sperms are all completely inactive (o), then they have been killed and not merely temporarily immobilized, for the spermicide is now at only one-quarter (or less) of the concentration present before. Most substances have little effect on sperms at one-quarter of the least concentration sufficient to immobilize them.

39. Enter the figures representing the activity of the sperms before and after dilution with a line between them, thus: o/o or o/1, etc. The figure after the line represents the activity after dilution (see especially Appendix III.).

40. If the activity of the experimental sperms was reduced to o/o in section 39, repeat the experiment with the substance at half the concentration used before. Repeat it again and again, if necessary, at lower and lower concentrations in the series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, etc., per cent., until active sperms are found.

41. If there is any activity whatever in the experimental sperms in section 30 or 38, repeat the experiment at double the concentration used before. Repeat it again and again, if necessary, at higher concentrations in the series $\frac{1}{2}$, 1 and 2 per cent. 2 per cent. need not be exceeded.

42. The lowest concentration which suffices to kill all sperms (*i.e.*, reduce them to o/o) is thus ascertained. Perform the test again at this concentration.

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43. If all sperms are killed, perform the test a third time at the same concentration.

44. If all sperms are killed, perform the test a fourth time at the same concentration.

45. If all sperms are killed, this concentration is recorded as the killing concentration. This may be defined as the lowest concentration in the series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, etc., per cent., that kills all cavy sperms in half an hour at 37° C. in four consecutive experiments.

46. If in any of the tests mentioned in sections 42, 43 and 44 all sperms are not killed, a higher concentration in the usual series must be tried.

47. Further tests at the appropriate concentrations must be carried out, until the killing concentration, defined in section 45, has been found.

48. All substances whose killing concentration is the same are placed in the same "grade" of spermicidal power.

If the killing concentration is $\frac{1}{2^x}$, the substance is said to be

in grade x . It is obvious that substances in each grade have, on the average, twice the spermicidal power of the substances in the next lower grade.

49. Perform the test altogether three times with each substance at half the killing concentration. This enables you to compare the spermicidal powers of substances which fall into the same grade.

50. Two or three different substances, or the same substance at different concentrations, may be tested at the same time against the same control. No substance may be tested more than once at the same concentration against the same control.

51. It is possible to run two tests concurrently, each involving three experimental tubes, if one test is started exactly twenty-three minutes after the other. The double test is almost impossible to carry out except by the use of a special timer, with two hands always separated from one another by twenty-three minutes. Each hand governs one test. Twenty-three minutes is chosen as the period because it enables the two tests to be performed concurrently without the necessity of anything being done in both tests at the same moment. The performance of two tests concurrently,

each involving a control and three experimental tubes, can only be performed when one has had a lot of practice with single tests.

The various substances which ordinarily crystallize with one or more molecules of water were weighed out with the water unless the contrary is stated. Thus zinc sulphate at $\frac{1}{4}$ per cent. means a $\frac{1}{4}$ gm. of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ to 100 c.c. of fluid.

When it is said, *e.g.*, that iodine with four times as much potassium iodide immobilizes at $\frac{1}{64}$ per cent., it means that the iodine is present at $\frac{1}{84}$ per cent.

The dilution with alkaline diluting fluid was only introduced recently into the technique. The great majority of the experiments were performed before this improvement was incorporated. Therefore, in the great majority of cases, only the lowest *immobilizing* concentration is known, not the lowest *killing* concentration (though it is becoming probable that these will be found to be the same with most substances). In this book the use of the expression "immobilizing concentration" indicates that dilution was not performed. It must be stressed that in the Appendices a line drawn between two figures thus, o/1, indicates that dilution was performed, and o/o indicates the death of all the sperms, not merely their immobilization.

The results of the standard test are as follows. The substances are arranged in order of spermicidal power, beginning at the most spermicidal. Substances bracketed together cannot be separated in spermicidal power. Full details of all the experiments are given in Appendix I.

RESULTS OF THE STANDARD TEST OF PURE
SUBSTANCES WITH CAVY SPERMS IN B.G.S.
(OR A.G.S.)

Grade 9 (immobilizing concentration $\frac{1}{312}$ per cent.).

Toluquinone.

Butylquinone.

{ Methoxyquinone.
Ethylquinone.
Parabenzquinone.
Paraxyloquinone.

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

Mercuric chloride.

{ Methoxyhydroquinone.
Formaldehyde.
Paraformaldehyde.
Methylhydroquinone.

Grade 7 (immobilizing concentration $\frac{1}{128}$ per cent.).

Malachite green.

{ Thymoquinone.
Butylhydroquinone.

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

{ Saponine.

Methoxytoluquinone.

{ Hexylresorcinol.

Dimethoxyhydroquinone.

Oxyhydroquinone.

{ Iodine (with four times as much potassium iodide).

Ortho-ethoxysalicylaldehyde.

{ Orthovanillin.

Hydroquinone.

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

Salicylaldehyde.

Crystal violet.

{ Thymol.

{ Sodium oleate.

Sodium palmitate.

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

{ Potassium permanganate.

{ Ethylharmol hydrochloride.

Grade 3 (immobilizing concentration $\frac{1}{8}$ per cent.).

Mercurochrome.

{ Carmine (with four times as much borax).

{ Harmine hydrochloride.

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

{ Sodium taurocholate.

{ Zinc sulphate.

{ Potassium aluminium sulphate (anhydrous).

{ Sodium glycocholate.

{ Dioxyquinoline sulphate.

{ Quinine urea hydrochloride.

Chinosol.

{ Cresol.

{ Potassium borotartrate.

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

{ Quinine hydrochloride.

{ Potassium oxyquinoline sulphonate.

{ Potassium cyanide.

{ Quinine bisulphate.

Catechol.

Phenol.

Grade 0 (immobilizing concentration 1 per cent.).

{ Hydroquinone methyl ether.

{ Acetaldehyde.

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{ Chloral hydrate.
{ Resorcinol.

Grade—1 (immobilizing concentration 2 per cent.).

Pyrogallol.

{ Potassium capronate.
{ Orthophenylene diamine.

Ungraded (these fail to immobilize at 2 per cent.).

Ammonium chloride.

Zinc sulphocarbolate.

Potassium butyrate.

Sodium citrate.

{ Hexamine.
{ Sodium tartrate.
{ Magnesium sulphate.
{ Borax.
{ Ethyl alcohol.

Metaphenylene diamine

Glycerol.

Sodium dichlorylsulphaminobenzoate.

Other substances, which were not fully tested, are mentioned in Appendix I.

STANDARD TEST OF PURE SUBSTANCES WITH HUMAN SPERMS IN HUMAN SEMEN. The test described above may be used for all substances except acids to determine their power to immobilize or kill sperms in the absence of the protection provided by semen. If fresh human semen is available, a series of tests may be carried out, using it instead of cavy sperm suspension in B.G.S. The semen should be kept cool after ejaculation, and the test should preferably be made within three hours of ejaculation. To send semen by post, place it as soon as possible after

ejaculation in a small specimen tube with waxed cork. Slip this inside a rubber tube, and place it with some ice and water in a vacuum flask (preferably a steel one).

Human sperms are often inactive unless near a surface. For this reason only one drop instead of two is transferred in sections 22 and 23, and in section 25 attention is directed to the outermost part of the cavity of the slide, where the cavity is shallowest. It is legitimate with human sperms to record the results of an experiment if the control sperms are only 2+ in section 29, instead of insisting on 3 or 3+. When it is clear, however, that the majority of the control sperms are not moderately active, the test must be unhesitatingly rejected.

The results of the tests with human sperms in human semen are given in detail in Appendix VII., and they are discussed in Chapter V. (p. 87).

STANDARD TEST OF PURE SUBSTANCES WITH CAVY SPERMS IN ALBUMEN-SALINE. Cavy sperms suspended in albumen-saline behave, to the most diverse substances, almost exactly like human sperms in human semen, as we shall see in Chapter V. A standard test is made with this suspension when it is desired to study the spermicidal powers of substances in the presence of a protein protector. This is the ordinary test to apply when it is desired to know whether a certain substance would be suitable for practical use. The test is carried out in precisely the same way as the standard test described above, except that albumen-saline (p. 21) is substituted for B.G.S.

The results of this test are as follows. Full details of all the experiments are given in Appendix II.

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Grade 8 (immobilizing concentration $2\frac{1}{6}$ per cent.).

{ Toluquinone.
Methylhydroquinone.
Ethyhydroquinone.

Grade 6 (immobilizing concentration $\frac{1}{4}$ per cent.).
Hexylresorcinol.

Grade 3 (immobilizing concentration $\frac{1}{8}$ per cent.).

{ Mercuric chloride.
Normal propylresorcinol.
Orthovanillin.

Grade 1 (immobilizing concentration $\frac{1}{2}$ per cent.).
Sodium oleate.
Chinosol.

Grade 0 (immobilizing concentration 1 per cent.).
Zinc sulphate.
Methylhydroquinone (with twice as much anhydrous sodium sulphite).

Grade - 1 (immobilizing concentration 2 per cent.).
Potassium borotartrate.

Ungraded (these fail to immobilize at 2 per cent.).

Sodium sulphite.
Borax.

Other substances, which were not fully tested, are mentioned in Appendix II.

These standard tests of pure substances, whether the sperms are in B.G.S. or in albumen-saline or in semen, give consistent results and may be trusted. On one occasion a friend who was preparing a second lot of ethylhydroquinone made a mistake in the synthesis without knowing it. He handed the

substance to Mr. Ranson and me as ethylhydroquinone. We tested it and found it definitely less spermicidal than the first lot. We felt certain that the substance was not ethylhydroquinone, and on analysis it turned out that he had prepared hydroquinone by mistake. The difference in spermicidal power (in albumen-saline) is not great, but it was sufficient to enable us to say that the substance tested was not ethylhydroquinone. On another occasion I was given "metamethoxysalicylaldehyde." I gave it the complete test in B.G.S., and found its spermicidal power the same as that of orthovanillin. Since its smell was similar, I made enquiries and found that I had tested two lots of the same substance. One's confidence in the tests is confirmed by these incidents.

THE LACTIC ACID TEST. Some substances, especially methylhydroquinone, are shown to be very spermicidal by the standard test, but their power is reduced by an acid environment. Since the vagina normally contains lactic acid, which may sometimes be present in sufficient quantity to acidify the alkaline semen, it is necessary to test substances for spermicidal power in a slightly acid medium, which is not sufficiently acid to kill the sperms by itself. This is achieved by substituting lactic-saline A (p. 24) for ordinary saline in sections 3 and 4 of the standard test. Cavy sperms are suspended in albumen-saline, made up with 0.9 per cent. sodium chloride solution, as usual, not with lactic-saline. It is not necessary to apply the lactic acid test to any substance which is itself so acid that red litmus is not turned blue at the killing concentration, before dilution, in section 31 of the

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standard test. To apply the acid test to such a substance would be superfluous, because one already knows that it kills in an acid environment.

The only differences from the standard test for pure substances, with cavy sperms in albumen-saline, are the following :—

Section 1. A bottle of lactic-saline A is included among the objects placed in the thermostat.

Sections 3 and 4. Lactic-saline A is substituted for ordinary saline.

Section 29. An activity of only 1+ (or more) is demanded. High activity is not to be expected in acid solution. The experiment must be discarded if the activity of the sperms is 0 or 1.

Section 30. Do not pass on to section 41 until section 31 has been performed, thus :—

Section 31. The contents of the control tube are tested with *blue* litmus paper. If it does not go red the experiment must be discarded. If it does go red and if the experimental sperms are not 0, record their activity and skip to section 41. If the experimental sperms are 0, proceed to find out whether they are really killed, or only immobilized, as usual (sections 32-39).

The results of the lactic acid test are discussed on p. 54.

THE STANDARD TEST FOR ACIDS. The spermicidal power of acids depends mainly on their ability to overcome the alkaline buffer of the suspension fluid. The suspension fluid must be alkaline if the control sperms are to be really active. The only reliable suspension fluid for a test of acids is a fluid with the same alkaline reserve as human semen. The normal test for acids is therefore carried out with cavy sperms suspended in phosphate-albumen (p. 23). When testing acids one should not make up a stronger solution than 1 per cent. in section 3

for the first trial. Otherwise an excessive amount of alkaline diluting fluid may be required in section 33.

The results of this test are as follows. Full details of all the experiments are given in Appendix III.

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

Acetic acid.

Sulphuric acid.

Grade 0 (killing concentration 1 per cent.).

Succinic acid.

Tartaric acid.

Citric acid.

SUMMARY. In a test designed to assess the spermicidal powers of substances, it is better to find what is the lowest concentration of each substance sufficing to kill all sperms in a standard time, than to find how long sperms take to die in a standard concentration of each substance. A test is described in detail, whereby the killing concentration of every sufficiently soluble substance may be determined. The killing concentration is the lowest concentration in the series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, etc., per cent., that kills all cavy sperms in half an hour at 37° C. in four consecutive experiments. The various suspension fluids discussed in the last chapter are used.

CHAPTER IV

THE MODE OF ACTION OF SPERMICIDES

It is convenient to speak of spermicides as "very strong" if they immobilize all sperms at $\frac{1}{256}$ per cent. or weaker concentrations, "strong" if they immobilize at $\frac{1}{128}$, $\frac{1}{64}$ or $\frac{1}{32}$ per cent., "moderate" if they immobilize at $\frac{1}{16}$, $\frac{1}{8}$ or $\frac{1}{4}$ per cent., and "weak" if they immobilize at $\frac{1}{2}$, 1 or 2 per cent. Substances which fail to immobilize all sperms at 2 per cent. may be spoken of as "non-spermicidal." The results of all the experiments are given in full in the Appendices. In this chapter the spermicidal powers referred to are those with cavy sperms in B.G.S., unless the contrary is stated.

Most substances have little or no effect upon sperms at one-eighth of the concentration at which they immobilize every time, and some are without effect at one-quarter. Most substances have considerable effect at one-half of the immobilizing concentration. With a few substances, however, the killing of sperms is almost an "all or none" reaction. Hydroquinone is an example. It always immobilizes at $\frac{1}{4}$ per cent. At half this concentration sperms showed the following grades of activity in three experiments: — 0, 3, 3+. The number of sperms present probably determines whether it immobilizes every sperm or has no effect whatever at this concentration. If there were many substances like hydroquinone, it might be necessary to devise some laborious means of standardizing the number

of sperms more accurately than is done in the standard technique. Methylhydroquinone behaves in the same way as hydroquinone. Zinc sulphocarbolate stands at the opposite extreme. Its behaviour is most remarkable. It fails to kill every sperm even at 2 per cent. (though at this concentration a trained eye is required to detect the feeble movements of a very small number). Nevertheless, it still affects the activity of sperms at the great dilution of $\frac{1}{128}$ per cent. No other substance so far fully tested has anything approaching this enormous "spread-over."

I wish to stress my indebtedness to Dr. J. M. Gulland for his collaboration in the study of the aldehydes, phenols and quinones. These groups were chosen for intensive study, and the work could not have been done without the skilled help of an organic chemist. The reader is referred to Dr. Gulland's paper (1932) for a review of the chemical aspect of the subject.

The only *element* tested was iodine, which is a strong spermicide, presumably too irritant for practical use.

ACIDS. As was shown on p. 23, the proper way to test acids is with cavy sperms in phosphate-albumen, for this requires the same amount of acid to neutralize it as human semen. If one uses any other artificial suspension fluid, the result will depend almost entirely on how much alkaline buffer one chooses to put into it. All the acids tested are weak spermicides. Sulphuric and acetic acids have almost exactly the same spermicidal power, both killing at $\frac{1}{2}$ per cent. Succinic and tartaric acids are very slightly more spermicidal than citric, all

three killing at 1 per cent. The acids have thus a very small range of spermicidal power. The word "kill" is used here, as throughout this book, to indicate that the sperms are really killed and not merely immobilized, which is proved by diluting and alkalinizing and then re-examining. The interesting fact emerges from a study of Appendix III. that when all sperms are completely immobilized in four consecutive experiments by an acid at a certain concentration, then they are generally not merely immobilized but killed. With sulphuric, acetic, tartaric and citric acids the immobilizing concentration is the same as the killing. With succinic acid further experiment might give an immobilizing concentration of $\frac{1}{2}$ per cent. as distinct from a killing concentration of 1 per cent. Although the dilution test is well worth carrying out, both with acids and with other substances, yet it is rare for there to be much difference between the immobilizing and killing concentrations. It is easy to show that sperms whose activity has been reduced by hydrogen ions may be restored to normal by increase of pH , provided that complete immobilization has not occurred.

It was thought possible, before acids were tested, that tribasic ones might be more spermicidal than dibasic, and these than monobasic. This is not so. The monobasic acetic is slightly more spermicidal than the dibasic succinic and tartaric, which are themselves slightly more spermicidal than the tribasic citric.

A special series of experiments was performed to find the effects of fluids at various pH on cavy sperms. Acetic, succinic, citric and lactic acids

were investigated. Each was dissolved at various concentrations in a glucose-saline solution containing phosphates, and the pH of each solution was determined. The technique was almost precisely the standard one for testing pure substances with cavy sperms in B.G.S. 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 3 or 3+ in every case. In these experiments all sperms were immobilized by fluids below pH 5. At pH 5.0-5.9 their activity was usually reduced to about 1 (less than 10 per cent. active), and at pH 6.0-6.9 to about 2 (less than half moderately active). These results are obtained whichever of the four acids is used. The concentration of hydrogen ions altogether outweighs the nature of the anions in determining the degree of activity of the sperms. This can also be shown in another way. Citric acid may be tested by the standard test for pure substances with cavy sperms in B.G.S. (although, as we have seen, this is not really a good test for it). It will be found to immobilize every time at $\frac{1}{16}$ per cent.; yet sodium citrate fails to immobilize at 2 per cent. The relative unimportance of the anion is obvious.

An apparent exception to the rule that the anion does not matter is afforded by boric acid. A solution of boric acid at pH 6.3 reduced the activity of sperms to 1 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 the solution at pH 6.3 was a very concentrated one, thirty-two times as concentrated as a solution of citric acid at approximately the same pH . Therefore the boric acid probably exerted its effects largely by osmosis.

Acids are weak spermicides in the presence of semen, and not likely to be of great practical use. Acetic, citric and lactic acids are said by Stopes (1925) to be used in douches. Lactic acid has been used in suppositories.

Lactic acid is a normal constituent of the vaginal fluid and is thought in some cases to cause sterility by action on the sperms. Since it may be present in sufficient quantity to acidify the alkaline semen, it is important that spermicides for practical use should be active not only in alkaline, but also in weakly acid lactic solution, not sufficiently strong to kill sperms itself. These considerations have led to the standardization of the lactic acid tests of pure substances and suppositories.

Only two pure substances have yet been tested by the lactic acid test, but the results are most interesting. These are two substances which had shown themselves especially much influenced by hydrogen ion concentration in preliminary tests, namely, methylhydroquinone and normal propyl-resorcinol. Methylhydroquinone ordinarily immobilizes (in albumen-saline) at $\frac{1}{256}$ per cent. In the lactic acid test its immobilizing (and killing) concentration is changed to $\frac{1}{16}$ per cent. It is remarkable that its spermicidal powers should be reduced sixteen-fold. The preliminary tests show that most substances which are affected at all by acidity are reduced in spermicidal power, but normal propyl-resorcinol is an exception. The immobilizing concentration of this substance in albumen-saline is $\frac{1}{8}$ per cent. In the lactic acid test its immobilizing powers are increased four-fold, for now it immobilizes at $\frac{1}{32}$ per cent. It is rather remarkable

that its killing (as opposed to immobilizing) concentration is the same in alkaline and acid media, namely, $\frac{1}{8}$ per cent.

INORGANIC SALTS. The only inorganic salt fully tested which proved to be a strong spermicide was mercuric chloride. This has an effect even at the extreme dilution of $\frac{1}{2048}$ per cent. No other substance tested has any definite effect at such dilution, though the actual immobilizing concentration of mercuric chloride is less dilute than that of most of the quinones. A full test would probably give mercury potassium iodide an immobilizing concentration of $\frac{1}{32}$ per cent. No other inorganic salt has been shown to be a strong spermicide. Potassium permanganate, zinc sulphate and alum (potassium aluminium sulphate) are moderate spermicides. Potassium cyanide is a weak spermicide. The salts of the heavy metals and alum presumably act by precipitating the cell proteins. If proteins are present in the suspension fluid, they are precipitated below the immobilizing concentration, and the precipitate clogs the action of the still living sperms in a remarkable way. This is a true clogging action, unlike the supposed clogging action of fats (p. 112). The reduction in spermicidal power caused by the presence of proteins is discussed on p. 75. Potassium permanganate and cyanide presumably interfere with the respiration of the sperms. It is perhaps remarkable that potassium cyanide does not immobilize every time below $\frac{1}{2}$ per cent. Stopes says that potassium permanganate is used as a douche at $\frac{1}{20}$ per cent., and that alum is mentioned as a contraceptive in Sanskrit writings.

Ammonium chloride, borax, magnesium sulphate,

potassium iodide and sodium sulphite are all non-spermicidal. (The last two were tested in albumen-saline.) Such effect as they exert in reducing the activity of sperms may be ascribed to osmotic pressure (see p. 71).

SALTS OF ORGANIC ACIDS OTHER THAN FATTY ACIDS. Of the eleven salts in this category that were tested, none is a strong spermicide. Cupric gluconate, suggested and kindly supplied by Dr. Ellice McDonald, is a moderate spermicide. The bile salts, sodium taurocholate and glycocholate, were suggested by Professor J. B. S. Haldane on account of their surface activity (see p. 74). They are moderate spermicides, about twice as effective as quinine bisulphate. Potassium borotartrate is a moderate spermicide, made into a weak one by the presence of proteins in the suspension fluid. It is a constituent of the foaming tablet "Semori." Sodium tartrate and citrate are non-spermicidal, but exert some slight effect, presumably by osmosis. Zinc sulphocarbolate has also to be classified as non-spermicidal if we adhere rigidly to the rule given at the beginning of this chapter. Its unexampled "spread-over" has already been mentioned. It will be remembered that it affects sperms, without reducing their activity to 0, at all concentrations from 2 to $\frac{1}{28}$ per cent. Sodium dichlorylsulphaminobenzoate, the supposedly active substance in the foaming tablet "Speton," is completely without effect upon sperms at 2 per cent., even when dissolved immediately before the experiment.

SALTS OF FATTY ACIDS. At the very outset of this investigation Professor Haldane suggested that substances reducing surface-tension, especially

soaps, should be tested for spermicidal power. When tested in B.G.S., sodium oleate is a strong spermicide, sixteen times as effective as quinine bisulphate and chinisol. With human or cavy sperms in human semen, or with cavy sperms in albumen-saline, its spermicidal power is much reduced. It now immobilizes every time at $\frac{1}{2}$ per cent. This is the concentration at which chinisol immobilizes human sperms in human semen and at which quinine bisulphate immobilizes cavy sperms in human semen. We may say, then, that with sperms suspended in human semen, sodium oleate has the same spermicidal power as chinisol and quinine bisulphate. Chinisol costs thirty-seven and quinine bisulphate twenty-seven times as much as dry sodium oleate (powdered Castile soap). Soap has the advantage of spreading to minute crevices, on account of its power of reducing surface tension. Sodium palmitate has almost exactly the same spermicidal power as the oleate, but is somewhat less soluble. The stearate is also less soluble.

The sodium soap of coconut oil has about the same spermicidal power as sodium oleate, and has the advantage of being more soluble in the presence of electrolytes. The principal fatty acid of coconut oil is lauric. The advantages of this soap encouraged study of the soaps of acids still lower in the acetic series, but there is a great falling off in spermicidal power when the number of carbon atoms is much reduced. In B.G.S., in which sodium oleate and palmitate immobilize at $\frac{1}{2}$ per cent., potassium capronate only immobilizes at 2 per cent. and potassium butyrate does not even immobilize at

that concentration. Zinc acetate is more effective, but its action is to be attributed to the cation. As with the sulphocarbolate of the same metal, there is a considerable "spread-over," for the activity of sperms is reduced to 1 at both $\frac{1}{2}$ and $\frac{1}{8}$ per cent.

The 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 chinisol shortly before coition, yet she would have no hesitation in soaking herself in a soap solution in a hot bath. Bath water certainly enters the vagina in 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. No doubt their efficacy depends partly on the soap.

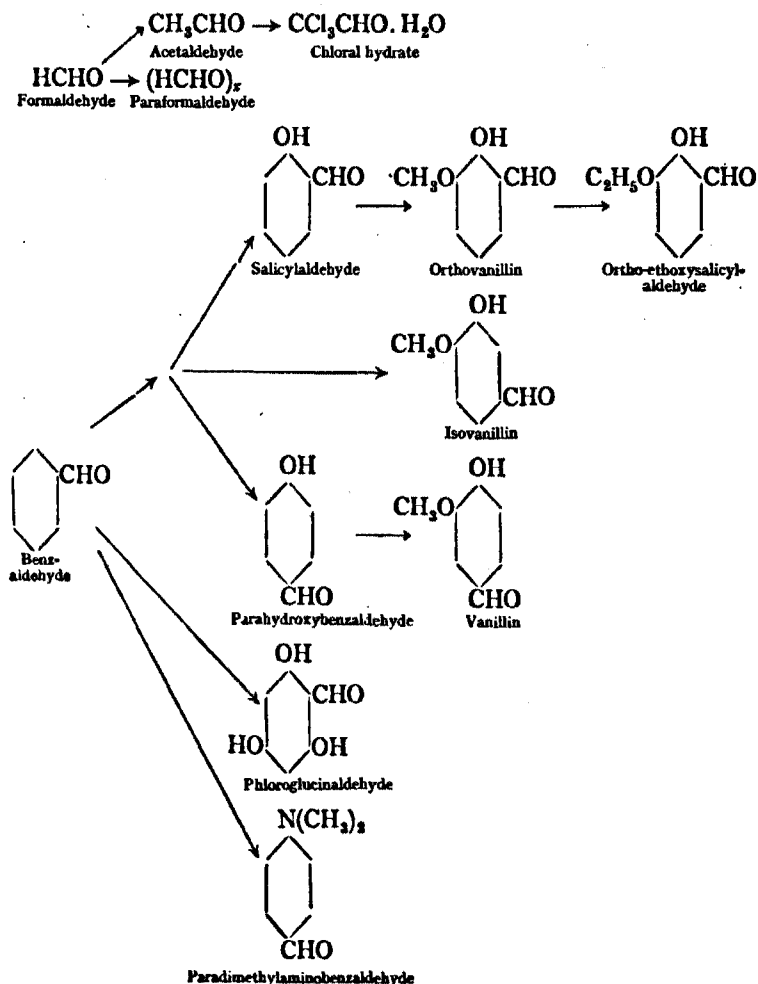
Although the fact that soaps are spermicidal was among the first discoveries of this investigation and was announced to the Sex Research Congress in 1930, it appears that practical use has not been made of it by manufacturing chemists and doctors interested in contraception. Possibly there has been a fear of upsetting the ordinarily acid reaction of the contents of the vagina.

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

ALCOHOLS. Ethyl alcohol and glycerol are non-spermicidal. Insufficient solubility prevented the test of menthol above $\frac{1}{16}$ per cent., at which con-

centration the activity of the sperms was reduced to 1+.

ALDEHYDES. The chemical constitution of the aldehydes tested is shown in the diagram. In this, as in the diagrams illustrating the phenols and quinones, the arrows indicate chemical relation-



ships, not methods of preparation. 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. One cannot detect formaldehyde if a solution at this concentration is taken into the mouth and swallowed. 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 is a strong spermicide, immobilizing at $\frac{1}{8}$ per cent., its two isomers, isovanillin and vanillin, are without effect at $\frac{1}{8}$ per cent. (It is vanillin that is the essential substance in the vanilla of commerce.) Salicylaldehyde was tried next, and found to be a strong spermicide. Salicylaldehyde and orthovanillin agree with one another, and differ from vanillin and isovanillin, in the hydroxyl and aldehyde groups being 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	Immobilizing concentration $\frac{1}{32}$ per cent.
Orthovanillin	Immobilizing concentration $\frac{1}{8}$ per cent.
Ortho-ethoxysalicylaldehyde	Immobilizing concentration $\frac{1}{8}$ per cent.
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 connexion 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.

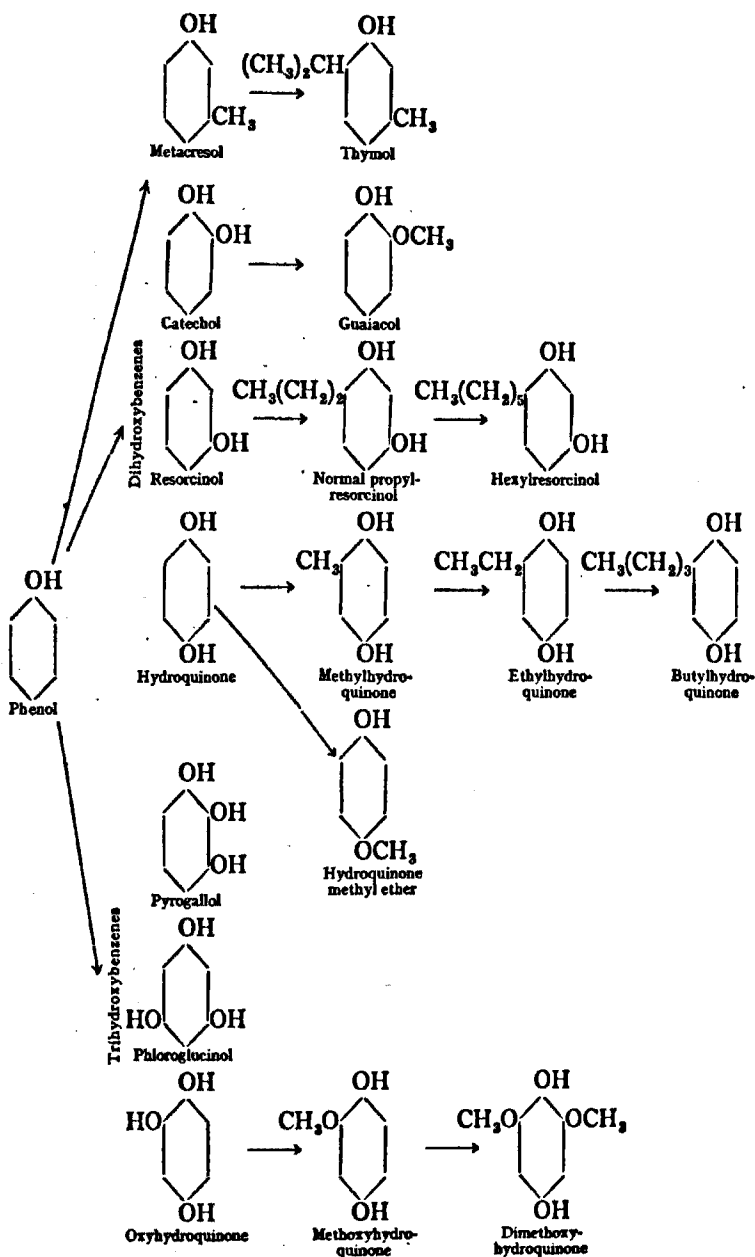
KETONES. Neither acetone nor camphor is a strong spermicide. It is thus clear that the extraordinarily strong spermicidal powers of the quinones, to which reference is made below, cannot be due

simply to $\begin{array}{c} | \\ \text{C} = \text{O} \\ | \end{array}$ groupings.

PHENOLS. The chemical constitution of the phenols investigated is shown on the next page. Those phenols which are easily oxidized were dissolved shortly before each test.

Phenol itself is a weak spermicide, only immobilizing at $\frac{1}{2}$ per cent. The addition of a methyl group doubles the spermicidal power, for cresol immobilizes at $\frac{1}{4}$ per cent. (The ordinary mixture of the three

62 . CHEMICAL CONTROL OF CONCEPTION



isomers was used, in which metacresol is said to predominate.) The addition of another alkyl group still further increases the spermicidal power, for thymol (isopropylmetacresol) is eight times as spermicidal as cresol. It is too insoluble and probably too irritant for practical use.

The effect of adding another hydroxyl group to phenol in each of the three possible positions was studied. Catechol (pyrocatechin), the ortho-compound, has almost exactly the same spermicidal power as phenol. (Guaiacol, or methylcatechol, is without effect at $\frac{1}{16}$ per cent., but was not further studied.) Resorcinol, the meta-compound, only immobilizes at 1 per cent. However, when the hydroxyl groups are placed in the para-position, an enormous increase in spermicidal power occurs, for hydroquinone immobilizes 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 (1907) 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.

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 hexylresorcinol immobilizes at $\frac{1}{64}$ per cent., as compared with 1 per cent. for resorcinol. (Normal propylresorcinol kills at $\frac{1}{8}$ per cent. in albumen-saline.) The idea of adding alkyl groups to hydroquinone, to produce substances of even greater spermicidal power, naturally suggested itself. This was impossible without the collaboration of an

organic chemist, and it was at this point that my work with Dr. J. M. Gulland started, which resulted in the discovery of some extremely spermicidal substances. The addition of a methyl group multiplied the spermicidal power by four, for methylhydroquinone immobilizes 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 Mr. C. E. J. Crawford therefore kindly undertook to prepare ethylhydroquinone, while Dr. Gulland synthesized butylhydroquinone for the first time. Ethylhydroquinone was found to have almost exactly the same spermicidal power as methylhydroquinone (when both are tested in albumen-saline), while butylhydroquinone is less spermicidal, with an immobilizing concentration of $\frac{1}{128}$ 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 resorcinols falls off when more than six carbon atoms are included in the side-chain (Dohme and others, 1926).

The mode of action of hydroquinone and its allies is not obvious. Since substances which are good photographic developers are sometimes strong spermicides, one is tempted to believe that the mode of action may be similar. It is true that acidity reduces the spermicidal powers of methylhydroquinone (see p. 54), as it also checks development by hydroquinone in photography; but the relations with sodium sulphite are quite different. This salt is commonly included in developing mixtures, to combine with newly formed oxidation products of the actual developer, forming nearly colourless

compounds. Thus staining is prevented, while the action of the developer is either accelerated, or at least not diminished (see Southworth and Bentley, 1933). In the killing of sperms, sodium sulphite behaves very differently. Methylhydroquinone, in the presence of twice as much sodium sulphite, is greatly reduced in spermicidal power. The immobilizing concentration (in albumen-saline) is now 1 per cent. instead of $\frac{1}{2}\frac{1}{56}$ per cent., and staining is not prevented.

Methylhydroquinone presents many great advantages as a spermicide for practical use. It has been shown by Dr. H. M. Carleton to be harmless to the vagina, and also harmless when injected into the blood-stream. It is a very soluble, stable, odourless solid. In the presence of proteins it equals toluquinone and ethylhydroquinone as the most spermicidal substance known. It has, however, two disadvantages. It is much less spermicidal in acid solution (p. 54), and in alkaline solution it stains semen brown. This staining is only preventable, so far as Mr. Ranson and I have been able to discover, by substances which reduce or destroy its spermicidal power. Ethylhydroquinone is somewhat less soluble, and does not stain quite so badly.

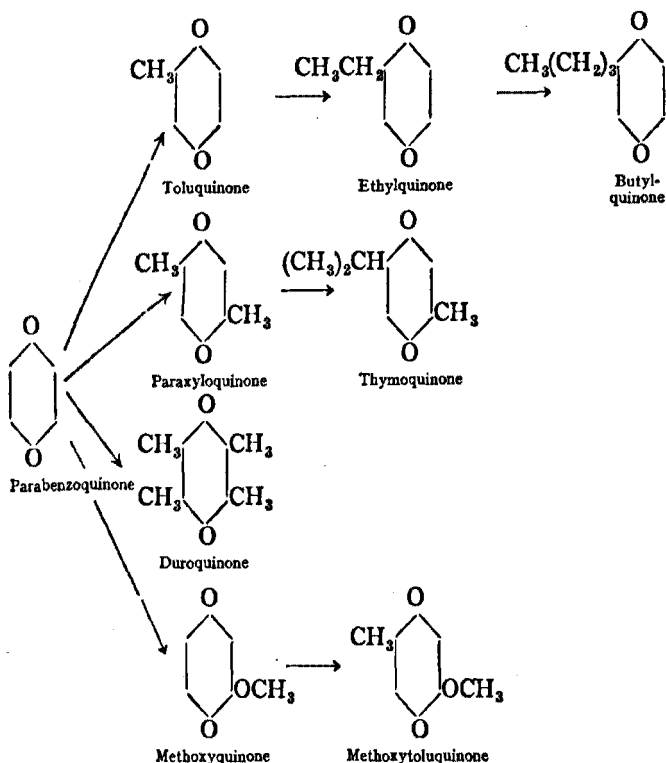
We tried substituting an alkyl group for the hydrogen of one of the hydroxyl groups of hydroquinone. Hydroquinone methyl ether was found only to kill at 1 per cent. It is evidently essential that the hydroxyl groups should remain intact if the spermicidal power of hydroquinone is to be retained.

When three hydroxyl groups co-exist in the molecule there are three isomeric phenols possible, namely, pyrogallol, phloroglucinol and oxyhydro-

quinone. 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 a powerful reducer. Phloroglucinol fails to kill at $\frac{1}{4}$ per cent. It is not soluble in saline at 1 per cent., and therefore cannot be tried at $\frac{1}{2}$ per cent. In contrast to the small spermicidal powers of its two isomers, oxyhydroquinone kills at $\frac{1}{8}$ per cent., the same concentration as hydroquinone. Thus two hydroxyl groups in the para-position confer high spermicidal powers, irrespectively 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 $\frac{1}{32}$ per cent. It comes next to mercuric chloride in order of spermicidal power. Dimethoxyhydroquinone, on the contrary, has the same spermicidal power as oxyhydroquinone.

QUINONES. The high spermicidal power of hydroquinone and its derivatives led us to try the quinones. The chemical constitution of those tested is shown on page 67.

We at once found a substance more spermicidal than any that had been tested before, for quinone itself (parabenzoquinone) immobilizes at $\frac{1}{32}$ per cent. The introduction of a methyl group increases the spermicidal power still further, although not sufficiently to put toluquinone into a higher grade. Thinking that a longer side-chain might result in even higher spermicidal power, Dr. Gulland prepared ethyl- and butylquinone. Butylquinone is slightly less spermicidal than toluquinone, and



ethylquinone slightly less again, equalling parabenzoquinone. So far as is known, toluquinone is the most spermicidal substance in the world in glucose-saline sperm suspensions. (In albumen-saline it is equalled by methyl- and ethylhydroquinone.) Toluquinone is probably insufficiently soluble and too irritant for practical use.

The substitution of two methyl groups for two of the hydrogen atoms of parabenzoquinone has no effect, for paraxyloquinone has the same spermicidal power. Rather unexpectedly, the substitution of an isopropyl group for one of the two methyl groups

reduces spermicidal power. The isopropyl compound, thymoquinone, only immobilizes at $\frac{1}{128}$ per cent., instead of $\frac{1}{512}$. This reduction of spermicidal power does not agree with the increase in spermicidal power in making the corresponding change from cresol to thymol. (It should be remarked that thymoquinone is not quite soluble enough to try at exactly $\frac{1}{128}$ per cent., but at a very slightly lower concentration than this (half-saturation) it immobilizes every time. At exactly $\frac{1}{256}$ per cent. it does not immobilize every time.)

When all the hydrogen atoms of parabenzquinone are replaced by methyl groups, spermicidal power is reduced. Duroquinone has no effect at $\frac{1}{512}$ per cent. Insufficient solubility prevents a test at higher concentrations.

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

GLUCOSIDES. The saponine tested was a strong spermicide, but probably too irritant for practical use. Arbutin has no effect on sperms at 2 per cent., and stimulates them at certain concentrations. It is evident that decomposition, with formation of hydroquinone, does not occur under the conditions of the experiment. Tannin is without effect at $\frac{1}{32}$ per cent.

AMINES. It was thought that hexamine might be a convenient producer of formaldehyde. Unfortunately it produces it very slowly, especially in alkaline solution, and is non-spermicidal.

The phenylene diamines are interesting for the light they throw upon the action of the dihydroxy-benzenes. It might be thought that the benzene ring would be strongly spermicidal if amine groups were substituted for OH groups in the para-position. This is not so. None of the three diamines is even a moderate spermicide. Paraphenylene diamine has roughly the same effect on sperms as orthophenylene diamine, which only immobilizes at 2 per cent. Thus the para-constitution of the molecule does not in itself confer spermicidal power, unless the attached groups are OH. Metaphenylene diamine has no effect upon sperms at 2 per cent., and generally stimulates them somewhat at $\frac{1}{4}$ per cent. (see Chapter IX.).

ALKALOIDS AND RELATED SUBSTANCES. No alkaloid has been shown to be a strong spermicide. The most effective of those tested are ethylharmol hydrochloride and harmine hydrochloride, both of which are moderate spermicides. The test of these was suggested by Professor J. A. Gunn.

The cinchona alkaloids are all weak spermicides of approximately the same spermicidal power. Quinine bisulphate is practically unaffected by the presence of proteins in the suspension fluid, and this will probably be found to apply to the others. Some of them have not been fully tested, but it is clear that the following all have almost exactly the same spermicidal power: quinine hydrochloride, quinine bisulphate, quinidine hydrochloride, quinidine acid hydrochloride, cinchonine hydrochloride, cinchonidine hydrochloride and cinchonidine acid hydrochloride. The immobilizing concentration of these is $\frac{1}{2}$ per cent., but the activity of the sperms at $\frac{1}{4}$ per

cent. is often 0 and never more than 1. Cinchonine acid hydrochloride is slightly less spermicidal than the ones mentioned above, but the difference is very small. For contraceptive purposes there is no advantage in separating quinine from these other cinchona alkaloids, though no alkaloid except quinine has yet been used to my knowledge. The acid hydrochlorides especially commend themselves on account of their great solubility.

Quinine urea hydrochloride is very slightly more spermicidal than the simple salts of the cinchona alkaloids.

Strychnine hydrochloride has no effect upon sperms at 1 per cent. It cannot be tried at 2 per cent., as it is not soluble in saline at 4 per cent. As is shown in Chapter IX., the hydrochlorides of strychnine and brucine are powerful stimulants of sperms at certain concentrations.

Since quinoline may be obtained from quinine and cinchonine by distillation with caustic potash, it is rational to consider quinoline derivatives with the alkaloids. Dioxyquinoline sulphate immobilizes at $\frac{1}{4}$ per cent. The exact chemical constitution of chinosol appears to be doubtful. It has been regarded as a double sulphate of potassium and oxyquinoline, and also as a mixture of potassium sulphate and oxyquinoline sulphate in non-molecular proportions. On biological grounds I am inclined to regard it as nearly pure dioxyquinoline sulphate. Its immobilizing concentration is $\frac{1}{4}$ per cent. At $\frac{1}{8}$ per cent. it reduces the activity of sperms very nearly, but not quite, as much as pure dioxyquinoline sulphate. Chinosol has probably been used as a contraceptive to a greater extent than any other

substance, except perhaps quinine ; but the reason is not obvious, for it is a weak spermicide. With human sperms in human semen its immobilizing concentration is $\frac{1}{2}$ per cent. At certain dilutions it appears to stimulate sperms. Potassium oxyquinoline sulphonate, which must not be confused with chinisol, has the same spermicidal power as quinine bisulphate and the other cinchona alkaloids mentioned with it above.

AMIDES AND RELATED SUBSTANCES. Urea has no effect at $\frac{1}{4}$ per cent. Caffeine and sodium veronal have no effect at $\frac{1}{16}$ per cent. These substances have not been tested further.

NITRO-COMPOUNDS. Neither nitrobenzene, metadinitrobenzene nor paranitrosodimethylaniline has any effect at $\frac{1}{4}$ per cent. Artificial musk has no effect at half-saturation.

DYES. Despite the differences in their chemical composition, it is convenient to consider these together. They are of theoretical rather than direct practical interest. The only strong spermicides among the eight dyes tested are malachite green and crystal violet. Mercurochrome and carmine (with borax) are moderate spermicides. Acriflavine and auramine are less spermicidal, and hæmatein (with alum) has no effect at half-saturation. Pyronin G. has no effect at $\frac{1}{84}$ per cent., but it has not been further studied. It will be noticed that three of the four strongly and moderately spermicidal dyes (malachite green, crystal violet and mercurochrome) are quinonoid compounds.

OSMOSIS

A series of experiments was performed to find to what extent cavy sperms are susceptible to various osmotic pressures of a substance which is not

chemically destructive to them. For this purpose sperms were suspended in different 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 at 37° C. ; 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 cavy 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 each in some 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 were 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 fluid not being alkaline. The following results were obtained :—

Concentration of sodium chloride, per cent.	Activity of sperms.
0	0, 0, 0
0.5	1+, 2, 3
0.9	2+, 2+, 2+, 2+, 3, 3, 3
1.5	1, 1, 1+
2	1, 1, 1
5	0, 0, 0

The results show that sperms are rather susceptible to changed osmotic pressure. The increase from 0.9 to 1.5 per cent. results in the immobilization of the great majority of the sperms.

No doubt many of those substances which only immobilize sperms at high concentrations do so largely by their osmotic pressure. It has been suggested that in the standard tests arrangements should be made that each substance should always be dissolved with the proper amount of some indifferent substance, such as sodium chloride, to make the osmotic pressure the same as that of mammalian blood. I have not done so, because it is as legitimate for substances to kill by high osmotic pressure as in any other way. It would be no more rational to disallow osmotic killing than, *e.g.*, to disallow killing by hydrogen ions, and to insist on the exact neutralization of acids before test. For this reason all substances are dissolved in 0.9 per cent. sodium chloride solution, and the solutions are therefore hypertonic. Except with very weak spermicides, the increase of osmotic pressure is negligible at the killing concentration.

It is often remarked what a splendid spermicide water is, since sperms die in it; but this is a very thoughtless statement, for sperms always die at once in every unmixed liquid! It would be equally sensible to advocate nitrogen as a poison gas for use in war. It would be quite legitimate to mix water with 0.9 per cent. saline at 1, 2, 4, 8, etc., per cent., and to test its spermicidal power like that of any other substance. It would come out very poorly in such tests: it would not immobilize all sperms at 32 per cent.! One could put a gram of water in

the vagina with very little likelihood of interfering with conception, even if it were not absorbed. Water may, of course, be used as a douche after coition, but sperms may then already have entered the uterus.

SURFACE TENSION

Professor J. B. S. Haldane suggested that substances which reduce surface tension might be good spermicides, and this resulted in the discovery of the high spermicidal powers of soaps and hexylresorcinol. It was discovered that substances which reduce surface tension are commonly strong spermicides.

To find whether there was a direct correlation between surface activity and spermicidal power, each substance was dissolved in 0.9 per cent. sodium chloride solution at $\frac{1}{32}$ per cent., and the surface tension of each solution was determined by a simple dropping method. Each solution was transferred drop by drop with a pipette to a measuring cylinder, and the number of drops required to make 10 c.c. was counted. 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

	No. of drops.	Immobilizing concentration, per cent.
Hexylresorcinol	741	$\frac{1}{64}$
Sodium taurocholate . . .	542	$\frac{1}{4}$
Sodium glycocholate . . .	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 per cent., alone	385	—

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.

Hexylresorcinol is the most surface active and the most spermicidal (with saponine) of the substances tested in this connexion, but the list shows that there is no direct relation between capacity to reduce surface tension and spermicidal power. The bile salts are very surface active, but only moderate spermicides.

Substances which reduce surface tension should be included in suppositories, 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 PROTECTIVE ACTION OF PROTEINS

To study the protective action of proteins it is only necessary to test substances first on sperm suspensions in B.G.S. and then on sperm suspensions in albumen-saline. The results with nine substances, differing widely in chemical composition and spermicidal power, are shown on page 76. Cavy sperms were used.

None of the substances is more active in the presence of proteins than in their absence. Methylhydroquinone and hexylresorcinol are unaffected. The spermicidal powers of toluquinone and chinol are reduced to one-half by the presence of proteins; of zinc sulphate to one-quarter; of orthovanillin and potassium borotartrate to one-eighth; of sodium

Immobilizing concentrations, per cent.	In B.G.S.	In albumen-saline.
$\frac{1}{32}$ $\frac{1}{64}$	Toluquinone Mercuric chloride Methylhydroquinone	Toluquinone Methylhydroquinone
$\frac{1}{16}$ $\frac{1}{32}$	Hexylresorcinol Orthovanillin Sodium oleate	Hexylresorcinol
$\frac{1}{8}$ $\frac{1}{16}$ $\frac{1}{32}$		Mercuric chloride Orthovanillin
$\frac{1}{4}$	Zinc sulphate Chinosol Potassium borotartrate	
$\frac{1}{2}$		Sodium oleate Chinosol
1		Zinc sulphate
2		Potassium borotartrate

oleate to one-sixteenth; and of mercuric chloride to one-thirty-second. It will be noted that the phenol derivatives are scarcely affected by the presence of proteins.

Seven of these substances have been tested also with cavy sperms in human semen and with human sperms in human semen, and the results are very similar. Further information on this subject is given in the next chapter. When it is desired to know how spermicidal a substance will be in practice, it should be tested with cavy sperms in albumen-saline, or with human sperms in human semen if fresh semen is available.

Sodium oleate was tested with cavy sperms in B.G.S. in the presence of gelatine at $\frac{1}{2}$ per cent. The sperms showed an activity of 1 with the oleate at $\frac{1}{32}$ per cent. This shows that gelatine has some slight power of protecting sperms, for they are always 0 with sodium oleate at $\frac{1}{32}$ per cent. in the absence of proteins.

Saliva was tested as follows: B.G.S. was prepared, using human saliva instead of distilled water. The amount of

glucose and phosphates was the same as in ordinary B.G.S., but the amount of sodium chloride was cut down to 0.05 gm. to allow for its presence in the saliva. Sodium oleate was then tested, the cavy sperms being suspended in this fluid instead of ordinary B.G.S. At $\frac{1}{32}$ per cent. the sperms showed an activity of 2, instead of the 0 which is always obtained in the absence of added proteins. At $\frac{1}{8}$ per cent. the activity of the sperms was reduced to 0. It is clear that saliva protects sperms, but not nearly as much as albumen-saline or human semen, in which sperms show some activity in the presence of sodium oleate at $\frac{1}{8}$ per cent. It is therefore unlikely that it is the mucin of semen that exerts the strong protective action on sperms.

CHANGES IN THE STRUCTURE AND BEHAVIOUR OF SPERMS CAUSED BY SPERMICIDES

Experiments were performed to find out in what way some of the substances investigated kill sperms. Six substances were investigated from this point of view, namely, formaldehyde, hexylresorcinol, sodium oleate, lactic acid, dioxyquinoline 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 cavy sperm. The best is that of Meves (1899), 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 cavy sperm (see Figs. 1 and 3).

The nucleus is shaped like the bowl of a spoon. Meves called 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

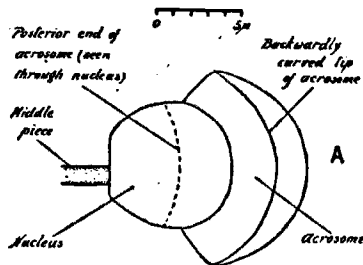


FIG. 3.*

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

* Figs. 3 and 4 reproduced by permission from "The Journal of Hygiene."

careful focussing has made me almost certain that this line is only seen by transparency when the sperm is viewed from "above."

Fig. 3 was drawn from numerous observations upon fresh sperms. The section B is an optical

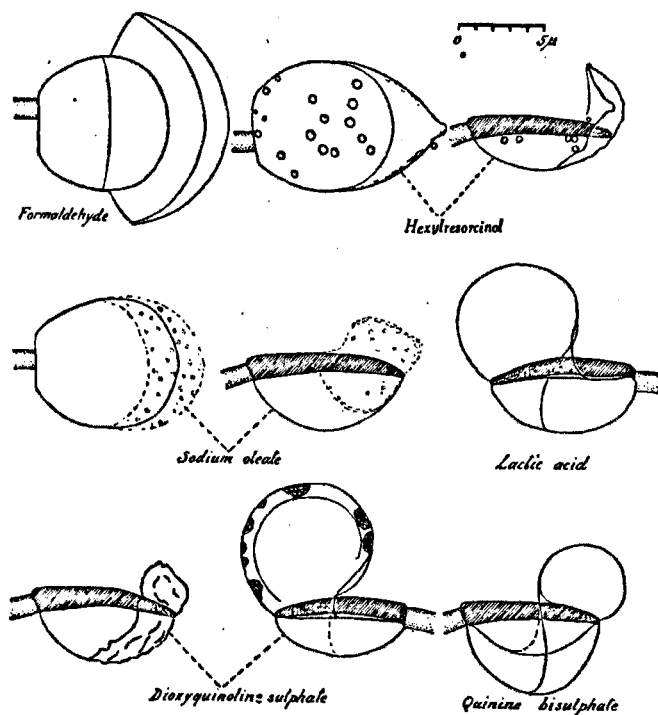


FIG. 4.

section. B and C are to be regarded as expressions of my opinion as to the structure of the acrosome, not as pictures of what can be seen with certainty 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. 3 and 4 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 17, the substance being present at *half* the concentration which always immobilizes 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 4-mm. objective on the hot stage, and at intervals up to a quarter of an hour later. The movements of those sperms which were active were carefully observed to find whether special types of movement characterized 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 immobilizes 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. The sperms were then subjected to critical examination under an oil-immersion objective to find what structural alterations, if any, had taken place in them. Controls were always examined.

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. 4 for representations of sperms acted upon by the six substances studied at twice the immobilizing concentrations.

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

HEXYLRESORCINOL. Since very few sperms remain active at half the immobilizing concentration, hexylresorcinol was tried at one-quarter 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 immobilizing 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 hexylresorcinol causes the acrosome to swell up and burst, for at one-quarter of the immobilizing concentration the acrosomes of the immobile sperms are commonly swollen. The shrunken acrosome seen at twice the immobilizing concentration is probably the collapsed remnant.

SODIUM OLEATE. Rapid oscillation through a small distance is characteristic of those sperms that are not immobilized by sodium oleate at half the immobilizing concentration. At twice the immobilizing concentration the acrosome is very much shrunk and transparent, and lacks a clear outline. It appears to contain small, indistinct granules. As with hexylresorcinol, it has apparently swollen up and burst, for swollen acrosomes are seen at half the immobilizing concentration.

LACTIC ACID. At half the immobilizing concentration the tails of the active sperms tend to curl up slowly and then straighten out quickly, which results 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 immobilizing 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.

DIOXYQUINOLINE SULPHATE. At half the immobilizing concentration quite active lashing movements of the tail occur, but very few sperms progress spirally. There is no oscillation. At twice the immobilizing 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 immobilizing 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 immobilizing 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 may be 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 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 dioxyquinoline sulphate and quinine bisulphate, but this is not to be regarded with certainty as a characteristic reaction. It is the acrosome that is the most vulnerable part of the sperm, so far as structural changes are concerned. Professor G. T. Popa, of the Histological Laboratory at Jassy, Rumania, 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.

THE IDEAL SPERMICIDE FOR PRACTICAL USE

The ideal spermicide for practical use would be a very soluble, stable, non-volatile, inodorous, non-irritant, inexpensive solid substance, moderately or strongly spermicidal in the presence of proteins and in both alkaline and acid media within the physiological limits. It would not stain tissues or fibres, nor be capable of giving rise to staining substances. It would be without remote effect if absorbed into the blood-stream. The ideal spermicide need not itself reduce surface tension, for a surface-active substance could be included with it in the suppository or ointment. The characters of the ideal suppository are given on p. 113.

Whether any of the substances discussed in this chapter will be found to conform to the ideal remains to be seen. Further work on some of them is in progress. At least we now know which substances do not deserve further investigation. It is hoped that a basis for the understanding of the mode

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of action of spermicides has been firmly laid, and that this will facilitate the discovery of the ideal.

SUMMARY. This chapter does not lend itself to compression, but the following are some of the

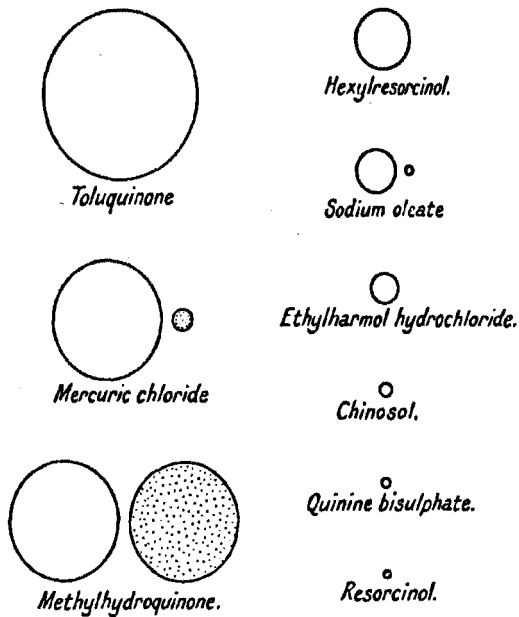


FIG. 5.—Diagram showing the spermicidal powers of certain substances with cavy sperms in B.G.S. (white circles) and in albumen-saline (dotted circles). The spermicidal powers are proportional to the areas of the circles.

outstanding facts. Fig. 5 shows some of them diagrammatically.

Most substances have little or no effect upon sperms at one-eighth of the concentration at which they immobilize every time, but zinc sulphocarbolate is most remarkable in reducing the activity of sperms without immobilizing them completely at all concentrations from 2 to $\frac{1}{128}$ per cent.

THE MODE OF ACTION OF SPERMICIDES 85

With acids the hydrogen ion outweighs the anion in importance. In the presence of a fluid buffered as human semen is, acids are weak spermicides. They reduce the activity of sperms reversibly above about pH 5.0, and kill below. Tribasic acids are not more spermicidal than dibasic, nor dibasic than monobasic. Certain substances are altered greatly in spermicidal power by an acid environment.

Inorganic salts of heavy metals vary greatly in spermicidal power. Those of other metals are often non-spermicidal, and exert their small effects by osmosis.

Soap (sodium oleate) is as spermicidal as chinosol and quinine bisulphate, which are respectively thirty-seven and twenty-seven times as expensive. Substances which reduce surface tension are often spermicidal, but there is no direct relationship between surface activity and spermicidal power.

In benzaldehyde derivatives the presence of OH in the ortho-position to CHO confers moderately high spermicidal power. In the dihydroxy- and trihydroxybenzenes the presence of two OH groups in the para-position confers strong spermicidal power. A short alkyl side-group increases spermicidal power. Methylhydroquinone is a very strong spermicide and presents many great advantages for practical use as well as some disadvantages. The first six substances in order of spermicidal power are all quinones. Toluquinone, which immobilizes every time at $\frac{1}{512}$ per cent. and generally at $\frac{1}{1024}$ per cent., is the most spermicidal substance known.

Of the alkaloids tested, ethylharmol and harmine are the most spermicidal. The cinchona alkaloids are all weak spermicides of very nearly the same

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power. Strychnine has no effect upon sperms at 1 per cent. Chinosol is a weak spermicide in the presence of proteins.

Quinonoid dyes are moderately or strongly spermicidal.

Proteins protect sperms against some substances (especially mercuric chloride and soap), but slightly or not at all against others (*e.g.*, alkaloids and phenols).

The acrosome is the most obviously vulnerable part of the cavy sperm.

CHAPTER V

THE ACTION OF SPERMICIDES UPON THE SPERMS OF OTHER SPECIES AND UPON BACTERIA

IF adequate supplies of human semen were always available, it would have been best to carry out a large series of tests with human sperms, although it would not have been possible to free them from the secretion of the accessory glands for the important tests of the spermicidal powers of substances in the absence of protecting proteins. Unfortunately adequate supplies of fresh human semen are not available in the laboratory, but, thanks to the co-operation of two volunteers, sufficient human material has been available for a small series of comparative tests. The two volunteers were both married men of young middle age and both had families, so their sperms may be regarded as likely to be normal.

The way in which the sperms were obtained and kept is described on p. 44, where an account is also given of the small departures from the standard technique which are made when human sperms in human semen are used instead of cavy sperms in albumen-saline.

Seven substances, differing widely in chemical composition and spermicidal power, were selected for the test with human sperms. The results are given in full in Appendix VII. and shortly overleaf, with the previously determined results with cavy

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sperms in albumen-saline beside them for comparison.

	Immobilizing concentrations, per cent.	
	Human sperms in human semen.	Cavy sperms in albumen-saline.
Toluquinone	$\frac{1}{512}$	$\frac{1}{3000}$
Hexylresorcinol	$\frac{1}{64}$	$\frac{1}{64}$
Orthovanillin	$\frac{1}{8}$	$\frac{1}{8}$
Sodium oleate	$\frac{1}{2}$	$\frac{1}{2}$
Chinosol	$\frac{1}{2}$	$\frac{1}{2}$
Potassium borotartrate	2	2
Borax	Fails at 2	Fails at 2

The close resemblance between the two columns is most remarkable, and the actual resemblance is more remarkable than the columns show, for toluquinone killed all sperms in three out of four experiments at $\frac{1}{512}$ per cent. with cavy sperms in albumen-saline. In the fourth experiment there was the slightest possible activity (1). If there had been no activity, there would have been identity between the two columns. This close resemblance appears very remarkable when one considers that our common ancestor with the cavy must have lived before the Eocene. Throughout the millions of years since then, the susceptibility of sperms to chemicals has scarcely changed, or has changed almost exactly equally in the two lineages. Despite this constancy in susceptibility to chemicals, the sperms of the two species have come to differ from one another markedly in size and form (see Fig. 1).

It is very convenient that cavy sperms in albumen-saline may be used in laboratory tests without any likelihood that the results would have differed widely if human semen had been used instead.

It will be remembered that Gellhorn found that frog sperms differ greatly from cavy sperms in their responses to various cations when suspended in solutions of single salts (see p. 5).

Some experiments were performed to find whether the sperms of a species belonging to another phylum of the animal kingdom behave at all similarly to cavy sperms. The sea-urchin, *Echinus esculentus*, was chosen for the investigation, as being common and producing an abundance of sperms, which are easily obtained and extremely active. The work was done at the Marine Biological Laboratory at Plymouth, by kind permission of Dr. E. J. Allen, F.R.S.

To make a standard sperm suspension for the experiment, 1 c.c. of testis was snipped up in 4 c.c. of sea water. (The testis is so soft that it may be measured by volume.) When the fragments of testis had settled, the supernatant sperm suspension was removed with a pipette, and the fragments discarded. The substances tested were dissolved in sea water instead of 0.9 per cent. sodium chloride solution, and the experiment was carried out in the open at room temperature instead of in a damp-chamber at 37° C. In all other respects the test was carried out exactly as with cavy sperms.

The three dihydroxybenzenes were chosen for the test on account of their having been found to differ from one another in a surprising way in their effects on cavy sperms.

The results are given in full in Appendix IX.

The immobilizing concentrations are shown below, with those of the cavy for comparison.

	Immobilizing concentrations, per cent.	
	Sea-urchin sperms in sea water.	Cavy sperms in B.G.S.
Hydroquinone . . .	$\frac{1}{32}$	$\frac{1}{64}$
Catechol	$\frac{1}{4}$	$\frac{1}{2}$
Resorcinol. . . .	$\frac{1}{2}$	1

It will be observed that with the sperms of the sea-urchin, as with those of the cavy, hydroquinone is much more spermicidal than catechol, which is slightly more spermicidal than resorcinol.

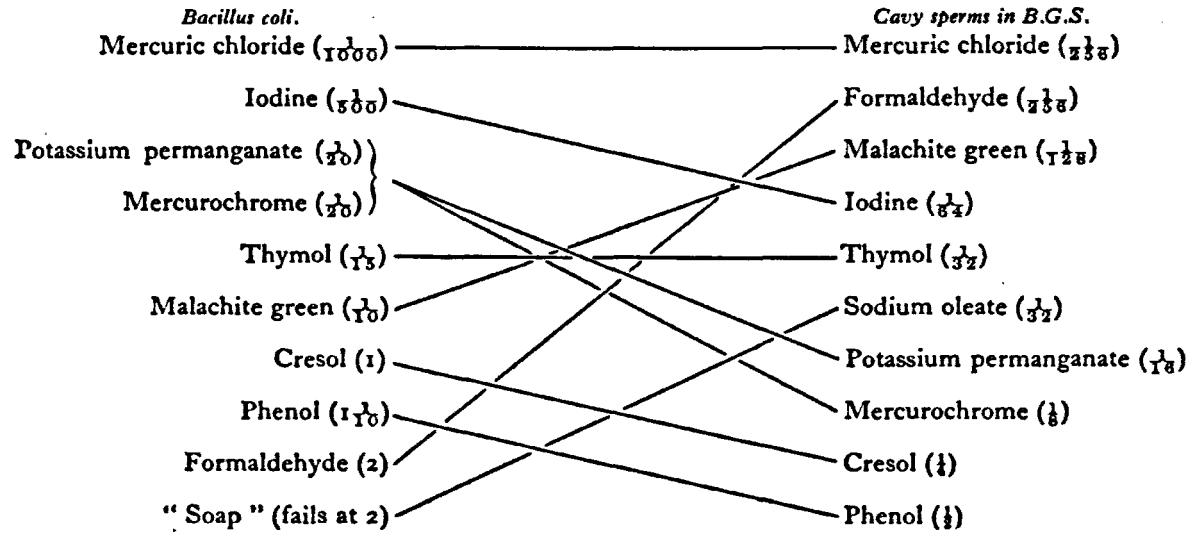
It was thought advisable to test also some quite unrelated substance. Chinosol was chosen. The full results are given in Appendix IX. The immobilizing concentration was found to be $\frac{1}{4}$ per cent., which is the same as with cavy sperms in B.G.S.

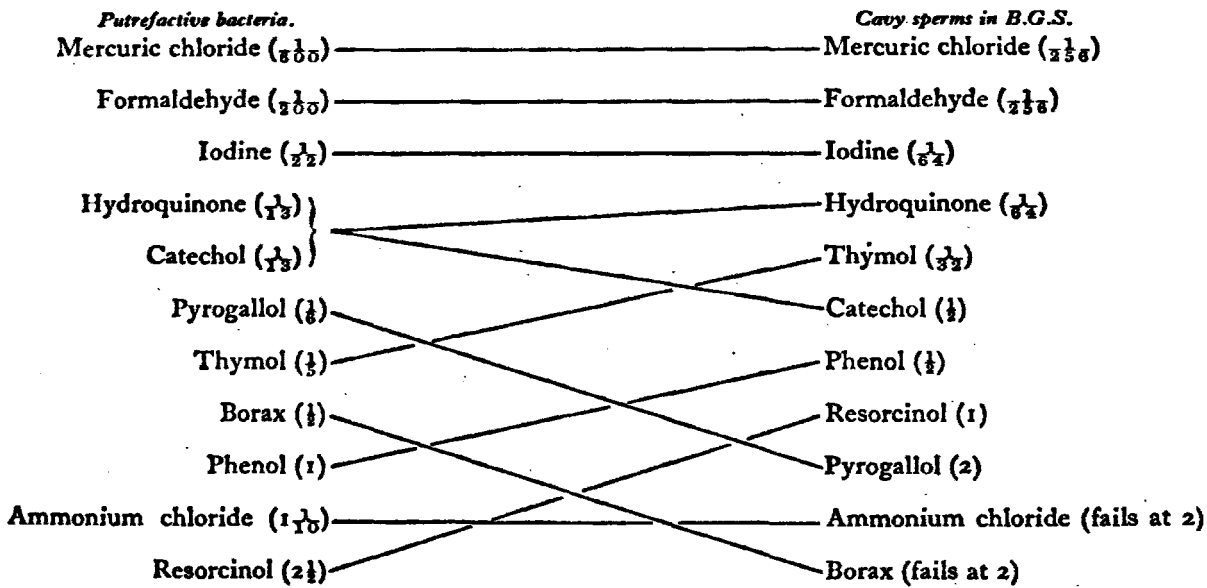
The close correspondence in the reactions of the sperms of completely unrelated species is most remarkable and was quite unexpected.

The manufacturers of chemical contraceptives have relied mostly on known germicides. This has perhaps been done partly because there has long been some knowledge of germicides: there have been tests of germicidal power, but until recently no reliable ones of spermicidal power. Manufacturers have relied upon sperms behaving like germs. They have also sometimes been directly interested in germicidal power, in order to provide safety against venereal diseases at the same time as against conception.

The most perfect tests of germicidal power are the Rideal-Walker test and its derivatives, but unfortunately they have been applied more to proprietary disinfectants than to pure substances. Martindale (1929), however, tested a large number of pure substances by a simplified test of the same sort. He found the lowest concentration of various substances dissolved in water which sufficed to kill *Bacillus coli* in two and a half minutes, their death being shown by inability to multiply subsequently in a nutrient medium at the proper temperature. Those substances, which Martindale tested sufficiently accurately for their germicidal powers to be assessed, and which I also tested for spermicidal power, are shown overleaf arranged in the order of their germicidal powers with *B. coli* and their spermicidal powers with cavy sperms. The killing and immobilizing concentrations are given in brackets, expressed as percentages.

There is not much correspondence between the two lists, except that mercuric chloride is first in each list, iodine high or fairly high, thymol intermediate, and cresol and phenol low. Two substances, which have too little effect on *B. coli* for it to be possible to grade them, are nevertheless moderate and strong spermicides respectively. Sodium taurocholate fails to kill *B. coli* even in half an hour at 20 per cent., but it immobilizes sperms at $\frac{1}{4}$ per cent. Crystal violet fails to kill *B. coli* in half an hour at 1 per cent., but immobilizes sperms at $\frac{1}{3}$ per cent. Formaldehyde and soap are equally remarkable, for they have very little power of killing *B. coli*, but are very strong and strong spermicides respectively. It is clear that it would





be unsafe to guess the spermicidal power of any substance from a knowledge of its power of killing *B. coli*.

Friedenthal (1919) made a most exhaustive study of the chemical inactivation of putrefactive bacteria. He infected alkalized broth with putrefactive bacteria, and added this to about three times its volume of water containing the substance under test at known concentration. The least amount of each substance required to prevent any increase in the number of bacteria within forty-eight hours was ascertained. The result was expressed as the number of litres of dilute broth that can be kept sterile by 1 gm. of each substance. Accurate controls were kept. In the list (p. 93) the inactivating concentrations are once again given in brackets as percentages.

It will be seen that putrefactive bacteria show some resemblances to cavy sperms, but the differences are great.

It is, perhaps, not surprising that different cells are affected differently by the same poisons. It is instructive to examine phenol and its allies in this connexion. The amounts of these substances necessary to kill cavy by subcutaneous injection is known (Solis-Cohen and Githens, 1928). They act upon the cells of the nervous system controlling respiration. One may express the killing power of phenol as 1, and thus compare its derivatives with it. In Fig. 6 the toxicity of phenol and its derivatives for cavy sperms (white), whole cavy (black), and putrefactive bacteria (dotted) is compared, phenol being taken as unity in each case. It will be seen that there is no general rule governing the relationship of the constitution of the molecule to its toxicity for different sorts of cells, except that the meta-

position of the OH groups (in resorcinol) does not confer toxicity. On the whole the para-position (in hydroquinone) confers toxicity, though it is not high with whole cavies.

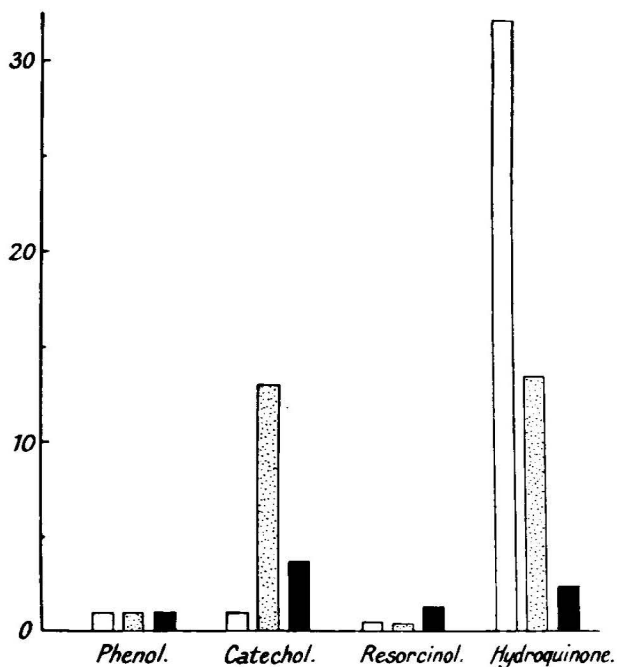


FIG. 6.—The toxicity of phenol derivatives for cavy sperms (white), putrefactive bacteria (dotted) and cavies (black). The toxicity of phenol is expressed as unity in each case.

SUMMARY. Human sperms in human semen react to the most diverse substances of widely different spermicidal power in almost exactly the same way as cavy sperms in albumen-saline. Sea-urchin sperms in sea water react not very differently from cavy sperms in B.G.S. The germicidal powers of substances are not a reliable indicator of their spermicidal powers.

CHAPTER VI

SUPPOSITORIES AND OINTMENTS

It is customary to say that the only tests of chemical contraceptives that are of any use are the clinical tests, and that tests made in glass tubes are unreliable. If this means that a chemical contraceptive is of no use unless it works in practice, the statement is a truism ; but beyond that it will not bear criticism. Clinical tests are open to innumerable objections. If suppositories are given out to women for clinical test, the responsibility of the investigator ends at the moment that they leave his possession. In most cases he does not and cannot know how often copulation takes place ; how long before ejaculation the suppository is inserted on each occasion, or even whether it is inserted on every occasion ; whether any other contraceptive or abortifacient method is used ; whether the woman or man is temporarily or permanently sterile ; at what period in the œstrous cycle the copulations take place ; how much vaginal fluid is present on each occasion, and whether it is acid or alkaline at the time ; or whether the amount of semen is normal. The experiment is entirely uncontrolled. The investigator knows nothing, and can only record how many babies, if any, the woman produces after the suppositories have been given to her. To say that that is the only proper test of a chemical contraceptive is like saying that new sorts of machine-guns

should only be tested in wars ; that mere tests on the range are useless ! To me it seems most improper to ask any woman to test a chemical contraceptive unless it has already been shown in laboratory tests to be strongly spermicidal and harmless.

In laboratory tests the investigator performs a controlled experiment, and knows what is happening from start to finish. If the critic distrusts the results, he should point out in what respect the test is badly designed, and not criticize it simply on the ground that it is a laboratory test. He should find fault, if he can, with the temperature at which the experiment is carried out ; the length of time during which the spermicide is allowed to act ; the pH or protein content of the suspension fluid ; the number of tests made with each make of suppository ; or with the accuracy of the observations. His criticism might then have value.

During the course of the investigation, several different tests for suppositories have been used, which have been subjected to valuable criticism. The standard test described in this chapter is intended to be free from the disadvantages of the earlier tests, and to provide a body of knowledge upon which clinical workers may rely.

In this book the word "suppository" is used for any solid object intended to be placed within the vagina and to prevent conception by chemical means. It thus includes foaming tablets as well as types with fatty or gelatine vehicles.

The suppository is first tested at what is postulated as being the "standard" (S) or ordinary concentration, namely, in the proportion of one suppository

to 2.5 c.c. of saline, representing the vaginal fluid, and 5 c.c. of cavy sperm suspension in albumen-saline, representing the semen. The suppository is allowed to disintegrate completely in the saline representing the vaginal fluid, which is maintained at body temperature and filtered when disintegration is complete. The filtrate is mixed with the fluid representing the semen, and five minutes later the activity of the sperms is recorded. If all are immobile, the fluid is diluted with an alkaline fluid favourable to the activity of the sperms, and it is thus discovered whether they have really been killed or only temporarily immobilized. If they have really been killed, further tests are carried out at $\frac{S}{2}$, $\frac{S}{4}$, $\frac{S}{8}$, etc., until the lowest concentration in this series which kills all sperms every time is discovered. This is called the killing concentration. The lower concentrations are obtained by diluting the filtrate to the appropriate degree with saline. The diluted fluid is mixed with the semen in the proportion of 1 : 2 (2.5 : 5).

It is not thought necessary to grade the spermicidal powers of suppositories which fail to kill all sperms at S concentration, though tests at 2S or 4S could easily be made by throwing two or four suppositories instead of one into the 2.5 c.c. of saline representing the vaginal fluid.

If perfectly fresh human semen is available, it may, of course, be substituted for cavy sperm suspension in albumen-saline in the standard test, but it is improbable that there would be large differences in the results.

With those suppositories that are not themselves

so acid as to give an acid reaction to the mixed fluids at the killing concentration, it is necessary to perform an acid test (p. 106), in addition to the ordinary test, so as to make sure that they are spermicidal also in the acid environment which may occur in the vagina. A suppository which was only spermicidal in alkaline solution would be unreliable, for there may be so much lactic acid in the vagina as to acidify the alkaline semen. In the lactic acid test the vaginal fluid is represented by lactic-saline B (p. 25), which is just strong enough to acidify the sperm suspension, but too weak to kill the sperms. (Lactic-saline B is not strong enough if human semen is substituted for cavy sperm suspension in albumen-saline, and it is therefore best not to use human semen in the acid test, though one could make up a special lactic-saline with about twice as much lactic acid in it as lactic-saline B contains.)

Since suppositories rarely rely merely on acidity for their results, it is not thought necessary to make a special test with sperms suspended in phosphate-albumen (p. 23), but each make of suppository is tested for acidity or alkalinity by titration with alkali or acid. Special tests of rate of disintegration are also made.

A detailed description of the standard test for suppositories is given on p. 102.

In the older tests for suppositories too long a time (fifteen or thirty minutes) was allowed for the spermicide to act. For the reasons already stated (p. 29) a fairly long time is necessary in the test of pure substances, but in the test of suppositories, for immediately practical purposes, a short time is

better. There is no branch of pharmacology where quick action is so necessary as in chemical contraception, for if action is slow sperms may have time to swim through the cervix into a region free from spermicide. Another improvement in the technique is that the real killing power of the suppository is tested, not merely its immobilizing power. Again, the new tests enable one to attach a figure to all reasonably powerful suppositories, proportional to their spermicidal powers.

A point which aroused criticism in the older tests, but which is not changed in the new, is the fixing of the standard amount of vaginal fluid at 2.5 c.c., which has been regarded as too high. No measurement of the amount of vaginal fluid has ever been made, though some workers have measured how much fluid they have obtained from the vagina. Such measurements leave out of account the relatively large amount that must be required to wet the whole surface of the vagina and leave it wet after the removal of the excess. In different women and in the same woman at different times the amount of vaginal fluid varies considerably, and we must put ourselves on the safe side by choosing a reasonably high figure.

The representation of the vaginal fluid by saline is unnatural but unavoidable. It is impossible to represent the fluid accurately. If one were to use a protein solution, the filtration, which is a necessary part of the experiment, would take an inconveniently long time. Saline has the great advantage of simplicity, and since there is plenty of protein present in the albumen-saline, it is unlikely that the use of saline affects the results materially.

There are not very many records of the amount of the human ejaculate, but the existing ones seem to justify one in taking 5 c.c. as the standard amount. During the course of this investigation a number of ejaculates, all from one man, were measured. The semen was caught at coition in a rubber sheath and measured to the nearest tenth of 1 c.c. The decimal figure is not quite dependable, as some of the semen tends to remain in the sheath. In a first series of twenty-three measurements, the mean amount was found to be 6.7 c.c. In a second series of forty-eight ejaculates the mean amount was 5.4 c.c. Thus the mean of the seventy-one ejaculates was 5.8 c.c. Since the man was considerably above the average size of the population, 5 c.c. seems a fair figure to choose. In the second series of measurements the largest ejaculate was 8.9 c.c. and the smallest 2.2 c.c. The latter was ejaculated less than an hour after one of 6.7 c.c. The amounts were distributed as follows :—

	Number of ejaculates.
2.0-2.9 c.c.	2
3.0-3.9 c.c.	7
4.0-4.9 c.c.	6
5.0-5.9 c.c.	17
6.0-6.9 c.c.	10
7.0-7.9 c.c.	5
8.0-8.9 c.c.	1
	—
	48

At a period when ejaculates were not being measured, an ejaculate was obtained which was measured on account of its obviously large volume, namely, 12.4 c.c. It is quite certain that this represents a single ejaculate, not two mixed together,

This large amount is not included in the figures given above, because it was only measured on account of its being extraordinary, and it would therefore weight the mean unfairly. It was produced after a period of thirty days during which no ejaculation occurred. Such large ejaculates must be kept in mind when one is deciding on the margin of safety required of a suppository.

The details of the way in which the standard test of suppositories is carried out are as follows:—

THE TECHNIQUE OF THE STANDARD TEST FOR SUPPOSITORIES

1. Regulate a thermostat to 37° C., and place in it two glass capsules with covers; a bottle of 0.9 per cent. sodium chloride solution (called saline throughout the rest of this description of the test); a bottle of alkaline diluting fluid (see p. 34); a filter with paper and receptacle; a damp-chamber containing a rack holding two specimen tubes about 12 mm. in internal diameter and about 5.5 cm. long; four cavity slides, two marked control and two marked experimental; coverslips; and pipettes (graduated and ordinary).

2. Regulate a thermostatic heater to deliver a constant stream of water at 37° C. through a hollow microscope stage.

3. Open a hen's egg. Place the white in a beaker. Remove the chalaza. Beat the white for about a minute with forceps to break the membranes. It is preferable, but not necessary, to break the egg a day or two before the experiment, and to keep the beaten white in a covered glass vessel. (The beaten egg-white may be kept a month or more in a refrigerator maintained near freezing-point.)

4. Transfer 2.5 c.c. of warm saline with one of the graduated pipettes to one of the glass capsules in the thermostat. This represents the vaginal fluid, which is postulated to amount to 2.5 c.c. (see p. 100). An indifferent solution is used to represent the vaginal fluid.

5. Throw one suppository into the saline in the capsule. It is legitimate to use twice or several times as much saline, and to throw a proportionate number of suppositories into

it. This is sometimes necessary with foaming tablets in order to get sufficient filtrate to use in section 17. Two suppositories are used instead of one, when it is directed that two suppositories are to be used at each coition (*e.g.*, with Finil).

6. Kill an adult male cavy by a blow on the head, and damp the fur in the pelvic region to prevent hairs from getting in the way. Set it aside so that post-mortem reflexes shall not interfere with the dissection.

7. Meanwhile mix 2·3 c.c. of egg-white, prepared in section 3, with 0·7 c.c. of 0·9 per cent. sodium chloride solution in a capsule (not one of the capsules in the thermostat). In this mixture, called albumen-saline, sperms behave to the most diverse chemicals almost exactly as they do in human semen.

8. Cut out the tails of both epididymides of the cavy and place them in the albumen-saline.

9. Cut each across once with scissors. Press each fragment several times with flat-pointed forceps to cause the sperms to come out. Lift the fragments of epididymis out of the suspension and throw them away.

10. Suck the fluid into a pipette and press it out again three or four times to break up any clumps of sperms and form an even suspension.

11. Transfer 0·5 c.c. of sperm suspension to each of the two empty specimen tubes in the damp-chamber in the thermostat, and leave it to warm up. These two tubes are called respectively the control tube and the experimental tube. Note the time.

12. Shake the capsule containing the suppository, holding the cover firmly in position. (With foaming tablets it is best to use a watch-glass placed concave side up as a cover, as it is less easily displaced by the effervescence than a flat cover.)

13. If the suppository has not completely disintegrated, shake the capsule from time to time until it has done so.

14. When the suppository has completely disintegrated, filter the contents of the capsule in the warm filter in the thermostat (so as to prevent insoluble matter from obscuring the sperms during the subsequent microscopical examination).

15. At least fifteen minutes after section 11 (*i.e.*, when the sperm suspension in the control and experimental tubes

has had time to warm up), transfer 0.25 c.c. of warm saline to the control tube mentioned in section 11. The saline represents vaginal fluid in the *absence* of any spermicide; 0.25 c.c. is used, with 0.5 c.c. of sperm suspension. These amounts represent, on the scale of one-tenth, the 2.5 c.c. of vaginal fluid and 5 c.c. of semen, which are postulated as being present in normal cases.

16. Suck part of the contents of the control tube into a warm pipette and press out again three times to mix the fluids. With the same pipette bubble air through the contents of the tube three times to aid the respiration of the sperms.

17. Transfer 0.25 c.c. of the filtrate obtained in section 14 (experimental vaginal fluid) to the experimental tube containing sperm suspension, which was mentioned in section 11. The experimental tube now contains, on the scale of one-tenth, the soluble part of one suppository to 2.5 c.c. of vaginal fluid and 5 c.c. of sperm suspension. The spermicide is now said to be present at standard (S) concentration.

18. Treat the contents of the experimental tube as those of the control tube were treated in section 16.

19. Note the time at once. It is convenient to have a timer, which one may set at 0 minutes.

20. Three minutes later bubble air three times through the contents of the control tube with a clean pipette, and with the same pipette transfer two drops to the hollow of the cavity slide marked control.

21. Apply a warm coverslip. Two drops of fluid do not fill the hollow of the slide. A bubble of air is included below the coverslip, which prevents the sperms from becoming inactive rapidly from inability to respire.

22. Leave the slide on the floor of the thermostat.

23. Three and a half minutes after section 19, repeat sections 20, 21 and 22 with the contents of the experimental tube, transferring two drops of it to the cavity of the slide marked experimental.

24. Four minutes after section 19 determine the activity of the sperms on the control slide by examination under a 4-mm. objective on the hot stage mentioned in section 2.

25. Record the activity of the control sperms on the usual principles. If they show an activity of 3 or 3+,

proceed to section 26. If they show an activity of less than 3, it is clear that an immature or diseased cavy has been used, so discard the experiment.

26. Five minutes after section 19 determine the activity of the sperms on the experimental slide by examination as before, and record the result.

27. Dip strips of red and blue litmus paper in the experimental tube, and record whether its contents are alkaline or acid.

28. If the experimental sperms show any activity whatever (1 to 3+) the experiment is at an end. It should be repeated once with sperms from another cavy.

29. If the experimental sperms show no activity whatever (0), proceed to find out whether they are really killed or only temporarily immobilized as follows (sections 30-38):—

30. Add 2.25 c.c. of warm alkaline diluting fluid to both the control and the experimental tubes, and suck the mixtures in and out of the pipettes three times to mix thoroughly.

31. If the contents of the experimental tube were acid in section 27, test again now with red litmus paper. If the fluid is not alkaline, add more alkaline diluting fluid gradually with a graduated pipette, noting the amount, until the paper goes blue. Add the same amount of alkaline diluting fluid to the control tube.

32. Eight minutes after section 19 prepare a slide from the contents of the control tube, exactly as in sections 20, 21 and 22.

33. Eight and a half minutes after section 19 prepare a slide from the contents of the experimental tube in the same way.

34. Nine minutes after section 19 examine the activity of the control sperms on the control slide under the microscope on the hot stage.

35. If the control sperms show an activity of 2+, 3 or 3+, proceed to section 36. If they show an activity of less than 2+ (which has never happened yet), discard the whole experiment.

36. Ten minutes after section 19 examine the activity of the sperms on the experimental slide.

37. Record the activity of the sperms on the experimental slide. If the sperms are all completely inactive (0), then they have been killed and not merely immobilized, for the spermicide is now at only one-quarter (or less) of the

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concentration present during the first five minutes. Few substances have much effect upon sperms at one-quarter of the least concentration sufficient to immobilize them.

38. Enter the figures representing the activity of the sperms before and after dilution with a line between them, thus 0/0 or 0/1, etc. The figure before the line represents the activity before dilution.

39. If all the sperms were found in section 37 to have been killed, repeat the experiment at half the concentration ($\frac{S}{2}$ concentration; see section 17). This is done by diluting part of the filtrate obtained in section 14 with the same volume of warm saline, and using this diluted fluid instead of undiluted filtrate in section 17. It is in preparation for this dilution that two capsules are provided in section 1 instead of one.

40. If all the sperms are once more found to be killed, repeat the experiment at $\frac{S}{4}$ concentration. This is done by diluting part of the filtrate obtained in section 14 with three times its volume of warm saline.

41. If all the sperms continue to be killed, perform the experiment at $\frac{S}{8}$, $\frac{S}{16}$, $\frac{S}{32}$, etc., by diluting part of the filtrate with 7, 15, 31, etc., times its volume of warm saline.

42. Continue these experiments until active sperms are found in the experimental slide in section 37.

43. The lowest concentration, in the series S , $\frac{S}{2}$, $\frac{S}{4}$, $\frac{S}{8}$, etc., which suffices to kill (not merely immobilize) all sperms in three consecutive experiments is called the killing concentration of the suppository.

44. Perform the experiment twice at half the killing concentration, or once at half and once at a quarter of the killing concentration. The results enable one to compare suppositories whose killing concentration is the same.

THE TECHNIQUE OF THE LACTIC ACID TEST FOR SUPPOSITORIES

This test need only be applied when in section 27 of the standard test an alkaline reaction is given at the killing

concentration. If the reaction in section 27 is acid at the killing concentration, one already knows that the suppository is spermicidal in acid solution at that concentration and the acid test would be superfluous. If a suppository fails to kill at S concentration in the standard test, it is not considered worth while to perform the lactic acid test.

The only differences from the standard test described above are the following :—

In section 1 place in the thermostat a bottle of lactic-saline B (p. 25) in addition to the other objects mentioned.

In sections 4, 5, 15, 39, 40 and 41 substitute lactic-saline B for saline.

In section 25 the control sperms should show an activity of 1+, 2 or 2+. If they are 0 or 1 discard the experiment. High activity does not occur in acid media. If the sperms are 3 or 3+, it is clear that an egg with abnormally alkaline white was used in the preparation of the albumen-saline, and that the lactic acid has been neutralized.

Ordinary saline, not lactic saline, is used in section 7. This is most important.

The results of the standard and lactic acid tests of suppositories are given shortly in the table on p. 108, and in detail in Appendices V and VI.

THE RATE OF DISINTEGRATION OF SUPPOSITORIES

It is important that the rate of disintegration of suppositories should be known. In general, it is best for suppositories to disintegrate rapidly; but fairly slow disintegration would not necessarily be harmful if the proper time were allowed in practice.

In our former tests of the spermicidal powers of suppositories we allowed a definite time for disintegration, after which the fluid was filtered whether disintegration was complete or not. We now think it fairer to allow disintegration to be complete before filtration, and this is done in the new test. The rate of disintegration is measured in a

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series of experiments quite separate from the tests of spermicidal power.

The rate of disintegration is measured in distilled water, owing to the impossibility of representing the vaginal fluid accurately in a laboratory experiment. One suppository is thrown into 50 c.c. of distilled water in a glass vessel in the thermostat maintained at 37° C. It is very important to make sure that the water has reached 37° C. before the suppository is thrown in. A large volume of water is taken, because a cold suppository appreciably reduces the temperature of the water if only a small quantity is taken. The vessel is covered with a glass plate. The time taken to disintegrate completely is noted. If five minutes elapse before complete disintegration, the vessel is shaken ten times, and this is repeated every five minutes. Disintegration is watched through the closed door of the thermostat.

When disintegration is complete, the water is filtered. A measured part of it is titrated with decinormal alkali or acid, and the amount necessary to neutralize the soluble contents of the suppository is calculated.

The rate of disintegration and the degree of acidity or alkalinity of the suppository are tested three times, and the means calculated. The results are shown in the table, with the killing concentrations.

	Killing concentration,		Time of disintegration (mins.)	Reaction.	Amount of decinormal solution required to neutralize (c.c.).
	Standard test,	Lactic acid test.			
M ₃	$\frac{S}{32}$	S	5	Neutral	0
Rendell's wife's friend	S	Fails at S	20	Acid	2.4
Finil (two tablets)	S	Not tested	—	Acid	7.7
Vimule	Fails at S	Not tested	10	Acid	0.2
Speton	Fails at S	Not tested	—	Acid	0.6
Semori	Fails at S	Not tested	—	Acid	1.5
Prorace (chinosol)	Fails at S	Not tested	14	Acid	0.3
Martindale's lactic acid pessaries.	No effect at S	Not tested	14	Faintly acid	0

The results of this new, improved test are different from those of the old test (Baker, 1931). The much shorter time during which the spermicidal substances are allowed to act is probably the main cause of the difference.

M₃ is a product of our own, which is not on the market. It is prepared by mixing 2 gm. of methylhydroquinone with 98 gm. of a proprietary fat called "cocola," and casting in a "15-gr." mould. Each suppository weighs approximately 1 gm. It is enormously more spermicidal in the standard test than any of the commercial products, but its spermicidal power is greatly reduced in the acid test. It has the disadvantage of staining semen brown except in acid solution.

None of the commercial suppositories tested is sufficiently spermicidal for complete reliance to be placed upon it in the absence of other methods of contraception. Rendell and Finil are the most spermicidal of the few that we have so far tested.

Rendell immobilizes nearly all sperms even at $\frac{S}{16}$ in the standard test. Its spermicidal power is reduced by acidity, although the suppository itself is acid. It is remarkable that the specimens of Martindale's lactic acid pessaries which we tested not only had no spermicidal power, but also were so nearly neutral that each required less than one-tenth of 1 c.c. of decinormal alkali to neutralize it. (See p. 148.)

It is worth remarking that permission has never been granted to any firm of manufacturers to use extracts from my papers in advertisements, except in letters sent in envelopes to qualified medical men. Two German firms,

whose products came out well in the early, less reliable tests, have ignored my wishes in this respect. One of these firms has even gone so far as to enclose a leaflet mentioning my name with each packet of its product, giving an extract from one of my papers differing from the original in a material point. This was done without my knowledge. It suffices to say that the product in question is shown by the new standard technique described in this chapter to be a weak spermicide. Probably it is its slow action that condemns it, for it was successful in the earlier tests, in which the time allowed was longer.

THE STANDARD TESTS FOR OINTMENTS

The practice of smearing a contraceptive ointment over the upper (cervical) side of a rubber occlusive pessary has several advantages. To test these ointments, it would not be proper to try them in the proportion of an average "dose" to 5 c.c. of semen and 2.5 c.c. of vaginal fluid, because it is intended that the rubber pessary should keep the semen away from the ointment as far as possible. Special tests are therefore required for ointments.

Mr. Ranson and I are subjecting ointments to two separate tests. The object of the first test is simply to find *how much* spermicidal substance there is in each. To do this the ointment is tested with cavy sperms in albumen-saline exactly as though it were a pure substance, except that tests are made up to 8 per cent. (instead of only 2 per cent.). The ointment is left overnight in saline in the thermostat at 37° C. in order to allow all spermicidal substances time to dissolve out completely before the test is made. The results are expressed as the lowest concentration in the series 8, 4, 2, 1, etc., per cent., which suffices to kill all sperms in half an hour in three consecutive experiments.

The object of the second test is to find *how quickly* the spermicidal substances diffuse out of the ointment. Sufficient of the ointment is introduced into one of the standard specimen tubes (p. 34) to make a column 1 cm. high. This is placed in the damp-chamber in the thermostat.

Some standard cavy sperm suspension in albumen-saline is mixed with an equal quantity of 0.9 per cent. sodium chloride solution and also placed in the damp-chamber. When both have had time to warm up, 1 c.c. of sperm suspension is placed in an empty specimen tube and another 1 c.c. is carefully introduced into the tube containing the ointment in such a way as not to disturb the ointment and thus cause mechanical mixing. A drop of the control and a drop of the experimental sperm suspensions are examined on the hot stage of the microscope five minutes, fifteen minutes, half an hour and one hour later, and subsequently at intervals of one hour. The experiment is stopped if the control sperms are not 3+, 3 or 2+. The shortest length of time sufficing to immobilize all sperms in each of three consecutive experiments (using sperms from different cavies in each experiment) is recorded. (See p. 149.)

VEHICLES FOR SPERMICIDES

It is usually inconvenient to place a pure substance in the vagina by itself. A pure substance would usually be difficult to insert unless in crystalline form or compressed into a tablet, and in these conditions would in most cases take far too long to dissolve, and might also act in too high a concentration in a very localized area, and thus threaten damage to the vagina. It is therefore usual to suspend the active spermicide in some vehicle which melts or dissolves in the warmth or dampness of the vagina.

Quinine urea hydrochloride has been used with no other vehicle than sodium chloride, the value of which is very doubtful.

Cocoa-butter has been largely used as a vehicle on account of its melting a little below body-temperature. It has the disadvantage of a slight smell. The proprietary product "cocola" is without smell and keeps indefinitely. A firm of manu-

facturers was asked to suspend chinisol in these two vehicles, so that tests of spermicidal power might be made. The tests appeared to show that chinisol is less spermicidal when suspended in cocoa-butter than in cocoa, and a report to this effect was published. Since then I have myself made chinisol suppositories in the two vehicles, and found no difference in their spermicidal power. It must be concluded that in the first lot, made by the manufacturer, the amount of chinisol was greater in the cocoa suppositories than in the cocoa-butter ones.

The British Drug Houses Ltd. are introducing a new suppository vehicle, consisting of cocoa mixed with a small proportion of one of the higher alcohols. The mixture has the advantage of melting rather sharply at 30° C. It is almost devoid of odour.

There is evidence that fats may give some protection against conception, even in the absence of any spermicide. This might be due to a film of melted fat closing the aperture of the cervix. More than one author has said that the melted fat clogs the sperms or entangles them, but sperms can no more be clogged or entangled by melted fat than a human being can be clogged or entangled by a brick wall. Anyone who looks down a microscope at sperms and melted fat suspended in suitable aqueous media will see how surface tension completely prevents the fat globules from spreading over the minute sperms or in any way inconveniencing them. Fatty vehicles have the disadvantage of causing the deterioration of rubber.

Gelatine gels are used as vehicles, but they are only suitable for use with those spermicides that are

not rendered markedly less effective by the presence of proteins, and that do not undergo decomposition from prolonged contact with water.

Foaming mixtures, generally consisting of tartaric acid and sodium bicarbonate in the appropriate proportion, are suitable for use as vehicles if the vagina is damp, but they may act too late if the vagina is dry and the moisture necessary for the reaction thus only arrives at the moment of ejaculation.

Foaming mixtures have themselves weak powers of immobilizing sperms. A series of tests was carried out by the standard test for pure substances, using cavy sperms in B.G.S. A small departure had to be made from the usual routine, the suspension fluid being mixed with the dry foaming mixture at the beginning of the experiment. The results are shown in full in Appendix XI. Both tartaric and citric foaming mixtures immobilize every time at 8 per cent., but fail at 4 per cent. They are thus very weak, and would probably appear still weaker if tested in albumen-saline and if the dilution test were applied. Although a great deal of foaming mixture may be placed in the vagina without danger of damage, it would not be safe to rely on it for the killing of sperms in the absence of other spermicide.

THE IDEAL CHEMICAL CONTRACEPTIVE

In 1931 I published a list of the characters of an ideal chemical contraceptive (Baker, 1931). A year later Dickinson and Bryant (1932) published a scarcely different list. In the list given below three of my previous requirements are omitted, and the tenth is different in that it is expressed in terms of the new test for suppositories. Otherwise the list is that which I gave in 1931. Dickinson's and Bryant's requirements are given in their own words in small type after mine. (I have taken the liberty

of changing the punctuation to make it conform with English usage.) The order in which their requirements were given is changed to agree with mine.

The ideal chemical contraceptive should have these characters :—

1. It should be inexpensive.

It must be cheap and generally procurable.

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

It should require no special appliance to introduce, or only some simple appliance.

3. It should be small.

It must be small in bulk.

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

It should keep well in moist or hot climates and under usual circumstances of storage, sale and home care.

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

It should be non-staining.

6. It should contain no volatile or odorous substance.

It should not be unpleasant in odour.

7. It should be absolutely non-irritant to the vagina, uterus and penis.

It must be free from smarting and irritation of vagina or male urethra, and free from possibilities of disturbing the normal chemical and bactericidal balance in the vagina.

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

It must be free from poisonous or disturbing effect if absorbed.

9. It should contain a substance reducing surface tension, to ensure the smallest crevices in the folds of the vagina being reached.

This agent should spread readily into the minute vaginal crevices and the internal os, and therefore it should possess low surface tension.

10. It should kill sperms at $\frac{S}{8}$ or lower concentration in the standard and acid tests for suppositories.

It should not merely paralyse but kill spermatozoa, and it must do this within one minute on very vigorous human sperms, and this not alone in the laboratory but in the vagina. . . .

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

It must be unaffected by acidity or alkalinity or protein content within the ranges ordinarily encountered.

11. It should contain no substance which could induce any genetic modification in those sperms which were not killed outright by it.

Dickinson and Bryant also require that it must be not easily displaceable, and independent of individual variation in amount of moisture present.

With regard to the second requirement, pastes may be used by those who have no æsthetic objection to the use of special appliances for insertion. Ointments may be smeared over the cervical side of rubber pessaries ; but the ideal chemical contra-

ceptive would not require the use of a rubber pessary, except when the cervix was lacerated or when it was known that semen was directly injected into it at coition, as is known to happen with some couples.

In the seventh requirement, harmlessness to the uterus is mentioned to cover the possibility of the contraceptive passing through the cervix, which would not be unlikely if it were lacerated through childbirth.

In the tenth requirement it will be noticed that a "factor of safety" of eight is required; *i.e.*, the spermicide is required to be at least eight times as powerful as is necessary to kill all sperms in laboratory experiments. In aeroplane design, to guard against death, a factor of safety of about eight is commonly demanded. The various parts are made about eight times as strong as they need to be theoretically for the purpose of normal flight. It seems just to demand a factor of safety of eight to guard against life in the design of chemical contraceptives.

Nothing is known upon the subject of the eleventh requirement beyond the fact that there are no statistics showing an increase in the birth rate of abnormal persons since the use of chemical contraceptives became widespread.

A slight protest may be made against the common habit of saying that a given chemical contraceptive fails in, say, 50 per cent. of cases. The trouble with such a statement is that no meaning can be attached to it. Does it mean that women have a level chance (50 : 50) of becoming pregnant every time coition takes place if they use the contraceptive

in question? Or does it, on the contrary, mean that if a woman uses the contraceptive all her life the chances are equal whether she has one baby or no baby? Or does it mean that 50 per cent. of the women to whom the doctor has recommended the contraceptive have had a baby between the time of recommendation and the time of the making of the statement? If so, it means almost nothing unless the average time elapsing is stated.

SUMMARY. Laboratory tests provide accurate knowledge which cannot be obtained clinically. In the standard laboratory test described in this chapter the suppository is allowed to act for five minutes, after it has had time to disintegrate. The standard (S) concentration, at which each is tested first, is in the proportion of one suppository to 5 c.c. of cavy sperm suspension in albumen-saline to 2.5 c.c. of fluid representing the vaginal fluid. If all sperms are killed (not merely temporarily immobilized) at this concentration, further experiments are performed at one-half ($\frac{S}{2}$), one-quarter ($\frac{S}{4}$), one-eighth ($\frac{S}{8}$), etc., of the standard concentration, until the lowest concentration in this series is found which suffices to kill all sperms every time. Tests are carried out in both alkaline and acid media. The amount of acid or alkali contained in each suppository and its rate of disintegration are measured. Of the seven commercial makes of suppository so far tested, five fail to kill at the standard concentration and none kills below it. Special tests are required for ointments. A list of requirements for an ideal chemical contraceptive is given.

CHAPTER VII
THE PATHOLOGY OF CONTRACEPTION

By H. M. CARLETON

INTRODUCTION

To write on the pathology of contraception is of necessity to write under a disadvantage. For, as yet, a literature on the subject is lacking, and one is forced largely to rely on personal observations without the advantage of comparison with other work.

The following account is partly based on nearly 350 individual animal experiments. To these must be added another 137 observations on the pathology of the Gräfenberg ring, thus making a total of close on 500 observations.

In addition, an attempt has been made to apply the accredited findings of general pathology to the limited sphere under consideration. This particularly applies (1) to the degree of certainty with which vaginal examinations with the speculum may be relied upon to show minute and incipient lesions following applications of a chemical contraceptive; (2) to the advisability (without careful pathological tests) of applying a chemical contraceptive to the *os uteri*, and then retaining it there by means of an occlusive cap; (3) to the possible pathological danger of the Gräfenberg ring method for controlling conception. That this technique involves

chemical as well as mechanical factors is shown by the observations of Carleton and Phelps (1932) which are summarized at the end of this chapter.

The absence of human material is scientifically regrettable, but such is the case, and the nearest available approach has been the examination of Gräfenberg rings kindly submitted to me, after prolonged sojourn in the human uterus, by Dr. Helena Wright.

The possibility of vaginal damage following the repeated use of chemical contraceptives must be envisaged. In this connection a number of proprietary preparations were tested. Since the latter are often mixtures it is usually impossible to assess the degree to which their individual components are harmful or not. Similar difficulties in estimating the spermicidal powers of many commercial contraceptives were experienced by Dr. J. R. Baker for precisely the same reason.

METHODS

The technique adopted was that of Carleton and Florey.¹ Since it is impracticable in animal experiments to employ a routine lasting over many years, recourse is had to daily (or even more frequent) injections over a limited period. The substance to be tested is dissolved in normal (0.9 per cent.) saline and injected with a syringe to which is attached a short, soft rubber catheter. If the substance is incorporated in a fatty base, sufficient tablets are melted to fill the syringe, the contents of which are re-melted for subsequent injections

¹ *Journal of Obstetrics and Gynaecology*, 38, 1931.

by placing the syringe in warm water. Pure substances are kept in solution (usually in 0.9 per cent. saline) and injected as required.

The first subjects, chosen on the dual grounds of convenience and economy, were rabbits. But, owing to the more delicate (columnar) type of epithelium forming their vaginal mucosa, this species is very susceptible to damage. Thus, either a commercial contraceptive or a pure substance harmful to the vagina of the rabbit may be harmless to that of the bitch. After a number of experiments had been made on cats this species was rejected owing to the difficulty of inserting a catheter without resistance sufficient in itself to damage the vaginal epithelium.

A frequent procedure was to test substances out *first* on the hypersensitive vagina of the rabbit, it having been found that substances harmless to the delicate vaginal mucosa of the rabbit were equally so in regard to the dog; hence a negative result with the former could be interpreted as negative for the latter.

It should here be stressed that of the three animal species used (rabbit, cat and dog), the stratified squamous epithelium of the vagina, together with the corium, far more closely resembles that of the human female in the case of the bitch. Of the available laboratory animals it therefore is the most suitable.

CONTROL OBSERVATIONS

A number of control experiments were made to make sure that insertion of a catheter could not, in itself, cause damage. In one case (rabbit) it was

passed daily for twenty-seven consecutive days. The vaginal mucosa was normal both to the naked eye and the microscope. The daily injection of 0.9 per cent. saline through such a catheter gives, however, different results. In some rabbits the changes are limited to distension of the lymphatics of the corium and slight œdema of the latter. These are probably due to absorption of the saline through the thin columnar epithelium. But sometimes definite inflammation is elicited and leucocytes, after infiltrating the mucous membrane, pass into the lumen of the vagina. The reason for this hypersensitivity in some rabbits is not known.

TESTS ON PROPRIETARY COMPOUNDS

A large number of tests were made on rabbits, cats and dogs; daily injections in the latter species were made over periods up to three months. Details of this work are to be found in the paper by Carleton and Florey already cited (1931).

Taking the results obtained with dogs (these being regarded as the nearest approach to human experimentation) the following substances were found to be harmless:—

“*Antibion*,” “*Chinosol*,” “*Finil*,” “*Permfoam*,” *Quinine* (a well-known brand of suppository of the type described as double strength), “*Semori*,” “*Speton*,” “*Spetonex*.”

The only change sometimes found was slight desquamation of the superficial epithelial layers; this probably was within physiological limits.

The only preparation found to be definitely harmful was *Quinine urea hydrochloride*. Three

rabbits received daily injections over a period of three weeks. There was intense vaginitis in all. In view of the delicacy of the rabbit's vagina the tests were extended to three dogs which received 19, 25 and 62 daily injections respectively. One tablet was dissolved in 1.5 c.c. of 0.9 per cent. saline and 1 c.c. of this injected.

All three subjects showed a marked vaginitis, in one case the necrotic epithelium forming a false membrane in the cavity of the vagina. Areas of ulceration with attempt at repair of the epithelium were sometimes seen and the corium was congested and infiltrated with white blood corpuscles. (Figs. 7 and 8.)

It is thus clear that, under the conditions of these experiments, quinine urea hydrochloride is an irritant. Possibly the vagina of the dog is more sensitive than that of the human female; even so, its advocacy by birth control clinics seems definitely contra-indicated.

THE RELATIONSHIP BETWEEN CLINICAL AND EXPERIMENTAL OBSERVATIONS

Comment must now be made on the following point. Even under the ideal conditions for observation at necropsy on an experimental subject in which the uterus and vagina are removed *in toto*, slit up and examined in a good light, little or no damage may be seen with the naked eye. Yet the microscope often reveals small but definite areas of damage. These comprise localized infiltration of the submucosa (or corium) with leucocytes; small but definite areas of infiltration of the epithelium; discrete patches of desquamation of the latter;

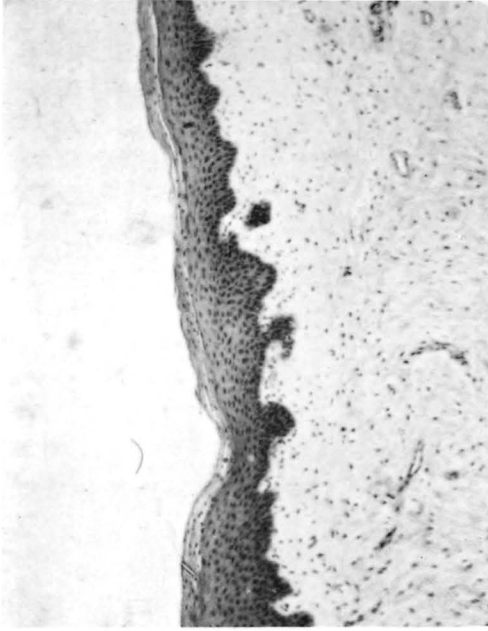
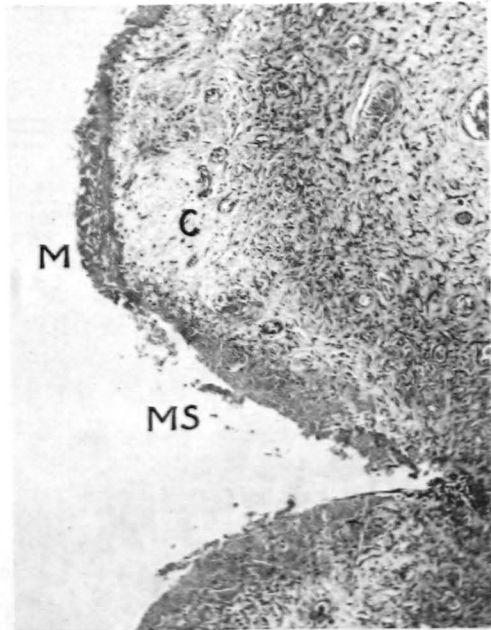


FIG. 7.—Section of normal vagina of bitch.

FIG. 8.—Section of the vagina of a bitch which had received 25 daily injections of quinine urea hydrochloride. Note extensive damage. C, corium, infiltrated with leucocytes; M, necrosis, and MS, sloughing of the epithelium.



Both figures reproduced from the Journal of Obstetrics and Gynaecology, by kind permission.

vascular congestion and lymphatic distension of varying degree in the submucosa. Even with the experience of 350 necropsies on experimental subjects the naked-eye detection of small and isolated areas of vaginal damage requires care and is sometimes restricted to small areas between the folds or rugæ of the mucous membrane.

It therefore follows that the detection in women of such lesions during a routine examination of the vagina with a speculum is unlikely.

Contraceptive substances and proprietary compounds are tried out, and sometimes permanently adopted as routine methods, by birth control clinics in the absence of adequate experimental tests. In many cases it is thus not known whether a product is harmful or not. It is therefore urged that chemical contraceptives should be experimentally tested as to possible pathological effects *before* clinical application.

Another subject for discussion is the practice, advocated by some birth control authorities, of interposing a layer of a chemical contraceptive between the *os uteri* and a cervical cap. This is advised in the interest of contraceptive safety—should the cap be an imperfect fit the chemical contraceptive will deal with the sperms.

One of the few incontrovertible facts of the causation of many forms of carcinoma is the insidious part played by irritation over a prolonged period. And, although the *rôle* of irritation in squamous carcinoma of the cervix is undecided, it is significant that this condition chiefly affects multiparæ who have had cervical lesions (laceration or erosion). The retention by a cervical cap of a chemical substance against the *os* (and in many cases its highly

probable penetration into the cervical canal itself) should be regarded as a potentially dangerous procedure. For, quite apart from a possibly carcinogenic effect, inflammation of the cervical mucosa might be elicited by certain chemical products. It must be remembered, moreover, that the abrupt ending of the columnar epithelium of the cervix and its point of replacement by the squamous epithelium of the vagina is very variable (Cullen; Strachan).¹ In some cases the cervical mucosa extends down to the external os, in others the squamous epithelium of the vagina extends upwards into the cervical canal. Probably, therefore, the lower the extension of the cervical mucosa the greater the danger of chemical trauma to it—columnar epithelia being more susceptible to physical and chemical trauma than squamous epithelia.

Another aspect of the possible danger associated with the retention of a chemical contraceptive between the os and a cap is the following: it may, and has been, argued that this procedure is entirely harmless, as proved by the absence of irritative or pre-cancerous changes in or around the os. Such argument has for its corner-stone a blissful ignorance of the length of the time factor. Out of the enormous mass of evidence on the various types of irritation-carcinomata one incontrovertible fact emerges—the prolonged period necessary for the production of a tumour. The average time for a new growth of this type in man is about twelve years and it is an interesting fact that in the tar-cancers of mice, a roughly proportionate time (130 days) in relation to

¹ Cullen: "Cancer of the Uterus." New York, 1900. Strachan: *Journal of Obstetrics and Gynaecology*, 40, 1933.

the far smaller life-span is required.¹ It is thus clear that the long latent period preceding the appearance of such tumours is a fundamental fact.

It is therefore concluded that the inclusion of a paste or jelly between the os uteri and a cervical cap is to be regarded as a potentially dangerous procedure, and that women adopting this practice should not so much be encouraged by experts as warned of its eventual pathological possibilities.

TESTS ON PURE SUBSTANCES

Although a large number of experiments were made on both dogs and rabbits a detailed account would be out of place here. Many of the substances described in Chapter IV by Dr. J. R. Baker were tested in different concentrations; many of the most spermicidal compounds unfortunately proved very harmful to the vaginal mucosa.

Thus, *toluquinone*, which as shown by Baker is, *in vitro*, the most spermicidal substance known, causes a vaginitis. (This substance immobilizes sperms every time at $\frac{1}{512}$ per cent. and usually at $\frac{1}{1024}$ per cent. !)

Methylhydroquinone was the subject of many tests. When dissolved in normal saline and injected in varying concentrations *per vaginam* in rabbits, it is an irritant. But a 1 per cent. suspension in the fat base cocola is harmless. In histological sections lymphatic distension was noted in rabbits after the injection of cocola in these and other experiments. Frozen sections stained with Scharlach R revealed

¹ For an excellent summary of recent work on the irritation cancers, see Hadfield and Garrod, "Recent Advances in Pathology," 1934.

abundant fat in the lymphatics. Fat can therefore be absorbed in rabbits through the vaginal columnar epithelium and passed on to the lymphatic system. There is no evidence, however, that this occurs in the bitch.

Experiments on bitches with methylhydroquinone showed that concentrations up to 1 per cent. could be incorporated in the base cocola and injected daily for periods up to a month without damage. A concentration of 4 per cent. in cocola proved, however, to be irritant.

Since methylhydroquinone is a very powerful spermicide, immobilizing sperms at $\frac{1}{256}$ per cent., its use would appear to be indicated. But unfortunately, as shown by Baker, it (1) turns semen brown and (2) is less spermicidal in the presence of acids.

The following test of the general toxicity of methylhydroquinone was made. A 2-kilogram rabbit received 2 c.c. of a 4 per cent. solution intravenously. No effects were noticed. Four per cent. hydroquinone was similarly injected into another rabbit. One c.c. was sufficient to cause immediate shivering and rapid respiration lasting for five minutes.

Chinosol, when tested on rabbits in concentrations in saline of from $\frac{1}{16}$ to 4.0 per cent. gave varying results; at the most, however, it never caused more damage than a transitory infiltration of the mucosa with leucocytes. In view of the extreme sensitivity of the delicate and relatively thin epithelium of the rabbit's vagina it may be taken that *chinosol* is comparatively innocuous. The experiments on dogs, mentioned on p. 121 of this chapter, certainly

bear this out. Unfortunately, as shown by Baker, chinisol is a weak spermicide, only immobilizing sperms at a $\frac{1}{4}$ per cent.

Sodium oleate (see Chapter IV) is "as spermicidal as chinisol and quinine bisulphate, which are respectively thirty-seven and twenty-seven times as expensive." Extensive tests were therefore made with this substance on both rabbits and dogs. Even in the latter species concentrations of 8 per cent. to 25 per cent. of sodium oleate in coccola caused an intense mucopurulent vaginitis, with patchy necrosis and desquamation of the mucosa, after seven or more daily injections.

The foregoing and very condensed account of some of the experiments on pure substances shows that the combination of a really potent chemical contraceptive which is also devoid of any pathological effects has yet to be found.

THE EFFECTS OF COMMON CHEMICAL CONTRACEPTIVES ON THE ENDOMETRIUM

It is possible that certain pathological conditions of the *cervix uteri* allow access of contraceptive substances to the uterus. It is even likely that occlusion of a chemical contraceptive between a cervical cap and the *os* allows some penetration of the substance into the cervical canal. It has also been stated (on, scientifically, the slenderest evidence) that suction during coitus is exerted by the uterus on the vaginal contents in the human female.

For a complete investigation, therefore, as to possible harmful effects, substances should be tested not only on the vaginal epithelium but also on the endometrium.

The following method¹ (Carleton and Florey) is employed :—

“ Direct injection of the *os uteri* is impossible in most laboratory animals owing to the curvature and narrowness of the vagina. Another method of approach was therefore used.

“ The uterine horns were exposed in cats and rabbits through a median laparotomy wound. The other abdominal viscera were packed off by gauze sponges wrung out in hot saline. It was then possible to inject one horn through a hypodermic needle with the contraceptive to be tested and the other with saline as a control. The sites of injection were marked by fine black silk sutures in the sero-muscular layer of the uterus. The aim of this procedure was to ensure a lower segment of the uterus being microscopically examined, for some traumatic abrasion of the mucosa was to be expected at the point where it was touched by the needle. The abdomen was then closed in layers with full asepsis.

“ In the course of the manipulations described it was noted that the exposed uterus and broad ligament became congested ; this was due partly to exposure, partly to unavoidable traction. It thus became necessary to find out the influence of the manipulation in order that the changes produced by it should not be attributed to the contraceptive injected. A simple operation was planned to test this matter. Both uterine horns

¹ I am greatly indebted to Sir Comyns Berkeley, F.R.C.P., F.R.C.S., F.C.O.G., for permission to quote widely from publications by Florey, Phelps and myself in the *Journal of Obstetrics and Gynaecology of the British Empire* (Vols. 38, 1931; 40, 1933).

were identified; one of them was then brought out of the abdomen, and both it and the broad ligament were gently massaged for two minutes by the gloved hand wetted with warm sterile saline. It was then replaced and the abdomen closed. On comparing the sections of both horns with the microscope no damage could be seen in the horn which had been subjected to massage as compared with the other horn which had lain untouched in the abdominal cavity. The conclusion was drawn that no harm could have been caused by the far more delicate manipulation during actual injection of the uterus with a contraceptive."

All operations were done under open ether after a preliminary injection of atropine. Naked-eye and histological examinations of the uteri and vaginæ were made eighteen to seventy-two hours after operation. Details of the findings are omitted, but the results are summarized below :—

(1) *Chinosol* (a well-known brand of suppository in a cocoa butter base);

(2) *Quinine* (another proprietary compound termed double strength);

(3) *Semori* (a commercial foaming contraceptive).

No lesions were detected; the uterine lymphatics were engorged with the fatty base after the injection of (1) and (2), indicating absorption by the endometrium. (A similar phenomenon, only seen in rabbits, has been noted on p. 126 in regard to the lymphatics in the vaginal wall.)

Formaldehyde. Baker has made the interesting observation that this substance immobilizes sperms

in as low a concentration as $\frac{1}{256}$ per cent. A number of tests were made with varying concentrations in order to determine at what strength formaldehyde becomes a danger to tissues.

$\frac{1}{64}$ per cent. "Two experiments were performed on rabbits with formol of this concentration. In each case both the uterine horns were injected in each subject, but one was tied off low down in order to retain the formol. No damage was recorded.

"A concentration of $\frac{1}{8}$ per cent. would appear to be on the borderline from the records of two experiments. There was some congestion and œdema of the uterine mucosa, and some leucocytic infiltration. Although the injection must have passed, at any rate partly, into the vagina, no changes were recorded here other than œdema of the corium.

"Formol in concentration of $\frac{1}{2}$ per cent. is already definitely harmful. Both the uterus and vagina suffer: their mucosæ desquamate, leucocytes are present in large numbers in both lumen and corium, and the latter is markedly œdematous. The innocuity¹ of formol of $\frac{1}{64}$ per cent. was demonstrated in another way. Two guinea-pigs of almost equal weight received four daily injections, each of 10 c.c. The mode of administration was intraperitoneal, one animal receiving formol of the above concentration in normal saline, and the other normal saline only. Five hours after the last injection both animals were killed. The one which had received the

¹ *Apparent* innocuity would be a better expression (H. M. C.).

formol appeared to have very slightly more peritoneal fluid. The number of cells in the latter appeared to be the same, so far as it could be estimated by the rough method of examining films stained by Leishman's method. The abdominal organs were normal. Spread-out pieces of mesentery after fixation and staining did not show any signs of inflammation."

Quinine urea hydrochloride. This substance induced necrosis and sloughing of the endometrium in twenty-four hours. Under the conditions of these experiments quinine urea hydrochloride was equally damaging to both vagina and uterus.

EXPERIMENTS ON THE GRÄFENBERG RING

This device is a ring of fine, spirally wound silver wire and its insertion can only be performed by a gynæcologist. Such a ring, when in position, should lie entirely in the fundus and *not* in the cervical canal. The method was originally claimed to be certain as a contraceptive and harmless in action, while the ring, when removed by the gynæcologist, allowed normal conception to take place later if desired.

At first sight this method would seem to act purely on some physical basis, but (as is shown later) the Gräfenberg ring has chemical implications, whence its discussion in this chapter.

The method, as applicable to animals, has been described by Carleton and Phelps ¹ and is as follows :

"The material for these experiments consisted of rabbits. These animals were used as the

¹ *Journal of Obstetrics and Gynæcology*, 40, 1933.

absence of an œstrous cycle combined with the high fertility and breeding rates renders them particularly suitable for such studies.

“ The introduction *per os* of a ring (as in the human female) is not possible in the animal referred to, owing to the length of its vagina. Laparotomy is hence the only feasible means, and this was performed as follows, under open ether preceded by injection of atropine.

“ The abdomen is opened by a median sub-umbilical incision, the skin edges of which are attached by small Michel clips to sterile gauze sponges wrung out of 1/5000 mercuric chloride. A sterile field is thus so far as possible secured. Both uterine horns are exteriorized and laid upon gauze sponges wrung out of hot saline. A small longitudinal slit is made in the upper third of one horn; the ring or other object is gently pushed through towards the cervix; the upper end of the ring is provided with a loop of fine black silk to each end of which a needle is threaded. The ring is then anchored by a loose stay suture at least one cm. above the incision through which it was introduced. This is necessary for two reasons: in the first place, a ring or spiral free in the uterine cavity of a rabbit is nearly always expelled by peristalsis. Secondly, the upper end of a contraceptive device, unless placed above the site of insertion, is liable to pierce it. The slit is then repaired by one or two fine sero-muscular stitches.

“ There being a possibility that the sutures at the site of insertion might occlude the uterine cavity and thus render the horn sterile, a control

operation was always performed on the other horn. A similar short incision was made, a probe passed twice down the uterine cavity, and the slit repaired. Only experiments in which the control horn became pregnant were regarded as satisfactory. The abdominal wound is finally closed in layers."

These experiments were divided into two groups : *Breeding Experiments*, in which the animals were allowed to copulate and examined post-mortem at half-term or later ; *Histological Observations*, in which the subjects were kept segregated from the males for periods from three days to six months in order to study such changes as might be induced by the ring.

The breeding experiments showed that rings and spirals of silver, gold or nickel were efficient contraceptives, *provided* that they occupied the greater length of the uterine horn. A curious feature is that when a short spiral is inserted, so as to occupy only the middle third of the uterine *cornu*, pregnancy is more prone to occur above than below it. This would suggest that the device "works" by hindering the passage of ova *down* (but not the passage of spermatozoa *up*) the uterine horn.

It was also found that many other foreign bodies would inhibit conception provided that they occupied the greater length of the uterine cavity. Thus, the rubber-coated ring devised by Dr. Helena Wright for use in human beings, strips of rubber, plaits and loops of silkworm gut or celluloid thread—all these were effective. That no specific chemical factor can be involved is evident from the diversity of

the substances tested. That a silver Gräfenberg ring, however, does *chemically* affect the tissues is certain and is the reason for discussion of the method here.

Histological examination showed, from four months onward, the following changes: (1) flattening of the uterine mucosa and obliteration of the glands due to pressure of the ring against the endometrium; (2) a variable degree of endometritis; (3) the presence of innumerable, small, dark-brown granules—mostly extra-cellular—in the corium. The ring itself in such cases is black, owing to the formation of silver sulphide. The granules lying beneath the endometrium were shown by Dr. H. J. Phelps to be silver sulphide also; Dr. John Phelps of the Royal Mint submitted fragments of the endometrium to spectographic examination: unmistakable silver lines, absent in the control specimens, were then revealed. This, coupled with the slate-grey colour of the tissues, undeniably defines the condition as a localized Argyria.¹

The significance of this must now be considered; a grave disadvantage of the blackened and sulphided ring is its brittleness, and a number of human rings kindly submitted to me by Dr. Helena Wright showed this defect. This suggests the wholly undesirable possibility of fragmentation of the device in the uterine cavity.

A factor which has already been stressed must be emphasized yet once again, namely, the possible carcinogenic effect of a silver ring in the *fundus uteri*. It may be argued that it is a far cry from the rabbit to the human female; that the

¹ That silver wire and plates are slowly attacked in the body is not new. Ballance and Edmunds recorded this fact in 1891 in their book "Ligation in Continuity."

former does not menstruate and the latter does. All this is perfectly true, but the experiments described above receive added confirmation from two operations in which silver rings were inserted into two normally menstruating monkeys.¹ When the animals were chloroformed seven and eight months respectively after insertion of the rings, it was noticed at necropsy that, in one, the endometrium was dark grey and the ring blackened. *Histological examination of this subject revealed changes like those already described, including the presence of innumerable dark granules, mostly extra-cellular, lying below the endometrium. These granules were again conclusively shown by Drs. H. J. and John Phelps to be silver sulphide.*

This result shows that in one monkey the ring underwent a change similar to that noted by Dr. Helena Wright in rings removed from certain human patients. No uteri of women bearing Gräfenberg rings have come my way, and I am hence unable to give any account of the endometrial changes. But that such changes do occur seems highly probable. The picture of a sulphiding ring, possibly fragmented, stirring up an endometrial reaction is not reassuring. All the evidence to hand points to the method as one to be condemned.

This short survey of the pathology of contraception is now ended. I have indicated where animal experimentation and the findings of general pathology appear to me to bear on human contraception. It remains for the clinician to apply or discard the suggested applications.

¹ I am much indebted to Professor Hindle for these animals (H. M. C.).

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My grateful acknowledgments are due to the Birth Control Investigation Committee for financing the experiments; to Dr. C. P. Blacker, its Secretary, for his support and active interest; to Dr. Mary Fraser for comments on the clinical aspects of the problem; to Messrs. Jesse Wheal, Thomas Marsland and A. S. Chapman for surgical and histological assistance.

CHAPTER VIII

THE STIMULATION OF SPERMS BY DRUGS

DURING the investigation of the spermicidal powers of pure substances, it was noticed that a few substances at certain concentrations had the effect of making sperms more active than the controls. A special study was made of this subject. Only a preliminary note (Baker, 1931c) has been published up till now.

Fresh cavy sperms are so extremely active that any increase in activity caused by chemical stimulation is difficult to notice. It was therefore decided to allow the sperms to remain for many hours in the thermostat at the temperature of the body before adding the stimulant. By this time their activity will be noticeably reduced, and any stimulant effect can easily be seen.

The experiment is carried out according to the standard technique for cavy sperms in B.G.S., except that the drug solution and control saline are only added to the tubes of sperm suspension in B.G.S. five to ten hours (instead of fifteen minutes) after the suspensions have been placed in the thermostat, and the sperms are examined fifteen minutes (instead of half an hour) later. If the experimental sperms are more active than the controls, it is concluded that the drug has stimulated them in the fifteen minutes during which it has acted. The writing on the slides is covered up, as usual, so

that prejudice or expectation cannot bias the result. It is only after one has assessed the activity of the sperms on the two slides that one turns back the blank label and discovers which is the control and which the experimental.

The results of the experiment are shown in full in Appendix XII. The activity of the experimental sperms is printed above the activity of the corresponding controls. Thus $\frac{3}{2}$ indicates that the sperms stimulated by the drug had an activity of 3, while the control sperms were only 2. The results may be summarized thus :—

Strychnine hydrochloride stimulates sperms strongly at concentrations of $\frac{1}{16}$ and $\frac{1}{64}$ per cent., and rather less strongly at $\frac{1}{256}$ per cent.

Brucine hydrochloride stimulates sperms strongly at $\frac{1}{4}$ and $\frac{1}{16}$ per cent., and rather less strongly at $\frac{1}{64}$ per cent.

Chloral hydrate stimulates, not very strongly, at $\frac{1}{16}$ per cent.

Metaphenylene diamine generally stimulates somewhat at $\frac{1}{4}$ per cent.

In addition to these, chinisol sometimes stimulates at $\frac{1}{16}$ and $\frac{1}{64}$ per cent., but it cannot be relied upon to do so. Arbutin stimulates from $\frac{1}{8}$ to $\frac{1}{256}$ per cent., but it has not yet been carefully studied.

Further tests with strychnine and brucine hydrochlorides showed that stimulation is maintained half an hour after mixing the drug with the sperms.

In another set of tests the sperms were not fatigued before mixing with the drug, but were examined two hours after mixing. The drug tested

in this way was brucine hydrochloride at $\frac{1}{16}$ per cent. The sperms were found to be considerably more active in the tube containing the drug than in the control tube. It is thus clear that brucine does not only stimulate already fatigued sperms, but increases the activity of fresh ones over a considerable period.

Strychnine and brucine hydrochlorides were further tried with human sperms. The sperms were fatigued by leaving the semen for some hours at room temperature instead of in the thermostat, but in other respects the experiment was carried out in exactly the same way as the one reported on in Appendix XII. Strychnine hydrochloride gave $\frac{3}{2}$ at $\frac{1}{16}$ per cent. and $\frac{2+}{2}$ at $\frac{1}{8}$ per cent. Brucine hydrochloride gave $\frac{2+}{2}$ at $\frac{1}{16}$ per cent.

Tests were made to find whether cavy sperms, whose activity had been reduced by acidity, could be stimulated to more active movement by strychnine hydrochloride. For this purpose the sperms were suspended in a very weak solution of citric acid in saline. The experiment need not be described in detail, because the result was negative. Strychnine does not stimulate sperms whose activity is being kept low by acidity.

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. The egg secretions of many marine invertebrates contain a substance which stimulates

sperms, and Carter thinks that it may be a substance allied to thyroxin. The reaction of the sperms to thyroxin may thus be adaptive. The reactions of cavy and human sperms described in this chapter are obviously non-adaptive.

It might be thought that strychnine would interfere with the capacity of sperms to achieve fertilization, or with their genetic constitution. An experiment was performed with the collaboration of Mr. Ranson to test these points. Female cavies were artificially inseminated with cavy sperm suspension in B.G.S., containing strychnine hydrochloride at $\frac{1}{84}$ per cent. The sperms from the tails of both epididymides of a cavy were suspended in 1.5 c.c. of the solution, and 1 c.c. of the suspension was injected into the vagina. Control females were inseminated in exactly the same way, except that the strychnine was omitted. The inseminations were made in late pro-œstrus. Five experimental and five control females were used. (Two of the experimental females died of diseases unconnected with the injection of the strychnine, namely, internal hæmorrhage and tumours of the intestine. These two were replaced by others.) Every time a female came into late pro-œstrus, it was inseminated. Pro-œstrus was experienced altogether thirteen times by the experimental animals, and three of them became pregnant and produced families of one, three and one. The controls experienced pro-œstrus sixteen times, and two of them became pregnant and produced three offspring each. The young resulting from sperms which were suspended in strychnine hydrochloride at $\frac{1}{84}$ per cent. were normal in every respect. It is clear from this

experiment that strychnine at this concentration does not damage the sperms in any way.

The stimulation of sperms by drugs might well be tried with human beings in cases where sterility is thought to be due to the feeble activity of the sperms. 50 mg. of strychnine hydrochloride should be dissolved in 100 c.c. of B.G.S. (p. 15). No doubt the ingredients could conveniently be made up in tablets by manufacturing chemists, so that it would only be necessary to dissolve one tablet in 100 c.c. of distilled water. Semen should be caught in a sheath, and an ordinary ejaculate of about 5 c.c. should be mixed with 2 c.c. of the strychnine B.G.S. solution. The 2 c.c. of this solution should preferably be at the temperature of the body, in a glass specimen tube of about 10 c.c. capacity. The physician could easily have the solution at the right temperature by keeping the tube under his clothes until the sheath containing the semen was produced. The semen could then be poured into the warm tube, mixed with the strychnine B.G.S. solution with a glass rod, and injected without delay into the vagina or uterus.

If these instructions were followed, 1 mg. of strychnine hydrochloride would be injected into the woman, and the drug would be at a concentration of about $\frac{1}{64}$ per cent. before injection and somewhat less afterwards. This is a suitable concentration for stimulating sperms strongly. According to the British Pharmacopœia, the normal dose of strychnine hydrochloride is 2 to 8 mg. Thus the amount injected would be half the least normal dose, and it is therefore unlikely that there could be any harmful effects. It is even probable that 100 mg.

of strychnine hydrochloride, instead of 50, could advantageously be dissolved in 100 c.c. of B.G.S. The dose would then be the least normal dose recommended by the British Pharmacopœia.

Brucine hydrochloride might be substituted, but it has the disadvantage that its dosage for human beings has not been so authoritatively stated. It is definitely less toxic than strychnine, but against that there is the fact that it should be made up at a higher concentration (200 mg. to 100 c.c. of B.G.S.) to get the greatest sperm-stimulating effect when 2 c.c. are mixed with the ejaculate. Chloral hydrate could be used at the same concentration (200 mg. to 100 c.c. of B.G.S.), 2 c.c. of the solution being mixed, as before, with the ejaculate. The lowest normal dose of chloral hydrate given by the British Pharmacopœia is 300 mg., and the maximum 1.2 gm., so there could be no conceivable danger in injecting the 4 mg. that the 2 c.c. of solution would contain. When mixed with the semen the chloral hydrate would be at approximately $\frac{1}{18}$ per cent. This is about the concentration at which it is most effective at stimulating sperms, but it is not such a powerful stimulant as strychnine or brucine at their best concentrations. It is a remarkable fact that chloral hydrate kills all sperms every time at sixteen times the concentration at which it stimulates them. Strychnine hydrochloride has no effect on sperms at 1 per cent. Not being soluble at 4 per cent., it cannot be tried at 2 per cent. by the standard test.

Chemical stimulation provides a means of attacking only one of many causes of sterility, and its limited application makes it of practical importance

to comparatively few people. To those few, however, it might mean a great deal. The method might find application also in veterinary practice. Apart from the possibility of its practical use, the fact that sperms may be stimulated by certain drugs is of interest in relation to their other pharmacological effects. It is remarkable that of the few substances known to stimulate sperms, two should be powerful excitants and one a powerful depressant of the central nervous system.

SUMMARY. Strychnine and brucine hydrochlorides, chloral hydrate and metapheylene diamine have the property of stimulating sperms at certain concentrations. Cavy sperms stimulated by strychnine hydrochloride are in no way damaged. They can fertilize eggs and give rise to normal offspring. It is suggested that stimulation of sperms by strychnine should be used in human beings when sterility is due to feeble motility of the sperms.

CONCLUSION

In contrast to the limited application of chemical stimulation, the chemical killing of sperms is of very wide significance, and the discovery of a perfect spermicide would be of value not only to those who wish to prevent pregnancy because it might be fatal to them, but also to the large body of people who wish to space the births in their families to the best advantage of both mother and children. The spread of contraceptive knowledge into over-populated regions may be of enormous consequence for the peace of the world. May this book contribute a little to the final discovery of the perfect chemical contraceptive, by giving some insight into the mode

of action of spermicides and providing a trustworthy means of assessing their effectiveness.

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POSTSCRIPT

by John R. Baker and R. M. Ranson

SINCE this book was written, we have obtained the results shown below.

PURE SUBSTANCES

We have found no cause to alter the technique. It is important not to beat the egg-white for more than a minute when making up albumen-saline, because prolonged beating results in a loss of protein in the froth, and the results of tests on substances which are susceptible to the presence of proteins are materially altered.

The following are our latest results in the standard test of pure substances with cavy sperms in albumen-saline :—

Acetyl choline bromide	1/0, 3.
Antrypol	2/0, 3.
Diacetin	1/0, 3.
Kerol	1/8/0, 0/0, 0/0, 0/0, 0/0 ; 1/16/0, 0/0, 1.
Sodium ethyl mercuri-thiosalicylate (merthiolate)	1/32/0, 1.
Trypan blue	1/4/0, 3.

Lactic acid test of pure substances with cavy sperms in albumen-saline :—

Chinosol	1/2/0, 0/0, 0/0, 0/0, 0/0 ; 1/4/0, 0/0, 1+ ; 1/8/0, 1/3, 2.
Quinine bisulphate	1/0, 0/0 ; 1/2/0, 1/1 ; 1/4/0, 1.
Sodium oleate	1/2/0, 1/0, 3.

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It is interesting to note that quinine is rendered less spermicidal by acidity. Sodium oleate is decomposed by the lactic acid, with the production of oleic acid.

Standard test of pure substance with cavy sperms in human semen :—

Neokharsivan . . . 1/8%, 0, 0, 0, 0; 1/16%, 0, 0, 0, 1;
1/32%, 0, 1, 1, 1; 1/64%, 1, 1.

Neokharsivan is thus a moderate spermicide with a wide "spread-over."

Mr. R. C. Burbank has estimated the number of cavy sperms present per cubic centimetre of suspension under the conditions of the standard test of pure substances. It will be recollected that the standard test is performed with the sperms present in a concentration equal to that of the contents of the tails of two epididymides in 6 c.c. of fluid. Mr. Burbank has used the hæmocytometer method in a series of counts of the sperms of ten cavies, all of which were used in standard tests. The sperm suspension was diluted before counting, one volume of suspension being made up to 100 volumes with formol-saline. The number of sperms in 0.1 c.mm. was counted, and an average of seven such counts was used to estimate the total number present. The lowest number of sperms per cubic centimetre in this series of ten cavies was 22.7 million, and the highest 63.4 million. The mean of the ten was 47.1 million.

When toluquinone, the most spermicidal substance known, is present at its immobilising concentration (1/512 per cent.), there are about 10^{17} molecules of it per cubic centimetre. Mr. Burbank's counts, therefore, show that it takes about

2,000 million molecules of the most spermicidal substance known to kill one sperm in half an hour under the conditions of the standard test.

SUPPOSITORIES

For convenience of reference we show below all our results with suppositories, including the ones already reported on in the book. The following are the results of the standard test of suppositories with cavy sperms in albumen-saline :—

	S	$\frac{S}{2}$	$\frac{S}{4}$	$\frac{S}{8}$	$\frac{S}{16}$
Fletcher's bijou	—	0/0, 0/0, 0/0	0/0, 1	1	—
Quinine urea hydrochloride	—	0/0, 0/0, 0/0	0/0, 1	1	—
Rendell's wife's friend	0/0, 0/0, 0/0	0/0, 0/0, 0/1	0/1, 1	1	1
Finil (two tablets)	0/0, 0/0, 0/0	1, 1	—	—	—
Bircon	0/0, 0/0, 0/0	1, 1	—	—	—
Vimule	0/0, 1	1+, 1	1	—	—
Lambert's soluble	0/1, 0/1	2	—	—	—
Speton	0/1, 1	1	—	3	—
Semori	1, 1	1+	—	3	—
G.P. soluble	1, 1	2, 3	—	—	—
SM's	1, 2	—	—	—	—
Prorace	1, 2	—	3	—	—
Lambert's double strength	2, 2+	3	—	—	—
Chinobut soluble	3, 3	3	—	—	—
Martindale's lactic acid pessaries	3, 3	3	—	—	—

The following are the results of the lactic acid test of suppositories with cavy sperms in albumen-saline :—

	S	$\frac{S}{2}$	$\frac{S}{4}$	$\frac{S}{8}$
Quinine urea hydrochloride	—	0/0, 0/0, 0/0	0/0, 0/0, 0/1	1
Fletcher's bijou	—	0/0, 0/0, 0/0	0/1, 0/1	—
Bircon	0/0, 0/0, 0/0	1, 1	—	—
Rendell's wife's friend	0/0, 0/1	1	—	—

Messrs. Martindale ask us to make it clear that the lactic acid suppositories made by them are made to the prescription of a birth control clinic, and are not recommended by Messrs. Martindale for contraceptive purposes. Messrs. Martindale are not to be regarded as in any way responsible for their lack of spermicidal power. No doubt the lactic acid uses itself up by reacting with the fatty vehicle and setting free the fatty acids.

Messrs. Gilmont Products inform us that G.P. solubles are intended for use in conjunction with a rubber occlusive pessary, and not for use alone. The intention is to make it unnecessary to use a douche before removing the occlusive pessary.

We now no longer apply the time-of-disintegration test to foaming tablets or to tablets which disintegrate simply by solution, for with these the time of disintegration is mainly dependent on the amount of moisture in the vagina, which is very variable. We regard the disintegration test as well worth carrying out with suppositories which contain a vehicle which melts when warmed. The test has been misinterpreted by some. We have never suggested that the suppositories take exactly the same length of time to disintegrate in the vagina as in the test. Nevertheless, the time taken in the test shows which suppositories disintegrate slowly and which rapidly. The body may be taken to have thermal qualities not very different from those of water. It must be repeated that slow disintegration is not necessarily a bad quality in a suppository, provided that the user is aware of it. The times of disintegration and acidity of all the suppositories tested are shown below :—

	Time of disintegration (mins.)	Amount of deci-normal alkali required to neutralise (c.c.)
Fletcher's bijou	29	4.0
Quinine urea hydrochloride	—	3.3
Rendell's wife's friend	20	2.4
Finil (two tablets)	—	7.7
Bircon	—	0.4
Vimule	10	0.2
Lambert's soluble	15	0.7
Speton	—	0.6
Semori	—	1.5
G.P. soluble	20	1.3
SM's	—	—
Prorace	14	0.3
Lambert's double strength	(3½ hours)	1.0
Chinobut soluble	6	0.1
Martindale's lactic acid pessaries	14	0

The samples of Lambert's double strength suppositories which we tested disintegrated extremely slowly in this test, and it was not possible to determine the exact end-point. This is the reason why these suppositories appear less spermicidal than Lambert's ordinary solubles in the standard test, despite the fact that they contain more of the spermicide. Possibly the spermicide affects the melting-point of the vehicle.

OINTMENTS

The results of the standard test of the total spermicidal power of ointments, carried out as explained on p. 110, are shown below. Cavy sperms were used. They were suspended in albumen-saline, except when the amount of decinormal alkali required to neutralise 2 gm. was 4.0 c.c. or more. In such cases it appears only fair to test in phosphate-

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albumen (p. 23), because the ointment in question is evidently relying largely upon its acidity for its spermicidal power. This applies to Spetonex, Milsan and Pasta.

	Amount of decinormal alkali required to neutralise 2 grams (mean of 2 tests). c.c.	8 per cent.	4 per cent.	2 per cent.
G.P. ointment . . .	1.9	0/0, 0/0, 0/1	0/2	2
Contraceptalene . . .	3.5	0/2, 1	1	—
Spetonex	6.2	1+, 2	—	—
Orthogynol	0.1	2, 2	2	—
G.P. jelly	0.0	2, 2	2+	—
Milsan	7.2	2, 2	3	—
Pasta	5.1	2, 2+	—	—

The following result was obtained by the lactic acid test, using cavy sperms in albumen-saline :—

		8 per cent.	4 per cent.	2 per cent.
G.P. ointment . . .	—	0/1, 1	0/2	—

The results of the diffusion test for ointments, carried out as explained on p. 110, are shown below. With Spetonex, Milsan and Pasta, phosphate-albumen was once again used as suspension fluid for the cavy sperms, owing to the high acidity of these ointments. The others were tested with cavy sperms in albumen-saline.

	5 mins.	15 mins.	30 mins.	1 hour	2 hours	3 hours
G.P. ointment	3, 3, 3	3, 3, 3	1, 2, 3	1, 1, 0	0, 0, 0	0, 0, —
G.P. jelly	3, 3, 3	3, 3, 3	3, 2, 3	1+, 1, 0	0, 0, 0	0, 0, —
Spetonex	3, 3, 3	3, 3, 3	3, 1+, 3	1, 2+, 1	0, 1, 2+	0, 0, 0
Contraceptalene	3, 3, 3	3, —, 3	3, 3, 3	3, 3, 0	2, 1, 0	0, 0, —
Milsan	3, 3, 3	3, 3, 3	3, 3, 3	2+, 3, 3	2+, 0, 2+	0, 0, 0
Pasta	3, 3, 3	3, 3, 3	2+, 3, 2	1+, 3, 1	0, 2, 0	0, 1, 1
Orthogynol	3, 3, 3	3, 3, 3	3, 3, 3	2, 3, 2	1, 2, 2	1, 2, 2

It will be noticed that G.P. ointment is the most spermicidal of the ointments studied both in the test of total spermicidal power and in the diffusion test.

The results of the diffusion test are less consistent than those of our other tests. The reason for this is that mixing of the fluid is intentionally avoided.

APPENDICES

THE significance of the symbols 0, 1, 1+, 2, 2+, 3 and 3+ used in the appendices is explained on pp. 37 and 39.

The reader is referred to p. 41 for remarks on water of crystallization and on the test of one substance in the presence of another (*e.g.*, iodine with four times as much potassium iodide).

In the columns showing the activity of the sperms at one-half, one-quarter, etc., of the immobilizing concentrations, the activities are arranged in order, beginning with the least active. No attention has been paid to the order in which the experiments were performed, which is of no interest.

Experiments performed at concentrations higher than what was subsequently found to be the immobilizing concentration are not recorded.

APPENDIX

STANDARD TEST OF PURE SUB-
(A.G.S. was substituted for B.G.S.)

Concentrations, per cent.	2	1	½	¼	⅛	★
Toluquinone . . .	—	—	—	—	—	—
Butylquinone . . .	—	—	—	—	—	—
Methoxyquinone . . .	—	—	—	—	—	—
Ethylquinone . . .	—	—	—	—	—	—
Parabenzoquinone . . .	—	—	—	—	—	—
Paraxyloquinone . . .	—	—	—	—	—	—
Mercuric chloride . . .	—	—	—	—	—	—
Methoxyhydroquinone . . .	—	—	—	—	—	—
Formaldehyde . . .	—	—	—	—	—	—
Paraformaldehyde . . .	—	—	—	—	—	—
Methylhydroquinone . . .	—	—	—	—	—	—
Malachite green . . .	—	—	—	—	—	—
Thymoquinone* . . .	—	—	—	—	—	—
Butylhydroquinone . . .	—	—	—	—	—	—
Saponine . . .	—	—	—	—	—	—
Methoxytoluquinone . . .	—	—	—	—	—	—
Hexylresorcinol . . .	—	—	—	—	—	—
Dimethoxyhydroquinone . . .	—	—	—	—	—	—
Oxyhydroquinone . . .	—	—	—	—	—	—
Iodine (with 4 times as much potassium iodide) . . .	—	—	—	—	—	—
Ortho-ethoxysalicylaldehyde . . .	—	—	—	—	—	—
Orthovanillin . . .	—	—	—	—	—	—
Hydroquinone . . .	—	—	—	—	—	—
Salicylaldehyde . . .	—	—	—	—	—	—
Crystal violet . . .	—	—	—	—	—	—
Thymol . . .	—	—	—	—	—	—
Sodium oleate . . .	—	—	—	—	—	—
Sodium palmitate . . .	—	—	—	—	—	—
Potassium perman- ganate . . .	—	—	—	—	—	o, o, o, o
Ethylharmol hydro- chloride . . .	—	—	—	—	—	o, o, o, o

* See remark

STANCES WITH CAVY SPERMS IN B.G.S.

when this was desirable. See p. 16)

st	st	st	st	st	st	st
—	—	—	—	0, 0, 0, 0	0, 0, 0, 1+	3+, 3+
—	—	—	—	0, 0, 0, 0	0, 1, 3	3
—	—	—	—	0, 0, 0, 0	2, 3+	—
—	—	—	—	0, 0, 0, 0	2+, 3, 3+	—
—	—	—	—	0, 0, 0, 0	2+, 3, 3+	3+
—	—	—	—	0, 0, 0, 0	2+, 3, 3+	—
—	—	—	—	0, 0, 0, 0	0, 0, 0, 1+	1+, 1+, 2+
—	—	—	—	0, 0, 0, 0	0, 0, 1, 2	2+, 3, 3+
—	—	—	—	0, 0, 0, 0	1, 1, 1+	—
—	—	—	—	0, 0, 0, 0	1+, 1+, 1+	—
—	—	—	—	0, 0, 0, 0	0, 3, 3	—
—	—	0, 0, 0, 0	0, 0, 0, 1	1+, 2	—	—
—	—	0, 0, 0, 0	0, 0, 1	3+, 3+	—	—
—	—	0, 0, 0, 0	0, 0, 2	0, 2, 3	—	—
—	0, 0, 0, 0	0, 0, 1	1+	2+	—	—
—	0, 0, 0, 0	0, 0, 1	1, 2	2+, 3	—	—
—	0, 0, 0, 0	0, 0, 1	1+	3	—	—
—	0, 0, 0, 0	0, 1, 1	1, 1+	—	—	—
—	0, 0, 0, 0	0, 0, 0, 1, 1	2, 2+	3	—	—
—	0, 0, 0, 0	0, 1, 2	—	—	—	—
—	0, 0, 0, 0	1, 1, 1+	—	—	—	—
—	0, 0, 0, 0	1+, 1+, 1+	—	—	—	—
—	0, 0, 0, 0	0, 3, 3+	3+	—	—	—
0, 0, 0, 0	0, 1, 1	—	2	—	—	—
0, 0, 0, 0	0, 0, 2	3, 3, 3	—	—	—	—
0, 0, 0, 0	1+, 2, 2	—	—	—	—	—
0, 0, 0, 0	2, 2, 2+	—	—	—	—	—
0, 0, 0, 0	2, 3, 3	—	—	—	—	—
0, 1, 1	0, 1+, 2+	—	—	—	—	—
1, 1, 1	—	—	—	—	—	—

on p. 68.

STANDARD TEST OF PURE SUB

Concentrations, per cent.	2	1	1	1	1	1
Mercurochrome.	—	—	—	—	0, 0, 0, 0	0, 0, 1+
Carmine (with 4 times as much borax)	—	—	—	—	0, 0, 0, 0	0, 1+, 2+
Harmine hydrochloride	—	—	—	—	0, 0, 0, 0	1+, 1+, 1-
Sodium taurocholate.	—	—	—	0, 0, 0, 0	0, 0, 0, 1	1+
Zinc sulphate	—	—	—	0, 0, 0, 0	0, 0, 1	1, 1, 1+
Potassium aluminium sulphate (anhydrous)	—	—	—	0, 0, 0, 0	0, 0, 1	2, 2+
Sodium glycocholate.	—	—	—	0, 0, 0, 0	0, 0, 1	2, 3, 3
Dioxyquinoline sulphate	—	—	—	0, 0, 0, 0	0, 1, 1	—
Quinine urea hydro- chloride	—	—	—	0, 0, 0, 0	0, 1, 1+	—
Chinosol	—	—	—	0, 0, 0, 0	1, 1+, 1+	—
Cresol	—	—	—	0, 0, 0	1, 2, 2	—
Potassium borotartrate	—	—	—	0, 0, 0, 0	1, 2, 2+	—
Quinine hydrochloride	—	—	0, 0, 0, 0	0, 0, 0, 1	1+, 1+	2+
Potassium oxyquinoline sulphonate	—	—	0, 0, 0, 0	0, 0, 0, 1	1+, 2, 2	—
Potassium cyanide	—	—	0, 0, 0, 0	0, 0, 1	1	2
Quinine bisulphate	—	—	0, 0, 0, 0	0, 0, 1, 1	1, 1	3
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	—	—	—
Potassium capronate	0, 0, 0, 0	1, 1, 1	2	—	—	—
Orthophenylene diamine	0, 0, 0, 0	0, 1, 3+	2	—	—	—
Ammonium chloride	0, 1, 1	2	3	—	—	—
Zinc sulphocarbonate	1, 1, 1	1, 1	1, 1	1	1+	2
Potassium butyrate	1, 1, 1	—	3	—	—	—
Sodium citrate	1+, 2, 2	—	—	—	—	—
Hexamine	2+, 2+, 2+	2+	—	—	—	—
Sodium tartrate	2, 2, 3	—	—	—	—	—
Magnesium sulphate	2, 2, 3	—	—	—	—	—
Borax	2, 3, 3	—	—	—	—	—
Ethyl alcohol	2+, 3, 3	—	—	—	—	—
Metaphenylene diamine	3, 3, 3	—	3+	—	—	—
Glycerol	3, 3, 3+	—	—	—	—	—
Sodium dichlorylsulph- aminobenzoate	3, 3, 3+	—	—	—	—	—

Incomplete tests were made of the substances which are listed below in alphabetical order. Insolubility prevented a full test of some of them. No substance can be tried above half-saturation by the standard test. Cavy sperms in B.G.S. were used in all these incomplete tests. It would have been better to have used A.G.S. with mercury potassium iodide and copper acetate, and perhaps also with the gluconates.

	Activity of sperms.
Acetone	3 at $\frac{1}{32}$ %.
Acriflavine	3 at $\frac{1}{16}$ %.
Auramine	3 at $\frac{1}{32}$ %.
Caffeine	3 at $\frac{1}{16}$ %.
Camphor	3 at $\frac{1}{16}$ %.
Cerium gluconate	2 at $\frac{1}{8}$ %.
Chloroform	3 at $\frac{1}{16}$ %.
Copper acetate.	Fails to immobilize at $\frac{1}{32}$ %.
Cupric gluconate	0, 0 at $\frac{1}{8}$ %; 3 at $\frac{1}{32}$ %.
Duroquinone	3+ at $\frac{1}{32}$ %.*
Guaiacol	3 at $\frac{1}{16}$ %.
Hæmatein (dissolved in $\frac{1}{32}$ % potassium alum in saline)	3 at half-saturation.
Isovanillin	3 at $\frac{1}{16}$ %.
Lead gluconate	2, 2+ at $\frac{1}{16}$ %; 1, 3, 3 at $\frac{1}{32}$ %.
Menthol.	1+ at $\frac{1}{16}$ %.
Mercury potassium iodide	0, 0 at $\frac{1}{32}$ %; 2+ at $\frac{1}{64}$ %.
Metadinitrobenzene.. . . .	3+ at $\frac{1}{32}$ %.
Musk, synthetic	3 at half-saturation.
Nitrobenzene	3+ at $\frac{1}{32}$ %.
Paradimethylaminobenzaldehyde	3 at $\frac{1}{16}$ %.
Parahydroxybenzaldehyde.	2+ at $\frac{1}{16}$ %.
Paranitrosodimethylaniline	3 at $\frac{1}{32}$ %.
Paraphenylene diamine	1, 2, 2+ at 1%.
Phloroglucinaldehyde	0, 0, 1 at $\frac{1}{16}$ %; 2+, 2+, 2+ at $\frac{1}{32}$ %.
Phloroglucinol	1, 1, 1 at $\frac{1}{8}$ %* ; 1+, 2 at $\frac{1}{16}$ %.
Potassium chlorate	3 at $\frac{1}{16}$ %.
Potassium dichromate	3 at $\frac{1}{32}$ %.
Pyronin G	3 at $\frac{1}{32}$ %.
Sodium nitrite	2 at $\frac{1}{16}$ %.
Sodium veronal	3+ at $\frac{1}{16}$ %.
Strychnine hydrochloride	No effect at 1%.
Tannin	3 at $\frac{1}{32}$ %.
Urea	3 at $\frac{1}{16}$ %.
Vanillin	0 at $\frac{1}{8}$ %; 3 at $\frac{1}{16}$ %.

* Not sufficiently soluble for test at higher concentration.

APPENDIX

STANDARD TEST OF PURE SUBSTANCES

Concentrations, per cent.	2	1	1	1
Toluquinone	—	—	—	—
Methylhydroquinone	—	—	—	—
Ethylhydroquinone	—	—	—	—
Hexylresorcinol	—	—	—	—
Mercuric chloride	—	—	—	—
n-Propylresorcinol	—	—	—	—
Orthovanillin	—	—	—	—
Sodium oleate	—	—	0, 0, 0, 0	0, 1, 1,
Chinosol	—	—	0, 0, 0, 0	0, 0, 2
Zinc sulphate	—	0, 0, 0, 0	—	1, 1
Methylhydroquinone (with twice as much a-hydrous sodium sulphite)	—	0, 0, 0, 0, 0, 0, 0, 0	1, 1, 1	1+
Potassium borotartrate	0, 0, 0, 0	0, 0, 1	2	—
Sodium sulphite (a-hydrous)	1, 1, 1	2+	—	—
Borax	2, 2, 2	—	—	—

Incomplete tests were made of the substances which are listed

Arbutin
Chloral hydrate
Potassium iodide
Potassium permanganate
Pyrogallol
Sodium perberate
Vanillin
Zinc acetate

APPENDIX

STANDARD TEST OF ACIDS WITH

Concentrations, per cent.	2	1	1	1	1
Sulphuric acid	—	—	0, 0, 0, 0, 0, 0, 0, 0	1, 1, 1+	—
Acetic acid	—	—	0, 0, 0, 0, 0, 0, 0, 0	0, 2+, 1, 1	3
Succinic acid	—	0, 0, 0, 0, 0, 0, 0, 0	0, 0, 0, 0, 1+	—	—
Tartaric acid	—	0, 0, 0, 0, 0, 0, 0, 1	—	1+, 2+	—
Citric acid	—	0, 0, 0, 0, 0, 0, 0, 1	1, 1, 1+	1+	—

II

WITH CAVY SPERMS IN ALBUMEN-SALINE

†	$\frac{1}{8}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	$\frac{1}{1024}$	$\frac{1}{2048}$
—	—	—	—	—	o, o, o/o, o/o	o, o, o, I	2, 2	—
—	—	—	—	—	o, o, o, o	o, o, o, I	I, 2, 2, 2+	3
—	—	—	—	—	o, o, o, o/o	o, o, I	I+, 2, 2	—
o, o, o, o	o, o, o, I	I, 2, 2+	3	o, o, o, o	I, I, I+	—	—	—
o/o, o/o, o/o, c/o	o/o, o/o, I	2+	—	—	3	—	—	—
o, o, o, o	o, I, I	I	—	—	—	—	—	—
I, I, I	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	2	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—

below in alphabetical order :—

										Activity of sperms
.	3 at 2%.
.	o at 2%.
.	2 at 2%.
.	o, I at 1%.
.	o at 2% ; I at 1%.
.	2+, 2+, 2+ at $\frac{1}{2}$ %
.	3 at $\frac{1}{2}$ %.
.	I at $\frac{1}{2}$ % ; I at $\frac{1}{8}$ %

III

CAVY SPERMS IN PHOSPHATE-ALBUMEN

★							
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
3+	—	—	—	—	—	—	—

APPENDIX

LACTIC ACID TEST OF PURE SUBSTANCES

Concentrations, per cent.	2	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$
Methylhydroquinone	—	—	—	—	—
<i>n</i> -Propylresorcinol	—	—	—	—	0/0, 0/0, 0/0, 0/0

APPENDIX

STANDARD TEST OF SUPPOSITORIES

Concentrations.	S	$\frac{S}{2}$	$\frac{S}{4}$	$\frac{S}{8}$	$\frac{S}{16}$
M3	—	—	—	—	—
Rendell's " wife's friend "	0/0, 0/0, 0/0	0/0, 0/1	0/0, 1	1	1
Finil (two tablets)	0/0, 0/0, 0/0	1, 1	—	—	—
Vimule	0/0, 1	1, 1+	1	—	—
Speton	0/1, 1	1	—	3	3
Semori	1, 1	1+	—	3	3
G.P. soluble	1, 1	3	—	—	—
Prorace	1, 2	—	3	—	—
Martindale's lactic acid	3, 3	3	—	—	—
Chinobut soluble	3, 3	3	—	—	—

APPENDIX

LACTIC ACID TEST OF SUPPOSITORIES

Concentrations.	S	$\frac{S}{2}$	$\frac{S}{4}$		
M3	0/0, 0/0, 0/0	1/0, 1/1	1/2	—	—
Rendell's " wife's friend "	0/0, 0/1	1	—	—	—

IV

WITH CAVY SPERMS IN ALBUMEN-SALINE

$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$					
o/o, o/o, o/o, o/o o/o, o/i, o/i	o/o, o/o, I o/i, o/2, o/2	I, I I	--	--	--	--	--

V

WITH CAVY SPERMS IN ALBUMEN-SALINE

$\frac{S}{32}$	$\frac{S}{64}$	$\frac{S}{128}$	$\frac{S}{256}$			
o/o, o/o, o/o	o/o, I	1, 2	3	--	--	--
--	--	--	--	--	--	--
--	--	--	--	--	--	--
--	--	--	--	--	--	--
--	--	--	--	--	--	--
--	--	--	--	--	--	--
--	--	--	--	--	--	--
--	--	--	--	--	--	--

VI

WITH CAVY SPERMS IN ALBUMEN-SALINE

--	--	--	--	--	--	--
--	--	--	--	--	--	--

APPENDIX

STANDARD TEST OF PURE SUBSTANCES

Concentrations, per cent.	2	1	½	¼	⅛
Toluquinone	—	—	—	—	—
Hexylresorcinol	—	—	—	—	—
Orthovanillin	—	—	—	—	0, 0, 0, 0
Sodium oleate	—	—	0, 0, 0, 0	0, 0, 0, 1+	2, 3, 3,
Chinosol	—	—	0, 0, 0, 0	0, 0, 1	2
Potassium borotartrate	0, 0, 0, 0	0, 0, 2	2	—	—
Borax	2, 2+, 2+	—	—	—	—

APPENDIX

STANDARD TEST OF PURE SUBSTANCES

Concentrations, per cent.	2	1	½	¼	⅛
Toluquinone	—	—	—	—	—
Hexylresorcinol	—	—	—	—	—
Orthovanillin	—	—	—	—	0, 0, 0, 0
Sodium oleate	—	—	0, 0, 0, 0	0, 0, 1	2
Quinidine acid hydrochloride	—	—	0, 0, 0, 0	0, 1, 1	1
Cinchonidine acid hydrochloride	—	—	0, 0, 0, 0	1, 1, 1	1+
Quinine bisulphate	—	—	0, 0, 0, 0	1, 1, 1	2
Chinosol	—	0, 0, 0, 0	0, 0, 1	1+	—
Potassium borotartrate	0, 0, 0, 0	1, 1, 2	2	—	—
Borax	1+, 1+, 2	—	—	—	—

Incomplete tests were made of the substances which are listed

- Cinchonidine hydrochloride.
- Cinchonine acid hydrochloride
- Cinchonine hydrochloride
- Methylhydroquinone
- Quinidine hydrochloride

APPENDIX

TEST OF PURE SUBSTANCES WITH SPERMS OF THE

Concentrations, per cent.	2	1	½	¼
Hydroquinone	—	—	—	—
Catechol	—	—	—	0, 0, 0, 0
Chinosol	—	—	—	0, 0, 0, 0
Resorcinol	—	—	0, 0, 0, 0	0, 0, 0, 1, 1

VII

WITH HUMAN SPERMS IN HUMAN SEMEN

1	2	3	125	250	512	1024
—	—	—	—	—	0, 0, 0, 0	1, 2+, 2+
0, 0, 0, 1	1+, 1+, 2	0, 0, 0, 0	0, 0, 1	2	—	—
—	—	—	—	—	—	—
—	—	—	—	—	—	—
—	—	—	—	—	—	—

VIII

WITH CAVY SPERMS IN HUMAN SEMEN

1	2	3	125	250	512	
—	—	—	—	0, 0, 0, 0	0, 1+, 2	—
1, 1, 1	1+	0, 0, 0, 0	0, 0, 0, 1	0, 2, 2	—	—
3	—	—	—	—	—	—
—	—	—	—	—	—	—
2, 2+	—	—	—	—	—	—
—	—	—	—	—	—	—
—	—	—	—	—	—	—

below in alphabetical order:—

Activity of sperms

- 1 at $\frac{1}{2}\%$; 1+ at $\frac{1}{2}\%$.
- 0, 0, 0, 1 at $\frac{1}{2}\%$; 1, 1 at $\frac{1}{4}\%$; 2 at $\frac{1}{2}\%$.
- 0 at $\frac{1}{2}\%$; 1+ at $\frac{1}{2}\%$.
- 0, 0, 0 at $\frac{1}{256}\%$; 1+, 2 at $\frac{1}{512}\%$.
- 0 at $\frac{1}{2}\%$; 1 at $\frac{1}{2}\%$.

IX

SEA-URCHIN, *ECHINUS ESCULENTUS*, IN SEA WATER

1	2	3	125	250	512
—	—	0, 0, 0, 0	0, 0, 0, 1, 1	1	0, 3
1+, 1+, 2	3	2+	—	—	—
1, 2+, 3	—	—	—	—	—
2+, 2+	—	—	—	—	—

APPENDIX

TEST OF SODIUM OLEATE WITH CAVY SPERMS IN

Concentrations, per cent., of sodium oleate.	1	2	3
Volumes of egg-white to one volume of B.G.S.			
0	—	—	—
0.2	—	—	—
2	—	—	—
3	—	—	0, 0, 0, 0
3.5	—	0, 0, 0, 0	0, 1, 1
4	0	1	1
Egg-white alone	0, 0	0, 1	1, 3
Human semen . . .	—	0, 0, 0, 0	0, 0, 1

APPENDIX

TEST OF FOAMING MIXTURES

Concentrations, per cent.	3
Citric foaming mixture	0, 0, 0, 0
Tartaric foaming mixture	0, 0, 0, 0

APPENDIX

STIMULATION EXPERIMENT

Concentrations, per cent.	1	2
Strychnine hydrochloride	—	2
Control	—	2+
Brucine hydrochloride	1+	3 3 3+ 3 3
Control	2	2', 2', 2+', 2+', 2+', 2'
Chloral hydrate	—	2
Control	—	2+
Metaphenylene diamine	—	3 3 2+ 2+
Control	—	2+', 3', 2', 2'
Chinosol	—	—
Control	—	—

X

VARIOUS PROPORTIONS OF B.G.S. AND EGG-WHITE

1	2	3	4
—	—	0, 0, 0, 0	2, 2, 2+
—	0, 0, 0	3	—
0	1	—	—
0, 1	—	—	—
—	—	—	—
2	—	—	—
3	—	—	—
2	3	—	—

XI

WITH CAVY SPERMS IN B.G.S.

4	2	1
1, 1, 1	1, 1+	1+
1, 1, 1	2+, 2+	2+

XII

WITH CAVY SPERMS IN B.G.S.

1	2	3	4
$\frac{3}{2+}, \frac{3}{2+}, \frac{3+}{1+}, \frac{3+}{2}, \frac{3}{2}$	$\frac{3}{2+}, \frac{3}{2+}, \frac{2}{1+}, \frac{3}{1+}, \frac{3}{2}, \frac{3}{2}$	$\frac{3}{2+}, \frac{3}{1+}, \frac{2}{1+}, \frac{2}{2+}, \frac{2+}{2+}$	$\frac{1+}{1+}$
$\frac{3}{2+}, \frac{3}{2+}, \frac{3+}{2+}, \frac{3}{2}, \frac{3+}{2}$	$\frac{2+}{2}, \frac{2+}{2}, \frac{3}{2}$	$\frac{2+}{2+}$	—
$\frac{3}{2+}, \frac{3}{2+}, \frac{2+}{2}, \frac{2}{2+}, \frac{2+}{2}$	$\frac{2+}{2+}$	—	—
$\frac{2+}{2+}, \frac{3}{2+}, \frac{2}{2+}, \frac{1+}{2}$	$\frac{2}{2+}$	—	—
$\frac{1}{1+}, \frac{2}{1+}$	$\frac{2+}{1+}, \frac{2+}{1+}, \frac{2}{3}, \frac{1+}{1+}, \frac{1+}{2}$	$\frac{2}{1+}, \frac{2}{1+}, \frac{2}{3}, \frac{1+}{1+}, \frac{2}{2}$	—

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