THE SPERMICIDAL POWERS OF CHEMICAL CONTRACEPTIVES

VII. APPROVED TESTS

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INTRODUCTION

OUR tests have been submitted from time to time to valuable criticism, especially from the Medical Sub-Committee of the National Birth Control Association, to whom we are very grateful. In particular, we wish to acknowledge helpful advice from Dr Margaret Jackson, Dr Cecile Booysen, and Dr Helena Wright. Our tests of spermicidal power have been modified until they have the general approval of these clinical workers. Since other clinical workers and manufacturers naturally want to know exactly how these approved tests are carried out, we have thought it advisable to give full details of them. Our work has been financed by the Birth Control Investigation Committee, whose Secretary, Dr C. P. Blacker, has continually given us encouragement and good advice.

All our tests are nowadays done with human semen, and we cannot sufficiently thank our regular donors, Messrs A, C, D, E, F, G, H, M, R and S, who have made the work possible. We have also used occasional specimens from Messrs B, K, P and Q.

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RATE OF DISINTEGRATION AND ACIDITY OR ALKALINITY OF CHEMICAL CONTRACEPTIVES

We have not found it necessary to change the technique given in *The Chemical Control of Conception* (Baker, 1935). So far as rate of disintegration is concerned, the test is not applicable to foaming tablets, for with these the time of disintegration is mainly dependent on the amount of moisture in the vagina, which is very variable; nor is it applicable to tube-jellies or ointments, which are already semi-fluid. It is especially applicable to suppositories made of gelatin gels or of solid fats. It is not suggested that the rate of disintegration in the vagina is the same as in the test, but those suppositories which disintegrate most rapidly in the test are likely to do so also in practice. Other things being equal, rapid disintegration is beneficial. So far as acidity or alkalinity is concerned, the test is applicable to all chemical contraceptives.

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 5 min. elapse before complete disintegration, the vessel is shaken ten times, and this is repeated every 5 min. Disintegration is watched through the closed glass 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 of this test are given in Appendix I.

ALKALINE TEST OF (TOTAL) SPERMICIDAL POWER

This test is applicable both to pure substances and to commercial spermicidal products, i.e. suppositories (including gelatin gels, products with fatty vehicles, and foaming tablets), pastes and ointments.

The principle of the test as applied to pure substances is as follows. The substance is dissolved in saline at some concentration in the series 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, etc. per cent. 0·3 c.c. of this solution at 37° C. is mixed with 0·3 c.c. of human semen at the same temperature. The substance is now at half the concentration at which it was first made up. Sperms are examined on the hot stage of the microscope after the lapse of 5 and of 30 min. and their activity recorded. If they are immobile at 30 min., the suspension is diluted with three times its volume of an alkaline fluid favourable to the activity of the sperms, to find whether they were only temporarily immobilized (e.g. by weak acid), or really

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dead. An exact control is also examined. The notation used in recording the result of this test is described in §§ 18 and 36 in the detailed description below.

We postulate that in coition there are present on the average 5 c.c. of semen and 1 c.c. of vaginal fluid. These are the figures which the Medical Sub-committee wishes us to use. We ourselves are inclined to think that the usual amount of semen is somewhat less and of vaginal fluid rather more, at any rate during sexual excitement. Nevertheless, the total amount, 6 c.c., cannot be far wrong. We therefore use the expression "standard" or "S" concentration to indicate one suppository to 6 c.c. of sperm suspension, and we make tests at S, $S/2$, $S/4$, $S/8$, etc. A test at $S/2$ means a test in the proportion of the soluble part of one suppository to 12 c.c. of sperm suspension. Each product is tested first at $S/2$. One suppository is thrown into 6 c.c. of saline at 37° C. and allowed to disintegrate. The solution is filtered. 0·3 c.c. of the filtrate is mixed with 0·3 c.c. of semen at the same temperature. The sperms are examined at the same periods as in the test of pure substances. Further tests are made at other concentrations, as found necessary. This test can also be applied to pastes and ointments, 2 g. being postulated as the normal dose. No attempt is made in this test to copy natural conditions exactly, but a very accurate statement of total available spermicidal power is obtained. A closer approximation to natural conditions is given by the diffusion test (p. 481), which, however, from its nature cannot give such consistent results. In the test of total spermicidal power of commercial products only *available* spermicidal power is considered, i.e. only that part of the product that is soluble in 6 c.c. of saline is considered. If this were not done, it would be possible, by using a rather insoluble spermicide, to make a product which would kill at low concentrations in the test, but which would have little spermicide available for actual use in practice.

The detailed description given below in numbered paragraphs applies to commercial spermicidal products. The small changes necessary to adapt the test to pure substances are referred to in § 48.

Human semen is obtained as follows. Each donor is provided with a glass specimen tube, 4 × 1 in., with waxed cork, fitting inside a wooden container. Semen is caught at coition in a clean rubber sheath. With as little delay as possible (not exceeding 5 min.), the contents of the sheath are transferred to the glass tube, which is corked and kept in its container at room temperature. A specimen which has remained overnight in a rubber sheath rarely contains any active sperms. Human sperms in semen remain alive much longer at ordinary room temperature than at body temperature or near freezing point. (Probably the temperature of the average American bedroom is above the optimum temperature for sperm survival.) For this test of total spermicidal power we have usually used semen between 2 and 15 hours after ejaculation. Sperms become progressively easier to kill by spermicides as time elapses after ejaculation. After the lapse of 15 hours, if the controls are still active, the sperms are not usually twice as easily killed as fresh sperms (i.e. the fresh

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sperms do not require twice the concentration of spermicide necessary to kill the stale ones). The freshness or staleness of the semen, within these limits, does not, therefore, greatly affect the result of the test.

We find that sperms from different men have different powers of resistance to spermicides, and most specimens from any one man (at the same freshness) show roughly the same resistance. In general A's sperms require about twice as much spermicide to kill them as M's, and the other donors are intermediate, some being close to A. The special resistance shown by the sperms of A and certain other donors applies to all spermicides, so far as we know, with the curious exception of acids. The resistance is not simply a matter of the number of sperms present per c.c. Thus M has considerably more than the average sperm abundance per c.c., but is exceptionally non-resistant. D, on the contrary, has rather few, but unusually resistant, sperms.

The results of the tests of commercial products are given in Appendix II. Tests performed with A's sperms are marked with an asterisk.

Detailed description of the alkaline test of (total) spermicidal power

Note: Where cross-references to numbered steps in the procedure occur in the text, the sign § precedes the number of the paragraph in the description.

1. Regulate a thermostat to 37° C., and place in it the following: cavity slides, cover-slips, graduated and ordinary pipettes, a bottle of alkaline diluting fluid (see Appendix III), a damp chamber, a measuring cylinder, a corked specimen tube (3 × 1 in.), three glass capsules with covers, one bottle of 0·9 per cent sodium chloride (250 c.c.), and a filter funnel with filter and receptacle. The damp chamber is of glass with a glass lid. 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 two small test-tubes and two 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. Make sure that the semen specimen shows good activity by examination of one drop on a slide on the hot stage of the microscope.

4. Transfer 6 c.c. of warm saline to the corked specimen tube. Throw in one suppository (or two if two are directed to be used at the same time). If an ointment or tube-jelly is to be tested the standard dose is 2 g. One g. is the dose for a contraceptive powder. Leave until the suppository has disintegrated completely, or for 1 hour, whichever is less, shaking occasionally. Filter through the warm filter funnel (any spermicide which does not dissolve in 6 c.c. at 37° C. being thus got rid of). We postulate that normally 5 c.c. of semen and 1 c.c. of vaginal fluid are present after copulation. The filtrate will subsequently be mixed with an equal volume of semen, and the soluble parts of the suppository will be present in the proportion of one suppository to 12 c.c. of fluid. This is half the standard concentration of one suppository to 6 c.c., and the test is always first performed at this concentration ($S/2$).

5. Introduce 0·3 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.

6. Introduce 0·3 c.c. of the filtrate obtained in § 4 to another test-tube in the damp chamber.

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7. Transfer 0·3 c.c. of the semen 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.

8. Set a timer at 0 min. The fluids are left to warm up.

9. Fourteen min. later transfer the contents of the test-tube mentioned in § 5 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.)

10. Put the point of the 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.

11. 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 semen which may have stuck to the side of the tube when it was introduced. The control tube now contains human semen mixed with an equal volume of saline.

12. When the timer records 15 min., transfer the contents of the test-tube mentioned in § 6 to the experimental tube, which will then contain semen and the filtrate under investigation in equal volumes. The filtrate will now be at half the standard concentration ($S/2$). (If two or three suppositories or tube-jellies are being tested at the same time, in accordance with § 45, the process described in this section should be started when the timer records about $14\frac{1}{2}$ min.) Replace the empty test-tubes which have been used by two clean specimen tubes in readiness for § 29.

13. Treat the contents of the experimental tube exactly as those of the control tube were treated in §§ 10 and 11. The performance of the process described in § 11 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 § 16 or 25, and thus vitiate the results.

This section must be complete when the timer records 16 min.

14. When the timer records 17 min. bubble air three times through the contents of the control tube with a pipette. (This is in preparation for a quick examination of the activity of the sperms when the timer has recorded 20 min., i.e. 5 min. after the mixture of the semen with the filtered contraceptive.)

15. With the pipette used in § 14 transfer two drops of the fluid in the control tube to the hollow of one of the slides. Apply a warm cover-slip. Two drops of fluid do not fill the hollow of the slide. A large bubble of air is included below the cover-slip, which prevents the sperms from becoming inactive quickly, owing to inability to respire. Leave the slide on the floor of the thermostat.

16. Apply the process described in §§ 14 and 15 to the contents of the experimental tube and to another slide.

17. When the timer records 19 min., remove one of the slides containing sperms from the thermostat and examine it under the microscope with a 4 mm. objective and a low-power eyepiece. (With a low-power objective it is not possible to detect minute movements of the sperms.) This, with § 19, constitutes the "5 min. examination".

(If two or three substances are being tested at the same time, the examination of the slides may begin when the timer records 18 min., but it must be complete when it records 22 min. The object is to examine the slides as exactly as possible 5 min. after the mixture of the semen with the substance under investigation.)

18. Record the activity of the sperms 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

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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 delay which would arise from prolonged indecision:

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, and 1* to indicate that only a single sperm was seen to move in ten fields of view. The sign ° (thus, 1°) indicates that movement was confined to sperms situated in clumps, into which the spermicide diffuses with difficulty.

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 at the stated time of examination. Quickness is, of course, particularly important when several substances are being tested at the same time.

19. Repeat §§ 17 and 18 with the other slide containing sperms.

20. If the control sperms are 2+, 3 or 3+, proceed to § 21. If the control sperms show an activity of 2 or less, discard the whole test.

21. Distinguish four hollowed microscopical slides with diamond or grease-pencil marks, two being marked "*C*" for control and two marked "1" for experimental. (When two or three substances are being tested at the same time it is convenient to have two sets of slides marked "*C*", "1", "2", "3".)

22. 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 § 26.

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

24. When the timer records 40 min. repeat the process described in §§ 14 and 15, using one of the labelled slides marked "*C*".

25. Apply the process described in §§ 14 and 15 to the experimental tube, using one of the slides marked "1".

26. 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 § 45. It is thought unnecessary to perform the covering-up of identification marks and shuffling before the 5 min. examination, since in this test the main interest is focussed upon the half-hour examination.)

27. When the timer records 44 min. repeat the process described in §§ 17–19, recording the activity of the sperms on the blank labels. This is the "half hour examination".

28. Turn the blank labels aside to disclose the identity of the slides. If the controls show an activity of less than 2+, discard the experiment. If the controls are 2+, 3 or 3+ and the activity of the sperms in the experimental tube is 0, proceed to § 29. If the controls are 2+, 3 or 3+ and the experimental sperms are not 0, proceed to § 38.

29. Transfer 0·3 c.c. of the contents of the experimental to the clean tube mentioned in § 12. Add a drop of B.D.H. "Universal" indicator and record the *p*H. Repeat the process for the control tube.

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30. Add 0·9 c.c. of (warm) alkaline diluting fluid to the remaining contents of both the control and experimental tubes.

31. When the timer records 55 min., prepare a slide from the contents of the control tube, exactly as in §§ 14 and 15.

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

33. When the timer records between approximately 59 and 61 min., determine the activity of the sperms on the control and experimental slides as in §§ 17–19.

34. If the activity of the control sperms is 2, 2+, 3 or more, proceed to § 35. After dilution an activity of 2+ is not insisted upon. If the activity of the control sperms is less than 2 discard the experiment.

35. Record the activity of the experimental sperms. 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 of the concentration present before. Most substances have little effect on sperms at one-quarter of the least concentration sufficient to immobilize them.

36. Enter the figures representing the activity of the sperms at 5 min., half an hour, and after dilution with lines between them thus: 2/0/0 or 2/0/1, etc. The first figure represents the activity at 5 min., the second at half an hour, and the third after dilution.

37. If the activity of the experimental sperms was 0 at half an hour and after dilution repeat the experiment with the substances at half the concentration used before. Repeat it again and again, if necessary, at lower and lower concentrations in the series $S/4$, $S/8$, $S/16$, etc., until active sperms are found.

38. If there is any activity whatever in the experimental sperms in § 28 repeat the experiment at S. This is achieved by putting one suppository into 3 c.c. of saline (instead of 6 c.c.) in § 4. (It is, of course, permissible to put two suppositories into 6 c.c.)

39. The lowest concentration which suffices to kill all sperms in half an hour is thus ascertained. Perform the test again at this concentration.

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

41. If all sperms are killed, this concentration is recorded as the killing concentration. This may be defined as the lowest concentration in the series S, $S/2$, $S/4$, $S/8$, etc., that kills all human sperms in half an hour at 37° C. in three consecutive experiments.

42. If in any of the tests mentioned in §§ 39 and 40 all sperms are not killed, a higher concentration in the usual series must be tried.

43. Further tests at the appropriate concentrations must be carried out, until the killing concentration defined in § 41 has been found.

44. Perform the test altogether at least once at half the killing concentration and once at one-quarter.

45. Two or three suppositories or the same suppository at different concentrations may be tested at the same time against the same control. No suppository may be tested more than once at the same concentration against the same control.

46. It is possible to run two tests concurrently, each involving three experimental tubes, if one test is started exactly 23 min. 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 23 min. Each hand governs one test. 23 min. 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.

47. The performance of two tests concurrently, each involving a control and three experimental tubes, is only possible after much practice with single tests, and requires the assistance of another person from the time when the hand controlling the first experiment records 40 min. until it records 47 min.

48. Exactly the same test is used for pure substances, except that they are tried at 2, 1, ½, etc., per cent instead of S, $S/2$, $S/4$, etc. This is done by dissolving the pure substance at 4, 2, 1, etc., per cent in § 4.

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ACID TEST OF (TOTAL) SPERMICIDAL POWER

It is possible that the amount of lactic acid in the vagina may sometimes be such as to acidify the semen without killing the sperms. Some spermicides are less active in acid solution. Methylhydroquinone is actually sixteen times as spermicidal in alkaline as in acid solution. It is therefore necessary to perform a test in a weakly acid medium. We have simplified our former acid test, and now carry it out by acidifying the semen at the start. In other respects the test is exactly the same as the alkaline test of (total) spermicidal power.

The necessary acidification is carried out as follows:

Prepare a stable lactic acid solution by adding 3 c.c. of strong lactic acid (sp. gr. 1·21) to 100 c.c. of distilled water and boiling for 24 hours with reflux condenser. This weak ("3 per cent") lactic acid may be kept for months and filtered if moulds grow. Semen is acidified by adding this solution. It is essential to have really active sperms (3 or 3+), for relatively inactive ones are too easily killed by slight acidity to withstand the test. The object is to add just enough acid, so that, after the lapse of a few minutes, the semen is slightly acid (*p*H 5·5–6·5) and the sperms show an activity of 1+, 2 or 2+. Different specimens of semen will be found to require between 0·13 and 0·23 c.c. of the weak lactic acid solution. It is easy to add the necessary amount with a graduated capillary pipette. For each c.c. of semen add 0·13 of weak lactic acid. Draw the fluid in and out of a pipette three times to mix it thoroughly, and then transfer one drop to a cavity slide and one drop to a small specimen tube. Leave the slide on the floor of the thermostat for a few minutes to warm up. Meanwhile determine the *p*H of the drop of semen in the specimen tube by diluting with 1 c.c. of saline and adding a drop of B.D.H. "Universal" indicator. If the *p*H is too high and the sperms fully active, add another 0·02 c.c. of the weak lactic acid, and go on repeating the determination of activity and *p*H and the addition of lactic acid in increments of 0·02 c.c. until the sperms are 1+, 2 or 2+, and the *p*H between 5·5 and 6·5. The control sperms should show this activity also at the 5 min. and half hour examinations, but should recover to 3 or 3+ after the addition of the alkaline diluting fluid. (If they are not 2+ or more after dilution, too much acid was added and the test must not be counted.)

The alkaline reserve of the semen, which varies considerably with the different donors, is not an index of the amount of acid required to reduce the activity of the sperms. At a given *p*H (e.g. 5·5) the sperms of some individuals are all immobilized, while those of other individuals show an activity of 1+ or 2. Those whose sperms are easily immobilized by acids are not necessarily those with a small alkaline reserve. Thus D has a normal alkaline reserve but his sperms are immobilized with particular ease by acidity. As previously mentioned, the sperms of A, which are especially resistant to most spermicides, are easily immobilized by acidity. For further remarks on the alkaline reserve of semen, see p. 486.

DIFFUSION TEST

This test is applicable to suppositories, ointments and tube-jellies, and measures how quickly the spermicide escapes from the vehicle under conditions which resemble the natural ones. To make the test as severe as possible,

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mechanical mixing is avoided. It is useless to put a lot of spermicide in a chemical contraceptive, if it cannot diffuse out rapidly enough to kill the sperms.

To economize semen the test is performed on the scale of one-quarter, i.e. one-quarter of a suppository to 0·25 c.c. of vaginal fluid and 1·25 c.c. of human semen. The product is warmed to the temperature of the body in a glass capsule and then smeared over the bottom of it. 0·25 c.c. of warm artificial vaginal fluid is added. Five min. later 1·25 c.c. of semen are squirted into the capsule with a pipette. Sperms are examined at 2, 5, 15, 30, 60, 120 and 180 min. after the addition of the semen. At each examination time the sperms are examined once if they show any activity at all. If they are immobile, they are diluted and examined again. If they are dead, another sample of sperms is at once examined and dilution performed if necessary. If they are once more found to be dead, the experiment is at an end. A control is kept to ensure that normal sperms are being used.

It may be asked why the vaginal fluid is not put in first, and the suppository afterwards. The reason is as follows. We wish the suppository to have a definite time (5 min.) in contact with the vaginal fluid before the addition of the semen. Glass is such a poor conductor of heat that if a cool suppository were placed in a warm glass capsule containing warm vaginal fluid, it would be a long and variable time before the proper temperature were regained. In natural conditions the specific heat and conductivity of the living tissues are such that the whole suppository must rapidly attain the temperature of 37° C.

For the diffusion test we do not use semen which has been ejaculated more than 6 hours previously, because it becomes stringy and unlike fresh semen, and gives inconsistent results. The results of the diffusion test are given in Appendix IV.

Detailed description of the diffusion test

(See *Note* to heading on p. 477)

1. Arrange the thermostat and the warm stage for the microscope as for the test of total spermicidal power.

2. Place the following in the thermostat: one damp chamber containing a rack with about half a dozen test-tubes of the standard size, and a metal plate about 4 mm. thick, with a glass capsule exactly 3 cm. in internal diameter standing on it; some graduated and ordinary pipettes; slides and cover-slips; and a bottle of alkaline diluting fluid (see Appendix III).

3. Prepare artificial vaginal fluid (A.V.F.) as follows. To 10 c.c. of beaten egg-white add 1·4 c.c. of "3 per cent" lactic acid (made by adding 3 c.c. of strong lactic acid of specific gravity 1·21 to 100 c.c. of distilled water). Filter. Place in the thermostat to warm up.

4. Place the specimen of semen (at least 1·75 c.c.) in a corked specimen tube in the thermostat and leave to warm up.

5. Divide a suppository longitudinally into four quarters and put one of them into the glass capsule in the damp chamber in the thermostat.

6. Half an hour after § 5, or when the suppository has melted, whichever is later, smear it evenly over the bottom of the capsule with a glass rod. Transfer 0·25 c.c. of A.V.F. to the

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capsule, and 0·1 c.c. of the same fluid to one of the test-tubes in the rack. If a suppository fails to melt, do not leave it for longer than 1 hour before continuing with § 6.

7. Four min. later transfer 0·5 c.c. of semen to the test-tube containing 0·1 c.c. of A.V.F. To economize semen the control is carried out on the scale of one-tenth.

8. Exactly 5 min. after the addition of the A.V.F. to the suppository, squirt 1·25 c.c. of semen into the capsule with a pipette.

9. Set the timer at 0 min.

10. At 1½ min. after § 9, with a pipette take a drop from each of three places in the capsule and transfer them to one of the test-tubes in the damp chamber. Mix by sucking into the pipette and pressing out again twice, and transfer one drop only of the mixture to a warm slide. Cover with a warm cover-slip.

11. At 2 min., examine the slide on the warm stage of the microscope. (There is not time to prepare and examine a control slide in 2 min.) Record the activity of the sperms in the usual way (see § 18 of the alkaline test of (total) spermicidal power).

12. At 4½ min., repeat § 10.

13. At 5 min., repeat § 11. If the sperms show any activity whatever, skip to § 17. If the sperms are immobilized, proceed to § 14.

14. Add to the two remaining drops in the test-tube six drops of warm alkaline diluting fluid, mix, and prepare a slide.

15. Examine and record the activity of the sperms. If the sperms show any activity whatever skip to § 17.

16. If the sperms in § 15 show no activity whatever repeat immediately §§ 12, 13, 14 and 15.

17. With a pipette take a drop from the control tube and transfer it to a warm slide; cover with a warm cover-slip. Examine the slide on the warm stage of the microscope. Record the activity of the sperms.

18. If the control sperms are 2+, 3 or 3+, proceed to § 19. If the control sperms show an activity of 2 or less, discard the whole test.

19. If the experimental sperms showed no activity in § 16, the experiment is at an end.

20. If in § 16 the sperms show any activity whatever, repeat §§ 12 to 19 at 14½, 29½, 59½, 119½, and 179½ min.

21. At the end of the experiment record the pH of the contents of the capsule by adding 10 c.c. of saline and 2 drops of B.D.H. "Universal" indicator.

PATHOLOGICAL TEST

Bitches are used, on Dr H. M. Carleton's recommendation, on account of the fairly close (though not exact) histological resemblance of the vagina to the human. Adult bitches of the size of an ordinary spaniel are the best, and we prefer those which have had young. Rodents are unsuitable on account of the cyclical changes in the vaginal epithelium, and the vagina of the cat is so bent as to make the insertion of a suppository difficult.

The standard dose is one-half a suppository. The latter is divided longitudinally. One half-suppository is inserted into the vagina daily (except Sundays) for a fortnight. The bitch is held in a standing position on a sloping table in such a way that the hind-quarters are slightly higher than the head. Two people are required. One holds the bitch by the collar and by a hand placed below the abdomen. The other damps the vulva with warm saline, spreads it open with two fingers of the left hand, and inserts a glass rod damped

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with saline to clear away any debris. He then inserts the half-suppository wit his fingers. Owing to the configuration of the vestibule, it is necessary t direct the suppository almost vertically upwards for the first 2 or 3 cm. Th glass rod, which is flat ended, is used to push it through the vestibule and int the vagina, which runs slightly downwards anteriorly, so that its vestibula end is its highest part. A cork on the glass rod stops the insertion when th base of the half-suppository is $4\frac{1}{2}$ cm. from the exterior. The rod is held i place for 3 min.; it is then removed and the bitch released.

The bitch is killed by humane killer or chloroform on the day after the las injection. (It has received 12 injections.) The pubic symphysis is sawn throug and the bones forcibly pulled apart. The vagina is cut out, laid open dorsally and examined with the naked eye. It is then fixed in Heidenhain's Sus fixative and embedded in paraffin. Transverse sections about 8μ thick ar made about 1 cm. from the uterine and vestibular ends. The sections, usuall stained in haematoxylin and eosin, are critically examined by Dr Carleton, wh looks especially for sloughing and necrosis of the epithelium, infiltration wit leucocytes, and vascular disturbances. A very small number of leucocytes i the epithelium is not necessarily regarded as pathological.

When tube-jellies and ointments are studied, the product is brought t 37° C. and injected through a rubber or gum-elastic No. 8 male catheter attache to an all-glass syringe. The daily dose is 1 c.c. (approximately half the human)

The fact that the vagina is highest at its vestibular end prevents an considerable outflow of the product. This makes the test very stringent, as doe also the fact that no semen is present to dilute the spermicide.

The vagina of the bitch is much larger than in the human being relatively t their respective body sizes. We think it probable that the surface area of th vagina of an "old breeder" bitch is not much less than half that of a woman To be on the safe (i.e. stringent) side, we use half the human dose on the bitch

Measurement of the *p*H of human semen

By the use of the glass electrode we have calibrated a colorimetric metho of determining the *p*H of semen rather accurately by the "Universal" indicato of the British Drug Houses. We wish to thank Prof. R. Robinson, F.R.S., fo kindly allowing us to do this work in his Department, and Dr G. J. Marriot fo giving valuable advice.

It requires at least 6 c.c. of fluid to cover the glass bulb of the electrod completely, and in order to be able to use most specimens of semen it wa therefore necessary to dilute with saline. We decided always to dilute th semen in the proportion of one volume to four volumes of 0·9 per cent sodiur chloride solution. At the outset we showed that this dilution does no appreciably affect *p*H.

Specimens of diluted semen were changed in *p*H through a wide range b the addition of isotonic solutions of sodium hydroxide or bicarbonate or o lactic acid. Very small additions of these solutions sufficed. When the *p*H of

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diluted specimen of semen, with or without the addition of alkali or acid, had been accurately determined by the glass electrode, we put one drop of "Universal" indicator in a colourless glass specimen tube 12 mm. in internal diameter and added 3 c.c. of the tested sperm suspension. We next compared the colour with Ridgway's standards (Ridgway, 1912). The tube was held for observation with a strip of white paper touching it behind. It was illuminated by daylight from above.

In this way we gradually accumulated the data recorded in Table I.

TABLE I

Plate no. in Ridgway	Colour	Approximate *p*H
XLII	Delft blue	9·5
XLII	Deep bluish grey green	9·0
XXXIII	Terre verte	8·8–8·9
VI	Antique green	8·5–8·6
VI	Meadow green	8·3–8·5
XVIII	*Hay's green*	*8·0–8·3*
VI	Grass green	7·8–7·9
VI	Parrot green	7·6–7·8
V	Yellowish oil green	7·4–7·6
V	Oil yellow	7·0–7·2
IV	Pyrite yellow	6·8–7·0
IV	Sulphine yellow	6·7–6·8
XVI	Yellowish citrine	6·5–6·7
XXX	Olive ochre	6·4–6·5
IV	Aniline yellow	6·0–6·4
XV	Yellow ochre	5·6–6·0
XV	Ochraceous orange	5·1–5·4
XIV	Apricot orange	4·9–5·1
XIV	Rufous	4·6–4·9
I	Strawberry pink	4·3–4·5

The unalkalinized and unacidified human semen specimens had the following *p*H, by glass electrode: 7·4, 7·4, 7·4, 7·5, 7·6, 7·6, 7·6, 7·7, 7·8, 7·8, 7·8, 7·9, 7·9, 8·1, 8·2, 8·4. Mean, *p*H 7·8. Specimens from one man (D) ranged in *p*H from 7·4 to 8·2.

We hope that this colorimetric method of finding the *p*H of human semen will be of use not only to laboratory workers, but also to clinicians. If it were thought that sterility was caused by excessive acidity of the vaginal fluid, it would be instructive to remove the contents of the vagina after coition and determine the *p*H. (If this were done at night, it would be important to use a daylight lamp when noting the colour.) The semen should be diluted with four times its volume of 0·9 per cent sodium chloride solution, and care taken to use a colourless glass tube of the proper diameter.

REPORTS ON SEMEN SPECIMENS

We undertake to report upon semen specimens from two classes of patients in addition to our regular donors. These are (1) men who fear they may be infertile, and (2) men who have reason to think that their sperms may be particularly resistant to spermicides. We report under the following heads: volume (c.c.); millions of sperms per c.c.; activity of sperms; abnormality of sperms; debris in semen; resistance of sperms to spermicides; *p*H; number of

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c.c. of $N/100$ HCl required to neutralize 1 c.c. of semen; and ditto, to acidify to pH 6·7.

The normality or otherwise of the specimens submitted is judged by comparison with our usual donors. Table II brings together all the data obtained from these donors, the means being recorded below.

We find that when a sheath is emptied into a tube, about 0·1 c.c. of semen remains behind in the sheath. In the volumes given in the second column we have allowed for this average loss.

In determining the number of sperms we have used Burbank's (1935) opacity method. We have excluded semen specimens in which many cells were present in addition to the sperms, for the opacity method gives unreliable results with these. We are now finding it convenient to use the haemocytometer method, with Rheinberg chequered squares in the plane of the diaphragm of the microscope eyepiece. (Ruled cover-slips are far less convenient.)

The highest density of sperms that has occurred in our specimens so far is 396 millions per c.c. (by haemocytometer). The total number of sperms in the ejaculate was 1030 millions. This was a specimen from F, who has the highest average number per c.c. of all our donors, but produces specimens of small volume. Presumably the secretion of his accessory glands is less than the usual, while his testes produce more than the usual abundance of sperms.

Table II. *Means of observations on human semen specimens*

(The figures in parentheses represent the numbers of specimens from which the means were calculated)

Donor	Volume c.c.	Millions of sperms per c.c.	Total no. of sperms per ejaculate (millions)	Volume of $N/100$ HCl required to bring 1 c.c. to pH 7·1 (neutrality) c.c.	Volume of $N/100$ HCl required to bring 1 c.c. to pH 6·7 c.c.
A	4·7 (32)	149 (18)	700	2·7 (13)	4·4 (13)
B	4·0 (3)	—	—	—	—
C	5·8 (84)	74 (9)	429	3·4 (8)	5·2 (8)
D	2·6 (36)	107 (7)	278	2·9 (4)	4·6 (4)
E	6·9 (9)	120 (9)	828	2·9 (7)	4·8 (7)
F	3·1 (28)	165 (18)	511	3·1 (9)	4·9 (9)
G	4·1 (5)	114 (4)	467	2·7 (3)	4·2 (3)
H	5·0 (17)	107 (9)	535	3·6 (2)	5·1 (2)
K	4·5 (2)	118 (2)	531	2·7 (2)	3·9 (2)
M	3·5 (27)	152 (13)	532	2·5 (10)	4·4 (10)
P	1·8 (1)	131 (1)	236	3·5 (1)	5·0 (1)
Q	2·0 (1)	146 (2)	292	3·0 (2)	4·6 (2)
R	2·2 (2)	87 (1)	192	4·3 (1)	5·7 (1)
Means	3·9	122	461	3·1	4·7

Summary

1. All tests of spermicidal power are done with human semen at 37° C.

2. The total spermicidal power of pure substances and commercial products is measured by a test which is not intended to represent natural

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conditions very closely, but which gives consistently reliable results. The activity of the sperms is determined at 5 and at 30 min.

3. A commercial product of high total spermicidal power may be useless on account of the slow rate of diffusion of the spermicide out of the vehicle. A special test measures the rate of diffusion. This test is designed to represent natural conditions as well as is possible in the laboratory. The test is made as stringent as possible by avoiding mechanical mixture of the product with the semen. The activity of the sperms is determined at 2, 5, 15, 30, 60, 120, and 180 min.

4. The rate of disintegration and acidity or alkalinity of commercial products is measured.

5. The pathological test consists in inserting one-half the prescribed dose of a commercial product into the vagina of a bitch daily (except Sundays) for a fortnight, and then sectioning the vagina and examining microscopically.

6. A method is described whereby the pH of human semen may be determined colorimetrically rather accurately.

7. The average ejaculate of the human semen we have studied, from 13 donors, measures 3·9 c.c., contains 461 million sperms, and requires 3·1 c.c. of $N/100$ HCl to neutralize it.

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APPENDICES

The data given in the Appendices are intended to show the notation used in recording the results of our tests. During the last year we have performed the alkaline test of spermicidal power 520 times and the diffusion test 50 times, mostly on our own research products.

APPENDIX I

Results of the tests of rate of disintegration and of acidity (or alkalinity)

Name of product	Time of disintegration min.	Reaction	Amount of $N/10$ alkali to neutralize c.c.
Finil (two tablets)	—	Acid	7·7
Prentif	22	Acid	6·5
Prensol	15	Acid	4·9
Rendell's wife's friend	20	Acid	2·4
Semori	—	Acid	1·5
Prorace (chinosol)	14	Acid	0·3
Vimule	10	Acid	0·2
Lactic acid pessaries*	14	Only very faintly acid	—

* Made by a well-known manufacturer.

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Appendix II

Results of alkaline test of (total) spermicidal power

Name of product	S	$S/2$	$S/4$	$S/$
"P. 242"*	—	—	0/0/0	2/1/1, 2,
Prorace (chinosol)	—	0/0/0, 0/0/0, 0/0/0	0/0/0, 0/0/0, 3/3/–	—
Chinobut	—	1/0/0, 0/0/0, 0/0/0	1/0/0, 2/1/1, 0/0/0	—
Prensol	0/0/0†, 0/0/0, 0/0/0	1+/1/–	2+/2+/–†	—
Prentif	0/0/1, 0/0/0	3/3/–	—	—
Rendell's wife's friend	0/0/0, 0/0/0, 1/0/0†	1/1/1	1/0/0	2+/2/–
Bircon	0/0/0, 1/1/0†	2/1/1	2/2/0	—
Milsan (2 g.)	0/0/1, 0/0/2+, 0/0/2†	0/0/2	1/1/1	—
Lam-butt	2/1/1, 1/1+/0	—	—	—
Orthogynol (2 g.)	2+/4/1, 3/3/–†	—	—	—
G.P. soluble	3/3/–, 2+/2+/–, 2+/2+/–†	—	—	—
G.P. ointment (2 g.)	3/3/–, 3/3/–	—	—	—
G.P. jelly (2 g.)	3/3/–, 3/3/–	—	—	—

* A product of our own, not on the market.

† Test with A's sperms.

Appendix III

Formula for making alkaline diluting fluid

Glucose	1·5 g.
Sodium chloride	0·1 g.
$Na_2HPO_4 . 12H_2O$	3·3 g.
KH_2PO_4	0·005 g.
Distilled water	100 c.c.

Filter if moulds grow.

Appendix IV

Results of the diffusion test

	2 min.	5 min.	15 min.	30 min.	1 hour	2 hours	3 hours
"P. 242"	0	0/0, 0/0	—	—	—	—	—
Prensol	0	1/2	0/1	0/1	0/0, 0/0	—	—
Prentif	1+	1/3	2+/3	0/3	0/2+	0/1	0/1
Rendell's wife's friend	3	3	3	3	2+	1	0/0, 0/0
Prorace (chinosol)	3	3	3	3	3	1+	1
Chinobut	3	3	3	3	3	3	1

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