A NEW METHOD OF COMPARISON OF SPERMICIDAL ACTIVITIES

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Summary. A new method of comparison of spermicidal activities of compounds is presented. The method depends on the fact that spermatozoa, when suspended in a sub-lethal concentration of a spermicidal compound, show a reduction in speed of movement. This speed when expressed as a percentage of the speed of the untreated spermatozoa varies with the concentration of spermicidal compound; comparisons of spermicidal activity are made by including a standard material in each test series. The spermicidal activities of a number of substances were compared and the protective effect of seminal plasma on spermatozoa was demonstrated quantitatively.

INTRODUCTION

Previous work has shown that the method of determining spermicidal power due to Baker, Ransom & Tynen (1937), though of great practical value in deciding whether any contraceptive substance reaches a required minimum standard, does not lend itself to the comparison of substances of closely similar spermicidal power. This limitation of the Baker method became apparent when an attempt was made to compare the spermicidal activity of the three octyl cresol condensates having respectively 10·5, twelve and fifteen ethylene oxide units (Harvey & Stuckey, 1962). It was impossible to distinguish quantitatively between the compounds with twelve and fifteen ethylene oxide units, although there was some indication of greater spermicidal activity in the tests with the twelve ethylene oxide compound. The compound with 10·5 ethylene oxide units was possibly more spermicidal than the other two, but the poorer results gave a range that overlapped that of the other two compounds. It was clear that some more refined method of comparing spermicidal power was necessary.

In the course of a study of sperm speeds (Harvey, 1960) it had been found that, during the prolonged incubation of sperm suspensions, not only the percentage motility but also the average speed of the spermatozoa declined as the time of incubation increased. Percentage motility is difficult to measure quantitatively, but the changes in average speed can be determined with accuracy, and as each mean can be based on twenty individual sperm speeds, the differences between one set and another can easily be assessed by a variety of statistical techniques. If, therefore, it could be shown that, in a suspension

of spermatozoa in a sub-lethal concentration of a spermicidal compound, the speed was reduced, and if such reduction compared with a control suspension was proportional to the concentration of spermicide in the test suspension, then the degree of reduction in speed would be a convenient method of assessing the relative spermicidal power of different compounds.

TECHNIQUE

In order to study the effect on spermatozoa of any chosen spermicide, a range of dilutions of the compound in normal saline solution is first prepared. By mixing a drop of diluted spermicide with a drop of semen and examining under a microscope, an approximation is obtained to the highest concentration that does not immobilize spermatozoa in 2 min, and the series of dilutions to be used in the test are made in a range below this point. Usually four or five dilutions are used. The sperm suspensions are made in small glass tubes (2 in. $\times$ $\frac{3}{8}$ in.), one tube being required for each concentration of the spermicide. Each tube contains 0.45 ml of solution, and two control tubes are used, each containing 0.45 ml of saline solution. A further tube contains 0.5 to 1.0 ml of active semen. All the tubes are placed in an incubator at 37°C for 15 to 30 min to reach that temperature.

The method of mixing and speed determination has been previously described (Harvey, 1960). The suspensions are made by adding 0.05 ml of semen to each tube of saline or spermicide solution, giving a mixture of one part semen to nine of suspending fluid. A control suspension is prepared first and the speed of the spermatozoa in this determined immediately. The tubes containing the test solutions are then used in turn, the speed of the spermatozoa in each being determined immediately after mixing. Finally, the second control is used. The average speed of the spermatozoa in each solution of spermicide is expressed as a percentage of the mean speed of the two controls. Occasionally a significant fall in speed occurs in the semen between the beginning and end of the experiment. If this is the case, the fall may be plotted against time, and each of the speeds in the tests may be expressed as a percentage of the estimated speed of the spermatozoa at the time of observation, as read from the graph. In practice, however, even when the difference between the speed in the two control tubes is significant, the results obtained by averaging the speed in the two control suspensions are so close to those obtained by using a graph, that the discrepancy may be ignored. Of course, if the speed in the second control should be so much slower than that in the first that it indicates rapid deterioration of semen motility during incubation, the experiment should be discarded. Such a fall has not been observed in the course of the work here reported.

Text-fig. 1 shows a graph obtained for a non-ionic surface-active agent (octyl cresol condensate with fifteen ethylene oxide units). In the most concentrated of the dilutions, 0.02% before mixing with semen the spermatozoa were dying rapidly and speeds could not be determined. The remaining points show a gradual increase in speed up to 88% of the control, which speed was not significantly different from the control itself.

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Similar experiments with other substances, e.g. phenyl mercuric acetate and hexyl resorcinol, gave similar results. Concentrations greater than those giving a reduction in speed to 50% of the control produce rapid immobilization of the spermatozoa, but with lower concentrations speeds between 60% and 90% of the control are obtained. It is obvious that, for comparisons of the effect of different spermicides on the speed of the spermatozoa, concentrations giving speed reductions between 60% and 80% of the control are required.

No deductions can be made from values obtained in a single experiment with one compound, as the effect of a given concentration of spermicide differs widely with the resistance of the spermatozoa in different samples of semen. With a 0.01% solution of the octyl cresol condensate with fifteen ethylene oxide units, and suspensions of one part semen to nine of solution, speeds varying

TEXT-FIG. 1. Speed-reduction curve for octyl cresol condensate (fifteen ethylene oxide units). The speed of spermatozoa is given as a percentage of the speed in normal saline solution.

from 54% to 82% of the corresponding control have been obtained with different specimens of semen (see Table 1). In the calculation, the lethal concentration of the octyl cresol condensate (fifteen ethylene oxide units) is taken as being 0.06%. It is probably rather higher. The speeds in the standard solution are included to show the variation in speed reduction with different samples of semen.

It follows that in order to compare different substances either the same semen must be used in each case or the different compounds must each be tested against a standard. As changes in the activity and resistance of spermatozoa during incubation make it undesirable to use semen that has been incubated for more than 1½ hr, it is impracticable to test more than two compounds at a time, and reference to a suitable concentration of a standard substance is the more satisfactory method. In the present series, the octyl condensate (containing fifteen ethylene oxide units) at 0.01% in saline solution has been included in all experiments. When the speed-reduction/concentration curve has been plotted for the substance being tested, a point on the curve is found corresponding to the speed reduction of the standard, and from the

concentration corresponding to this point the relative spermicidal activity of the two compounds is calculated. Text-fig. 2 shows the curve for sodium lauryl sulphate.

TABLE 1

CONCENTRATIONS OF SPERMICIDE (METHOD OF BAKER *ET AL.*) REQUIRED TO KILL AS OBSERVED AND AS CALCULATED FROM SPEED REDUCTION TESTS

Compound		Speed in 0.01% octyl cresol condensate (15 units) as % of speed in normal saline	Concentration of test soln. giving same reduction in speed (%)	Calculated concentration lethal in Baker test (%)	Observed concentration lethal in Baker test (%)
Octyl cresol condensate 10.5 ethylene oxide units	1.	82	0.005	0.03	0.06 to 0.03
	2.	64	0.0066	0.04	
12 ethylene oxide units	1.	82	0.007	0.04	0.06 to 0.03
		64	0.008	0.03	
		54	0.008	0.03	
Phenyl mercuric acetate		58	0.00045	0.003	0.0036 to 0.0018
Sodium lauryl sulphate		79	0.014	0.08	0.25 to 0.125
		75	0.02	0.12	
Texafor 60		80	1.0 approx.	6.0	Not killed by 1

TEXT-FIG. 2. Speed-reduction curve for sodium lauryl sulphate. The speed of spermatozoa is given as a percentage of the speed in normal saline solution. The horizontal line shows the mean speed in 0.01% octyl cresol condensate.

From the classical test of spermicidal power (Baker *et al.*, 1937), we know the approximate activity of the different compounds. Table 1 shows these values and the corresponding values calculated from the speed-reduction

experiments, assuming the concentration of the octyl cresol condensate (fifteen ethylene oxide units) which immobilize all spermatozoa in 5 min when mixed with an equal volume of semen to be 0.06%. It will be seen that the two values are compatible, and that where the tests have been repeated the results are consistent.

PROTECTIVE EFFECT OF SEMINAL PLASMA

In the above determinations of sperm speed, the proportion of semen to saline or test solution has been one to nine. In the test of Baker *et al.*, semen and test solution are mixed in equal parts. The proportion of seminal plasma in the suspension has considerable effect on the absolute amount of spermicide

TABLE 2

EFFECT OF VARYING THE PROPORTION OF SEMINAL PLASMA IN THE MEDIUM ON THE SPEED REDUCTION GIVEN BY A STANDARD CONCENTRATION OF SPERMICIDE

Test	Tube under test conditions					Observed speed as:	
	Whole semen (ml)	Sperm-free plasma (ml)	Normal saline (ml)	0.05% spermicide in saline (ml)	Plasma in susp. (%)	Speed (μ/sec)	% of control
A	0.1	—	0.8	0.1	10	no activity	0
B	0.1	0.1	0.7	0.1	20	12	28
Control B	0.1	0.1	0.8	—	20	43	
C	0.1	0.3	0.5	0.1	40	19	45
Control C	0.1	0.3	0.6	—	40	42	
D	0.1	0.5	0.3	0.1	60	22	50
Control D	0.1	0.5	0.4	—	60	44	
E	0.1	0.8	—	0.1	90	21	50
Control E	0.1	0.8	0.1	—	90	39	50

required to immobilize spermatozoa, presumably because of adsorption of the spermicide by the colloids in the plasma or by chemical reaction. Table 2 shows the effect of varying the proportion of plasma in the system on the reduction in speed caused by the same spermicidal solution. The spermicidal power of this solution decreases as the proportion of plasma in the medium increases up to a level of 60% plasma to 40% saline solution. The sperm-free plasma was obtained by centrifuging part of the semen sample, and the quantity of uncentrifuged semen and the final concentration of spermicide was the same in each case.

Two further experiments with plasma at the rate of 10% and 50% of the suspending medium have confirmed the results in Table 2.

In one case, the spermatozoa were immobilized in the suspension containing 10% plasma, but had a speed equal to 50% of the control in that containing 50% plasma. In the other test, with the smaller proportion of plasma, a few

spermatozoa were moving, but their speed was only 20% of the control, while the speed in the suspension with 50% plasma was 75% of the control.

It follows from these results that the proportion of plasma to saline solution must be the same in any tests that are to be compared directly. It was still necessary to discover if the relative spermicidal power was affected by the proportion of plasma in the suspension used in the test.

Suspensions of three octyl cresol condensates were made such that the proportion of plasma was 50% of the volume and the ultimate concentration of the spermicide after adding the whole semen was 0.005%. This concentration of spermicide produced no detectable reduction in speed within 5 min of mixing, but after the sperm suspensions had been incubated for 1 hr, the speeds for the octyl cresol condensates containing 10.5, twelve and fifteen ethylene oxide units were respectively 53%, 66% and 80% of the speed of spermatozoa similarly incubated in a suspension in saline solution. These results give the proportion of spermicide required to give the same result as 0.66, 0.82 and 1.0, values almost identical with those obtained under the routine conditions.

DISCUSSION

The relationship established by these experiments between the average speed of human spermatozoa and the nature of the suspension in which they are moving does not seem to have been previously recorded. The sensitivity of the speed of the spermatozoa to factors in the environment, the precision with which the average speed can be determined, and the ease with which the results can be tested statistically, make this technique an extremely useful one. The small numbers of spermatozoa in most human ejaculates, as compared with the numbers in the ejaculates of experimental animals, makes it impossible to use changes in the rates of fructolysis or impedance-change frequency to assess their reaction, and the determination of speed reduction is a satisfactory alternative to these methods of assessing sperm activity.

REFERENCES

- BAKER, J. R., RANSOM, R. M. & TYNEN, J. (1937) The spermicidal powers of chemical contraceptives. VII. Approved tests. *J. Hygiene*, **37**, 474.
HARVEY, C. (1960) The speed of human spermatozoa and the effect on it of various diluents, with some preliminary observations on clinical materials. *J. Reprod. Fertil.* **1**, 84.
HARVEY, C. & STUCKEY, R. E. (1962) Spermicidal activity of surface-active agents. *J. Reprod. Fertil.* **3**, 124.