Diffusion Times of Spermicidal Compounds. (22962)

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Evaluation of contraceptive materials by observations of resulting pregnancy rates is difficult and time consuming. It is, moreover, uncertain because of unreported omissions or incorrectness of use. For this reason laboratory tests have been developed based on measurements of the power to immobilize sperm in human semen. To approach conditions in the postcoital vagina semen has been placed in contact with contraceptive preparations, and duration of motion of sperm observed (Baker *et al.*, 1, Davidson,3, Gamble,4). Large differences in actions of commercial contraceptive preparations have been observed.

To determine individual activities of chemical compounds used in such preparations, rates of immobilization by one of these tests (Gamble,4) have been measured after incorporation in active jellies. The results of these assays are reported in this paper.

Materials used. Each compound was incorporated into a jelly having a consistency approximating that of commercial contraceptive jellies. Since it seemed possible that jellies of different types would release included compounds at different rates, 2 jelly-forming materials were used, each had, by itself, little or no immobilizing effect. Rice flour in a concentration of 20% water present, was used for all compounds except 15% lactic acid, which required 30% of flour for the desired consistency. Ten % tragacanth was used. *Compounds chosen.* Some compounds were among those in commercial contraceptive mixtures (Gamble,4). Others were: acetic acid which has been advocated for contraceptive use with a tampon; zinc sulfocarbolate, and sodium tartrate, which have been incorporated in contraceptive foam tablets (Gamble,8); hydroquinone (Baker,2); and chlorhydrol and zephiran. *Concentrations tested.* Most compounds were tested in 2 concentrations. One termed "normal", was approximately the highest found in known formulas of commercial contraceptive mixtures. The other, a "high" concentration, was about 10 or more times "normal", or a solution saturated at approximately 23°C. The concentrations used are given as % of water present. The more concentrated mixtures, tested to determine the possibility of increasing spermicidal activity, may well be too concentrated to be tolerated by human tissues, and are not suggested for contraceptive use. *Procedure.* Semen was secured from 6 medical students 22 or 23 years of age, at least 3 days after previous ejaculation. It was kept in an ice bath. Before it was 3 hours old a column of semen 5 mm long (4.8 to 5.2 mm) was placed in a glass tube of 2 mm inside diameter, in contact with a column of jelly 10 or more mm long. The tubes were sealed with paraffin and held in a horizontal position. Motion of sperm was observed through 10X objective and 10X eyepiece. Indirect illumination was by coiled filament of small headlight bulb placed at an angle of about 45° from the axis of microscope. Partial optical correction for the cylindrical surfaces was secured by smearing the specimen tube with immersion oil and placing it between a glass microscope slide and a cover glass. For convenience 2 parallel tubes were used to support the coverglass in horizontal position. Except during observation the tubes were kept in incubator at 37°C. Time was recorded from first contact of semen with jelly to immobilization of last sperm discoverable in the column between jelly and semen-air meniscus. Motion of sperms in semen around the hemispherical meniscus was disregarded. Spermicidal time with a given mixture varied with donor, ejaculate, age of ejaculate, and to a lesser extent with repetition of observation. Immobilization time of each compound was, therefore, measured in each of 2 jellies, with semen from 4 or more donors. The average of all tests with a given compound and jelly is recorded in the accompanying Table, except for

TABLE I. Spermicidal Times of Compounds in Rice or Tragacanth Jellies.

Compound*	Normal concentration %	Normal concentration Rice jelly, min.	Normal concentration Tragacanth jelly, min.	High concentration %	High concentration Rice jelly, min.	High concentration Tragacanth jelly, min.
NaCl				Sat.	31	32
"				30	29	24
"				20	42	40
"	10	110	70			
Sodium tartrate	10			Sat.	46	80
Quinine hydrochloride	5	120	100			
Phenylmercuric acetate				Sat.	100	110
" nitrate	.06	140	130	"	140	70
Trioxymethylene	.15	190	90	3.75	60	44
p-Diisobutylphenoxy-polyethoxyethanol	1.	190	220	12.5	130	170
Acetic acid	1.	230	160	10	80	60
p-Triisopropylphenoxypolyethoxyethanol	1.25	260	190	12.5	80	110
Dextrose	45	300	310			
Chloramine T	2	320	180	12	90	120
Triethanolamine	10.5	330	210	50	100	100
Phenol	.24	360	200	5	90	80
Zinc sulfocarbolate				25	190	190
Chlorocresol	.15	420 + 1	280	Sat.	410	180
Hexylresorcinol				Sat.	420	160
Lactic acid	1.5	420 + 2	400	15	90	190
Methyl p-hydroxybenzoate	Sat.	420 + 2.6	320			
Sulfonated castor oil				Sat.	360	420 + 3.
Polyethylene glycol monoiso octyl phenyl ether	.3	420 + 3.4	140	7.5	410	190
Dodecaethylene	5		420 + 1	†		70
Propyl p-hydroxybenzoate	Sat.	420 + 4	420 + 3			
Duponol C	.2	420 + 4.9	420 + 5	5	420 + 5	290
Zephiran chloride	.2	"	360	2.5	420 + .9	330
Hydroxyquinoline sulfate	.9	420 + 5	280	Sat.	220	210
Hydroquinone	1	"	320	10	80	80
Glyceryl monoricinoleate	1	"	420 + 2.6	10	420 + .2	420 + 2
Hydroxyquinoline benzoate	.9	"	420 + 3	Sat.	290	260
Aluminum acetotartrate	3.125	"	420 + 5	30	180	180
Aminoacetic acid	.1	"	"	10	420 + 5	420 + 5
p-tert amylphenol	.2	"	"	Sat.	"	420 + 3.2
Benzethonium chloride	.2	"	"	5	230	280
Butyl p-hydroxybenzoate	Sat.	"	"			
Cetyl alcohol	1.	"	"	Sat.	420 + 0.5	420 + 5
Cetyl dimethyl benzyl ammonium chloride	.25	"	"	"	420 + 5	420 + 3
Chlorhydrol	10	"	"	100	360	230
Chlorothymol	Sat.	"	"			
Dioctyl sodium sulfosuccinate	.5	"	"	Sat.	"	420 + 1.5
Nonaethylene glycol monolaurate	1.	"	"			
p-Chloro-m-xylenol	Sat.	"	"			
Ricinoleic acid	"	"	"			

* Ranked according to times of normal concentrations in rice jelly.
† Pure compound without water or tragacanth.
The figures following + give mm of semen column in which sperm continued in motion.
Stand. dev. of each time given is approximately 17%.

preparations that showed motility in one or more tests at the end of 7 hours. For these the median value is used. If this exceeded 7 hours, length in mm of semen column still showing motility is also given.

Results. Immobilization times in the Table of more than 50 minutes have been rounded off to nearest 10 minutes. Results are approximately the same with each jelly-forming material. Of comparable averages or medians 35 are shorter with tragacanth, 15 with rice jelly, and 18 are equal. This suggests that

tragacanth may release spermicidal compounds a little more rapidly, or that it may have slight additive spermicidal effect.

Correlation of ranking by 2 materials is significant, being 0.60 for "normal" concentrations, and 0.61 for those that were "high".

Spermicidal times of pure compounds were relatively long, when compared to those of commercial contraceptive mixtures, of which the median time of 87 brands was 226 minutes(Gamble,5). Only 5 of the 35 compounds in contraceptive jellies, creams, or suppositories, had times less than this in "normal" concentration in rice jelly. The greater activity of commercial mixtures may be due in part to the fact that they contain more than one compound having spermicidal activity.

Even when "high" concentrations are considered, however, some of which are many times "normal" strength, the spermicidal time of pure compounds is not much shorter than commercial mixtures. Only 18 of 33 tested in rice jelly were below 226 minutes, the median of commercial mixtures.

The shortest spermicidal time was found with NaCl. In rice jelly containing 10% salt, sperm were immobilized in 110 minutes, and when jelly was saturated, in 31 minutes.

Commercial contraceptive materials containing NaCl had previously been found more active than most(Gamble,6).

Saturated sodium tartrate had relatively low spermicidal time. This is the end product after exposure to moisture of contraceptive which were clinically effective(Gamble,8).

Quinine hydrochloride, 5%, was also active, 120 and 100 minutes in rice and tragacanth. This activity was greater than a suppository, containing 7.74% of quinine, 401 minutes(Gamble,7), perhaps because of greater solubility of the salt.

Paraformaldehyde, which was present in 9 of 87 commercial mixtures previously studied, and phenyl mercuric compounds which occurred in 20 of 87 preparations(Gamble,4) were relatively active.

Dextrose in 45% concentration had median time of 300 minutes in rice jelly and 310 in tragacanth. This was much longer than the corresponding 110 and 70 minutes for 10% NaCl which has about the same osmotic effect, suggesting that the spermicidal action of NaCl is not entirely osmotic.

Less extensive tests with semen from 2 donors showed that saturated KCl, with time of 50 minutes, and saturated sodium sulfate with time of 150 minutes were less active than NaCl or sodium tartrate.

Discussion. In clinical contraception semen is presumably deposited in droplets which are partly or completely surrounded by contraceptive mixture. The time required for immobilization of sperm in the droplets is dependent upon a number of unrelated factors: 1. A higher concentration of immobilizing compound in the cream, jelly or suppository will result in shorter spermicidal time. Concentration is limited by solubility of the compound, and by possibility of damage to tissues exposed to the mixture. 2. A compound which diffuses rapidly from surface of mixture into the contiguous semen will stop motion of sperm more rapidly. Such diffusion is faster for compounds of low molecular weight, and may depend upon the nature of the surface of jelly-forming material. 3. The greater the area of semen exposed to contraceptive mixture, the more rapid is diffusion of the compound into the semen. This is dependent upon unknown size of semen droplets, which is probably independent of the nature of contraceptive mixture. 4. The shorter the distance from surface of semen droplets to center, the more rapidly will the last sperm be immobilized. As with surface area this distance is dependent upon unknown and unreproducible size of semen droplets. 5. The more rapidly an immobilizing compound is transported throughout the droplet, the shorter will be immobilization time. Molecular diffusion will contribute to this, but in droplets of the size to be expected in postcoital semen, diffusion is much less effective than convection currents. This was shown by the finding that when semen was placed above contraceptive jelly in a vertical tube, the zone in which sperm had been immobilized increased with extreme slowness in comparison with the same preparation in a horizontal tube(Gamble,4).

When these factors in clinical action of

contraceptive compounds are considered, it is clear that ranking of presumed clinical activity based upon spermicidal times, is not necessarily that which would be found, could immobilization be observed in a postcoital droplet of semen. The droplets may well be smaller than the column 5 mm long and 2 mm in diameter used in the test, and are exposed to contraceptive material over their entire surface. Rapidly diffusing compounds may, therefore, be more effective clinically than ranking by this test indicates.

As distance to center of droplet is probably less than 5 mm distance from jelly to the most distant sperm, ability to cause convection may be less important in clinical use.

In spite of these unavoidable divergences, our laboratory test appears to provide a useful approximation to postcoital conditions. It is reasonable to conclude that the compounds with a relatively short spermicidal time in concentration which does not cause irritation, are desirable for inclusion in contraceptive mixtures.

Sodium chloride with other compounds. From the above, it was inferred that NaCl in high concentration would produce active convection in the column of semen, and such convection was observed (Gamble.4). It, therefore, seemed possible that spermicidal activity of a compound might be greatly increased if sodium chloride were added to aid in spreading it through the semen column. Tragacanth jellies were, accordingly, prepared containing a "normal" concentration of each of the 15 compounds found most active, together with 10% NaCl, and tested with semen from 2 donors. The average spermicidal time was, in most instances, slightly less than that of 10% NaCl alone. For no substance, however, was the time found with added 10% NaCl as short as that of 20% NaCl. Consequently, the hope of finding an unusually short spermicidal time resulting from including sodium chloride in a jelly with other substances was not fulfilled.

Summary and Conclusions. 1. To estimate comparative contraceptive effectiveness of chemical compounds, under conditions approximating those of clinical contraception, each was incorporated in rice flour or tragacanth jelly. Time to immobilize all sperm in a column of human semen in contact with jelly was then observed. 2. The time of various compounds in commercial contraceptive mixtures, and of others, was compared in this way, both in concentrations used in commercial preparations and in concentrations 10 or more times as great. 3. NaCl had the shortest time observed, probably due in part to convection currents it creates in semen. Time of saturated sodium tartrate was also short. 4. Only a small proporation of compounds in concentrations used in contraceptive mixtures showed times shorter than the median of a series of commercial preparations. 5. Addition of compounds used in contraceptive mixtures to 10% NaCl jellies did not result in times much shorter than that of NaCl alone. From these results it is believed that in contraceptive mixtures greater spermicidal activity is to be expected from compounds of low molecular weight. Diffusion of such compounds from jelly into semen, which must necessarily precede spermicidal activity, will be more rapid than that of heavier compounds. Sodium chloride and sodium tartrate, which are nontoxic, appear desirable compounds for contraceptive mixtures.

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