THE SPERMICIDAL POWERS OF CHEMICAL CONTRACEPTIVES.

V. A COMPARISON OF HUMAN SPERMS WITH THOSE OF THE GUINEA-PIG.

By JOHN R. BAKER, M.A., D.Phil.

(With 2 Figures in the Text.)

CONTENTS.

Introduction.

Most of the experiments described in this series of papers have been carried out with the sperms of the guinea-pig. For many reasons these sperms are very convenient to work with, but there has always been the possibility that human sperms might react differently towards chemicals. It is true that I had found a general agreement between the reactions of the sperms of the two species, but it was felt that a precise investigation of this matter was necessary.

The work has been carried out, as before, in the Department of Zoology and Comparative Anatomy at Oxford, and I once more express my indebtedness to Prof. E. S. Goodrich, F.R.S., and to the Birth Control Investigation Committee.

Recapitulation.

This section is included for those who have not read the second and fourth papers in this series (Baker, 1931 and 1932). In the technique described in those papers, guinea-pig sperms from the epididymis are suspended in a buffered glucose-saline fluid (called B.G.S. for short). The purpose of the technique is to find what is 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 immobilise 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 killing concentration. Each substance is also tried three times at one-half of the killing concentration, and the activity of the sperms is graded. The

https://doi.org/10.1017/S0022172400018271 Published online by Cambridge University Press

highest activity is 3+. Complete absence of any movement is 0. 3, 2+, 2, 1+ and 1 are intermediate grades of activity.

METHOD.

For the comparison of the two sorts of sperms seven different substances were chosen, whose spermicidal powers with guinea-pig sperms in B.G.S. varied from borax, which never kills at 2 per cent., to the most spermicidal substance known, namely toluquinone, which kills every sperm every time at $\frac{1}{512}$ per cent. The seven substances were borax, potassium borotartrate, chinosol, sodium oleate, orthovanillin, hexylresorcinol and toluquinone.

These seven substances were retested, using human semen instead of the guinea-pig sperm suspension in B.G.S.

Only very small changes in the technique were necessary. They were as follows:

Section 13 (introduction of semen into the control tube). The semen is transferred with a pipette. The point of the pipette is then put at the bottom of the tube and the bulb squeezed. This is done three times, to mix the fluids and oxygenate the sperms. Part of the mixed fluid is then sucked into the pipette, and distributed all round the inside of the control tube near its top. In falling down to the bottom of the tube, this mixed fluid catches any semen which may have stuck to the sides of the tube when the semen was transferred. This would not matter much with the control, but with the experimental tube it might upset the results, for the sperms sticking to the side of the tube might be mixed with the others in the process described in section 21. They might then be examined after having only been acted upon by the chemical under investigation for a few minutes instead of half an hour. The contents of the control tube are treated in the same way so as to make sure that they constitute a precise control.

Section 21 (preparation of microscopical slide). Only one drop is transferred to the cavity of the microscopical slide, instead of three. Human sperms are often inactive unless close to a surface. Only the extreme edge of the cavity is therefore examined under the microscope.

Sections 27 *and* 28 (rejection of experiment if control sperms are inactive). The experiment is recorded if the control sperms show an activity of 2+, 3 or 3+. The sperms were not always very active, because many hours had usually to elapse between ejaculation and testing. During this period they were kept cool, but nevertheless their activity became somewhat reduced, and it was not possible to insist on the controls showing an activity of 3 or 3+. In a large proportion of cases it was not possible to determine, without actual counting, whether the majority of the control sperms were moderately active or not. When this doubt exists, the activity is graded as 2+, and such experiments were recorded. When it was clear that the majority were not moderately active, the experiment was unhesitatingly rejected, and the result not recorded.

RESULTS.

The results with human sperms are shown in tabular form in Appendix I. They are given shortly below, with the previously determined figures for guinea-pig sperms placed beside them for comparison.

https://doi.org/10.1017/S0022172400018271 Published online by Cambridge University Press

Killing concentrations.

	Human sperms (in human semen) %	Guinea-pig sperms (in B.G.S.) %
Toluquinone	$\frac{1}{512}$	$\frac{1}{512}$
Hexylresorcinol	$\frac{1}{64}$	$\frac{1}{64}$
Orthovanillin	$\frac{1}{8}$	$\frac{1}{64}$
Sodium oleate	$\frac{1}{2}$	$\frac{1}{32}$
Chinosol	$\frac{1}{2}$	$\frac{1}{4}$
Potassium borotartrate	2	$\frac{1}{4}$
Borax	Fails to kill at 2 %	Fails to kill at 2 %

These figures are expressed graphically in Fig. 1. The heights of the columns show the killing concentrations. The black columns represent the results with human sperms, and the dotted columns those with guinea-pig sperms. It must be noticed that the scale is proportional, not to numbers, but to powers of numbers.

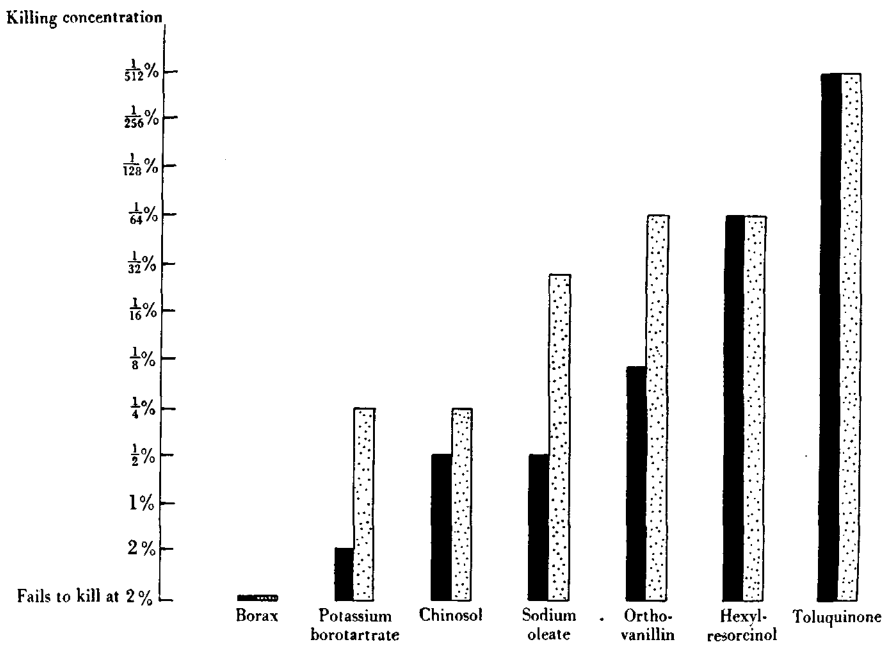

Fig. 1. Killing concentrations with human sperms in human semen (black), and with guinea-pig sperms in B.G.S. (dotted).

It will be observed that while certain substances have exactly the same killing concentration with human as with guinea-pig sperms, others are far less spermicidal with human sperms than with those of the guinea-pig. This applies especially to sodium oleate, which is no less than sixteen times more spermicidal with guinea-pig sperms than with human ones. Orthovanillin and potassium borotartrate are eight times as spermicidal with guinea-pig sperms, and chinosol is twice as spermicidal.

https://doi.org/10.1017/S0022172400018271 Published online by Cambridge University Press

Two explanations present themselves. Either the guinea-pig sperm is inherently different from the human sperm, or the difference lies between B.G.S. and semen as suspension fluids. To test this, a complete investigation was made of the spermicidal powers of the same seven substances with guinea-pig sperms suspended, not in B.G.S., but in human semen. The standard experiment was performed, human semen being substituted for B.G.S. The contents of the tails of two epididymides were suspended in 3 c.c. of human semen. In judging the activity of the sperms, attention was paid only to those of the guinea-pig. The latter are much bigger and quite differently shaped. They were found to be very active in human semen. The human sperms seemed unaffected by their presence.

The results were as follows (see Appendix II). The results with human sperms in human semen, which have already been given, are here repeated for comparison.

Killing concentrations.

	Human sperms (in human semen) %	Guinea-pig sperms (in human semen) %
Toluquinone	$\frac{1}{512}$	$\frac{1}{256}$
Hexylresorcinol	$\frac{1}{64}$	$\frac{1}{64}$
Orthovanillin	$\frac{1}{8}$	$\frac{1}{8}$
Sodium oleate	$\frac{1}{2}$	$\frac{1}{2}$
Chinosol	$\frac{1}{2}$	1
Potassium borotartrate	2	2
Borax	Fails to kill at 2 %	Fails to kill at 2 %

These figures are expressed graphically in Fig. 2. The black columns represent the results with human sperms in human semen, and are of course the same as in Fig. 1. The dotted columns here represent the results with guinea-pig sperms in human semen.

One cannot fail to be struck by the close correspondence between the two columns in each case. Hexylresorcinol, orthovanillin, sodium oleate, potassium borotartrate and borax now show exactly the same killing concentrations with human as with guinea-pig sperms, while toluquinone and chinosol are only twice as spermicidal with one as with the other. The experiment proves two things: (1) that human sperms are affected by widely different chemicals to almost exactly the same degree as guinea-pig sperms; and (2) that there exists a substance in human semen which protects sperms from certain chemicals (especially sodium oleate, orthovanillin and potassium borotartrate).

The close parallel between human sperms and guinea-pig sperms was quite unexpected, and appears very remarkable when one considers that our common ancestor with the guinea-pig must have lived before the Eocene. Throughout the millions of years since then, the susceptibility of sperms to chemicals has not changed, or has changed almost exactly equally in the two lineages. Despite this constancy in susceptibility to chemicals, the sperms have come to differ markedly from one another in size and form.

With regard to the protective action of human semen against sodium

https://doi.org/10.1017/S0022172400018271 Published online by Cambridge University Press

oleate and the other substances mentioned, it is simplest to suppose that these substances are adsorbed by one or more of the constituents of the semen. I was anxious to find whether one could get similar results by adding various proteins to B.G.S., on the assumption that the adsorbing agents might be proteins. 20 c.c. of fresh egg-white was therefore added to 100 c.c. of B.G.S., and this fluid was used instead of ordinary B.G.S. in a series of experiments with sodium oleate. The killing concentration was found to be $\frac{1}{16}$ per cent., instead of $\frac{1}{32}$ per cent., which is its killing concentration with B.G.S. without egg-white. Sperms showed full activity with sodium oleate at $\frac{1}{32}$ per cent. when egg-white was present. The killing concentration was also increased

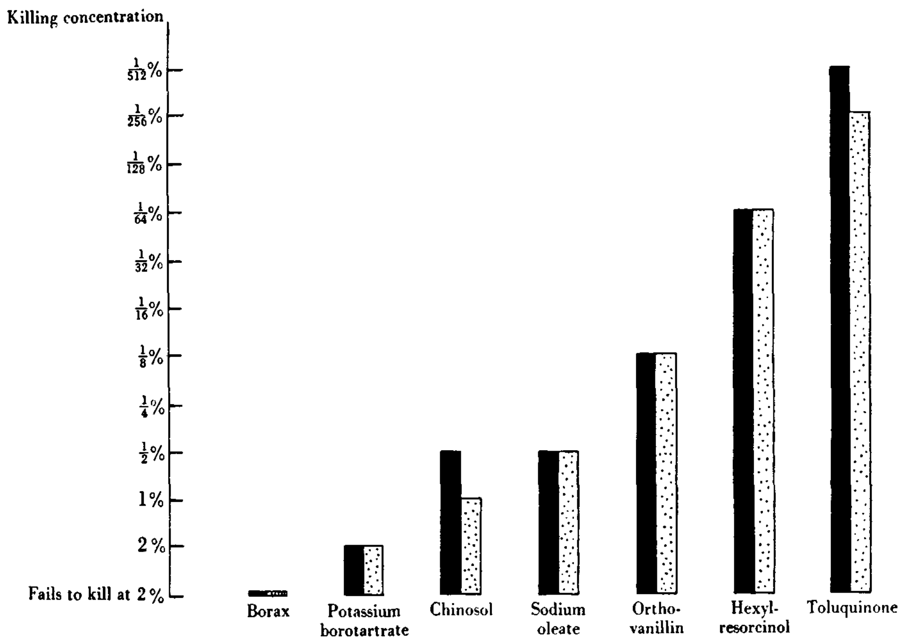

Fig. 2. Killing concentrations with human sperms in human semen (black), and with guinea-pig sperms in human semen (dotted).

when 1 per cent. gelatine in B.G.S. was substituted for B.G.S. Neither gelatine nor egg-white, however, approaches human semen as a protection to sperms. To find whether the mucin in semen was the protective agent, some of the commercial product was dissolved in B.G.S. at $\frac{1}{4}$ per cent. Unfortunately this markedly reduced the activity of the control sperms, and it was therefore impossible to carry out the test.

Since the most diverse substances have the same or nearly the same killing concentrations with guinea-pig sperms in human semen as with human sperms, it appears legitimate to use the former when it is desired to know the spermicidal power of any substance. The test with guinea-pig sperms in human semen is very much more convenient than the test with human sperms. One

https://doi.org/10.1017/S0022172400018271 Published online by Cambridge University Press

can always be sure of getting absolutely fresh guinea-pig sperms, and one's controls are therefore nearly always very active. It is seldom possible to test human sperms immediately after ejaculation. The result is that the controls are often not very active, even though the semen has been kept cool until the time of the experiment; and the experiment has to be rejected. Guinea-pig sperms are highly active when freshly suspended in human semen which has been kept cool for a long time, and the protective properties of the semen are not affected. When human semen is available for test within 3 hours of ejaculation, the test should be performed with human sperms. When a longer period elapses between ejaculation and testing, the semen should be used as a suspension fluid for guinea-pig sperms.

Summary.

1. Human and guinea-pig sperms are susceptible to the most diverse chemicals to almost the same degree.

2. There exists a substance in human semen which protects both human and guinea-pig sperms against certain chemicals, especially sodium oleate, orthovanillin and potassium borotartrate.

3. When perfectly fresh human semen is not available, the spermicidal powers of substances may be tested by using guinea-pig sperms suspended in human semen which has been kept cool.

REFERENCES.

Baker, J. R. (1931). The spermicidal powers of chemical contraceptives. II. *J. Hygiene*, **31**, 189–214.

—— (1932). The spermicidal powers of chemical contraceptives. IV. *Ibid.* **32**, 171.

(*MS. received for publication* 2. iii. 1932.—Ed.)

POSTSCRIPT.

Four more substances have been tested, using guinea-pig sperms suspended in human semen. Quinine bisulphate and the acid hydrochorides of quinidine and cinchonidine all have a killing concentration of ½ per cent. The killing concentration of cinchonidine acid hydrochloride is 1 per cent., but it killed all sperms in three out of four experiments at ½ per cent.

https://doi.org/10.1017/S0022172400018271 Published online by Cambridge University Press

APPENDIX I.

Results of experiments with human sperms (in human semen).

Concentration (%)

	2	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	$\frac{1}{1024}$
Toluquinone	.	.	.	.	.	.	.	.	.	.	0 0 0 0	1 2+ 2+
Hexylresorcinol	.	.	.	.	.	.	.	0 0 0 0	0 0 1	2	.	.
Orthovanillin	.	.	.	.	0 0 0 0	0 0 0 1	1+ 1+ 2	.	.	.	.	.
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 II.

Results of experiments with guinea-pig sperms (in human semen).

Concentration (%)

	2	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$
Toluquinone	.	.	.	.	.	.	.	.	.	0 0 0 0	0 1+ 2
Hexylresorcinol	.	.	.	.	.	.	.	0 0 0 0	0 0 0 1	0 2 2	2
Orthovanillin	.	.	.	.	0 0 0 0	1 1 1	1+	.	.	.	.
Sodium oleate	.	.	0 0 0 0	0 0 1	2	3	.	.	.	.	.
Chinosol	.	0 0 0 0	0 0 1	1+	.	.	.	.	.	.	.
Potassium borotartrate	0 0 0 0	1 1 2	2	.	.	.	.	.	.	.	.
Borax	1+ 1+ 2	.	.	.	.	.	.	.	.	.	.

The figures (0, 1, 1 +, etc.) are explained in the section of this paper headed "Recapitulation."

https://doi.org/10.1017/S0022172400018271 Published online by Cambridge University Press