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THE MOTILITY AND VIABILITY OF RABBIT SPERMATOZOA AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

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A considerable literature has accumulated about the viability of mammalian spermatozoa under various conditions of storage in a great variety of media, but no systematic observations seem to have been made on the effects of varying the pH. Cole, Waletzky & Shackelford (1940) added 1–5% solutions of lactic acid, potassium dihydrogen phosphate or sodium bicarbonate to rabbit semen, keeping it at room temperature for 15 min. to 1 hr. before insemination. They record that no motility was seen outside the pH range 5·0–8·8, and that only slight undulatory non-progressive movements were usually observed outside the range 6·2–7·9. Casida & Murphee (1942) also say that the addition of 5% sodium bicarbonate solution to rabbit semen often immobilizes the spermatozoa. None of these authors records the actual amounts of the various chemicals that were added to the semen, or seems to have considered the possible influence of changes in tonicity. Baker (1931) must have seen the effects of variations in pH when deciding on his final diluting fluid for mammalian spermatozoa, but does not appear to have recorded his observations. His fluid has a pH of about 8·1, and is said to give optimal conditions for survival.

It is generally agreed that ejaculated spermatozoa are less viable in vitro than those taken directly from the epididymis or vas deferens. In the rabbit, this is confirmed by a comparison of the results of Hammond (1930) and Walton (1930). In Hammond's experiments, semen collected from the vagina after coitus was kept in stoppered tubes for varying periods prior to reinsemination, and in Walton's experiments spermatozoa from the vas deferens were collected under paraffin, and placed in tubes with paraffin seals. Under these fairly comparable conditions (the tests were meant by the authors to be comparable with one another) the fertilizing capacity of Walton's collections was retained considerably longer at low temperatures than was that of Hammond's, but the difference disappeared with storage at about 35–37° C. In view of this,

and for convenience in working, it was decided that rabbit ejaculates shielded from too free a gaseous interchange, and kept at room temperature, would suffice for the present studies. From Hammond's figures, it is to be expected that, under such conditions, undiluted semen should retain 50% motility for between 1 and 2 days and lose practically all motility within 4 or 5 days. Hammond found that the fertilizing capacity of semen collections ran parallel with their motility.

MATERIAL AND METHODS

Selection of ejaculates. Semen was collected from rabbits of various breeds with an artificial vagina and the jelly-like fraction, when present, was rejected. Specimens showing an obviously low sperm count or poor motility were also rejected, and thus the results reported below are not obtained from a random sample of ejaculates, but represent the best 50% or so of the several ejaculates usually obtained for test on any one occasion. It was necessary to make these rejections because only a good specimen has sufficient initial motility for the precise detection of the influences which tend to impair the movement of spermatozoa, and provides a sufficient density of cells for making counts in stained preparations at the dilutions used.

Diluents. Very soon after collection (always within 30 min.) the semen was partitioned between various buffered dextrose solutions for observations on viability. These were made in sterile 2 c.c. flat-bottomed glass tubes, each with a second slightly larger tube as a loose cover. As a standard procedure, either 0·05 or 0·1 c.c. of semen, well mixed by gentle pipetting up and down, was placed in 1·5 c.c. of the diluent and again gently mixed by means of a pipette. The resulting suspensions were at a density of from 10 to 60 millions/c.c. The volume of semen used depended on the original volume of the ejaculate, as observations in any one replication of a test were made with a single sample of semen. The diluents all contained 3% of dextrose, 0·2–0·4% of sodium chloride, and sufficient buffer to bring them to a tonicity between that of 0·9 and 1·0% sodium chloride. Preliminary tests of the effect of varying the tonicity of Baker's solution showed that, if anything, a slight increase in the sodium chloride content was beneficial to rabbit spermatozoa. The buffers used were: (*a*) sodium mono- and dihydrogen phosphate mixtures; (*b*) glycine acetate phosphate buffer (Northrop & de Kruif, 1921–2); (*c*) Sørensen glycine (glycocoll) buffer (as given in Clark, 1920); (*d*) Sørensen borate buffer (Clark, 1920); (*e*) sodium carbonate and bicarbonate mixtures. The pH of buffers or semen-buffer mixtures was determined with the B.D.H. capillator outfit, sometimes checked by a reading with the glass electrode.

Motility index. For the determination of motility, a drop of spermatozoal suspension was placed on a glass slide and examined under the microscope within a few minutes.

The usual method of scoring the activity of spermatozoa is to assess the percentage motility. This may be done by eye, or by more elaborate techniques by which the number of immotile spermatozoa is counted at a standard dilution, the specimen killed entirely, and then a full count made. None of these methods seemed adequate for the present purpose, as a specimen may continue to give approximately the same count of immotile cells over a long period, while those remaining motile are in fact becoming progressively less active. Occasionally a semen specimen exhibits a strong contrast between a number of immotile and apparently dead cells and a residue of actively motile cells, even after long storage, but the far commoner picture is of a general damping-down of activity culminating in a high percentage of cells with vibratile tails and stationary heads. In view of this, the following scheme (p. 473) was substituted in order to assess motility. These were found to be grades of activity which are readily carried in the mind and can be applied with little indecision to any specimen with which the observer is confronted, even in the absence of an opportunity for direct comparison. Moreover, it rarely happens that a specimen does not fall into one of the grades listed. When a series of tubes exhibits various grades of activity simultaneously, it is possible, but of doubtful general use, to interpolate quarter-grades to indicate differences between specimens showing very similar activity.

Index of motility	State of spermatozoa
4	Full activity, but there may be up to 30 or 40% of dead cells as in the fresh ejaculate
3½	A detectable damping of activity compared with 4
3	Sluggish, rate of motion about two-thirds that of 4
2½	Most cells progressing but many stationary with tails vibrating
2	Most cells stationary, many with tails vibrating
1½	Many motionless, none or almost none actively progressing
1	Hardly any motility, only tails moving (very rarely an occasional cell in actual motion)
½	Only a few cells per field showing any movement
0	Completely motionless

Staining methods. Lasley, Easley & McKenzie (1942) describe a method of staining semen smears which results in the living cells remaining unstained against a stained background of seminal debris, while the dead cells stain with varying degrees of intensity. These authors noted that several stains could be used, but the best results were obtained with a mixture of water-soluble eosin and opal blue. In the present investigation, it was found that eosin, although giving apparently excellent results, is unsuitable unless the slides are examined immediately, as within a few hours the once-living spermatozoa are stained by diffusion of the dye from the surrounding material. Equally good and more permanent results were obtained with a saturated aqueous solution of Congo red or a 1% aqueous solution of aniline blue.

A droplet of neat or diluted semen is placed on a clean slide and mixed with an approximately equal quantity of stain by means of a glass rod. The mixture is smeared immediately over the surface of the slide and dried rapidly but very gently, high over a flame, care being taken not to heat the slide appreciably. The whole process is complete within a minute. Slides may be examined without further preparation under the oil immersion lens, or may be rendered permanent by the application of a drop of Canada balsam and a cover-slip. If not made permanent, the preparation does not keep for more than a few days, as the smear is an excellent medium for the growth of bacteria and moulds.

All results reported here were obtained with Congo red, which is generally to be preferred to aniline blue because it is easier to see the unstained spermatozoa when this dye is used. Aniline blue gives a better staining of dead cells, but does not colour the background sufficiently well for one to be sure that unstained cells are not missed in making counts. That the stain does in fact differentiate between living and dead spermatozoa was checked by making smears of suspensions killed by heat. Immediately after being heated to 56° C. for a few minutes all cells in fresh suspensions of active spermatozoa are stained, whereas, before heating, the suspensions show a high percentage of living (unstained) cells. The reliability of the method is discussed in detail below.

RESULTS

The stability of buffered suspensions. The mean pH of 24 samples of rabbit semen was 7·6, with a standard deviation of 0·32. The extremes were 7·0 and 8·2. It was to be anticipated that a pH range covering perhaps 5·0–9·0, or even more, would be necessary for the exploration of the effects of hydrogen-ion concentration on viability. With this in mind, early trials were made with the glycine buffers, but both proved unstable at high pH levels. The systems eventually employed as a routine were (*a*) phosphate buffers for the pH range 5·0–8·0 approximately, and (*b*) bicarbonate-carbonate buffers for the range

8·9–11·0 approximately. The apparent gap between pH 8·0 and 8·9 was in practice bridged by the buffering action of normal semen, which sometimes shifted the pH of the carbonate system to as low as 8·1 or 8·2. These latter buffers proved more stable than others and did not exhibit any signs of toxicity (as did, for instance, borate systems). The greater relative stability of the carbonate system is shown in Table 1.

TABLE 1. Stability of buffered alkaline suspensions of rabbit spermatozoa

Buffer	Intended pH	Actual pH of suspension on day 1	2	3	No. of samples
Glycine-acetate-phosphate	7·5	7·4–7·6	7·3–7·6	6·6–7·1	4
	10·0	8·8–9·6	8·7–8·8	7·4–8·3	4
Carbonate-bicarbonate	8·9	8·2–8·6	8·1–8·4	7·6–7·9	3
	9·6	9·7–9·8	9·0–9·8	8·8–9·5	7

Even with the most stable buffers, some tests had to be rejected because changes in pH were too wide to be acceptable. It was rare for such changes to occur within the first 12 hr., and equally rare for them not to occur within 3 days. Fortunately, much of the later work involved measurements of motility within the first few hours only, and thus pH changes were not a trouble. Most of the work recorded here, however, covers test periods of up to 48 hr., and the rejection of tubes in which the pH did not remain reasonably constant has so upset the balance of tests that it has been impossible to analyse the results in as efficient a way as was hoped. It has not proved possible, for instance, to eliminate differences between ejaculates in estimating the effects of pH on motility or survival rate.

Effect on motility. Assessments of motility were at first made at varying intervals after the start of a test. It was soon found, however, that observations at ¼–½, 1–1½, 2½–3, 4–4½ and 5½–6 hr. were adequate for differentiating the effects of pH when the range studied was wide. Further observations were made at 24 and 48 hr. when necessary. Table 2 is an example of the scoring of a test.

TABLE 2. Motility indices from one replication of a test of the effects of pH on the activity of rabbit spermatozoa

	Buffer			
	Baker's solution	Glycine-acetate-phosphate		
pH of suspension at start	7·3	5·3	7·4	8·8
Score at: 0·5 hr.	4·0	0·0	4·0	3·5
1·5 ,,	4·0	0·0	4·0	3·5
3·0 ,,	4·0	0·0	4·0	3·5
4·2 ,,	3·5	0·0	3·5	3·0
5·5 ,,	3·5	0·0	3·0	2·5
24·0 ,,	2·5	0·0	2·5	1·0
48·0 ,,	1·5	0·0	2·0	1·0
pH of suspension at 48 hr.	7·2	5·8	7·1	8·3

The suitability of different buffering systems was assessed (in addition to considerations of their stability) by adding the total motility scores for the first 6 hr. and comparing that for each buffer with the results from control tubes containing Baker's solution. The results, taken only from tubes in which the pH did not change by more than 0·2 over the 6 hr. are shown in Table 3. Some later results with carbonate buffers have the phosphate system as control, it being virtually equivalent to Baker's solution. Table 3 lists only the preliminary tests with various buffer systems. When the influence of pH as

TABLE 3. Total motility indices with different buffers during the first 6 hr. of a test

Test no.	Baker's solution (pH of suspension 7·3–7·9)	Buffering agent	pH of suspension 6·4–6·5	7·1–7·9	8·5–9·0	9·6–9·8
1	11·0	Glycine-acetate-phosphate	7·0	16·5	14·0	—
2	15·5	Glycine-acetate-phosphate	9·5	15·5	15·0	14·5
3	12·5	Glycine-acetate-phosphate	—	12·0	14·0	—
		Glycine (Sørensen)	—	—	12·5	7·5
4	19·0	Glycine-acetate-phosphate	—	18·5	16·0	—
5	11·0	Glycine-acetate-phosphate	—	10·0	8·0	—
6	19·5	Bicarbonate-carbonate	—	—	18·5	17·5
7	—	Phosphate	11·5	20·0	—	—
		Bicarbonate-carbonate	—	—	17·0	15·0
8	—	Phosphate	12·0	17·5	—	—
		Bicarbonate-carbonate	—	—	17·0	13·8
9	—	Phosphate	12·3	18·3	—	—
		Bicarbonate-carbonate	—	—	15·5	13·8

observed within the same buffering system is taken into account, it will be seen that these buffers are about equally suitable for use. The differences between tests (in which different ejaculates were used on different days) are large. At a pH of less than about 5·8, no motility was seen, although revival of the spermatozoa was possible (see below). On the other hand, the spermatozoa can clearly tolerate a considerable degree of alkalinity.

Table 4 gives the mean motility index at different times after the start of the test, together with the standard error of that mean, for various hydrogen-ion concentrations.

TABLE 4. The effect of pH on the motility of rabbit spermatozoa in vitro

pH of suspensions	No. of tubes	Index of motility at hours 0·5	2·5	6	24	48
Up to 5·8	8	0·0	0·0	0·0	0·0	0·0
6·4–6·5	3	3·7±0·33	2·5±0·27	1·0±0·0	0·3±0·17	0·0
7·2–7·9	10	4·0±0·0	3·5±0·17	2·6±0·16	1·2±0·44	0·6±0·28
8·5–8·9	5	3·5±0·27	2·8±0·37	1·7±0·46	0·7±0·30	0·2±0·20
9·7–9·8	6	3·5±0·26	2·4±0·48	1·2±0·18	0·0	0·0
10·2 approx.	5	3·5±0·25	1·8±0·12	0·6±0·40	0·0	0·0

With the restriction that only suspensions in which the pH did not change by more than 0·5 in the course of 2 days are included (it was in fact nearly always

within 0·3 of its initial value), the data in Table 4 comprise all results obtained with the glycine, phosphate and bicarbonate-carbonate systems, prior to those to be reported in a further communication dealing with other variables.

The mean motility indices for pH 7·2–10·2 are also shown in Fig. 1 (those for pH 6·4 are omitted for clarity), and have been fitted, by eye, by a series of smooth curves. The standard error of each point is that applicable to the group of ejaculates with which the tests were made—ejaculates with an initial motility index of 4·0—and would be wider if entirely random samples had been made. However, the object of the tests was not the determination of the extent of variation in a normal population, but the effect of pH on active and fully-motile spermatozoa.

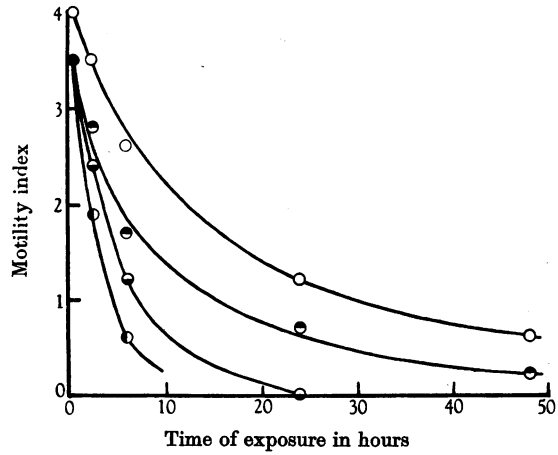

Fig. 1. Mean indices of motility of suspensions of rabbit spermatozoa after exposure to different hydrogen-ion concentrations (alkaline pH's). ○—○ pH 7·5; ◓—◓ pH 8·7; ◒—◒ pH 9·7; ◐—◐ pH 10·2.

Spermatozoa which are completely immotile are not necessarily dead. At pH 4·4–4·6, no motility is seen, but on adding excess of Baker's solution the spermatozoa are seen to revive very considerably unless they have been in the acid buffer for more than about 1 hr. A longer period during which revival is possible is found with progressively higher levels of pH. Above pH 6·5, little or no additional activity is seen when moribund suspensions are diluted with Baker's solution or with other buffered media designed to bring the pH to near 7·5; even above pH 9·8, revival is not particularly evident, but may be seen for up to about 6 hr. at pH 10·0 approximately. Sufficiently high degrees of alkalinity to inactivate the spermatozoa immediately have not been investigated, largely because of difficulties in finding a suitable buffer. In Table 5, the data concerning this phenomenon are summarized.

TABLE 5. The revival of rabbit spermatozoa after suspension in vitro at various hydrogen-ion concentrations

pH of suspension	Time of exposure (hr.)	Motility indices: Initial	Motility indices: After added Baker's solution	No. of samples examined
4·4–4·6	Up to 0·75	0	2–3	2
	1·25	—	0	—
5·3–5·4	Up to 1·0	0	2·5–3	2
	1·5	—	1–1·5	—
	3·0	—	0	—
5·5–5·8*	Up to 1·25	0	2·5–3	2
	3·0	—	1	—
	4·0	—	0·5	—
	5·0	—	0	—
6·4–6·5	4–6	1	1·5–2·5	5
	24	0	0·0–1·0	—
Above 7·0	At any time	—	Increases of 0·0–0·5, with Baker's solution or other buffers	—

* The specimen immotile at pH 5·8 would appear to be exceptional, as in subsequent work other ejaculates have retained some degree of motility for a few hours at this pH.

Effect on mortality. Smears from each tube under test were taken at approximately 0·5, 2·5, 6, 24 and 48 hr., or at 1·5 and 3·5 instead of 2·5 hr., in the case of the more acid suspensions. In addition, one or more smears were made from each fresh sample of semen before dilution. One hundred cells were counted on each slide, and the count was sometimes duplicated from the same slide or from a different slide made at the same time, as a check on technique. The variance of such counts can be partitioned in theory into two parts; the first is that associated with differences between samples, the second is that associated with random sampling. The second should, if the technique is entirely satisfactory, be determinable from the variance of the binomial distribution.

In the present series, technique was not entirely satisfactory. The mean variance for duplicate counts from the same slide was approximately 2·8 times that expected from the binomial distribution, while the mean variance for counts on different slides made at the same time was approximately 3·1 times that expected from theory. This indicates that the fault lies in the preparation or examination of the slides, different regions of one slide tending to be as variable as regions taken from entirely different slides. No significant improvement occurred with practice or after slight modifications in technique, and as the standard errors concerned were, although larger than expected, small enough to be unimportant in comparison with differences between ejaculates, no further steps were taken. Thus, the expected variance of counts of 100 cells at a mean of 72·5% alive is 19·9, the observed variance from duplicate counts was 62, while the variance between the ten tubes which gave this mean was 1165. These were ten suspensions of different ejaculates at the same pH, 24 hr. after the start of the test. Similar figures were obtained with all tests.

The estimated percentage of living (unstained) spermatozoa in the original ejaculate varied between 59 and 90%. The percentages estimated from samples taken from dilutions at various subsequent times were corrected so that the initial percentage living was equated to 100. The results are shown in Table 6, which lists the same series of tests as in Table 4. The standard error of each mean percentage of living cells follows that mean.

TABLE 6. The effect of pH on the mortality of rabbit spermatozoa in vitro. All percentages are adjusted to an initial percentage of 100 in the fresh ejaculates

pH of suspensions	No. of tubes	Percentage of living spermatozoa at hours						
		0·5	1·5	2·5	3·5	6	24	48
4·4–4·6	4	41±3	11±2	—	6±2	1±0·6	0	0
5·2–5·8	4	97±3	91±5	—	14±3	5±2	1±0·5	0
6·4–6·5	3	77±12	—	74±3	—	40±18	11±0·3	—
7·2–7·9	10	95±4	—	95±6	—	79±8	73±11	26±9
8·5–8·9	5	78±9	—	74±7	—	81±12	49±17	7±6
9·7–9·8	6	84±10	—	72±6	—	54±3	17±10	0·3±0·9
10·2 approx.	5	75±9	—	65±8	—	49±17	8±5	0

It will be noted that these means tend to be particularly variable at and after 6 hr. for pH levels above 6·4—a reflexion of the differences between ejaculates, which become more apparent later in the tests, and which cannot be systematically examined in the analysis owing to factors already discussed. However, as Figs. 2 and 3 demonstrate, the general picture is quite clear. In acid suspensions, the spermatozoa die rapidly. In alkaline suspensions they survive—just as they remained motile—considerably longer. Half the spermatozoa originally alive are killed at approximately the following times at various pH levels:

pH	4·5	5·5	6·5	7·5	8·7	9·7	10·2 approx.
50% mortality (hr.)	0·5	2·5	4·5	32	21	7·5	5·5

Spermatozoa in the original counts summarized in Table 6 were classed under four headings: (*a*) unstained, (*b*) head only stained, (*c*) tail only stained, and (*d*) both head and tail stained. Only those in class (*a*) have been scored as alive. Few cells show head staining alone, but it was observed that, as the cells die, their tails usually stain first, followed by the heads. This occurs independently of pH; a typical series from one test is shown in Table 7.

TABLE 7. An illustration of tail staining in spermatozoa as death occurs

Time after start of test (hr.)	pH 4·4				pH 7·8				pH 9·0			
	U	H	T	B	U	H	T	B	U	H	T	B
1½	12	4	30	54	—	—	—	—	—	—	—	—
3½	3	3	16	78	64	10	7	19	77	12	9	2
6	1	3	12	84	47	14	23	16	38	8	16	38
24	0	2	0	98	40	5	31	24	9	1	50	40

U = unstained; H = head stained; T = tail stained; B = both stained.

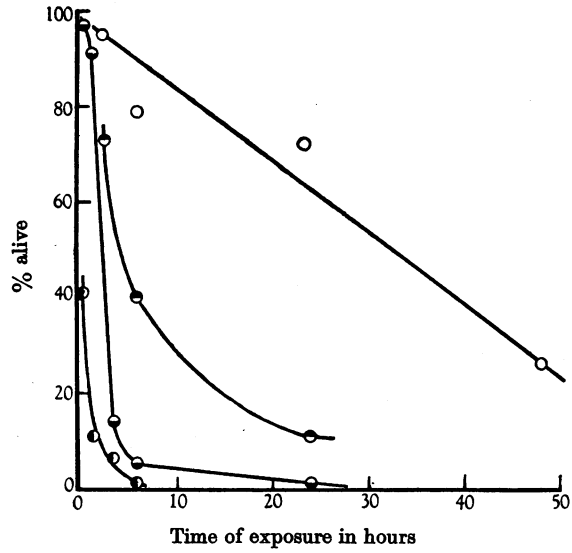

Fig. 2. Mean percentages of living rabbit spermatozoa after exposure to different hydrogen-ion concentrations (acid pH's). (Initial % living = 100.) O—O pH 7·5; ◓—◓ pH 6·5; ◒—◒ pH 5·5; ◐—◐ pH 4·5.

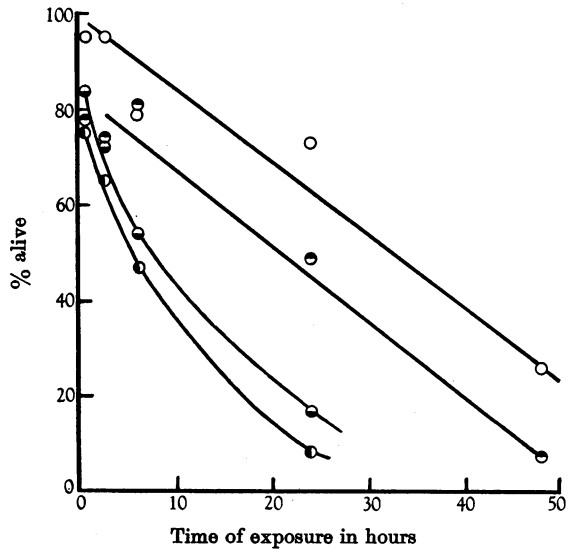

Fig. 3. Mean percentages of living rabbit spermatozoa after exposure to different hydrogen-ion concentrations (alkaline pH's). (Initial % living = 100.) O—O pH 7·5; ◓—◓ pH 8·7; ◒—◒ pH 9·7; ◐—◐ pH 10·2 approximately.

No increase in head staining occurs in this example and the trend of events is clearly death of the tail followed by death of the head. This is to be expected, as the cytoplasm of the tail is both furthest removed from the nucleus and most effectively exposed to the surrounding medium.

Relationship between motility and viability. The absence of movement does not mean that a spermatozoon is dead. Thus, spermatozoa at pH 5·5 are immotile, but may be revived if they have not been too long in acid. On the other hand, the data indicate that this immotility is soon followed by death. At pH 4·4–4·6, the cells cannot be revived by the addition of excess of Baker's solution after about 1 hr., at which time 80% stain with Congo red. At pH 5·2–5·8, they cannot be revived after about 3–4 hr., at which time they are again nearly all dead by the staining criterion. In more neutral or alkaline suspensions, revival on the addition of Baker's solution or other more acid buffered dextrose solutions is less striking or even absent, and under these conditions motility continues more or less parallel with the percentage remaining alive. A motility index of unity would appear to correspond roughly with a 50% mortality, the actual estimated mortalities at this level being:

pH	6·5	7·5	8·7	9·7	10·2 approx.
% dead at unit motility index	60	45	41	49	47

This approximate relationship is independent of pH between the limits shown.

It will be noted, however, that a motility index of unity implies no, or practically no, progressive motion on the part of the spermatozoa—those not yet dead are in process of dying. Suspensions still showing active movement, with an index of 2·5 have, however, suffered fair mortality in addition to that already observed in the fresh semen:

pH	6·5	7·5	8·7	9·7	10·2 approx.
% dead at a motility index of 2·5	26	13	21	28	32

In scoring motility we are, therefore, employing a sensitive index covering the mortality range up to about 50% in suspensions above pH 6. Beyond this, the motility index is relatively insensitive, and in suspensions below about pH 6 it is useless.

SUMMARY

1. The motility and survival rate of rabbit spermatozoa suspended at room temperature in buffered dextrose solutions has been studied at various levels of pH between 4·4 and 10·2. Suspensions were made only from active ejaculates with high counts, and were at approximate concentrations of between 10 and 60 million cells per c.c. Various buffers were used, but the proportion of dextrose was kept constant at 3% and the tonicity at between that of 0·9 and 1·0% sodium chloride.

2. Motility was scored by means of an arbitrary scale in which fully active specimens were given a rating of 4. The percentage of living spermatozoa was

estimated from smears stained with Congo red, in which cells alive at the time of making the smear remain unstained. Standard errors of estimate are given in the text.

3. Rabbit semen has a pH of between 7·0 and 8·2; the mean of 24 samples was 7·6. The spermatozoa are much more sensitive to acidity than to alkalinity. Below a pH of about 5·8 they are immotile and die rapidly, whereas at a pH of 9·5–10·0 they remain motile and survive for several hours, although with depressed motility. A motility index of unity corresponds roughly with a 50% mortality at pH levels between 6·5 and 10·0. The mean times at which unit index was reached and at which 50% of cells originally alive had died were:

pH	Index of unity (hr.)	50% mortality (hr.)
4·4–4·6	Immotile	0·5
5·2–5·8	Immotile	2·5
6·4–6·5	6	4·5
7·2–7·9	29	32
8·5–8·9	15	21
9·7–9·8	7	7·5
10·2 approx.	4·5	5·5

It should be noted, however, that unit motility index implies that hardly any activity is seen in the suspension, most cells being motionless, the rest moving their tails feebly.

4. Spermatozoa rendered immotile by acidity may be revived by the addition of excess of Baker's solution (pH 8·1 approximately) if they have not been too long at an adverse pH. The times at which they may be so revived correlate well with the mortality data, and no revival was seen where the death rate, as estimated from smears, exceeded 80%. Little or no revival is seen on adding Baker's solution or other more acid media to alkaline suspensions, in which the spermatozoa remain motile (if feebly) until dead or moribund.

5. As death occurs, at all pH levels, the tails of the spermatozoa tend to stain before the heads.

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