THE INTERACTION OF pH, TONICITY, AND ELECTROLYTE CONCENTRATION ON THE MOTILITY OF FOWL SPERMATOZOA

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Summary

The motility of fowl spermatozoa has been studied *in vitro* under various modifications of pH, osmotic pressure, and chemical composition of diluents. The glucose and sodium chloride content of the diluents has been varied to give tonicities ranging between that of 0·45 and 1·8 per cent. sodium chloride. These diluents were buffered with citric acid–disodium phosphate, sodium phosphates, or sodium carbonate–bicarbonate mixtures which were equally innocuous.

Fowl spermatozoa showed maximum motility at pH 7 and the pH of fowl semen was $7{\cdot}6 \pm 0{\cdot}42$. At a pH of 8·7–8·9 partial replacement of sodium chloride by glucose was favourable in hypotonic, isotonic, and hypertonic diluents.

Under all conditions hypotonic diluents were more deleterious than hypertonic ones of similar composition and the spermatozoa were able to partially adapt themselves to extreme osmotic pressures.

I. INTRODUCTION

Hydrogen ion concentration and tonicity are two of the most important factors influencing the survival of mammalian spermatozoa; the effect of electrolyte, in the form of sodium chloride, has, however, most probably been overrated by early workers.

The first systematic studies of all three factors was made by Emmens (1947, 1948) who observed the motility of rabbit spermatozoa at various pH levels with diluents of different tonicity and chemical composition. The optimal pH for motility of rabbit spermatozoa was found to be about 7 but they were at least partially motile between pH 5 and 10. Both hypotonic and hypertonic diluents were deleterious at all pH levels but the relative effects of hypo- and hypertonicity varied with pH. Thus in acid or neutral solutions the spermatozoa were more susceptible to hypothan to hypertonicity, but in alkaline media the situation was reversed. Contrary to what might have been expected from the early literature (see Anderson 1945) motility was not significantly affected by changes in the proportion of sodium chloride and glucose in buffered diluents of the same tonicity, except above pH 9.

More recently these studies have been extended to ram, bull, and human spermatozoa (Blackshaw and Emmens 1951). Ram and bull spermatozoa showed optimal motility at about pH 7 and human spermatozoa at about pH 8·5; the spermatozoa of all three species were at least partially motile within a pH range of 5–10 and bull spermatozoa were not completely immobilized even at pH 4·4. In these latter species hypertonic solutions were less harmful than hypotonic media at all pH levels and, furthermore, the relatively slight adverse effect of hypertonicity could be diminished by partial replacement of sodium chloride by glucose at alkaline pH's.

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There appears to be an almost complete lack of information on the effect of these factors on fowl spermatozoa, and it is the purpose of this paper to present similar studies using the spermatozoa of this species.

II. Material and Methods

Fowl semen was obtained by abdominal massage (Burrows and Quinn 1939). Only apparently normal specimens of good initial motility were employed, usually pooled with at least one other ejaculate.

The diluents used were glucose and sodium chloride mixtures buffered with (*a*) citric acid and disodium phosphate, (*b*) mono- and disodium phosphates, or (*c*) sodium bicarbonate–carbonate. The tables of Kolthoff and Rosenblum (1937) and

Table 1
COMPOSITION OF DILUENTS

Diluent	Relative Tonicity (0·9% NaCl = 100)	Glucose (%)	Added NaCl (expressed as %) at Nominal pH:				
			4·0	5·5	7·0	8·5	10·0
A	50	2·00	0·00	0·00	0·00	0·00	0·00
B	100	2·00	0·46	0·46	0·42	0·46	0·43
C	150	2·00	0·91	0·91	0·87	0·91	0·88
D	200	2·00	1·36	1·36	1·32	1·36	1·33
E	50	1·00	0·17	0·17	0·13	0·17	0·14
F	100	3·75	0·17	0·17	0·13	0·17	0·14
G	150	6·55	0·17	0·17	0·13	0·17	0·14
H	200	9·30	0·17	0·17	0·13	0·17	0·14

Vogel (1943) were used as a guide in preparing the buffers, the proportions of the constituents—which were all at a final concentration of 0·02M—being carefully adjusted to give the desired pH. Details of the composition of the diluents are given in Table 1, the relative tonicity being calculated on the assumption that all electrolytes were completely dissociated. Due to dissociation into varying numbers of ions the tonicity of the buffer mixtures varied slightly at different pH levels and this was compensated by adjusting the sodium chloride concentration of the diluent. All diluents were stored in a deep-freeze and the pH checked with a glass electrode meter before and after each experiment. The actual pH of suspensions sometimes differed slightly from the intended pH, particularly towards the end of some experiments, and the extent of this variation is shown in the tables by the standard deviation.

Semen was diluted 1 in 20 in small tubes and kept at room temperature. 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. Motility was scored by the system of Emmens (1947) at $\frac{1}{2}$, $1\frac{1}{2}$, $2\frac{1}{2}$, 4, and $5\frac{1}{2}$ hr unless otherwise

stated. Full motility was rated as 4 and complete immotility as zero, but in presenting results the actual scores have been multiplied by 4, since quarter-grades were frequently used.

The motility index referred to in the tables is the sum of the motility scores $\times$ 4 for each ejaculate over the experimental period. This has been used as unit observation (see Emmens 1948) for the analyses of variance which are presented in summary form.

TABLE 2

MOTILITY INDICES FOR FIVE FOWL EJACULATES IN DILUENTS CONTAINING VARIOUS BUFFERS

pH	Buffer	Diluent B						Diluent F						Grand Totals
		Ejaculate No.					Total	Ejaculate No.					Total	
		1	2	3	4	5		1	2	3	4	5		
6·1	Citric acid–phosphate	10	14	29	27	7	87	16	13	14	17	3	63	150
	Phosphate	15	16	24	37	6	98	17	18	20	21	7	83	181
8·4	Carbonate–bicarbonate	16	26	30	52	60	184	57	51	55	55	64	282	466
	Phosphate	28	41	39	50	64	222	45	53	24	57	64	243	465

Analysis of Variance

Source of Variation	Degrees of Freedom	Variance Ratios	
		Citric Acid–Phosphate *v.* Phosphate	Carbonate–Bicarbonate *v.* Phosphate
Between diluents	1	6·3	9·6*
Between buffers	1	4·0	0·0
Between ejaculates	4	19·2**	7·1*
Interactions:			
Diluent $\times$ buffer	1	0·3	4·0
Buffer $\times$ ejaculate	4	0·5	0·7
Diluent $\times$ ejaculate	4	4·3	1·8
Residual	4	*12*	*74*

*$P<0·05$. **$P<0·01$.

III. RESULTS

(*a*) *Preliminary Tests*

No one buffer would cover the wide pH range (4–10) required and preliminary tests were undertaken to find a combination of equally innocuous buffers. Fowl spermatozoa retained high motility in isotonic diluents B and F containing phosphate buffer and these were used as standards for the comparison of other buffers. All

TABLE 3

EFFECT OF pH, TONICITY, SODIUM CHLORIDE, AND GLUCOSE CONCENTRATIONS ON THE MOTILITY INDICES OF FOWL SPERMATOZOA

Diluents	Tonicity	pH = 5·7±0·1						pH = 7·1±0·1						pH = 8·8±0·2						pH = 9·5±0·2						Grand Totals
		Ejaculate No.					Total	Ejaculate No.					Total	Ejaculate No.					Total	Ejaculate No.					Total	
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		
A	50	1	5	0	5	2	13	31	51	22	33	41	178	18	38	4	35	19	114	0	0	0	0	0	0	305
B	100	12	8	1	15	7	43	61	62	28	61	77	289	8	6	1	9	9	33	1	0	0	2	2	5	370
C	150	18	16	9	34	22	99	67	45	32	71	76	291	3	3	2	9	6	23	2	1	8	1	1	13	426
D	200	3	7	2	26	10	48	50	0	13	67	55	185	3	3	1	12	2	21	0	5	0	1	1	7	261
E	50	0	0	0	1	0	1	38	53	34	54	62	241	2	11	0	3	0	16	0	0	0	0	0	0	258
F	100	12	15	1	25	11	64	65	55	42	67	70	299	37	51	12	60	43	203	1	4	0	2	5	12	578
G	150	5	24	2	32	4	67	70	58	27	73	75	303	63	27	15	18	42	165	1	6	1	4	3	15	550
H	200	0	4	0	4	1	9	18	3	3	68	9	101	0	2	0	7	3	12	0	4	0	1	2	7	129
Totals		51	79	15	142	57	344	400	327	201	494	465	1887	134	141	35	153	124	587	5	20	9	11	14	59	2877

comparisons were made at a common overlapping pH as close as possible to neutrality. The citric acid–phosphate *v.* phosphate comparison was made at pH 6·1 and the carbonate–bicarbonate *v.* phosphate comparisons at pH 8·4. Five ejaculates were used in each test and motility was scored at $\frac{1}{2}$, $1\frac{1}{2}$, 3, and 5 hr from the start of the test.

The results are shown with summary analyses of variance in Table 2. The motility score in carbonate–bicarbonate and citric acid–phosphate buffer did not differ significantly from that in phosphate and these three buffers were used in subsequent experiments.

TABLE 4

SUMMARY OF THE ANALYSES OF VARIANCE FOR THE DATA OF TABLE 3

Source of Variation	Degrees of Freedom	Variance Ratios
Between ejaculates	4	16**
Between pH levels	3	210**
Between diluents	7	15**
Interactions:		
Ejaculate × pH	12	4·5**
Ejaculate × diluent	28	1·1
Diluent × pH	21	5·6**
Residual	84	77

**$P<0·01$.

(*b*) *Systematic Studies*

The motility of fowl spermatozoa was studied at five pH levels (nominally 4·0, 5·5, 7·0, 8·5, and 10·0). At each pH level, the eight diluents of Table 1 were used, so that at four tonicity levels, the effect of substituting sodium chloride for part of the glucose could be examined. All experiments were repeated five times, all ejaculates (usually pooled specimens) being partitioned between tubes representing all possible combinations of the factors investigated.

The results are set out in Table 3, omitting the results for pH 4·0 as the spermatozoa were immotile in all diluents. Fowl spermatozoa had an optimum pH at about 7 and, although there was a rapid fall off in motility on either side of the optimum pH, at least partial motility was observed from pH 5·7 to 9·5. The pH of fowl semen lies close to the optimum pH for motility, thus the mean of 18 ejaculates was 7·6 (S.D.=0·42; range 8·1–6·9). The summary of the overall analysis of variance (Table 4) shows highly significant differences between pH levels and highly significant interactions. The results have therefore been analysed separately at each pH (Table 6) according to the scheme set out in Table 5.

The single significant difference in hypertonic diluents D and H at pH 5·7 may be fortuitous, as the greater part of the difference was contributed by ejaculate 4. All ejaculates did, however, show a slight advantage of diluent D over H.

At pH 7·1 fowl spermatozoa showed considerable tolerance to changes in tonicity, the optimum being between 100 and 150. At the extreme tonicities of 50

TABLE 5
SCHEME ADOPTED FOR ANALYSIS OF RESULTS IN TABLE 6

Source of Variation	Factorial Coefficients for Diluents:							
	A	B	C	D	E	F	G	H
Part replacement of glucose by NaCl, tonicity 50	+1	0	0	0	−1	0	0	0
Part replacement of glucose by NaCl, tonicity 100	0	+1	0	0	0	−1	0	0
Part replacement of glucose by NaCl, tonicity 150	0	0	+1	0	0	0	−1	0
Part replacement of glucose by NaCl, tonicity 200	0	0	0	+1	0	0	0	−1
Curve fitting:								
Linear regression	−3	−1	+1	+3	−3	−1	+1	+3
Simple curvature	+1	−1	−1	+1	+1	−1	−1	+1
Double curvature	−1	+3	−3	+1	−1	+3	−3	+1

and 200, they tended to adapt themselves to their unfavourable environment and, although their motility was very depressed at the start of the tests, it increased so that

TABLE 6
SUMMARY OF THE ANALYSES OF VARIANCE AT SEPARATE pH LEVELS FOR THE DATA OF TABLE 3

Source of Variation	Degrees of Freedom	Variance Ratios at pH:		
		5·7	7·1	8·8
Between diluents				
Glucose × NaCl (tonicity 50)	1	0·5	2·1	9·5**
Glucose × NaCl (tonicity 100)	1	1·5	0·1	28·6**
Glucose × NaCl (tonicity 150)	1	3·5	0·1	20·0**
Glucose × NaCl (tonicity 200)	1	5·2*	3·8	0·1
Curve fitting	3	44·4**	34·8**	22·7**
Between ejaculates	4	9·5**	9·2**	2·8**
Residual	28	*29*	*185*	*101*

*$P<0·05$. **$P<0·01$.

the 1½-hr scores were higher than the ½-hr scores. Thus when the ½- and 1½-hr scores for diluents A, D, E, and H and B, C, F, and G (Table 7) are subjected to analyses

of variance, diluents A, D, E, and H showed a significant rise ($P<0{\cdot}01$), and diluents B, C, F, and G a significant fall ($P<0{\cdot}05$), in motility. These effects are illustrated in Figure 1.

TABLE 7

SUM ($\times 4$) OF THE MOTILITY SCORES FOR FIVE FOWL EJACULATES AT $\frac{1}{2}$ AND $1\frac{1}{2}$ HR RESPECTIVELY IN DILUENTS AT pH 7·1

Individual ejaculate scores have been omitted from the table but were used in the analysis of variance

Unfavourable Conditions					Favourable Conditions				
Diluent	Tonicity	Time (hr)		Total	Diluent	Tonicity	Time (hr)		Total
		$\frac{1}{2}$	$1\frac{1}{2}$				$\frac{1}{2}$	$1\frac{1}{2}$	
A	50	34	43	77	B	100	73	68	141
D	200	27	40	67	C	150	69	68	137
E	50	44	59	103	F	100	73	69	142
H	200	17	18	35	G	150	74	68	142
Totals		122	160	282			289	273	562

Analysis of Variance

Source of Variation	Degrees of Freedom	Variance Ratios	
		Unfavourable Conditions	Favourable Conditions
Between diluents	3	32·9**	0·0
Between times	1	15·0**	5·7*
Between ejaculates	4	19·6**	13·3**
Interactions:			
Diluent × time	3	1·7	0·0
Diluent × ejaculate	12	10·8**	1·1
Time × ejaculate	4	3·3*	2·0
Residual	12	*2·4*	*1·5*

*$P<0{\cdot}05$. **$P<0{\cdot}01$.

At pH 8·8 there were highly significant differences between glucose and chloride diluents at tonicities of 50, 100, and 150 (Table 6). In all cases, the diluents containing little or no chloride were superior to the diluents of high chloride content. If diluents A and B, containing equal amounts of glucose, are compared it will be seen that the chloride-containing diluent B is less favourable than the chloride-free diluent A even though the tonicity of diluent B is more conducive to survival. This points to a detrimental effect of sodium chloride on the survival

of spermatozoa at this pH. Table 8 gives the results of confirmatory tests on four ejaculates using the carbonate–bicarbonate buffer at pH 8·6. The sodium chloride percentage was varied logarithmically from 0 to 0·17 at tonicity 50 and from 0 to 0·4 at tonicity 100, the tonicity in each diluent being kept constant by adjusting the glucose level. The summary analyses of variance (Table 8) showed in each case a significant linear fall in motility with increasing chloride concentration.

TABLE 8

EFFECT OF SODIUM CHLORIDE CONCENTRATION ON THE MOTILITY INDICES OF FOUR FOWL EJACULATES AT pH 8·6

Tonicity 50						Tonicity 100					
NaCl (%)	Ejaculate No.				Total	NaCl (%)	Ejaculate No.				Total
	1	2	3	4			1	2	3	4	
0·00	67	61	69	70	267	0·0	80	80	80	72	312
0·04	68	59	67	45	239	0·1	80	80	78	61	299
0·08	69	33	63	34	199	0·2	73	77	72	66	288
0·17	19	10	38	22	89	0·4	75	30	22	39	166

Analysis of Variance

Source of Variation	Degrees of Freedom	Variance Ratios	
		Tonicity 50	Tonicity 100
Between ejaculates	3	3·6*	1·6
Between diluents:	3	16·2**	7·8**
Linear	1	43·8**	17·1**
Quadratic	1	4·5	5·0
Cubic	1	0·5	1·1
Residual	9	*94*	*147*

*$P<0·05$. **$P<0·01$.

IV. DISCUSSION

Fowl spermatozoa are motile over a similar pH range (5–10) to that of the other species studied, viz. the rabbit, ram, bull, and human (Emmens 1947, 1948; Blackshaw and Emmens 1951) and have an optimal pH for motility of about 7. At high pH levels fowl spermatozoa tend to become susceptible to the harmful effect of increasing sodium chloride concentration and its replacement by glucose is beneficial. This effect has been previously observed with mammalian spermatozoa in hypertonic media (Emmens 1948; Blackshaw and Emmens 1951). The results reported here, however, are much more striking, and suggest that the phenomenon is a general characteristic of vertebrate spermatozoa. There is no obvious explanation as to why

sodium chloride should be particularly harmful at alkaline pH values; the beneficial action of replacing chloride by glucose in this and other work is, however, due to the maintenance of viability throughout the experimental period rather than to an initial increase in motility.

Fowl spermatozoa are particularly resistant to hypertonicity. They resemble ram, bull, and human spermatozoa in their response to changes in tonicity (Blackshaw and Emmens 1951) and do not show the interaction between pH and tonicity characteristic of rabbit spermatozoa (Emmens 1948).

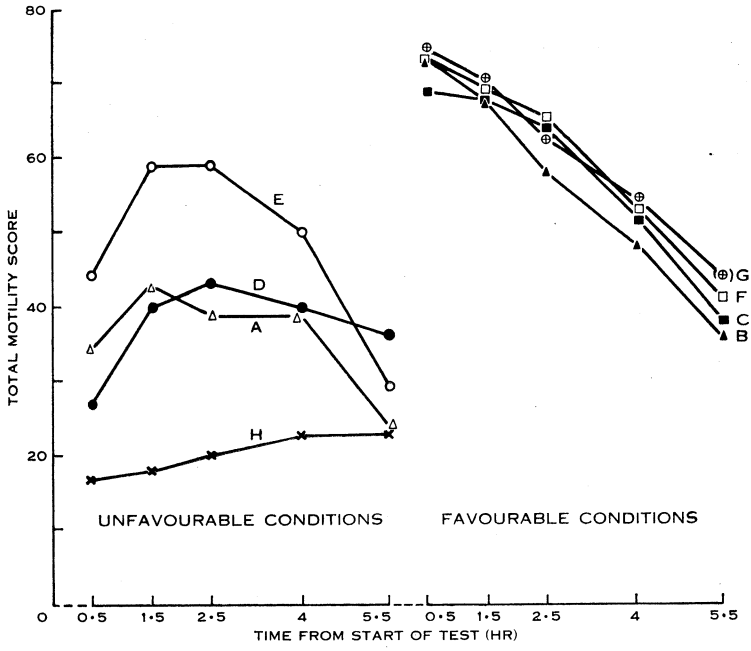

Fig. 1.—The motility scores (sum × 4) of five fowl ejaculates at pH 7·1, in diluents A–H (see Table 1), showing the adaption of fowl spermatozoa to unfavourable conditions during the first 1½ hr of the experiment.

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