TABLE 1

DISTRIBUTION OF FLUORIDE CONTENT OF THE NORMAL HUMAN PLACENTAL SAMPLES FROM ROCHESTER AND NEWBURGH, N. Y.

μg fluoride/ 100 g	Rochester		Newburgh	
	No. samples	Per-centage	No. samples	Per-centage
0– 49	7	58	2	17
50– 99	2	17	3	25
100–199	2	17	1	8
200–299	1	8	4	33
300–599	0	0	2	17
Totals	12	100	12	100

fluoride in the water resulted in a higher concentration of fluoride in the placental tissue, which may be related to the blood fluoride concentration. Also, it should be noted that both the Rochester and Newburgh placental samples had higher concentrations of fluoride than the respective blood samples. Two explanations are suggested for this observation. First, if fluoride is an essential trace element the placenta may act as a concentrating organ for fluoride, to ensure that the fetus will have adequate fluoride for the developing tissues. Second, since excessive fluoride is toxic, this organ may be acting as a barrier to prevent more than trace amounts of fluoride from reaching the fetus. How much of this accumulating fluoride passes from the placenta to the fetus is yet to be determined. At any rate, the placental concentrations are not of the order of magnitude to cause deleterious effects in the mother.

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Manuscript received September 24, 1951.

The Significance of Intravas pH in Relation to Sperm Motility[1]

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Important contributions in the field of reproductive physiology have concerned the relation of mammalian sperm activity to the hydrogen ion concentrations of the dilution media (*1–4*). These investigations were studies *in vitro,* generally on seminal sperm, in which the criteria of activity included motility, glycolysis, and oxidative respiration. Emmens' extensive work on rabbit spermatozoa indicated that the pH optimum for motility is in the pH range 7.2 to 7.9, whereas the results of Lardy and Phillips demonstrated a pH of 6.8 as optimum both for motility and respiration. Insofar as they go these data are of considerable practical importance, and probably the apparent discrepancy in results can be resolved on a basis of the chemical nature of the diluents and the relative dependability of the methods used in rating sperm motility. However, in these investigations two fundamental biological problems have been neglected, problems of more significance than the determination of the absolute pH values for optimum activity: (1) the mechanism by which pH changes affect sperm motility, and (2) the biological significance of pH in controlling the motility of sperm *in vivo,* particularly within the male reproductive ducts. This note concerns the second of these problems.

The spectacular achievement of motility by mammalian spermatozoa immediately following ejaculation represents a sudden change in metabolic pattern. When first obtained from the epididymis or from the vas deferens, sperm are immotile but rapidly become active under the new environmental conditions. The initiation of activity of vas sperm *in vitro* has prompted several suggestions as to the nature of the intravas inhibition of motility. Oxygen deficiency, high acidity caused by acid metabolites, and lack of space have been suggested as being mainly responsible for the immotility of sperm while still within the ducts, each of which conditions is modified at the time of ejaculation.

The data presented here indicate that a low pH is not normally found in the vas; an acid condition brought about by lactic acid or carbon dioxide accumulation does not prevail. On the other hand, we have evidence (to be presented elsewhere) that a very low intravas oxygen tension combined with a deficiency of carbohydrate substrate may indeed be the significant limiting factor for sperm motility and that this, rather than the hydrogen ion concentration, inactivates the vas sperm. This brings into line the motility regulation of mammalian sperm with that of sea-urchin sperm, in which it is now clear that oxygen lack is the important inactivating physical factor (*5*). Although the present suggestion is not new in regard to the relative insignificance of hydrogen ion concentration in inhibiting the motility of mammalian sperm in the vas deferens, no direct measurements of intravas pH have yet been recorded.

Mature, breeding buck rabbits were investigated under Nembutal anesthesia; pH determinations were made with the Beckman (Model G) pH meter, using a standard microglass electrode of approximately 5-μl capacity. The capillary containing the material to be measured was carried in a vessel of hydrochloric acid-quinhydrone solution connected to the instrument through an HCl-platinum wire junction. A calomel electrode completed the circuit. The electrodes were standardized against buffers at pH 7.00 and 4.00 before, and usually after, each determination. All measurements were made at room temperatures. Following the abdominal incision and exposure of the vas deferens, this duct was clamped off and removed. The

[1] Supported by a grant-in-aid from the Planned Parenthood Federation of America.

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capillary electrode was immediately introduced into the vas, sperm withdrawn into the capillary, and the pH measured. Determinations could be made within 2 min following the initial incision. In one series of experiments the circulation to and from the vas was closed off, and measurements were made on the sperm mass at various times up to 1 hr following the vascular constriction.

For comparative purposes the hydrogen ion concentration of semen, collected by means of an artificial vagina, was determined. Finally, the effect of pH on the motility of seminal sperm, resuspended in phosphate buffer, was observed in a series of buffers ranging from pH 5.6 to 7.4.

The motility of sperm—and motility rather than metabolic activity was under investigation here—was rated following the method of Emmens in which four degrees of motility from excellent (#4) to poor (#1) were noted, no motility having a value of zero. We have preferred to omit the half-steps in Emmens' index because of the necessarily subjective nature of the rating, although the method and its reliability are quite sound.

The pH of vas deferens sperm. The hydrogen ion concentration of the sperm mass transferred directly from the vas to the capillary electrode, without contact with air, ranged from pH 6.21 to 7.35 (Table 1).

TABLE 1

INTRAVAS PH OF NEMBUTALIZED RABBITS DETERMINED WITH THE MICROGLASS ELECTRODE

Experimental animal	pH	Time after incision (min)
1	7.10	15
2	6.92	25
3	6.40	5
4	6.35	7
5	6.81	5
6	6.88	5
7	6.91	7
8	6.21	5
9	6.83	5
10	6.61	20
11	6.85	9
12	6.55	14
13	7.10	3
14	7.35	2

A significant factor in the variation here proved to be the time lapse between the beginning of the operation—or more particularly the interruption of the blood supply to the vas—and the moment the pH was determined. In animals measured very soon after the beginning of the operation (Nos. 13 and 14) the intravas pH was high, approximating the pH of blood, 7.45. As the interval increased, the pH generally decreased, except in those animals in which the vas sperm were not very active (No. 1).

The possible accumulation of acid following an impairment of the blood supply was tested by clamping the vessels to the vas deferens and determining the pH after appropriate time intervals (Table 2). After the first determination of pH 6.34 following a 5-min interval, all the values were between pH 5.0 and 6.0 (Fig. 1).

TABLE 2

INTRAVAS PH FOLLOWING OCCLUSION OF BLOOD VESSELS TO THE VAS DEFERENS

Experimental animal	pH	Tie-off interval (min)
1	6.34	5
2	5.91	10
3	5.84	15
4	5.85	30
5	5.19	40
6	5.69	55

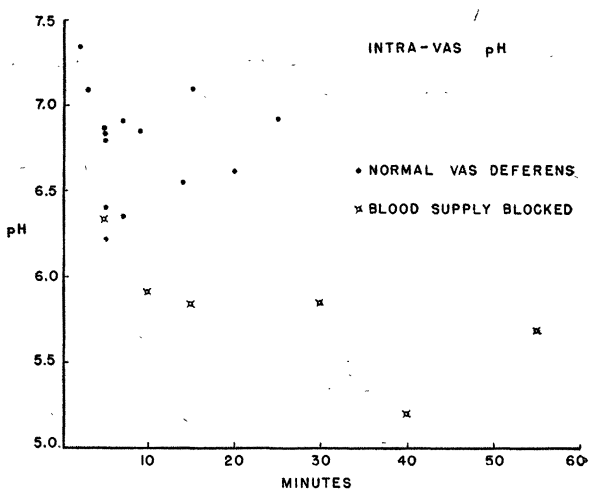

FIG. 1. Intravas pH determinations in the rabbit. Time intervals indicate time from beginning of operation (●), or from moment blood vessels are constricted (✣).

Although it was not possible to observe the sperm in every experiment in which a pH measurement was recorded, in those instances in which observations were made the vas sperm remained immotile until contact with air. Following the pH determination, the sperm in the capillary electrode became motile on exposure to air even though the pH had decreased to values as low as 5.85.

The pH of seminal sperm. It seemed desirable to verify, with this method, the effect of pH on the motility of seminal sperm. The average value for the rabbit sperm ejaculate was 7.3 to 7.4, with a range from 6.75 to 8.10. The variability is not unexpected, owing to the different and relatively independent sources of the seminal components, which vary widely in their own hydrogen ion concentrations (*6*).

In order to test the effect of pH on the motility of seminal sperm, the cells were isolated from the plasma by double centrifugation and resuspension in phosphate buffers over the pH range 5.61 to 7.39. Tribasic sodium phosphate (0.1 *M*) and monobasic potassium phosphate (0.1 *M*) were mixed to give the appropriate

buffer steps. These buffers obviously are not the ideal nutrient solutions, and there may well be some detrimental ionic effect, but they proved adequate to determine the immediate effect of pH per se on the sperm in glucose-free medium (Table 3). A subsequent series

TABLE 3

SPERM MOTILITY IN PHOSPHATE BUFFERS OF VARIOUS pH, WITHOUT GLUCOSE

(Motility ratings indicated from very motile [++++] to immotile [−])

Time (hr)	pH 7.39	6.81	5.91	5.61	Tyrode solution (pH 7.35)
0.0	++++	++++	++++	++++	++++
1.0	++++	++++	++++	++	++++
1.7	++++	++++	++++	+	+++
2.5	+++	++++	+++	+	+++
3.5	++	+++	++	−	++
4.5	++	+++	+	−	++
6.0	+	+++	−	−	++

of determinations was made in which glucose as substrate was added to the buffer mixtures (Table 4). The results not only show clearly that a pH of 6.0 will support motility for several hours, but indicate that the optimal pH is in the region of 6.8, somewhat less than the average pH of the ejaculate but precisely at the point previously demonstrated by Lardy and Phillips as the optimum pH for both motility and respiration of rabbit sperm.

TABLE 4

SPERM MOTILITY IN PHOSPHATE BUFFERS OF VARIOUS pH, WITH ADDED GLUCOSE

(M/100 final conc; motility ratings as in Table 3)

Time (hr)	pH 7.39	6.81	5.91	5.61	Tyrode solution (pH 7.35)
0.0	++++	++++	++++	++++	++++
0.3	++++	++++	++++	++	++++
0.6	+++	++++	++++	+	++++
1.6	++	+++	+++	−	++++
3.3	+	++	++	−	+++
5.0	−	+	+	−	++
22.5	−	+	+	−	++

These results minimize high hydrogen ion concentration as of any great consequence in inhibiting the motility of spermatozoa within the male tract. The sperm are immotile in spite of a pH which approximates that of neutrality. No lactic acid or carbon dioxide normally accumulates in any significant quantities because these metabolic end products are carried off by an adequate blood supply. However, even when these substances are allowed to increase, the potential motility of the spermatozoa is not lost; as air is admitted, high sperm motility is acquired. Likewise, decreasing the pH around seminal sperm does not immediately impair their motility unless a value of pH 6.0 or less is reached. Emmens demonstrated that rabbit sperm may be exposed to a pH of 5.8 before motility is lost; exposure to pH 4.5, although temporarily immobilizing the sperm, may be tolerated if the sperm are returned within an hour to pH 8.0.

The question of the source of the acid metabolites accumulated in the vas deferens, the circulation of which is restricted, is provocative in the light of the claim that there is little more than a trace of glycolyzable carbohydrate present within the tract (*7, 8*). However, as the intracellular glycolyzable and oxidative reserves of the spermatozoa cannot be utilized over too long a period of time, it is likely that some substrate, probably glucose, is made available by the blood supply previous to ejaculation to nourish the sperm while in the tract.

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Manuscript received September 4, 1951.

Inhibition of Sperm Motility by Tetrazolium Salts[1]

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The use of tetrazolium salts in cytochemistry, and specifically as indications of dehydrogenase activity, has been extensively described in a number of papers (*1–19*). When added to appropriate biological systems, these salts generally function as hydrogen acceptors and are irreversibly reduced to their colored derivatives: 2,3,5-triphenyltetrazolium chloride becomes red formazan, neotetrazolium chloride gives rise to a purple diformazan, and ditetrazolium chloride forms an intense blue diformazan. Besides dehydrogenation, tetrazolium reduction has also been associated with cysteine desulfurase activity (*20*) and reactions involving phosphate ions (*21*). Much of the tetrazolium literature has been surveyed recently by Smith (*22*).

A physiological characteristic of tetrazolium which bears reporting is its ability to inhibit motility of mammalian spermatozoa. In the presence of glycolyzable sugar (M/100), sperm which have been thoroughly washed and resuspended in Tyrode's solution are unaffected by the addition of 2,3,5-triphenyltetrazolium chloride in concentrations up to M/50, but in the absence of sugar a concentration of tetrazolium of M/10,000 rapidly inhibits motility of the sperm

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