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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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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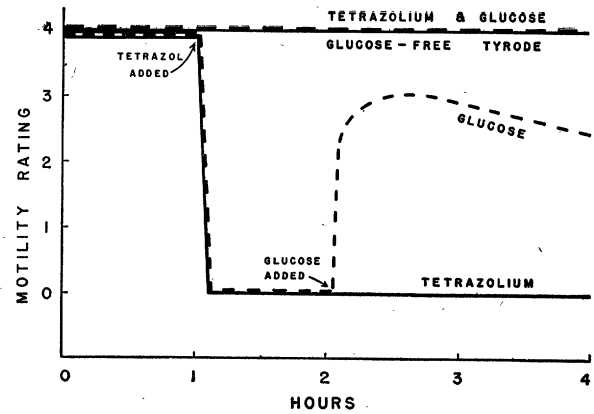

FIG. 1. Inhibition of rabbit sperm motility by 2,3,5-triphenyltetrazolium chloride in absence of glucose (lower curve). Addition of glucose to tetrazolium-treated sperm permits motility.

(Fig. 1). If motility is thus impaired, it may be reacquired by the addition of glucose within an hour following tetrazolium treatment. To this extent the reaction is reversible.

The direct counteraction by glucose of the tetrazolium suggested that the salt might be undergoing a reduction by the sugar, thereby rendering it unable to take part in the hydrogen transfer mechanisms of the cells. Tests with various sugars, however, and with photoreduced (red) tetrazolium indicate that this is not the case. Glucose and mannose are equally effective in preventing the inhibitory action of the tetrazolium; galactose and maltose are less effective; fructose, sucrose, and lactose are least effective; and ribose has no effect whatever. The molar ratios of glucose and triphenyltetrazolium chloride may be varied over a tenfold range without changing the reaction. A small quantity of tetrazolium inhibits motility; likewise a small amount of glucose, even in the presence of a large excess of tetrazolium, can maintain motility. No protective action against the tetrazolium was displayed by solutions ($M/100$–$M/1000$) of succinate, malate, fumarate, or malonate. The study has thus far been conducted primarily with 2,3,5-triphenyltetrazolium chloride and its effects on rabbit sperm, but both neo- and ditetrazolium chlorides produce the same types of response, and in fact give equivalent inhibition at lower concentrations. The reactions on rabbit sperm have been paralleled on human and bovine sperm.

Rabbit spermatozoa can generally maintain motility for periods up to 2 days at room temperature if provided with either glycolyzable energy sources (e.g., glucose, fructose, or mannose), or with an adequate oxygen supply. Under anaerobic conditions (tank nitrogen) sugar is essential (if sugar is absent oxygen is required) to support motility for more than a few minutes. These systems are sensitive to the glycolytic inhibitors, iodoacetate and fluoride, and to the oxidative poisons, cyanide and azide. If tetrazolium is used in combination with these compounds, motility is impaired in suspensions in which glucose is present but in which its utilization is prevented by iodoacetate or fluoride. On the other hand, so long as glucose is available, cyanide and azide sensitivity remains the same in the presence and absence of tetrazolium.

Attempts to elucidate the mechanism of action of tetrazolium salts on mammalian sperm have established these facts: (1) motility is impaired immediately following the addition of the salt to the sperm suspension; (2) the reaction need not proceed to the red formazan stage; (3) the inhibition of motility can occur if colored photoreduced tetrazolium is used; (4) the reaction can be reversed by the addition of suitable carbohydrate; (5) the reducing value of the sugar does not seem to be critical, although its enzymatic utilization by the cells may be; and (6) the inhibition is not counteracted by succinate, malate, fumarate, or malonate. It is justifiable to conclude at present, therefore, that the tetrazolium effect on sperm motility is not merely a reduction accompanying dehydrogenase activity, but is a physiological inhibition of a different order. The physical and physiological aspects of this impairment are being further investigated.

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On a Thiazolone Compound from Thiamine Disulfide

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In 1940 Zima *et al.* (*1*) obtained thiochrome (I) and a product melting at 233°–4° by heating thiamine disulfide (II) in ethylene glycol. From the analytical