experiments. It is evident that the employment of chemical procedures which would recover all of the metaphosphate from the yeast cell as one fraction would mask the existence of physiologically different usages for the same chemical compound. Such a procedure could result in false conclusions with regard to the metabolic significance and function of such a compound.

Detailed accounts of the experiments described here will be published elsewhere. This work is being supported by a grant from the American Cancer Society.

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Maintenance of Spermatozoal Motility in Dilute Suspension

THE rapid immobilization of mammalian spermatozoa which occurs when an ejaculate is diluted with a large volume of physiological saline is the basis of Milovanov's¹ test of 'resistance', used in assessing the value of a specimen for artificial insemination. Milovanov attributes this immobilization to the toxic action of chloride ions. Chang² also remarks on the low motility, rate of respiration and fertilizing capacity of rabbit spermatozoa in dilute suspension. Our own observations confirm these findings; rabbit spermatozoa at a concentration of 20 million/ml. in Baker's³ solution remain motile for two or three days at room temperature, whereas at a concentration of 0.4 million/ml. they are motionless within an hour or two. In our experience this rapid loss of motility is not attributable to a toxic action of chlorides, since chloride-free diluents have an identical action.

Dr. A. Walton had told us of an observation in his laboratory that an emulsion of liquid paraffin helps to maintain the motility of suspensions of spermatozoa. His suspensions were not, however, highly diluted. Emulsions of liquid paraffin with a little gum arabic added to assist emulsification in Baker's solution were therefore tried, and found to be highly effective in maintaining good motility for short periods of time (not more than 6-12 hours). However, it was then discovered that gum arabic alone, starch, glycogen, egg yolk, egg white, crystalline serum proteins and a number of other substances are equally effective. On the other hand, agar, gelatin, sodium silicate and catalase are entirely without effect. Supernatants from other suspensions of rabbit spermatozoa proved highly effective, and the sperm-free plasma from a vasectomized buck was also effective, but not as good as supernatants from whole semen, prepared from suspensions which had stood overnight.

Full details of these tests will be published elsewhere, but the following table is included to illustrate

the striking results obtained. The motility of suspensions kept at room temperature is given a score of between 0 and 4, according to the activity of the spermatozoa⁴.

SUBSTANCES EFFECTIVE IN MAINTAINING THE MOTILITY OF SUSPENSIONS OF RABBIT SPERMATOZOA IN BAKER'S SOLUTION AT 4×10^6 cells/ml.

Substance	Ejaculate	Motility score at hour						
		$\frac{1}{2}$	$1\frac{1}{2}$	$2\frac{1}{2}$	4	6	24	
Controls at 2×10^7 /ml.	1	4	4	4	4	4	3	
	2	3	2	2	2	2	$1\frac{1}{2}$	
Controls at 4×10^6 /ml.	1	0	0	—	—	—	—	
	2	1	$\frac{1}{2}$	0	0	—	—	
0.25% W/V starch	1	$3\frac{1}{2}$	$3\frac{1}{2}$	3	$3\frac{1}{2}$	$2\frac{1}{2}$	0	
	2	2	$2\frac{1}{2}$	$2\frac{1}{2}$	$1\frac{1}{2}$	$2\frac{1}{2}$	0	
0.25% W/V glycogen	1	$3\frac{1}{2}$	$3\frac{1}{2}$	$3\frac{1}{2}$	4	$2\frac{1}{2}$	0	
	2	3	2	$2\frac{1}{2}$	2	$1\frac{1}{2}$	0	
0.25% carbohydrate-free serum protein	1	$3\frac{1}{2}$	$3\frac{1}{2}$	$3\frac{1}{2}$	4	3	$\frac{1}{2}$	
	2	3	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$\frac{1}{2}$	
0.25% serum protein containing 7% carbohydrate	1	$2\frac{1}{2}$	$3\frac{1}{2}$	$3\frac{1}{2}$	$3\frac{1}{2}$	$2\frac{1}{2}$	0	
	2	3	2	2	2	2	0	

Two features of this table need comment. First, there was sometimes a small rise in motility during the test, attributable to a rise in room temperature. Secondly, there was little or no motility in any but the control suspensions at 20 million/ml. at 24 hours, which demonstrates that the action of the various effective substances is not long maintained.

The capacity of such widely differing materials to preserve motility in dilute suspension makes it highly probable that their action is physical and not chemical. Therefore, the possibility was explored that they adsorb a toxic agent present in the diluent. Baker's solution contains traces of copper, lead and arsenic, and these seemed the most likely agents. However, neither special purification of the diluent nor the addition of relatively large excess of these metals affected the spermatozoa in suspension at 20 million/ml., which, surprisingly enough, are unaffected for the first six hours by 160 parts per million of copper or lead, or 48 parts per million of arsenic. Only copper eventually reduced motility, at 24 hours, in comparison with the controls. We were thus inclined towards a second hypothesis, that the various effective substances in some way prevent the escape of essential material, perhaps an enzyme, from the sperm cells. This receives support from the observation that repeated washing in Baker's solution does not cause immotility in suspensions at 20 million/ml. at a stage when the concentration of seminal plasma is reduced to that equivalent to dilution to 0.4 million/ml., but does cause immotility when further washings are given (controls washed repeatedly in the original supernatant, rich in seminal plasma, remain motile).

It is therefore concluded that the immobilizing effect of high dilution is probably due to the loss of vital material from the spermatozoa. The mode of action of the various effective substances which prevent the phenomenon is unknown. It seems unlikely, but possible, that they all contain some common impurity (like biotin) which is responsible for their effectiveness, and if so, it might operate in some manner other than the prevention of loss of intracellular material.

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