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BRIEF COMMUNICATION

EFFECTS OF WASHING ON THE METABOLISM OF BULL SPERMATOZOA

R. G. WALES AND J. C. WALLACE

Department of Veterinary Physiology,
University of Sydney, Sydney, N.S.W., Australia

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In a recent communication (Wales & Wallace, 1964), it was observed that the oxidation of substrates other than fructose was greatly depressed by washing the spermatozoa free of their seminal plasma. This effect could be due either to the removal of protective macromolecules in the seminal plasma or to the removal of alternative substrates. The following experiments were undertaken to investigate these possibilities.

Veronal-buffered saline (pH 7·2) was the basic diluent used and the methods for preparing washed suspensions, for incubating the spermatozoa, for the assay of radioactivity and for the estimation of lactate and fructose, have been previously described (Wallace & Wales, 1964; Wales & Wallace, 1964). Sorbitol was estimated by an enzymic method (T. O'Shea and R. G. Wales, unpublished data). Acetate was estimated after steam distillation of metaphosphoric acid extracts (Annison, 1954). The results in Table 1 were submitted to analysis of variance after logarithmic transformation and the error variance was used to test the effects of treatments by means of a '*t*' test.

The first experiments were designed to establish whether inclusion of proteins in the basic diluent would help to prevent the deleterious effects of washing. The proteins were included in the basic diluent at 5 % w/v. In the case of dialysed seminal plasma proteins, a pooled sample of seminal plasma was used after dialysis for 24 hr in 'Visking' cellulose tubing against two changes of 100 vol. basic diluent. After dialysis the protein content of the dialysed plasma, as measured by the biuret reaction, was adjusted to 5 % by adding basic diluent. The respiration and aerobic fructolysis of unwashed cells, of cells washed in basic diluent containing casein, bovine serum albumin or dialysed seminal plasma proteins were compared with cells washed in basic diluent alone (Table 1). It was evident that the proteins were of little value and in a few cases appeared somewhat detrimental. In these experiments the effect of washing was mainly to depress the oxidative metabolism and it was the respiration attributable to substrates other than fructose which was more severely affected.

In the second experiment the oxidation of other substrates present in the unwashed ejaculate, as well as the oxidation of fructose, was investigated. Aliquots of bull semen were incubated in four flasks containing 5·4 μmole of added fructose. Fifty micromillicuries of U-^{14}C-fructose (flask 1), 1-^{14}C-lactate

(flask 2), 1-^{14}C-acetate (flask 3), or U-^{14}C-sorbitol (flask 4) were added as the carrier-free isotope, and, by analysis of initial samples, the specific activity of fructose, lactate, acetate or sorbitol in each flask was measured. From the $^{14}CO_2$ trapped in the corresponding flask, the contribution of each substrate to the total oxygen uptake was calculated and compared with metabolism of washed

TABLE 1

EFFECTS OF PROTEINS ON THE METABOLISM OF WASHED BULL SPERMATOZOA

Treatment	*Oxidative metabolism*			*Aerobic fructolysis*	
	Total O_2 uptake	*Fructose oxidation*	*Other O_2 uptake*	*Fructose utilization*	*Lactate accumulation*
EXPERIMENT A					
Washed in basic diluent (control)	0·66	0·081	0·18	1·55	1·62
Washed in casein diluent	0·66	0·078	0·20	1·39	1·87
Unwashed	1·27*	0·055	0·94**	1·94	2·09
EXPERIMENT B					
Washed in basic diluent	0·73	0·067	0·33	0·63	1·76
Washed in serum albumin diluent	0·22*	0·020*	0·09	0·61	1·32
Washed in dialysed seminal plasma diluent	0·43	0·041	0·19	0·84	1·04*
Unwashed	0·99	0·079	0·52	1·17*	1·87

Values are expressed as μmoles/10^8 cells and are the means for three ejaculates.

* Significantly different from the washed control, $P<0·05$.

** Significantly different from the washed control, $P<0·01$.

TABLE 2

OXIDATION OF SUBSTRATES IN UNWASHED SUSPENSIONS OF BULL SPERMATOZOA AND THE EFFECT ON OTHER OXYGEN UPTAKE

Treatment of spermatozoa	*Initial substrate conc. (μmole/10^8 cells)*	*Substrate oxidized (μmole/10^8 cells)*	*O_2 uptake due to exogenous substrates*	*Total O_2 uptake*	*Other O_2 uptake*
Washed	Fructose (1·39)	0·121	0·726	0·753	0·027
Unwashed	Fructose (3·17)	0·111	0·946	0·947	0·001
	Lactate (0·21)	0·038			
	Acetate (0·11)	0·081			
	Sorbitol (0·08)	0·0006			

All values are expressed as μmole/10^8 cells and are the means for three ejaculates.

spermatozoal suspensions incubated in the presence of fructose only. From the results for three ejaculates (Table 2) it is evident that the oxidation of various exogenous substrates in the seminal plasma accounts for practically all the oxygen uptake of unwashed cells and that the substantial oxygen uptake not attributable to fructose oxidation in unwashed cells is due to the presence of substrates in the seminal plasma.

The inclusion of macromolecules in washing diluents might have been expected to help guard against the detrimental effects of washing spermatozoa in ionic diluents since they have proved valuable in preventing the effects of dilution (Emmens & Swyer, 1948; Blackshaw, 1953; Wales & White, 1961, 1962), sudden temperature changes (Choong & Wales, 1962) and deep freezing (Choong & Wales, 1963; I.C.A. Martin, unpublished data). However, in the experiments reported here, these substances were surprisingly ineffective against the effects of the washing procedure. Thus more severe damage must have occurred during washing than could be controlled by the addition of macromolecules which are assumed to exert their beneficial effects by physical protection of the cell membrane.

It is clear that the depression in the oxidation of substrates other than fructose that occurs when bull spermatozoa are washed (Wales & Wallace, 1964) is due to the removal of various substrates by washing. This agrees with results for the ram (Wallace & Wales, 1964) where the addition of seminal plasma decreased fructose oxidation and increased other oxygen uptake of washed cells while the addition of dialysed seminal plasma caused no such change.

The results presented in Table 2 also indicate that both lactate and acetate are oxidized preferentially to fructose. In terms of μatoms of carbon, fructose made up 94% of substrate available to the unwashed cells while lactate and acetate made up 6 % and 1 % respectively. However, oxygen uptake due to fructose oxidation was only 70 % of the total, while lactate and acetate oxidation contributed 12 % and 17 % respectively to the oxygen uptake arising from oxidation of exogenous substrates. Sorbitol, on the other hand, was utilized least and presumably the step sorbitol→fructose is rate limiting under these circumstances.

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