Twenty-one days after denervation, the ability to contract is decreased. If it is accepted that contraction and proteolysis are separate and successive processes in structural breakdown, it is clear that, with a decreased ATPase activity and a diminished contractability, proteolysis will not be effective in breaking down the structure.

Fig. 1 shows an increase in the velocity of structural breakdown 42 days after denervation. Further investigations must be carried out to determine that this is not an experimental artefact and to find whether this increase is caused by secondary factors acting during this period or by primary factors.

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In vitro Maintenance of Rabbit Sperm at Room Temperature

MAMMALIAN germ cells have been utilized by several investigators for the investigation of the biochemical and physiological mechanisms of senescence in living cells[1]. Functionally and metabolically active bovine sperm have been successfully maintained and studied at room temperature for a period of 6 days or more[2]. The results of these studies led to the present work in which two chemically defined media were used to maintain large numbers of vigorously motile rabbit sperm. The two media tested here were 'Coconut Milk Extender' (CME)[3] and Norman-Johnson solution No. 2 (N-J-2)[4], a chemically defined medium. Over a 6 day period of storage at room temperature (24° ± 2° C), N-J-2 proved to be the more satisfactory medium for supporting survival and activity of the sperm.

The acrosome of the mammalian sperm is a relatively unstable structure which may become detached from the sperm. Bedford[5] reported that, as a result of *in vivo* ageing in the female reproductive tract, there was an increase in the loss of the acrosome cap from rabbit sperm. In our investigation it was found that *in vitro* ageing alone at room temperature in the two media did not increase acrosome cap loss from rabbit sperm.

The procedures used in this work were as follows: ejaculated sperm were obtained with an artificial vagina from ten hybrid male rabbits; the semen was pooled and the sperm were washed free of seminal plasma by gentle centrifugation (700*g* for 15 min) within 30 min of collection. The washed sperm were divided into two parts; one part was diluted with N-J-2 and the other part with sodium citrate buffered medium containing 15 per cent coconut milk, to give a final concentration of 10–12 × 10^6 sperm per c.c. Plastic vials (2 c.c. capacity) were filled completely with the suspended cells and tightly capped to maintain a relatively anaerobic state during a 6 day period of storage. The vials were kept in the dark[3] at ambient room temperature (22°–26° C). Fresh vials of extended sperm were removed every day for 6 consecutive days, thoroughly mixed and then examined and rated according to the following: (1) sperm motility, which was qualitatively rated by means of an arbitrary scale described by Norman *et al.*[6]; (2) concentration of the motile cells and concentration of the stored sperm—these were determined with the use of a haemocytometer; (3) loss of acrosome cap. To determine the number of cells which had lost their caps, samples of sperm were prepared as smears, fixed in formal-saline and stained in buffered Giemsa stain[7].

Table 1 summarizes the results of five replicates in the two different dilution media. The number of live cells appeared to be greater in CME after 6 days of storage than in N-J-2; however, a greater number of cells were found to be motile in N-J-2. The quality of motility of the cells dropped drastically after the second day of storage in CME but not in N-J-2. This may be the result of the presence of chloride ion in N-J-2, which may serve as a metabolic inhibitor, enabling the cells to conserve some of their energy for a later activity. CME contains calcium nitrate and it is known that nitrate enhances rabbit sperm metabolism[8]. It seems evident that in CME most of the energy source was used up during the first few days after which motility and the number of live cells decreased quickly.

Table 1. EFFECT OF NORMAN–JOHNSON SOLUTION NO. 2 AND 'COCONUT MILK EXTENDER' ON MOTILITY, SURVIVAL AND LOSS OF ACROSOME CAP OF RABBIT SPERM KEPT AT ROOM TEMPERATURE FOR 6 DAYS

Diluent	Sperm age in days	Percentage of sperm alive	Percentage of sperm motile	Quality of motility*	Percentage of sperm which lost acrosome cap
CME	0	91	92	5·0	10
	3	82	77	2·1	11
	6	72	64	1·3	13
N-J-2	0	89	91	4·9	9
	3	83	83	3·1	10
	6	69	72	1·8	12

* 0 (non-motile), 1 (vibratory), 2 (poor progressive), 3 (fair progressive) 4 (good progressive), 5 (excellent progressive).

After 6 days of storage some of the cells from both media were centrifuged and resuspended in fresh CME containing calcium nitrate and 5 per cent egg yolk. It was found that the motility of cells stored in CME could not be restored to better than "fair progressive". However, the motility of sperm stored in N-J-2 and later resuspended was restored to better than "good progressive". It was also found that a much higher percentage of the cells stored in N-J-2 responded favourably to resuspension than cells stored in CME. It was concluded that the prolonged maintenance of an economically usable number of functionally active rabbit sperm, at room temperature in N-J-2, can be of advantage in artificial insemination. N-J-2 was found to be a better diluent for storing rabbit sperm at room temperature because a greater number of cells remained motile over the storage period; and because a considerably larger percentage of the cells could be reactivated by resuspension.

In vitro ageing increased acrosome cap loss by only 3 per cent in both diluents; but this increase was not found to be significant ($P > 0.05$). These observations seem to contradict those of Bedford. However, it is possible that the increase in acrosome cap loss that Bedford observed did not result from ageing alone, but possibly from the combined effects of ageing and the physiological environment of the female reproductive tract.

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