BRIEF COMMUNICATION

PRESERVATION OF PRIMATE SPERMATOZOA
BY FREEZING

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As early as 1897, Davenport found that human spermatozoa would survive freezing to -17°C , but little progress in the freeze-preservation of spermatozoa was made until 1949 when Polge, Smith & Parkes reported that the use of glycerol made possible the revival of motility in fowl spermatozoa kept at -79°C for long periods of time. Later, freeze-preservation of the semen of several animals became a routine procedure (see Sherman, 1964). Success, however, appeared to depend upon closely controlled rates of freezing and thawing, which demanded the use of specialized equipment. It was first shown by Sherman (1963) with human semen, and by Roussel, Kellgren & Patrick (1964) with bovine semen, that successful freezing and storage of spermatozoa in liquid nitrogen vapour for indefinite periods of time could be achieved without instrumentation. The present report describes the employment of this procedure for primate semen.

Adult males of the following species were used: three *Macaca mulatta* (rhesus), two *M. speciosa* (stump-tail), three *Erythrocebus patas* (patas), three *Cercopithecus aethiops* (African green) and two *Pan troglodytes* (chimpanzee). The animals were anaesthetized with phencyclidine (Sernyl) at a dose rate of 2 mg/kg intramuscularly. Semen samples were collected by a modification of the electro-ejaculation technique described by Weisbroth & Young (1965): details of procedures, as applied to several different primates, together with observations on properties of the ejaculates obtained, are the subject of a separate report now in preparation. Immediately after collection, all samples were treated with 0.5% trypsin solution, which has been shown by Roussel & Austin (1967) to bring about liquefaction of primate seminal coagulum without deleterious effect to the spermatozoa. A small sample was then removed for estimation of the proportion of spermatozoa showing progressive forward motility. The extender used consisted of 20% egg yolk (avian), 64% of a 3.0% w/v sodium glutamate solution in double glass-distilled water, and 14% glycerol (egg yolk–glutamate–glycerol). Glutamate (sodium L- α -aminoglutarate) has been found to be a better buffer than sodium citrate for the storage of bovine semen at 5°C for long periods of time (J. D. Roussel, unpublished data). Semen was added to the extender in a ratio of 1:10 and equilibrated for 30 min at room temperature.

The extended semen (0.5 ml) was dispensed into 1.2 ml glass ampoules and sealed with a semi-automatic sealer. Two ampoules were placed on each cane and lowered for 3 min into the neck of a Linde liquid nitrogen refrigerator (35B) containing 8 in. of liquid nitrogen. Then, the canes were lowered further into the refrigerator so that the bottom ampoule was about 2 in. above the liquid nitrogen, and left for 5 min. Finally, the canes were placed in their assigned canister in the liquid nitrogen for 3 days. Frozen semen was thawed by placing ampoules in a 5° C water bath until thawing was complete, which generally took about 3 min. The proportion of spermatozoa showing progressive forward motility was again assessed.

A summary of the pre-freeze and post-thaw motility and the survival rate observed is set out in Table 1. The survival rate ranged from 37% to 75%

TABLE 1
MOTILITY AND SURVIVAL RATE OF SPERMATOZOA IN SEMEN FROZEN IN LIQUID NITROGEN VAPOUR

<i>Species</i>	<i>Animal No.</i>	<i>No. of samples</i>	<i>Per cent motility</i>		<i>Per cent survival</i>
			<i>Pre-freeze</i>	<i>Post-thaw</i>	
Rhesus	108	3	48	22	46
	208	3	58	32	55
	204	1	60	30	50
	Total and means	7	54	27	50
Stumptail	956	1	40	15	37
	1060	2	55	30	56
	Total and means	3	50	25	50
Patas	226	5	47	26	55
	224	1	40	20	50
	932	1	40	15	37
	Total and means	7	45	23	51
African green	116	6	55	31	56
	246	1	40	20	50
	244	1	45	20	44
	Total and means	8	53	28	53
Chimpanzee	332	1	50	30	60
	342	1	50	25	50
	Total and means	2	50	27	54

(overall mean 51%). There were no significant differences among the species investigated in the proportions of spermatozoa surviving after freezing, and the survival rate obtained is similar to that reported by Behrman & Sawada (1966) for human spermatozoa. Samples that had the highest percentage of spermatozoal survival after freezing generally were those that exhibited good initial quality. There were differences in sperm survival rates among males within a species; preliminary trials had revealed that rhesus No. 208, stumptail No. 1060, patas No. 226, and African green No. 116 yielded spermatozoa with a higher survival rate than others in the same groups and this result was obtained also in the present series. There was no apparent deleterious effect from the use of

trypsin before freezing. Tests showed that alpha-chymotrypsin had an adverse effect on sperm survival after freezing; this enzyme has also been found to produce ill effects on spermatozoa when these are stored at 5° C for 24 hr (Rousset & Austin, 1967).

Sadleir (1966) recently reported observations on a single ejaculate from a chimpanzee, diluted in three types of extender for freezing. The best motility after storage for 1 to 3 days was obtained from semen extended in egg yolk-citrate with 10% glycerol, milk-egg yolk with 10% glycerol and glucose-egg yolk with 7% glycerol. No figures for post-thaw motility or survival rates were given.

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