representing a $^1\Sigma - {}^1\Sigma$ transition, gave $B_1' = 0{\cdot}5747$ and $B_0' = 0{\cdot}5797$ cm.$^{-1}$. The agreement is good.

Our analysis shows that the red system represents a $^1\Sigma - {}^1\Pi$ transition. A more detailed paper on the red bands will appear in *Arkiv för Fysik*.

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Revival of Spermatozoa after Vitrification and Dehydration at Low Temperatures

THE effect on spermatozoa of vitrification at temperatures of −79° C. and below has been studied by several authors. Human spermatozoa appear to be the most resistant; a substantial proportion may show good motility on thawing after even prolonged vitrification. Revival is far better when semen is frozen in bulk than when minimal amounts in capillary tubes are used[1]. No explanation of this result is yet forthcoming, but it would appear that rapidity of freezing is less important than the avoidance of surface effects.

Positive results have also been obtained with frog and fowl spermatozoa[2,3], though in both these cases partial dehydration by the addition of sugar solutions was found to be necessary before any significant number of spermatozoa could be revived on thawing. Fowl spermatozoa thus treated showed some slight fertilizing power, but no live chicks were produced[4]. In our experience, revival of fowl spermatozoa after vitrification is negligible when the technique of addition of lævulose is followed.

The object of the present communication is to report the action of glycerol and related compounds, to which our attention was directed by a chance observation, in protecting spermatozoa against the effects of low temperatures. Experiments with human spermatozoa showed that diluents containing glycerol much increased the proportion of spermatozoa that could be revived after vitrification. With this material, the optimal final concentration was about 5 per cent (equal parts of semen and 10 per cent glycerol in Baker's fluid); lower concentrations were less effective, higher ones were immobilizing. Glycerol diluents did little to prolong the survival of spermatozoa thawed out after vitrification, which is usually inferior to that in control specimens. Experiments with propylene glycol and ethylene glycol gave similar results.

Results of a different order were obtained with rabbit spermatozoa. With this species no spermatozoa revive after bulk vitrification of semen. Glycerol diluents were found to be highly toxic, the maximum tolerated concentration being about 5 per cent. With this concentration there was some revival after vitrification (restricted movement of a considerable number of spermatozoa) but no effect, under the conditions employed, such as could be obtained with human spermatozoa.

Dramatic results were obtained with fowl spermatozoa. If fowl semen is diluted with equal parts of Ringer's solution and vitrified at −79° C. for 20 min., and then rapidly thawed, no significant revival of spermatozoa is observed. On the other hand, if the dilution is carried out with Ringer's solution containing 40 per cent glycerol, the spermatozoa resume full motility on thawing. So far as retention of motility is concerned, the specimen is indistinguishable from its unvitrified control; it shows even the wave motion characteristic of fowl semen. Decreasing the final concentration of glycerol below 10 per cent decreases the protection against vitrification. Increasing it above 20 per cent results in progressive immobilization of the spermatozoa, which cannot altogether be reversed by further dilution with Ringer's solution; but with these higher concentrations, no additional loss of motility is caused by vitrification. Specimens of spermatozoa have been found to resume motility completely after long periods (up to ten weeks) of vitrification. Other experiments showed that both propylene glycol and ethylene glycol were more toxic than glycerol, and in relation to their toxicity less protective against vitrification.

The fact that spermatozoa resumed full motility after vitrification under the conditions described above made it possible to investigate the effects of freeze-drying. 1 c.c. of fowl semen was diluted with 1 c.c. of 20 per cent glycerol in Ringer's solution, and vitrified as a thin layer in a 100-c.c. distilling flask at −79° C. The temperature was then allowed to rise to −25° C. and the flask connected to a high-vacuum distillation system of which the condenser unit contained liquid air. After 3 hr., when the distillation was stopped, the semen had the appearance of being dry, and 1·7 c.c. of water was thawed from the condenser. While still cold, the dehydrated semen was reconstituted with 1·8 c.c. of water and warmed to 40° C. On microscopical examination with a 4-mm. objective, active spermatozoa were seen in each field. Further experiments on these lines showed that recovery was better when 30 per cent glycerol in Ringer's solution was used, that the whole of the glycerol remained with the dehydrated semen, from which about 90 per cent of the water was removed by the distillation, and that spermatozoa could not be revived if the preparation was left at room temperature for 2 hr., presumably because of the toxic effect of concentrated glycerol. In each of eight consecutive experiments, motile spermatozoa were observed in the reconstituted semen, the best recovery being about 50 per cent of motile spermatozoa. No information is yet available as to the fertilizing power of spermatozoa thus resuscitated.

These experiments suggest that a high proportion of the water can be withdrawn from fowl semen at low temperatures without killing all the spermatozoa. To what extent this result depends on the retention of glycerol or on the unequal distribution of residual water is not yet clear. Certain obvious experiments on the factors involved and with the spermatozoa of other species are in progress.

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