## Preliminary studies on the behaviour of Limnaea embryos exposed to glycerol and to low temperatures

Amongst the trihydric alcohols, glycerol is well-known for its remarkable property to act as a shield against temperature shock.[1] Magnificent piece of work by Smith & Polge,[2] Polge[3] and Polge & Rowson[4] has revealed this property although the reason behind this still unknown. Polge, Smith & Parkes[1] in 1949 reported the protective power of glycerol in case of spermatozoa, against cold shock. In case of a number of other species—rabbit, guinea-pig and horse[2], bull and goat[5] the same phenomenon was observed. Polge[3] reported that fowl semen diluted to contain 15-20%. glycerol, frozen to -79°C and thawed afterwards at 40°C had unimpaired motility of the spermatozoa. Parallel experiment was done by Sloviter[6] with human red blood cells. All these attempts indicate the practical importance of storing the mammalian spermatozoa, human red blood cells in a freezing condition for months together in a potentially functional condition. The present study reports the behaviour of *Limnaea* embryos exposed to various concentrations of glycerol and to low temperatures.

Freshly laid eggs, from uncleaved condition to advanced veliger stages and upto organogenesis were subjected to the action of 0.5%, 1.0% 1.5% and 2% glycerol. Above the concentration of 2%, the egg masses died. All the solutions were prepared by using sterile distilled water.

In 0.5% and 1% solution, 80% of the uncleaved egg masses develop normally upto organogenesis. But difficulty arises with 1.5% and 2% solutions. They cleave normally in 1.5% but cannot proceed any further. In 2% most of them are arrested at the uncleaved stage. When left to water they cannot return to the original shape and subsequently disintegrate. In case of the eggs ranging from 2-cell stage to trochophore stage, there is normal development upto veliger in all the concentrations. After that stage the abnormality is prominent by the deformed shape and the slow heart beating. When left to water, most of the egg masses in 1% solution regain the original heart beating after 18 hrs. One or two become arrested at trocho, a few distintegrate and the others are subnormal veliger. The change is irreversible when the egg masses treated with 1.5% and 2% solution are left to water. The moving trochophore, however, does not find any resistance from either of the concentrations. But as soon as the eggs reach the "cashu" form they are vulnerable to the 'toxicity' of the glycerol solution and above the range of 1% solution, they all die within an hour. May be 'the anti-toxic power' which is so prominent in trochophore stage is gradually lost afterwards. Now, question may arise regarding the toxicity of the glycerol solution. Careful experiments using various concentrations of sucrose solutions show that 1% and 1.5% solutions are not isotonic for *Limnaea*. This was rechecked by using modified amphibian ringer solution. In the whole experiment, control egg masses were kept in sterile distilled water in which they grow normally. From time to time bacterial counts were taken from all the solutions using carbol fuchsin staining which shows negligible amounts of bacterial population in the control and treated solutions kept for a considerably long period.

Next, the glycerol-treated egg masses were subjected to cold shock. It was seen that like leukaemia cells and the active forms of nematodes, slow cooling is preferable. Another very important point is the formation of intracellular ice crystals as pointed out by Luyet[7]. The medium was solidified at -1.4°C. Presumably no ice crystals are formed within the cell at -1.4°C. The reaction with glycerol shows that 1% solution is most

effective for the viability of the eggs. Accordingly, before cold-treatment eggs in various stages were treated with 1% glycerol for 1 hour at room temperature. After that they were kept at 20°C, 13°C and-1.4°C in a gradual order and then thawed at 30°C. The quantitative results are shown in the table. Long standing at 13°C results in the abnormal development and sometimes disintegration of the egg masses whereas the untreated eggs, at the same temperature survive in most of the cases. It is presumed that at lower temperatures the glycerol treated egg masses suffer some adverse reaction lethal for them.

TABLE 1

**Effect of low temperature on glycerol treated egg masses**

| stages | temperature | | | | | | Duration of experiment |
|---|---|---|---|---|---|---|---|
| | 20° | | 13° | | — 1.4° | | |
| | Treated | Control | Treated | Control | Treated | Control | |
| Uncleaved | Normal development (90) | Normal development (95) | Normal development (88) | Normal development (90) | Disintegrates (90) | Disintegrates (100) | 1 hour |
| | —do— (88) | —do— (97) | Disintegretes (78) | Delayed (98) | —do— (98) | —do— (99) | 5 hour |
| | —do— (85) | —do— (96) | —do— (82) | —do— (85) | —do— (95) | —do— (98) | 18 hour |
| 2—cell to early trochophore | Normal development (77) | Normal development (80) | Normal development (60) | Normal development (70) | Arrested (95) | Dies (100) | 1 hour |
| | Arrested (65) | —do— (75) | Arrested (65) | Delayed (82) | Disintegrates (100) | Disintegrates (98) | 5 hour |
| | Disintegrates (72) | —do— (69) | Disintegrates (90) | —do— (60) | —do— (100) | —do— (98) | 18 hour |
| Tochophore | Normal development (100) | Normal development (80) | Slow moving (98) | Slow moving (90) | No movement (100) | Dies (100) | 1 hour |
| | —do— (99) | —do— (78) | —do— (99) | —do— (88) | —do— (98) | —do— (99) | 5 hour |
| | —do— (100) | —do— (85) | —do— (89) | Dies (90) | —do— (94) | Disintegrates (100) | 18 hour |
| Advanced veliger to organogenesis | Normal development (88) | Normal development (70) | Very slow movement (55) | Very slow movement (60) | Disintegrates (100) | Disintegrates (99) | 1 hour |
| | Dies (84) | —do— (78) | Disintegrates (70) | —do— (75) | —do— (100) | —do— (100) | 5 hour |
| | —do— (78) | —do— (62) | —do— (72) | Dies (180) | —do— (99) | —do— (100) | 18 hour |

Results indicate the mean of at least three sets of experiments. Percentage of eggs attaining the changes are given in parentheses. Number of eggs in each set of experiment was twenty.

shows that trochophore stage is unaffected by the cold shock although the movement is stopped altogether. The eggs regain their normal movement after an hour in water. The control trochophore eggs cannot survive the cold shock. Morulaes behaved peculiarly. After being thawed they rest as if in a dormant condition for a number of days. When left to water they disintegrate. The harmful effects of changes in freezing and thawing, the mechanical damage within the cell as pointed out by Smith[2]—all seem to be counterbalanced by glycerol, promisingly in the trochophore stage. The study may explore the possibilities of the successful applications of glycerol in the metabolic investigations of *Limnaea* embryos far below the normal temperature.

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