

Effect of Medium on Transplantability of Nuclei of *Bufo arenarum* Embryonic Cells

The method developed by BRIGGS and KING¹ for the transplantation of amphibian embryonic nuclei represents one of the most important contributions to experimental analysis of nuclear differentiation. It involves the transfer of embryonic nuclei into enucleated oocytes in order to test their capacities to promote normal development. Since no physiological solution is yet available to preserve living cellular nuclei, this method was devised in such a way as to insure nuclear protection by a coat of cytoplasm. This is attained by sucking the donor cell into a narrow pipette, where the membrane is broken without dispersion of the protoplasm. Even so, nuclei were seen to remain sensitive to influences of the medium.

BRIGGS and KING², after testing a series of inorganic and organic solutions, found that the best medium for *Rana pipiens* nuclei was the salt solution of NIU and TWITTY³ for the culture of amphibian embryonic cells. Another work of this type carried out on *Ambystoma mexicanum* nuclei⁴ has shown that the best results are obtained with STEINBERG's⁵ solution; and ELSDALE et al.⁶, working on *Xenopus laevis*, found good results and no differences between the solutions of BARTH⁷ and NIU and TWITTY.

On the basis of this information it seemed desirable to extend this type of control to the toad, *Bufo arenarum*. The results of the first transplantation experiments on this Anuran⁸, carried out in NIU-TWITTY solution, showed some differences in nuclear potentialities among mid-blastula, late blastula and mid-gastrula stages.

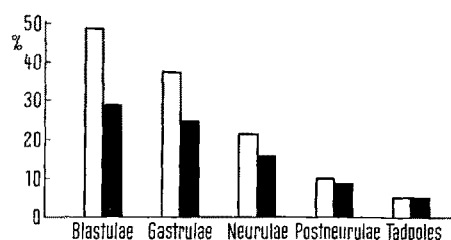
Concerning the cleavage promoting capacity of the nuclei tested, the higher proportion of regular cleaving blastulae was obtained with mid-blastula nuclei. As in this stage donor cells were of larger diameter than in the 2 other stages chosen, this result was provisionally ascribed to a better cytoplasmic protection of the nuclei during the transfer operation.

Although gastrula nuclei gave about the same proportion of complete blastulae as late blastula nuclei, their capacity to promote normal development in later stages appeared considerably restricted. As both nuclei were equally protected during transfer operation, some other reason must be looked for to account for the differences observed.

The purpose of the present study was to compare the influences of the operating media of BARTH and NIU and TWITTY on *B. arenarum* nuclei during transfer operation. The transplantation technique used was the same as that described previously⁸, and all the experiments were done with nuclei of the animal pole of late blastulae (stage 9), taken from the inner side of the centre of the blastocoel roof. The eggs, at the end of the operation, were always transferred into 10% amphibian Ringer's solution lacking bicarbonate in order to avoid any influence of the media tested upon later development. The results reported in this paper were taken from 350 transfer operations.

According to our data, the capacity of nuclei to promote normal cleavage was diversely affected by the 2 media tested. The best results were obtained with BARTH's solution in that the eggs operated in this medium gave the higher proportion of normal complete blastulae as shown in the histogram of the Figure. Nevertheless, the final proportion of normal tadpoles remained the same in both series of experiments. This suggests that larval development, which is known to depend on the quality of the nucleus transferred, would not be influenced in this case by the composition of the operating media. The higher proportion of cleaving eggs obtained with BARTH's

solution could be ascribed, as a tentative hypothesis, to a better protection of the cleavage centre that accompanies the injected nucleus. In this connection, it is known that while a cleavage centre suffices to elicit segmentation in amphibian eggs almost devoid of chromatin, a non-differentiated nucleus with a normal complete chromosome set is required to insure larval development⁹. This interpretation allows us to assume that BARTH's solution, in spite of improving the cleavage capacity of the transplanted nuclei, does not affect their capacity to promote larval development¹⁰.



Percentage of embryos (ordinate) reaching each of the stages listed on the abscissa. White columns: eggs operated in BARTH's solution. Black columns: eggs operated in NIU and TWITTY's solution. Total transfers taken as 100%.

Resumen. Se transplantaron núcleos de blástulas de *Bufo arenarum* en ovocitos enucleados de la misma especie operando en las soluciones salinas de BARTH y de NIU y TWITTY. Con la solución de BARTH se consiguió un porcentaje más alto de huevos con segmentación normal que el obtenido con la solución de NIU y TWITTY. La proporción final de renacuajos normales obtenidos, sin embargo, resultó idéntica en ambas series de experimentos.

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