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VARIATION IN THE NUCLEAR SODIUM CONCENTRATION OF NEWT OOCYTES DURING MATURATION

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SUMMARY

Measurements of the sodium content of single oocyte nuclei of *Triturus cristatus carnifex* at various stages of maturation, show a rise in concentration in eggs of the size 0·5–0·6 mm diameter. When the nuclei are isolated rapidly under paraffin, the absolute values obtained for the concentration are much lower than have been previously reported for this species.

INTRODUCTION

Riemann, Muir & Macgregor (1969) in their discussion of analytical results of the Na and K content of the nucleoplasm and cytoplasm of newt oocytes concluded that the absence of any detectable variation in these concentrations deprived Kroeger's (1966) postulated connexion between the ionic environment and puffing pattern of *Chironomus* salivary gland chromosomes of any validity in explaining the changes in form of the lampbrush chromosomes of newt oocytes. Their results were obtained on pooled samples of as many as 50 nuclei isolated from oocytes in arbitrarily divided size ranges. This method of sampling, necessitated by the quantity of material required for atomic absorption analyses, can result in the obscuring of quite large variations from one cell size to another if the arbitrarily chosen size ranges do not coincide with the variations occurring naturally. It was therefore desirable to re-examine material of this type, using an analytical method of sufficient sensitivity to enable variations between single nuclei to be detected with confidence.

MATERIALS AND METHODS

Oocytes from *Triturus cristatus carnifex* supplied by Messrs Haig Ltd, Beam Brook, Surrey, were obtained by partial oophorectomy. Measurements were made without regard to the time of year. The excised piece of ovary was kept in a small dish, sealed to prevent evaporation, which sat in an ice bath. Single oocytes were dissected out without the use of any saline, and transferred to medicinal paraffin. The diameter of the oocyte was measured using an eyepiece graticule calibrated against a stage micrometer, then opened by tearing the follicle and cell membrane by pulling with fine watchmaker's forceps. The cytoplasm was spread out into a thin ribbon through the paraffin by a swift movement of the remains of the cell held in one of the pairs of forceps. The nucleus usually appeared as a slight swelling in this ribbon from which it could readily be teased out and cleaned of adhering cytoplasm by rapid stirring and rolling

motions of a mounted needle. When clean the diameter of the nucleus or what remained of it if it had been damaged was measured using a Watson shearing eyepiece calibrated as before. The cleaned nucleus was drawn with the minimum volume of paraffin into a clean quartz capillary tube which was then sealed at both ends. The nucleus was heat-fixed by touching the side of the tube in the neighbourhood of the nucleus with a hot rod, to prevent it sticking to the side of the tube during subsequent treatment. No oocytes were used when the ovary fragment was more than 2 h old. The time taken from opening the oocyte till the completion of cleaning off the cytoplasm was not more than one minute and the subsequent time to the isolation of the nucleus in the capillary less than 0·5 min. Batches of nuclei were activated along with standards consisting of volumes of sodium chloride solution of such concentration that the quantity of sodium in the 5 μl used was about 1 μg. The standards were also sealed into clean quartz capillaries. The activation was by thermal neutron irradiation in the Scottish Research Reactor Centre's reactor at East Kilbride for a period of 6 h at a flux of 10^{12} neutrons/cm^2/s (or 3 times that flux after the reactor was upgraded). The short half life nuclides formed were allowed to decay for 18 h after which the contents of the capillaries were washed out with petroleum ether (or distilled water in the case of the standards) on to fibre-glass filter disks, supported on normal aluminium planchets. The nucleus was glued to the filter disk to prevent loss during handling. The gamma rays emitted were detected by a 7·6 × 7·6 cm sodium iodide crystal and recorded on a 100-channel analyser. Counting times were 100 min for samples and 10 min for standards. After initial examination of the paraffin in which no sodium could be detected in the volumes used in the experiment, blanks were not run. Further details of the analytical procedure may be found in Whitley & Muir (1971).

RESULTS

The average of the counts per channel of the background on either side of the ^{24}Na 1·37 MeV photopeak was subtracted from the counts in each channel of the

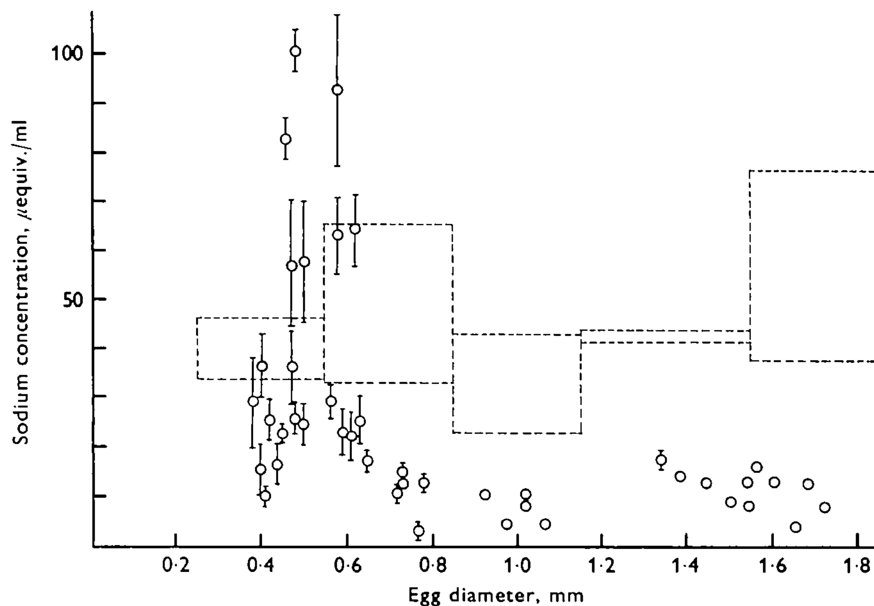

Fig. 1. Sodium concentration in single nuclei of various sizes. The boxes in pecked lines represent the results obtained for comparable pooled material by Riemann, Muir & Macgregor (1969). Each box indicates the size range and standard error of their results. The circles show the values obtained for single nuclei of eggs of that diameter, with error bars where the error exceeds the size of the circle.

photopeak and the remainders summed. This total for each sample was compared with those for the standards, when both had been corrected for decay time. The volume of the nuclear sample used was calculated from the diameter, and the sodium concentration computed from the two values. Fig. 1 shows the results of analyses of single nuclei from oocytes of different sizes with error bars. The boxes show the results obtained from pooled samples in the size ranges shown obtained by Riemann *et al.* (1969). It will be seen that the nuclear sodium concentration is not constant throughout the development of the oocyte.

DISCUSSION

Scatter of results

Unless the physiological stage of the cell is closely related to its physical size, and there seems to be no obvious reason why this should be so, the superimposition of instantaneous measures of a parameter fluctuating in a common pattern may well obliterate most of the evidence for such a pattern. It is then more convincing when despite the tendency to obliteration there remains indisputable evidence that at some egg sizes around 0·5 mm, sodium concentrations are found in many nuclei that are not found at smaller or larger sizes, and that within this size range, low sodium contents which are common at lower and higher parts of the size range are uncommon.

Absolute value

The length of the error bars shows that the analytical method employed is nearing the limit of its usefulness at the sodium quantities present in nuclei of oocytes of the smallest size examined. The errors represented in Fig. 1 take into account those arising in the measurement of low levels of activity of the samples, and the errors in measuring the diameter of the nuclei which have a considerable effect in calculating the volume. There seems, however, to be a genuine difference in the estimate of the concentration of sodium between that currently used and that of Riemann *et al.* (1969). The average concentration of sodium in nuclei of eggs over 1·5 mm in diameter found by the latter authors was 56 μequiv./ml and in our study nuclei from corresponding eggs gave 8 μequiv./ml. In this respect our present results resolve the anomaly between the results of Riemann *et al.* on *Triturus*, and others obtained on mature oocytes of various amphibians by Century, Fenichel & Horowitz (1970), who found nuclear sodium concentrations between 7·3 and 16·8 μequiv./ml. Century *et al.* suggest that the high results previously reported for *Triturus* might be due to the leakage of sodium into the nuclei from the cytoplasm where the concentration, at least in the mature oocyte, is believed to be much higher (Naora, Naora, Izawa, Allfrey & Mirsky, 1962; Century *et al.* 1970), while the nuclei are lying in the cytoplasm of the ruptured eggs prior to cleaning. As this process took several minutes in the procedure of Riemann *et al.* as opposed to the less than one minute of the present routine the suggestion seems to be well founded. The same reasoning could explain most of the disparity between the 2 sets of *Triturus* results, but one should also note that the size limit between the first and second pools of Riemann *et al.*'s samples, though arbitrarily

fixed, could hardly have been better chosen if the intention had been to obscure the transient rise in sodium concentration in the 0·5-mm size range found in our measurements. It appears then that the conclusion reached by Riemann *et al.* that 'it is unlikely that fluctuations in nuclear Na or K concentrations are responsible for extension or retraction of the lateral loops of lampbrush chromosomes' requires re-examination. The results of current determinations of potassium in the nucleoplasm are as yet insufficient to support or contradict previous measurements. The present authors have no competence to comment on the cytological significance of these results, but it is clear that the sodium content of the chromosomal environment is neither as stable nor of the exact magnitude previously reported for the oocytes of *Triturus* during maturation.

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