J. Cell Sci. 4, 299–304 (1969)
Printed in Great Britain

SODIUM AND POTASSIUM IN OOCYTES OF *TRITURUS CRISTATUS*

W. RIEMANN
Institut de Zoologie et d'Anatomie Comparée, Université de Genève, Switzerland

C. MUIR AND H. C. MACGREGOR
Department of Zoology, The University, St Andrews, Scotland

SUMMARY

Newt oocytes were dissected under liquid paraffin and known volumes of clean nucleoplasm and cytoplasm were obtained from oocytes in different stages of oogenesis. The samples were digested in redistilled nitric acid, diluted with deionized water, and their Na and K contents were measured by flame spectrophotometry. The results were expressed as micro-equivalents of Na and K per millilitre of nucleoplasm or cytoplasm. In oocytes of 0·3–0·5 mm diameter nucleoplasm and cytoplasm have similar Na and K concentrations, and the molar K:Na ratio is about 3:1. As the oocyte grows to maturity the nucleoplasmic Na and K concentrations do not change, but the cytoplasmic K concentration falls steadily until, in nearly mature oocytes, the cytoplasmic K:Na ratio is near 1:1. Measurements of Na and K concentrations in yolky and clear cytoplasm show that both fractions have the same K content and the concentration of K decreases in both fractions as the oocyte grows. The significance of these results is discussed in terms of the possible effects of changes in the intracellular ion balance on the morphology and synthetic activity of the chromosomes.

INTRODUCTION

According to Kroeger (1966) there are reasons for supposing that certain genetic loci in *Chironomus* salivary gland cells are 'activated', caused to puff, *in vitro* by a change in the intracellular Na:K ratio, and he argues that the same loci are activated by changes in ionic conditions during normal development. The polytene chromosomes of salivary gland cells from dipteran larvae and the lampbrush chromosomes from amphibian oocytes have several features in common, and it has been suggested that a puff or Balbiani ring on a salivary gland chromosome and a lateral loop on a lampbrush chromosome are examples of one and the same basic phenomenon, namely extension of DNA fibres allowing RNA synthesis to proceed (Callan, 1955; Gall, 1963), the former situation involving many chromosome strands, the latter only one strand. The loops of lampbrush chromosomes extend and retract at particular stages of oogenesis in Amphibia, and certain loops are larger, and hence more conspicuous, at one stage than at another (Callan & Lloyd, 1960). Therefore if the relative concentrations of sodium and potassium of the cell are causally connected with puff development or regression then some change in cellular sodium or potassium might be found during oogenesis in Amphibia, and this change ought to be particularly evident in oocytes nearing maturity when the chromosomes and their lateral loops shorten dramatically.

We have therefore measured the sodium and potassium contents of nucleoplasm from amphibian oocytes at different stages in oogenesis.

MATERIALS AND METHODS

All measurements were made on material obtained from oocytes of *T. c. carnifex* or *T. c. cristatus*. The standard method for obtaining nucleoplasm and cytoplasm from oocytes was as follows. All operations were carried out in medicinal liquid paraffin. No oocyte or oocyte nucleus was exposed to an aqueous medium at any stage in isolation. A piece of ovary consisting of oocytes in all stages of oogenesis was removed from a newt and placed in an embryo dish containing liquid paraffin. The oocytes were carefully separated from one another, measured under a stereoscopic microscope fitted with a micrometer eyepiece, and sorted into groups according to similarity in size.

Oocyte nuclei were isolated by tearing the oocytes open and leaving their contents to disperse. After about a minute the nucleus appeared as a 'blister' in a puddle of yolky cytoplasm. The nucleus was then picked up and cleaned of adherent yolk by repeatedly pumping it in and out of a fine-drawn glass pipette. It was then transferred by pipette to another embryo dish which contained nuclei from a particular size of oocyte. Sufficient nuclei were collected from each size range of oocyte to provide 5 μl samples of nucleoplasm. The nuclei were then ruptured by gently stirring them together with a fine glass needle. The 'bubble' of nucleoplasm so obtained was then drawn into a 5 μl 'Microcap' (Drummond Scientific Co., Inc., U.S.A.), carefully avoiding any contamination with paraffin. The microcaps were emptied into 10 ml calibrated quartz tubes and rinsed several times with deionized water. The water used at this and subsequent stages had been double glass distilled and passed through a 70-cm column of 'Biodeminrolit' mixed-bed resin (Permutit Co., Ltd.).

Cytoplasmic samples were obtained by rupturing whole oocytes under paraffin, removing their follicle tissue and nuclei, and drawing the remaining cytoplasm into 5 μl microcaps. Each 5 μl sample of nucleoplasm or cytoplasm was digested for 12 h with 2 ml redistilled nitric acid at 110 °C and subsequently diluted to 10 ml with deionized water.

In larger oocytes much of the cytoplasmic volume is occupied by yolk. For reasons which will be apparent later we decided to separate yolk from clear cytoplasm + nucleoplasm and measure the sodium and potassium contents of these fractions. Batches of 50–200 oocytes were gently homogenized under paraffin and transferred to a 1 ml cellulose acetate centrifuge tube. The tube was topped up with paraffin and spun for 1 h at 40000 rev/min in a 3×5 ml M.S.E. swinging-bucket rotor fitted with suitable adapters. After centrifugation 5 μl samples were taken from the clear supernatant of oocyte material and from the yolky pellet.

The apparatus used for ion determinations was a Hilger and Watts Uvispek spectrophotometer and atomic absorption attachment with hollow cathode lamps (Atomic Spectral Lamps Pty, Ltd., Melbourne, Australia) and a propane-air flame. The standard Hilger and Watts spraying unit was replaced by one with a concentric air annulus jet spraying into a 2×7 in. copper tube which was heated by an external nichrome

winding. The effect of this substitution was to heat the emergent gases to 105–110 °C and thereby increase the spraying efficiency from the 11% of the standard unit to nearly 100%. Consequently the amount of material needed for a single measurement could be reduced by an order of magnitude. The substituted sprayer also had the advantage of being less sensitive to air-flow fluctuations.

The measuring procedure was as follows. First, a series of standard solutions was sprayed in order of increasing concentration with reference to deionized water, and a calibration curve was obtained. The samples were then sprayed and their approximate concentrations determined. A standard was then resprayed to check that the response of the instrument had not changed. Samples and standards were then arranged in order of increasing concentration and the whole series was sprayed twice. Three readings were therefore obtained for each specimen.

Some measurements were carried out with a Carl Zeiss spectrophotometer PMQ II equipped with a flame attachment. A hydrogen/oxygen flame was used on this instrument. We wish to thank Ganz Optar of Zurich for providing the flame attachment and for assisting with its operation.

The samples measured came from oocytes of between 0·3 and 1·8 mm diameter. The oocytes were classified by size into 5 groups. Measurements were made on oocytes taken from newts in October and December of 1966 and in spring and summer of 1967.

OBSERVATIONS

In the smallest group of oocytes (0·3–0·5 mm diameter) nucleoplasm and cytoplasm have similar Na and K concentrations and the molar K:Na ratio is about 3:1. In the

Table 1. *Amounts of Na^+ and K^+ ($\mu eq/ml$) in nuclei and cytoplasm from Triturus oocytes*

	Oocyte diameter (mm)									
	0·3–0·5		0·6–0·8		0·9–1·1		1·2–1·5		> 1·5	
	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
Cytoplasm	72 ± 3	181 ± 50	59 ± 4	165 ± 20	82 ± 1	92 ± 3	52 ± 8	58 ± 2	56 ± 6	50 ± 3
	33 ± 3	143 ± 20	53 ± 5	173 ± 20	71 ± 2	68 ± 2	61 ± 2	57 ± 2	53 ± 2	50 ± 3
	36 ± 2	174 ± 30	82 ± 5	129 ± 5	52 ± 9	56 ± 1	87 ± 0	56 ± 2	58 ± 2	59 ± 5
	57 ± 2*	119 ± 2*	62 ± 4	125 ± 15	69 ± 7	75 ± 10	113 ± 15	80 ± 3	52 ± 1*	52 ± 1*
	72 ± 2*	123 ± 1*	81 ± 4	153 ± 10	57 ± 5	57 ± 5	51 ± 1*	46 ± 1*	58 ± 1*	51 ± 1*
	54 ± 1*	111 ± 1*	42 ± 2	128 ± 15	57 ± 3	80 ± 11	76 ± 1*	72 ± 3*	—	—
	—	—	74 ± 1*	93 ± 2*	49 ± 2*	57 ± 3*	75 ± 1*	49 ± 0*	—	—
Means	54	141	62	132	61	66	73	59	54	52
Nucleoplasm	46 ± 1	125 ± 30	65 ± 2	178 ± 20	40 ± 1	130 ± 12	42 ± 1*	96 ± 1*	63 ± 1	138 ± 20
	39 ± 1*	132 ± 1*	60 ± 5	180 ± 40	26 ± 0*	127 ± 2*	—	—	80 ± 4	138 ± 25
	35 ± 1*	126 ± 2*	34 ± 1	147 ± 15	—	—	—	—	37 ± 3	120 ± 27
	—	—	38 ± 1*	124 ± 2*	—	—	—	—	46 ± 1*	102 ± 2*
Means	40	127	49	157	33	129	42	96	56	124

Values marked * were obtained with a Carl Zeiss flame spectrophotometer.

Each value represents the mean of 3 measurements of a sample consisting of nucleoplasm or cytoplasm from several oocytes within the indicated size range.

next size range (0·6–0·8 mm diameter) the Na and K concentrations in the nucleoplasm are the same as in smaller oocytes. The cytoplasm, on the other hand has less K so that the K:Na ratio is a little over 2:1. In still larger oocytes the nucleoplasmic ion content changes only slightly, if at all; but the cytoplasm has still less K, and the cytoplasmic K:Na ratio approaches 1:1. Our detailed results are presented in Table 1 and Fig. 1.

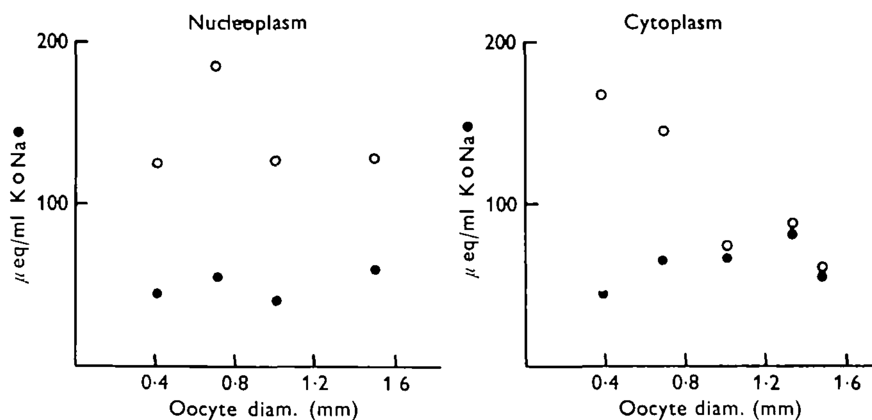

Fig. 1. Graphical representation of the mean values shown in Table 1.

As an amphibian oocyte grows larger, more and more of its cytoplasmic volume becomes occupied by yolk particles. It therefore seemed possible that the observed decrease in cytoplasmic K concentration as the oocyte grows larger was due to packing of the cytoplasm with relatively K-free yolk. Accordingly, the overall K concentration would apparently decrease as the oocyte became more yolky, but the K concentration in the clear cytoplasm might remain the same or even increase. Our measurements of Na and K concentrations of 'yolk' and 'clear cytoplasm' (Table 2) show that both fractions have about the same K content and that the concentration of K decreases in both fractions as the oocyte increases in size.

Table 2. *Amounts of sodium and potassium (μeq/ml) measured in centrifugally separated clear cytoplasm and yolk of Triturus oocytes*

	Oocyte diameter (mm)					
	0–0·7		0·75–1·1		1·5–1·75	
	Na	K	Na	K	Na	K
Whole oocyte clear layer	67	117	57	106	68	67
Whole oocyte yolk	—	95	54	74	47	39
Cytoplasmic clear layer	—	—	—	—	63	66
Yolk	—	—	—	—	54	36

DISCUSSION

According to our observations the concentrations of Na and K in nucleus and cytoplasm of small yolkless oocytes are about the same. In this respect our findings agree with those of Kanno & Loewenstein (1963), who found no electrical difference between cytoplasm and nucleoplasm of *Xenopus* and *Triturus* oocytes smaller than the smallest which we have examined.

Throughout oogenesis Na concentrations in the nucleus and cytoplasm are about equal, but as the oocyte grows the cytoplasmic K concentration decreases. In the largest oocytes only is there substantially more K in the nucleus than in the cytoplasm. These observations do not agree with the findings of Naoro *et al.* (1962). The latter found that in yolky oocytes from *Rana pipiens* the nuclear Na and K concentrations were respectively 3·2 and 2·4 times the cytoplasmic concentrations. They reported, moreover, that the Na:K ratio is 1·1 in the nucleus and 0·72 in the cytoplasm. Essentially, Naora *et al.* found, in frog nuclei, much more Na and K (250 and 227 μeq/ml respectively) and a much lower nuclear K:Na ratio (0·9) than we have found in newt nuclei. We cannot at the moment attribute any significance to these differences.

Kroeger (1963, 1966) claims to have demonstrated an association between puffing and specific ionic conditions in the nuclear sap of dipteran salivary gland cells. He has shown that salivary glands of *Chironomus thummi* explanted into artificial media having different Na:K ratios show different puffing patterns. Moreover, by stepwise replacement of Na^+ by K^+ in the explant medium a sequence of puffing is induced which matches the normal sequence of puffing between adult larva and middle pre-pupa stages. Kroeger argues that genetic loci which are activated *in vitro* by ions are also activated by ions during normal development, and that change in the internal ion balance of the cell is a primary agent in gene activation.

We have been unable to detect any significant changes in K or Na concentrations in the nuclear sap of newt oocytes. We therefore think it unlikely that fluctuations in nuclear Na or K concentrations are responsible for extension or retraction of the lateral loops of lampbrush chromosomes.

It is unfortunate that the amphibian oocyte is unsuitable for experiments of the type which Kroeger has performed on salivary glands of dipteran larvae, and that dipteran salivary cells are intractable material for ionic analyses. In our experience, however, lampbrush chromosomes isolated into NaCl appear no different from chromosomes of similar origin isolated into KCl of the same molar strength or from those isolated into any combination of NaCl and KCl having the same total molarity, and we have been unable to define any differences between chromosomes isolated from oocytes which have been incubated in NaCl and those taken from oocytes which have been similarly incubated in KCl of the same molar strength. Oocyte nuclei and lampbrush chromosomes undoubtedly do undergo changes when an oocyte, oocyte nucleus, or the chromosomes themselves are exposed to artificial media of different anionic *concentrations* (Duryee, 1941; Callan, 1952; Macgregor & Callan, 1962) but these changes are relatively drastic and irreversible and we do not think that anything comparable to them happens during normal development of the oocyte.

REFERENCES

CALLAN, H. G. (1952). A general account of experimental work on amphibian oocyte nuclei. *Symp. Soc. exp. Biol.* **6**, 243–255.

CALLAN, H. G. (1955). Recent work on the structure of cell nuclei. In *Symposium on the Fine Structure of Cells.* I.U.B.S. publ. series B, **21**, 89.

CALLAN, H. G. & LLOYD, L. (1960). Lampbrush chromosomes of crested newts *Triturus cristatus* (Laurenti). *Phil. Trans. R. Soc.* B **243**, 135–219.

DURYEE, W. R. (1941). The chromosomes of the amphibian nucleus. In *Cytology, Genetics and Evolution*, pp. 129–141. (University of Pennsylvania Bicentennial Conference, Philadelphia).

GALL, J. G. (1963). Chromosomes and cytodifferentiation. In *Cytodifferentiation and Macromolecular Synthesis* (ed. M. Locke), pp. 119–143. New York and London: Academic Press.

KANNO, Y. & LOEWENSTEIN, W. R. (1963). A study of the nucleus and cell membranes of oocytes with an intracellular electrode. *Expl Cell Res.* **31**, 149–166.

KROEGER, H. (1963). Chemical nature of the system controlling gene activities in insect cells. *Nature, Lond.* **200**, 1234–1235.

KROEGER, H. (1966). Potentialdifferenz und Puff-Muster, elektrophysiologische und cytologische Untersuchungen an den Speicheldrüsen von *Chironomus thummi. Expl Cell Res.* **41**, 64–80.

MACGREGOR, H. C. & CALLAN, H. G. (1962). The actions of enzymes on lampbrush chromosomes. *Q. Jl microsc. Sci.* **103**, 173–203.

NAORA, H., NAORA, H., IZAWA, M., ALLFREY, V. G. & MIRSKY, A. E. (1962). Some observations on differences in composition between the nucleus and cytoplasm of the frog oocyte. *Proc. natn. Acad. Sci. U.S.A.* **48**, 853–859.

(*Received* 29 *April* 1968)