The fixation and preparation for the SEM was made as previously reported[7]. The specimens were observed with a Jeol Scanning Microscope JMS 35 at 25 KV after being coated with gold in a sputering apparatus LWU (München).

Results and discussion. The supraependymal cells (SEC) in both sexes were localized on the nonciliated ependymal surface of the hypothalamus. This area corresponds to the projection of the arcuate nucleus and to the major part of the hypophyseotropic area[7]. The greatest concentration of these cells was found on the anterior part of the area, near the retrochiasmatic region and the transition zone between ciliated and nonciliated areas. The restriction of the SEC to this area was observed during all phases of the ovarian cycle. The cell number, however, varied from phase to phase as follows: Oestrus: between 5 and 8 supraependymal cells per ventricular surface; metoestrus: between 70 to 80 cells; di-oestrus: between 95 and 100 cells; pro-oestrus: 35 to 45 cells per ventricular surface. These variations were not observed in the males, which showed an average of 25 to 35 cells.

Four basic morphological types of SEC could be determined according to the number and shape of their processes, which take up differing degrees of contact with the ependyma. On some occasions the processes even protrude between the ependymal cells. These forms or types have been found in all phases of the ovarian cycle, although no phase shows exclusively one type. The type which has 2 or 3 processes and some cytoplasmic laminar folds (Figure 1) is the most common, followed by the type without the laminar folds (Figure 2). The type with enormous laminar folds that cover several ependymal cells was found more frequently during oestrus than in any other phase (Figure 3), whereas the one with many thin processes is more frequent during di-oestrus (Figure 4). This latter form is similar to the macrophages in vitro[14].

The outer borders of the laminar type (Figure 5) possess many thin finger-like as well as spherical protrusions which may be related to functions of particle uptake, cell locomotion and/or renewal of the cell membrane[15]. Although these laminar extensions are morphologically not similar to the ruffled borders of the macrophages in vitro, this cannot be used as an argument against the interpretation of the SEC as macrophages, because the formation of ruffled borders depends on the nature of the underlying substrate[16]. These four morphological types probably correspond to different behavioral expressions which each cell shows as an answers to environmental necessities by altering its shape and its processes. This assumption points to a similarity of the SEC to mesenchymal cells. Without experimental data to prove the contrary, the variations in cell number must be interpreted as being dependent upon such environmental factors as cyclic changes in the ependyma itself. On the basis of previous studies in vitro, the above-mentioned behavioral aspects are not comparable to those of the neurons and neuroglia[15]. It is also difficult to imagine the migration of neurons and neuroglia cells across the ependyma into the ventricle in adult animals. For these reasons, and according to the hypothesis of BLEIER et al[12], the SEC could be considered to be a specialized population of mesenchymal cells involved in the renewal of the ependyma as well as the transport of cell debris and foreign particles.

[14] J. P. REVEL, P. HOCH and D. Ho, Expl. Cell Res. *84*, 207 (1974).
[15] K. MELLER, P. MESTRES, W. BREIPOHL and M. WAELSCH, Cell Tiss. Res. *148*, 227 (1974).
[16] J. P. REVEL, Proc. 7th SEM Symp., Part III (IIT Research Institute, Chicago 1974), p. 542.

On the Mechanism of Water Uptake by the Developing Eggs of *Calotes versicolor*

SURESH C. GOEL

Department of Zoology, Poona University, Poona 411 007 (India), 18 March 1976.

Summary. Experiments show that osmotic gradient has a role in the absorption of water by the semi-cleidoic egg of *Calotes versicolor*. The observations also indicate that in nature the eggs need to be laid in water-saturated soil and can easily withstand flooding but not dryness.

The absorption of copious amounts of water (5 times the amount in freshly laid eggs) by the eggs of the lizard, *Calotes versicolor* during the entire period of incubation is a physiological necessity[1]. The present communication reports on the role of the osmotic gradient in the absorption of water, and also on some ecological parameters necessary for the development of the eggs of *Calotes*.

Materials and methods. Eggs, obtained from the uteri of gravid females, were incubated on cotton beds flooded with glass-distilled water[1] and embryos staged[2]. The surface area of eggs was calculated according to the formula for a prolate spheroid[3]. To study the role of osmotic pressure in water uptake, the eggs were incubated on cotton beds soaked with different concentrations of sodium chloride. Water flooding – a normal possibility in nature since the eggs are usually laid in the rainy season and deposited underground – was simulated by complete immersion of the eggs in water. The eggs were incubated on dry cotton beds in a glass chamber containing a beaker filled with water (environment of 100% humidity) to test whether the eggs laid after the end of the rainy season[2] can survive conditions where presumably only water vapour and no water is available in soil interstices. The incubation of egg in a desiccator (environment of 0% humidity) was made to ascertain the rate of water evaporation from the egg to elucidate the nature of egg shell. During incubation, the surface area of the egg increases from about 3.0 cm^2 to about 8.0 cm^2, and consequently eggs of different sizes were used in these experiments.

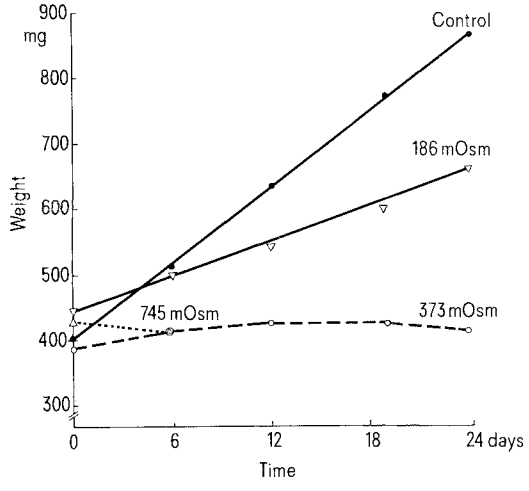
Results and discussion. The mechanism of water uptake by the eggs of *Calotes versicolor* appears to be passive, and mediated by an osmotic gradient, because a) the higher the osmotic pressure exerted by the environmental liquid, the less was the uptake of water (Figure, Table I); b) the eggs completely immersed in water did not differ

[1] S. C. GOEL, J. zool. Soc. India *25*, 37 (1973).
[2] V. MUTHUKKARUPPAN, P. KANAKAMBIKA, V. MANICKAVEL and K. VEERARAGHAVAN, J. Morph. *130*, 479 (1970).
[3] J. D. YOUNG, Proc. zool. Soc. London *120*, 455 (1950).

Table I. Effect of Osmotic pressure, created by different concentrations of sodium chloride, on the eggs and embryos of *Calotes versicolor*

Osmotic pressure (mOsm)	Egg weight on day 0 (mg)[a]	Parameters on day 24			Increase in egg weight during experiment	
		Egg weight (mg)	Embryo weight (mg)	Stage of embryo (mg)[b]	Absolute weight (mg)	Original weight (%)
0.0	406 (7)	869 (4)	106 (4)	38	463	114.0
186.3	444 (7)	664 (5)	83 (5)	38–[c]	220	49.5
372.6	393 (7)	415 (5)	43 (3)	34	22	5.6
745.2	438 (7)[d]	–	–	–[d]	–	–

[a] All eggs or embryos in a group were collectively weighed and the means calculated. Figures in parentheses indicate the number of eggs or embryos. [b] On day 0 the embryos were at stage 29. [c] Embryo with webbed digits, a feature of stage 36. [d] All eggs contained dead embryos when opened on day 12.



The effects of osmotic pressure, created by different concentrations of sodium chloride, on water exchange between the egg of *Calotes* and the environment.

from controls in the amount and rate of the water absorbed[4] (Table II); c) the eggs exposed to 100% or 0% humidity lost water at a characteristic rate (Table II). This seems to confirm the hypothesis of PACKARD[5] regarding the role of osmotic gradient in water absorption by reptilian eggs. However, we believe that the osmotic gradient is created due to the semipermeable nature of extraembryonic membranes, and not of the egg shell as suggested by PACKARD[5], because in *Calotes* the egg shell is freely permeable to water and also to urea and various dyes[6].

The considerable inhibitory effects on the development and growth of the embryos in the osmotic pressure experiment (Table I) appear to be either due to disturbed osmotic pressure inside the egg, or to low availability of water to the embryos. The low survival rate of the embryos forced us to terminate the experiment after 24 days.

[4] The complete immersion of egg does not lead to hypoxia, a distinct probability in cleidoic egg of hen.

[5] G. C. PACKARD, Am. Nat. *100*, 667 (1966).

Table II. Effects of different humidity environments on the rate of water exchange by the eggs of *Calotes versicolor*

Humidity of environment	Egg parameters at 0 h		Changes in egg weight after 24 h			Developmental stage of contained embryo[a]
	Sureface area (cm^2)	Weight (mg)	Absolute weight (mg)	Original weight (%)	Per cm^2	
Control	2.99	468	6	1.3	2.0	31– (1)
	6.96	1545	54	3.5	7.8	41 (1)
	7.89	2184	88	4.0	11.2	41– (1)
Water-immersed	2.99	478	17	3.6	5.7	31– (1)
	6.43	1388	53	3.8	8.2	40+ (1)
	7.95	2150	34	1.6	4.3	40+ (1)
100% humidity	2.99	439	–36	–8.2	–12.0[c]	32– (1)
	5.17	992	–56	–5.6	–10.8	35– (1)
	6.72	1528	–72	–4.7	–10.7	41– (1)
	6.96	1611	–81	–5.0	–11.6	41– (1)
	8.02	2114	–106	–5.0	–13.2	41– (1)
0% humidity	2.99	482	–272	–56.4	–90.9[b]	31 (1)
	4.48	800	–406	–50.8	–90.6	37+ (1)
	6.71	1534	–590	–38.5	–87.9	40+ (1)
	7.22	1708	–570	–33.4	–78.9	40+ (1)
	7.70	2017	–642	–31.8	–83.4	41– (1)

[a] Number of embryos in parentheses. [b] In these experiments the egg becomes soft and ultimately gets wrinkled. Stage 41-embryo morbid, others dead. [c] In another experiment (embryo stage 29+), after 72 h, the embryos had feeble heart beat, and retarded growth and development.

It is noteworthy that the rate of water exchange, expressed as mg water/cm²/day, showed little variation from experiment to experiment under 0% humidity (0.83–1) and 100% humidity (0.80–1), that is, when the egg actually lost water to the environment; on the other hand, the variance was high in control (0.11–1) and water immersed eggs (0.52–1), that is, when the egg absorbed water from the environment.

The eggs incubated under 0% humidity lost over 75% of their total water content within 24 h, and 95% within 50 h. The rate of water loss (75 to 91 mg/cm²/day) from the intact eggs of *Calotes* under 0% humidity is about 2 magnitudes higher than that from the eggs of the tortoise, *Testudo* (0.8–1.0 mg/cm²/day), and domestic hen (1.7 mg/cm²/day)[3]. The percentage losses in weight after 24 h in case of the lizard, tortoise and hen eggs are 32–56%, 0.15–0.18%, and 0.19% respectively. This is perhaps due to the presence of the numerous large pores in the egg shell of the lizard[3,6].

[6] Author's unpublished observations: the egg shell is freely permeable to trypan blue, methylene blue, eosin, urea and many other solutes.

La population germinale des gonades chez des embryons chimères obtenus par l'association de fragments de blastodermes de Caille japonaise et de Poulet domestique

Germinal Population of Gonads in some Chimerical Embryos Obtained by Connecting Pieces of Japanese Quail and Domestic Chick Blastoderms

E. Didier et N. Fargeix

Laboratoire de Biologie Animale, Université de Clermont, B. P. 45, F-63170 Aubière (France), 28 janvier 1976.

Summary. The experimental realization of chimerical embryos (Martin's[13] technic) permits a quantitative appreciation of the modalities of the colonization of quail gonads by chick germ cells. Results clearly show that nature and origin of the somatic part of the gonad areas settle the characters of the genital ridges populating, and specially the specific index of asymmetry expressed by the percentage of PGC colonizing the right gonad.

Chez les Oiseaux, les recherches expérimentales de Fargeix[1,2], Fargeix et Didier[3], Didier et Fargeix[4], Didier, Fargeix et Bergeaud[5] et de Bergeaud[6] ont montré que le nombre de gonocytes fixés par les crêtes génitales dépend ordinairement de l'importance quantitative du territoire gonadique et non pas du lot initial de cellules germinales (CGP) contenues dans le croissant de Swift, suggérant ainsi l'existence d'un contrôle exercé par le soma sur la population germinale intra-gonadique. Ce problème peut par ailleurs être abordé grâce aux possibilités d'associations entre des crêtes génitales et des CGP provenant d'espèces différentes, telles qu'en ont pu obtenir Simon[7], Reynaud[8] ou Tachinante[9].

Dans cette perspective, nous nous sommes proposés d'étudier d'un point de vue quantitatif la colonisation des gonades de la Caille par des gonocytes de Poulet. Les caractéristiques déterminantes du choix de ces 2 espèces sont, d'une part la structure nucléaire particulière de la plupart des cellules embryonnaires de Caille qui peuvent être utilisées comme de véritables «marqueurs biologiques» (Le Douarin[10]), d'autre part l'existence de différences spécifiques entre le Poulet et la Caille dans l'index de répartition asymétrique des CGP entre les deux crêtes génitales (Fargeix et Didier[11], Didier et Fargeix[12]).

La méthode utilisée est celle mise au point par Martin[13]; elle consiste à associer in ovo des fragments de blastodermes de Caille (souche Géromoise) et de Poulet (race Hubbard) prélevés aux stades 8 à 14 de Hamburger et Hamilton[14]. L'expérience réalisée est schématisée dans la Figure 1. Afin d'éliminer l'intervention éventuelle de gonocytes de Caille en migration dans la circulation sanguine, la majorité de l'aire vasculaire du fragment Caille est réséquée. Le développement des embryons chimères obtenus est poursuivi jusqu'au 5e jour de l'incubation (stades 22 à 27 de Hamburger et Hamilton).

Résultats. Malgré une mortalité très importante (95% environ) nous avons pu obtenir 27 embryons chimères suffisamment développés pour que les gonocytes puissent être dénombrés dans les crêtes génitales. Les résultats généraux sont consignés dans le Tableau I. Dans la plupart des cas, la morphogenèse gonadique est bonne, bien que le développement du mésonéphros soit parfois déficient.

Tableau I. Résultats généraux des expériences

Résultats obtenus	Nombre de cas	Stade moyen (selon H. et H.)	Nombre moyen de gonocytes
Morphogenèse mauvaise et agénésie gonadique	5	22	0
Gonades Poulet	1	26	1279
Gonades Poulet et gonades Caille	5	26	981
Gonades Caille	16	24	222

[1] N. Fargeix, Thèse Doct., A. O. CNRS No. 2920 (1968).
[2] N. Fargeix, Ann. Embr. Morph. *3*, 107 (1970).
[3] N. Fargeix et E. Didier, C. r. Acad. Sci., Paris *276*, 2067 (1973).
[4] E. Didier et N. Fargeix, Arch. Anat. Hist. Embryol. *56*, 33 (1973).
[5] E. Didier, N. Fargeix et Y. Bergeaud, J. Embryol. exp. Morph. *32*, 619 (1974).
[6] Y. Bergeaud, Thèse Doct. 3ème Cycle, Clermont (1975).
[7] D. Simon, Arch. Anat. microsc. Morph. exp. *49*, 93–176 (1960).
[8] G. Reynaud, J. Embryol. exp. Morph. *21*, 485 (1969).
[9] F. Tachinante, C. r. Acad. Sci., Paris *278*, 1895 (1974).
[10] N. Le Douarin, Bull. biol. Fr. Belg. *103*, 435 (1969).
[11] N. Fargeix et E. Didier, C. r. Acad. Sci., Paris *279*, 2099 (1974).
[12] E. Didier et N. Fargeix, J. Embryol. exp. Morph., sous presse (1976).
[13] C. Martin, C. r. Soc. Biol., Paris *166*, 283 (1972).
[14] V. Hamburger et H. Hamilton, J. Morph. *88*, 49 (1951).