

and pyrogallol (5 $\mu\text{g/ml}$) did not affect the contractions of the isolated organs induced by angiotensin II (1–5 μg) or prevent their responses after the injection of bradykinin into the lungs, but completely blocked the release of RCS by bradykinin, when injected into the lungs 5–10 min beforehand (Figure 3). DEDTC (80 $\mu\text{g/ml}$) did not prevent the release of RCS.

Discussion. We have recently demonstrated¹⁸ that sulphhydryl and antioxidant agents suppress the release of RCS, the hypotension and bronchoconstriction due to SRS-C and arachidonic acid. Blockade was obtained whether the antagonists were administered to the animals before SRS-C and arachidonic acid, or incubated for 15 min with the antagonists and then injected together.

The following hypothesis may be considered for the mechanism of action of the reference antagonists: 1. Rupture of vital S-S bridges belonging to an enzymatic system activated during the release of RCS: the relatively low activity of the otherwise strong S-S reducer dithiothreitol¹⁹ and the effectiveness of hydroquinone and of pyrogallol justify caution in accepting such a straightforward explanation;

2. Interference of sulphhydryl and other antioxidants upon the receptor, as described for TG inhibition of effects of oxytocin²⁰ or for 2–3 dimercaptopropanol inhibition of rat gut contraction due to bradykinin²¹.

3. RCS could be an unstable prostaglandin precursor²², which is rapidly inactivated^{9,10}, whereas SRS-C (a mixture of unsaturated fatty acids²³), arachidonic acid, and its peroxide²⁴ would be RCS precursor.

This hypothesis is compatible with the release of prostaglandins during experimental inflammation²⁶, with the blockade by non-narcotic antiinflammatory agents of the in vitro synthesis of prostaglandins from arachidonic acid²⁷ and with the suppression of the in vitro activity of SRS-C by triphenylphosphine, a lipid peroxide destructor²⁴.

Nevertheless, although they suppress in vivo bronchoconstriction and in vitro release of RCS, ME and the other antagonists did not prevent in vitro bronchoconstriction due to bradykinin (Figure 3). This apparent contradiction may be due to the direct spasmogenic effect of bradykinin, more important in vitro than in vivo, where the indirect mechanism predominates; alternatively, RCS may finally not be the common mediator of bronchoconstriction, but still be involved in

the mechanism of action of antiinflammatory drugs.

No definite choice between those or other explanations is possible at this stage. It remains that the effects of bradykinin, of RCS-C and of arachidonic acid that are inhibited by antiinflammatory drugs are also suppressed by various antioxidants, reinforcing the hypothesis of a common mechanism and/or a final metabolic pathway for those agonists.

Résumé. Plusieurs dérivés thiol et deux antioxydants inhibent la bronchoconstriction chez le cobaye, une partie de l'hypotension chez le lapin et la libération de «rabbit aorta contracting substance» (RCS) dues à la bradykinine. Il est proposé que les effets communs à la bradykinine, à la SRS-A, SRS-C et à l'acide arachidonique, qui sont bloqués par des antiinflammatoires acides, et par les dérivés thiol, sont dus à la formation de RCS, constituée par des peroxydes cycliques analogues aux précurseurs instables des prostaglandines E 2 et F 2 alpha.

B.B. VARGAFTIG²⁸ and NGUYEN DAO HAI

Organon S.A.,
F-60 Evagny-sur-Epte (France),
20 Septembre 1971.

¹⁸ B. B. VARGAFTIG and N. DAO HAI, *Europ. J. Pharmac.*, accepted for publication.

¹⁹ W. W. CLELAND, *Biochemistry* 3, 480 (1964).

²⁰ P. J. MARTIN and H. O. SCHILD, *Nature, Lond.* 196, 382 (1962).

²¹ A. CAMARGO and S. H. FERREIRA, *Br. J. Pharmac.* 42, 305 (1971).

²² S. BERGSTRÖM, *Science* 157, 382 (1967).

²³ W. VOGT, *J. Physiol, Lond.* 136, 131 (1957).

²⁴ T. DAKHIL and W. VOGT, *Arch. exp. Path. Pharmacol.* 243, 174 (1962).

²⁵ W. VOGT, T. SUZUKI and S. BABILLI, in *Endogenous Substances Affecting the Myometrium* (Eds. U. R. PICKLES and R. J. PATRICK; Cambridge Univ. Press 1966), p. 137.

²⁶ A. L. WILLIS, *J. Pharm. Pharmacol.* 21, 126 (1969).

²⁷ J. R. VANE, *Nature, Lond.* 231, 232 (1971).

²⁸ We thank Mrs B. CHARPENTIER and C. BELLEVAT for technical help, Prof. H. CLAUSER and J. R. VANE and Dr H. O. J. COLLIER for comments and Dr S. H. FERREIRA for advice concerning the superfusion technique and for BPP9. Squibb provided BPP5. This work is part of a thesis leading to the degree of Docteur-ès-Sciences, Orsay (B.B.V.).

Cytoplasmic Uniaxial Radial Symmetry During the Early Final Growth Period in the Oocytes of the Japanese Quail (*Coturnix coturnix japonica*)

In previous work¹ we have established the existence of peculiar subcortical cytoplasmic organelles in the oocytes of regularly laying Japanese quails just before and/or at the beginning of yellow yolk formation. These RNA-rich organelles, although Feulgen negative², seem to contain freshly synthesized DNA which can be demonstrated by autoradiography after H-Thymidine injection³. In the present work their shape and distribution in the germinal disc has been studied.

Materials and methods. 1. *In toto* study of germinal discs. Laying Japanese quails were killed by decapitation, their abdomen opened and the intrafollicular peduncular oocytes, with a diameter ranging from 5 to 7 mm, removed from their ovaries by cutting off their pedicle. The freshly excised oocytes were placed in Ringer's solution and opened by a circular incision round the germinal disc. The

yellow yolk and as much as possible the yolk underlying the germinal disc were removed. Thereafter, in a second bath of Ringer's solution, the theca interna and externa were peeled off from the membrana granulosa (follicle cell layer) overlying the germinal disc. The germinal disc supported by the membrana granulosa was then fixed in acetic acid-alcohol (1:3) for 1 h.

2. *Serial sections of quail oocytes.* After killing the birds by decapitation, the abdomen was opened and oocytes with a diameter ranging from 4.5 to 8 mm were removed

¹ M. CALLEBAUT, *Experientia* 26, 1134 (1970).

² M. CALLEBAUT, *C.r. Soc. Biol., Paris*, 164, 902 (1970).

³ M. CALLEBAUT, *Experientia* 27, 305 (1971).

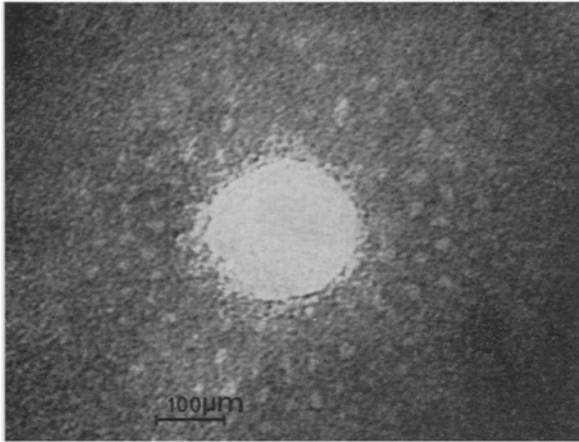


Fig. 1. General surface view of the germinal disc of a 5 mm quail oocyte, after fixation in acetic acid-alcohol. The germinal vesicle in the centre and the surrounding cytoplasmic organelles are transparent.

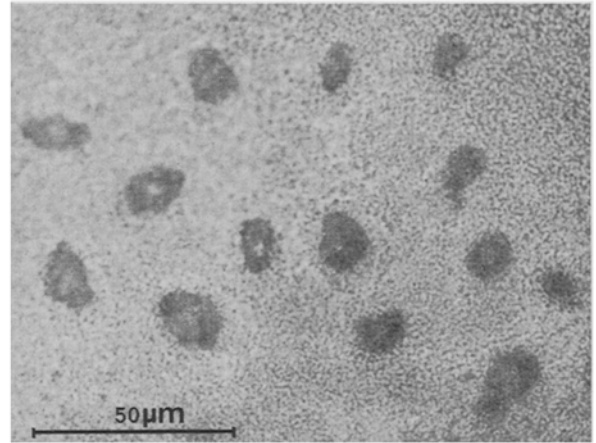


Fig. 2. Surface view of some of the cytoplasmic organelles from the germinal disc of Figure 1, after staining in toto with Unna.

from the ovary. The cicatricular region of these oocytes was labelled by application of carbon particles. They were then fixed by immersion in acetic acid-alcohol (1:3) for 3 to 4 h. After dehydration the oocytes were embedded in paraffin and sectioned at 5 to 7 μ m. The oocytes were then sectioned in a plane perpendicular to that of the germinal disc (perpendicular sections) or parallel with this plane (tangential sections). These paraffin sections were screened under dark ground illumination in order to select those containing some part of the germinal vesicle. Only these sections, which also contain part of the subcortical cytoplasmic organelles were employed in this study. After deparaffination the sections were coloured with toluidine blue O or with Unna.

Results. 1. *In toto study.* During fixation the general aspect of the germinal disc can easily be studied under a dissection microscope. The large nucleus (germinal vesicle) is surrounded by germinal cytoplasm in which numerous organelles are conspicuous (Figure 1). Most of these organelles are localized in concentric circles whose centre is also that of the germinal vesicle. After coloration with Unna, the cytoplasmic organelles are visible as pyroninophil, irregular, rounded bodies with a diameter ranging from 10 to 25 μ m. They usually contain a central colourless substance (Figure 2).

2. *Serial sections.* On tangential sections of germinal discs of oocytes with a diameter ranging from 4.5 to 7 mm, coloured with toluidine blue, we can observe numerous basophilic organelles disseminated in the subcortical cytoplasm surrounding the germinal vesicle (Figure 3). Some of them contain a central colourless substance and their disposition in concentric rings is striking. Moreover, in tangential sections of some oocytes, the organelles are often seen to lie in radially placed rows containing three or more, i.e. if we imagine the germinal vesicle to be represented by the central hub of a wheel, the organelles lie on the spokes radiating from it. The organelles found in the innermost ring (i.e. next to the nucleus) we have called perinuclear subcortical cytoplasmic organelles. These give the impression of being compressed and pushed aside by the nucleus. In some oocytes, and at certain stages of development, the perinuclear organelles have a regular form and on tangential sections they can then be seen as a ring of stirrup-shaped bodies surrounding the germinal vesicle (Figure 4).

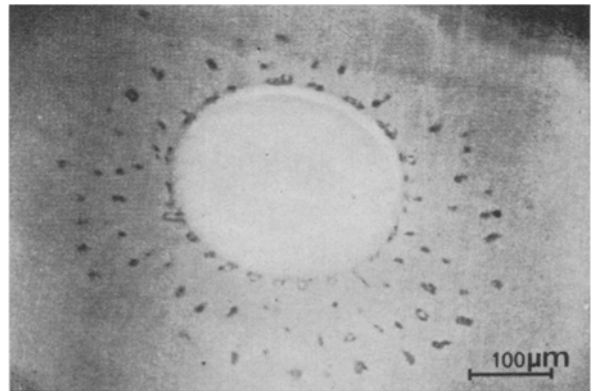


Fig. 3. Tangential section of the germinal disc of a 5.5 mm diameter quail oocyte (toluidine blue stain). Most of the subcortical cytoplasmic organelles are localized in concentric circles but also in radially placed rows. Note oval shape of germinal vesicle and surrounding concentric rings due to compression during sectioning.

On perpendicular sections through the cicatricular region of oocytes of approximately 4.5 mm (Figure 5), the subcortical cytoplasmic organelles are seen to form an interrupted layer of basophilic masses which, at the rim of this region, is continuous with the deepest part of the perigermlinal basophilic cortical layer. Extensions of these organelles penetrate through the neighbouring, more superficial clear layer of colourless yolk vacuoles. In this region the extensions are more or less in contact with the most superficial basophilic cortical layer. Comparison of tangential and perpendicular sections leads to the conclusion that the subcortical cytoplasmic organelles are usually basket-shaped, cylindrical or sometimes spherical. The axis of the cylinders or baskets is perpendicular to the surface of the oocyte and this explains their ring-shaped appearance as seen on the surface view (Figure 2). In oocytes of 5 to 6 mm diameter, the layer of the subcortical organelles is totally interrupted and their extensions have lost contact with the now much thinner, most peripheral basophilic cortical layer. In oocytes of 7 to 8 mm, the number and volume of the subcortical organelles decreases sharply. Their shape becomes rather irregular. The perinuclear

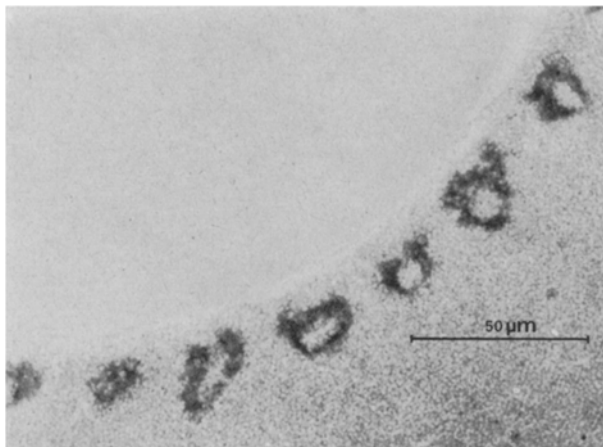


Fig. 4. High power view of part of the germinal vesicle (upper left hand corner) and of the perinuclear basophilic organelles of the same oocyte as in Figure 3. The cross-sectional area of most of these organelles is stirrup-shaped in this tangential section.

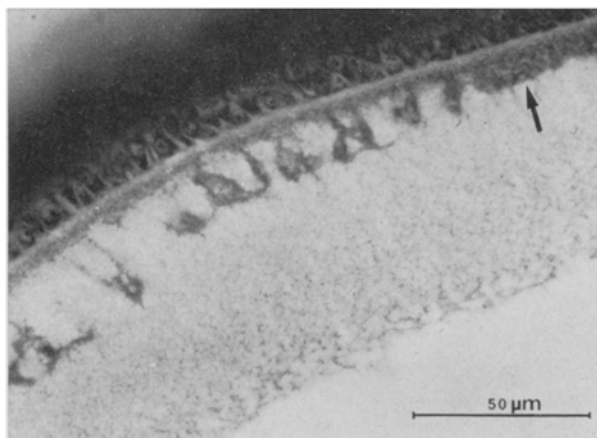


Fig. 5. High power view of perpendicular germinal disc section of an oocyte, 4.5 mm diameter after staining with toluidine blue. The perigerminal cortex is indicated by an arrow.

organelles are usually the last to disappear. In oocytes with a diameter greater than 8 mm (in a laying quail there are usually only some 3 oocytes of this size) these subcortical masses are usually no longer visible.

Conclusion. In Japanese quail oocytes of 4.5 to 7 mm diameter subcortical cytoplasmic organelles, situated about the germinal vesicle, show the singular property of being arranged in concentric circles (often also a radial disposition can be seen) about the dorsoventral axis of the future quail embryo (the dorsal side being situated at the surface of the cicatricular region, the ventral side towards the deeper part). This indicates an evident RNA- and probably also DNA-bound cytoplasmic polarity. As no other gradient is to be seen by this technique, it seems as though the quail oocyte in the stage studied exhibits symmetry around this axis only.

Résumé. Chez la caille japonaise pondeuse la région cicatriculaire des oocytes, au début de leur période de

grand accroissement, présente une symétrie radiale cytoplasmique uniaxiale. Cette symétrie radiale est caractérisée par l'emplacement d'organites cytoplasmiques subcorticaux sur des cercles concentriques autour de la vésicule germinative.

M. CALLEBAUT⁴

*Department of Anatomy and Embryology,
Rijksuniversitair Centrum Antwerpen,
Groenenborgerlaan 171, B-2020 Antwerpen (Belgium),
27 May 1971.*

⁴ The author is very grateful to Professor L. VAKAET, Laboratory of Anatomy and Embryology, Rijksuniversitair Centrum, Antwerpen, for his valuable suggestions and to Mr G. VAN DEN BROECK and Mrs E. HAEST for their skillful technical assistance.

Occurrence of Synapses in Olfactory Epithelium of Fish

The olfactory epithelium of vertebrates is known to comprise¹⁻⁵ olfactory or sensory cells intermingled with supporting cells. Other cellular elements present are the mucin-secreting goblet cells and the basal cells, the latter being arranged in a layer at the base of the epithelium. The structure of the olfactory epithelium in fishes has been studied by several workers⁶⁻⁹ and it has been concluded that it shows no fundamental variation from the general vertebrate pattern.

The commonly accepted picture of a sensory cell of the olfactory epithelium is that it is a bipolar primary neurone with its peripheral dendritic end swollen into an olfactory vesicle bearing sensory hairs or cilia. The greater part of the body of the sensory cell is occupied by its spherical nucleus, there being only scant cytoplasm present around it. The thin, centrally directed axons of sensory cells reach the olfactory bulb directly, there being no interconnecting neurones. This being so, the olfactory organ of vertebrates, as pointed out by KLEEREKOPER¹⁰, is 'the most primitive among the receptors which has remained un-

affected by evolutionary changes in its peripheral organization'. On reaching the olfactory bulb, the axons of sensory cells interlace with dendrites of mitral cells in structures called glomeruli. The axons of mitral cells gradually join to constitute, together with axons of spindle

¹ A. C. ALLISON, *Biol. Rev.* 28, 195 (1953).

² G. BLOOM, *Z. Zellforsch.* 47, 89 (1954).

³ W. E. LE GROS CLARK, *Yale J. Biol. Med.* 29, 83 (1956).

⁴ A. J. D. DE LORENZO, *J. Biophys. biochem. Cytol.* 3, 839 (1957).

⁵ D. FRISCH, *Am. J. Anat.* 127, 87 (1967).

⁶ O. TRUJILLO-CENÓZ, *Z. Zellforsch.* 54, 654 (1961).

⁷ L. H. BANNISTER, *Q. J. microsc. Sci.* 106, 333 (1965).

⁸ J. A. F. WILSON and R. A. WESTERMAN, *Z. Zellforsch.* 83, 196 (1967).

⁹ G. GEMNE and K. B. DOVING, *Am. J. Anat.* 126, 457 (1969).

¹⁰ H. KLEEREKOPER, *Olfaction in Fishes* (Indiana University Press 1969).