saccharide moiety on their surface, as do the Sponge collagen fibers[14] and the spongin[15]. As pointed out by MOSCONA[1], the interactions between the cell surface and the extracellular components appear of particular interest in the organization of cells into developmental patterns.

Besides its supposed aggregation properties, this mucopolysaccharide layer could assume a large range of functions. It is well known that sialic acid or acid mucopolysaccharides carry a high density of negative charges. Such an anionic distribution in the immediate vicinity of cells can act as an ion exchange system and effect the rates of diffusion of charged substances[16], thus controlling the metabolic and electrical[17] pathways of the cells. Modifications of this environment can cause distortions of cell interactions as is the case with malignant and some non-malignant cells[18]. Therefore, one could expect that the properties of the cell coat would not be the same in motile cells such as archeocytes, in tightly packed cells such as choanocytes or in surface pinacocytes. It has also been suggested that the mucopolysaccharide cell coat plays an active part in membranar aspects of collagen fibrillogenesis[19,20].

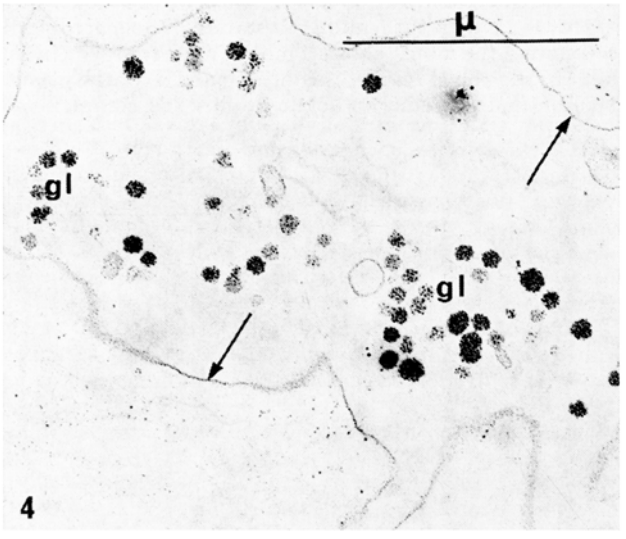


Fig. 4. *Hippospongia communis* Lmk. Silver proteinate staining. Arrows indicate the cell coat. gl, glycogen.

Résumé. A l'aide de diverses techniques (rouge de ruthénium, APT, acide périodique-TCH-protéinate d'argent), un revêtement de nature mucopolysaccharidique est mis en évidence à la périphérie des cellules d'Eponges. Le rôle et la signification de cette structure sont analysés.

R. GARRONE, Y. THINEY and M. PAVANS DE CECCATTY

Laboratoire d'Histologie et Biologie Tissulaire, Université Lyon-I, 16, quai Claude Bernard, F-69 Lyon (France), 6 April 1971.

[14] Y. THINEY and R. GARRONE, Proc. 7th Int. Congr. Electron. Microsc. (1970), Vol. 1, p. 589.
[15] R. GARRONE, J. Microscopie *8*, 581 (1969).
[16] M. PAVANS DE CECCATTY, Experientia *27*, 57 (1971).
[17] W. R. LOEWENSTEIN, Devl. Biol. *15*, 503 (1967).
[18] L. HAUSE, R. A. PATILLO, A. SANCES and R. F. MATTINGLY, Science *169*, 601 (1970).
[19] R. GARRONE and M. PAVANS DE CECCATTY, Proc. 7th Int. Congr. Electron. Microsc. (1970), Vol. 1, p. 639.
[20] This work was supported by the Centre National de la Recherche Scientifique (E.R.A. No. 183, R.C.P. No. 248).

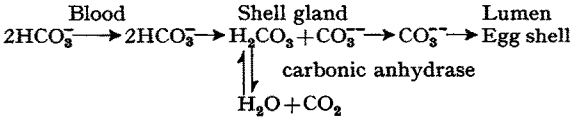
Uptake of Metabolic CO_2 by the Otoliths of the Chick Embryo

The otoliths of the chick embryo consist of calcium carbonate in the form of calcite[1], and of an organic matrix made up of a protein of non-collagenous nature and of a complex mucous substance, the structure of which contains various carboxylic and sulphuric radicals[2,3]. Earlier studies have shown that when chick embryos are treated immediately after the beginning of their morphogenesis, i.e. after 4 days of incubation, with carbonic anhydrase inhibitors (acetazolamide, dichlorophenamide, etoxizolamide, neptazane)[4,5], injected into the white of the egg, the formation of the otoliths is inhibited in a large proportion of the embryos treated.

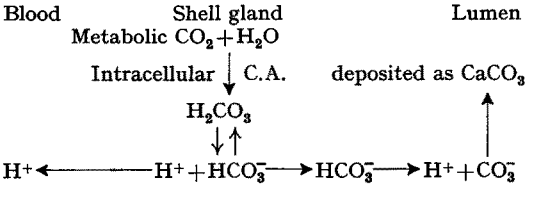
Carbonic anhydrase is demonstrable histochemically in the 5-day-old embryo and is confined to the epithelium of the endolymphatic sac. Following the administration of the above-mentioned inhibitors, the carbonic anhydrase is not detectable[6].

These findings indicate that carbonic anhydrase plays an important part in the morphogenesis of the otoconia of the chick embryo. The morphogenesis of the otoliths of the chick embryo appears similar to the formation of the shell of the chicken egg[7]. It has been demonstrated that carbonic anhydrase is present in the oviduct of hens, and that the development of the shell is inhibited when these animals are treated with specific inhibitors of carbonic anhydrase[8,9].

The most widely accepted hypotheses concerning the deposition of calcium carbonate in the egg shell are those of GUTOWSKA and MITCHELL[10]:



and of DIAMANTSTEIN[11] in which the shell glands derive the carbonate ions from their own metabolic production of CO_2



The latter theory is supported by the presence of carbonic anhydrase in the gland cells, with the pH values of the venous blood observed during the formation of the shell[12,13], and with the fluctuations of bicarbonate in the blood during this process. This theory postulates that

when the carbonate ion is formed from bicarbonate, a proton is formed.

Recent investigations by CAMPBELL and SPEEG[14] tend to confirm the concept that this proton is neutralized according to the following reaction:

$$NH_3 + H^+ \rightleftharpoons NH_4^+$$

Thus, the role of NH_3 in the biological deposition of $CaCO_3$ (both in molluscs and in the formation of the shell of avians) can be summed up in the following reaction:

$$NH_3 + HCO_3^- + Ca^{2+} \longrightarrow CaCO_3 + NH_4^+$$

Material and methods. In an attempt to verify whether the hypothesis advanced for the formation of the egg shell might also be valid for the formation of the otoliths, and in order to elucidate the origin of the CO_3^{--} ion during this process, chick embryos have been treated with $NaHC^{14}O_3$ or with $C^{14}H_3C^{14}OC^{14}OOH$ (pyruvate uniformly labelled in the carbon).

We know that pyruvic acid is completely oxidized according to the reaction:

$$CH_3\,COCOOH + 5O \longrightarrow 3CO_2 + 2H_2O$$

with the formation of 3 CO_2 molecules (the first one produced initially in the formation of actyl COA, and the other two during the various reactions of the Krebs cycle)[15].

The $NaHC^{14}O_3$ and the $C^{14}H_3C^{14}OC^{14}OOH$ were inoculated in doses of 25 μC into embryos of 7 and of 11 days' incubation (the stage of development during which the morphogenesis of the otoconia takes place). The embryos were then fixed in alcohol, 2, 4, 6 and 24 h after the injections.

Before the autoradiograms were made, some sections, of material from animals treated with pyruvate and from animals treated with bicarbonate, were subjected to slow decalcification with physiological solution at pH 6.7 at 37°C for 17 h[16].

Differences of the uptake of C^{14} into otoliths of embryos treated with $NaHC^{14}O_3$ or with $C^{14}H_3C^{14}OC^{14}OOH$ on the 11th day of incubation, and sacrificed 6 h after the injection

	$\bar{x} \pm$ S.E.	S.D.
$C^{14}H_3C^{14}OC^{14}OOH$	69.900 ± 2.426	15.343
$NaHC^{14}O_3$	4.175 ± 0.441	2.791

t, 26.652; $P < 0{,}001$. $\bar{x}$, arithmetical mean of the grains present in every otolith; *s.e.*, standard error; S.D., Standard deviation.

Autoradiograms were then prepared using the NTB-3 emulsion of KODAK[17]. The exposure times varied from 20 to 40 days.

Results. In the Table we have listed the results of one of a number of experiments, all of which have given identical results (in each experiment, the grains associated with each individual otolith were counted. 100 otoliths were examined per embryo).

The number of grains present over every otolith increased in correspondence with the prolongation of time interval between the isotope injection and the sacrifice of the embryo. The studies of the material treated in the manner described above have revealed a slight and selective uptake of C^{14} by otoliths of embryos treated with labelled bicarbonate. In decalcified material, this uptake was only slightly discernible, demonstrating the utilization of $C^{14}O_3^{--}$ in calcification. A greater uptake of C^{14} was found in the otoconia of embryos treated with pyruvate. However, this uptake in previously decalcified material was only about 1/60th of that found in non-decalcified material. This can be explained if we postulate that the greater portion of the C^{14} derived from the pyruvate is utilized in the formation of the CO_3^{--}, and a smaller portion is incorporated into the organic matrix of the otoconia.

Discussion and conclusion. These findings indicate that the CO_3^{--} of the calcium carbonate of the otoliths originates from blood bicarbonate and metabolic CO_2. The proportionally larger utilization of pyruvate (14 times greater than bicarbonate) indicates the metabolic source as the most important analogous to the formation of the skeleton of corals[18].

Furthermore, the findings corroborate the hypothesis of DIAMANTSTEIN which, accordingly, may also be used to explain the mechanism of deposition of calcium carbonate in the otoliths of the chicken embryo. This hypothesis is also in agreement with the presence of carbonic anhydrase in the membranous labyrinth[19] and with the results of experiments carried out with inhibitors of carbonic anhydrase.

Riassunto. Viene condotto uno studio sulla utilizzazione dell' $NaHC^{14}O_3$ e $C^{14}H_3C^{14}OC^{14}OOH$ nel processo di calcificazione degli otoliti dell'embrione di pollo. I risultati delle indagini condotte hanno messo in evidenza la prevalente utilizzazione della CO_2 metabolica in tale processo.

F. MARMO

Istituto di Biologia generale e Genetica,
II Cattedra di Istologia ed Embriologia,
Università di Napoli, I-80134 Napoli (Italy),
26 April 1971.

[1] D. CARLSTRÖM and D. H. ENGSTRÖM, Acta oto-lar. *45*, 14 (1955).
[2] M. DE VICENTIIS and F. MARMO, Boll. Soc. ital. Biol. sper. *42*, 2049 (1966).
[3] G. BALSAMO, M. DE VICENTIIS, F. MARMO and G. MATERAZZI, Riv. Istochim. norm. pat. *14*, 567 (1968).
[4] M. DE VICENTIIS and F. MARMO, Experientia *24*, 818 (1968).
[5] G. BALSAMO, M. DE VINCENTIIS, F. MARMO and G. PARISI, Experientia *25*, 292 (1969).
[6] M. DE VINCENTIIS and F. MARMO, Acta med. romana *4*, 43 (1966).
[7] R. D. HODGES and K. LÖRCHER, Nature, Lond., *216*, 609 (1967).
[8] I. DICULESCO and A. DINESCO, Ann. Histochim. *5*, 321 (1960).
[9] R. BENESCH, N. S. BARRON and C. A. MAWSON, Nature, Lond. *153*, 138 (1944).
[10] M. S. GUTOWSKA and C. A. MITCHELL, Poultry Sci. *24*, 159 (1945).
[11] T. DIAMANTSTEIN, Arch. Geflügelkunde *30*, 310 (1966).
[12] R. D. HODGES, Proc. Thirteenth World's Poultry Congr. (1966), p. 314.
[13] R. D. HODGES, *Physiology of the Domestic Fowl* (Edit. C. HORTON-SMITH; Oliver and Boyd, London 1966), p. 191.
[14] J. W. CAMPBELL and K. V. SPEEG, Nature, Lond. *224*, 725 (1969).
[15] A. CANTAROW and B. SCHEPARTZ *Biochemistry* (W. B. Saunders Company, Philadelphia, London 1962).
[16] G. PALLADINI, L. ALFEI and A. CONFORTI, Riv. Istochim. norm. pat. *11*, 3 (1965).
[17] B. MESSIER and C. B. LEBLOND, Proc. Soc. exp. Biol. Med. *96*, 7 (1957).
[18] V. B. PEARSE, Nature, Lond. *228*, 383 (1970).
[19] F. MARMO, Acta Embryol. Morph. Exp. *9*, 118 (1966).