Amino acid compositions of the 4 biliverdin compounds

	P_0	P_1	P_2	P_3
Asp	9.1	15.1	14.8	18.1
Thr	7.0	9.3	11.6	8.3
Ser	6.8	13.8	19.3	19.1
Glu	10.9	7.2	7.3	7.9
Pro	3.3	4.3	3.7	6.4
Gly	6.3	6.2	11.6	4.9
Ala	5.1	5.8	6.3	5.9
Val	5.6	4.0	0	0
Cys/2	3.0	7.9	6.9	20.9
Met	5.8	0	0	0
Ile	4.8	2.6	0	0
Leu	7.6	8.3	0	0
Tyr	2.3	0	0	0
Phe	4.7	4.0	4.1	0
Lys	8.5	5.6	4.8	3.1
His	4.5	3.5	6.9	5.4
Arg	4.7	2.4	2.7	0

Values are given as moles-% of amino acids found.

peptide mixture (P_3) together with 3 spots which were positive to Greig-Leaback reagent for peptide[12]. P_3 was extracted with 6 N HCl for amino acid analysis.

The amino acid compositions of the 4 biliverdin fractions were compared in molar percentage to total amount of amino acids found (see table). With the progressive shortening of the protein moiety of the pigment in the order P_0, P_1, P_2 and P_3, remarkable increases in molar percentage were found in aspartic acid, serine and half cystine. The rest of the amino acids showed no increasing tendencies, suggesting that the above 3 amino acid residues were bound to or located very near to the biliverdin.

The second approach to the elucidation of linkages was application of some specific splitting reactions. Neither acid acetone[13] nor boiling potassium hydroxide-methanol[3] could liberate the chromophore from the purified biliverdin-protein. However, on application of the acetic acid-silver sulphate method[14] employed in the splitting of haematohaeme from cytochrome c, the chromophore was released in a chloroform-soluble form. This was evidence that at least one of vinyl side-chains of biliverdin IX_α is linked to the sulfhydryl group of cysteine of the protein moiety through a thioether bond. In this connection, Köst-Reyes et al.[15] proved recently the occurrence of a thioether linkage in B-phycoerythrin mainly by the Edman degradation following pepsin digestion.

Finally, the nature of the linkages of the 4 pyrrole rings in the chromophore was examined by the chromic acid-dichromate degradation technique developed by Rüdiger[16]. On chromic acid oxidation at 20 °C, the biliverdin-protein yielded only half as much haematinic acid imide as that obtained at 100 °C, indicating that ring B or C is also linked to the protein moiety (see figure). Since the most probable partner is seryl residue on the basis of amino acid analysis, it may reasonably be deduced that the linkage should be an ester bond[17]. Furthermore, the fact that oxidation at 20 °C also yielded methyl vinyl maleimide indicates that at least one of the rings A and D is in a free state, and that even if an N-acyl bond[17] with aspartyl residue, the last possible partner, might exist, it should be located in the same pyrrole ring that is linked by the thioether bond. In addition, the dichromate degradation of the biliverdin-protein yielded pyrrole-dialdehyde, revealing that ring B is in an unbound state, so that the ester bond with seryl residue, if any, lies in ring C. In conclusion, a presumed mode of linkages between chromophore and apoprotein in the biliverdin-protein is given in the figure.

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Calcite growth under controlled diffusion[1]

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Summary. The growth of calcite was studied in a gelatin-gel medium under variable environmental conditions by 2 different methods. The results suggest that the organic matrix, the temperature, the diffusion fluctuation depending on ionic concentrations, and the presence of additives exert a fine control on the evolution of single crystals, polycrystalline aggregates, and highly structured concretions of calcite.

The environmental conditions that trigger biological calcification have been of considerable interest. In biomineralization, the organic matrix is considered as the prime substrate on which the mineral phase develops[4–6]. Recently, we reported the in vitro growth of organized calcite concretions in a gelatin-gel medium by slow diffusion of ions[7]. In view of these findings, the formation of these concretions in nature could be regarded as a chemical event, induced by some fibrous element and further dictated by diffusion kinetics and environmental conditions. This report describes the effect of the temperature, the ionic concentrations, and the presence of formaldehyde on the growth behavior of calcite crystals as studied by 2 different laboratory methods of crystal growth in gelatin-gel.

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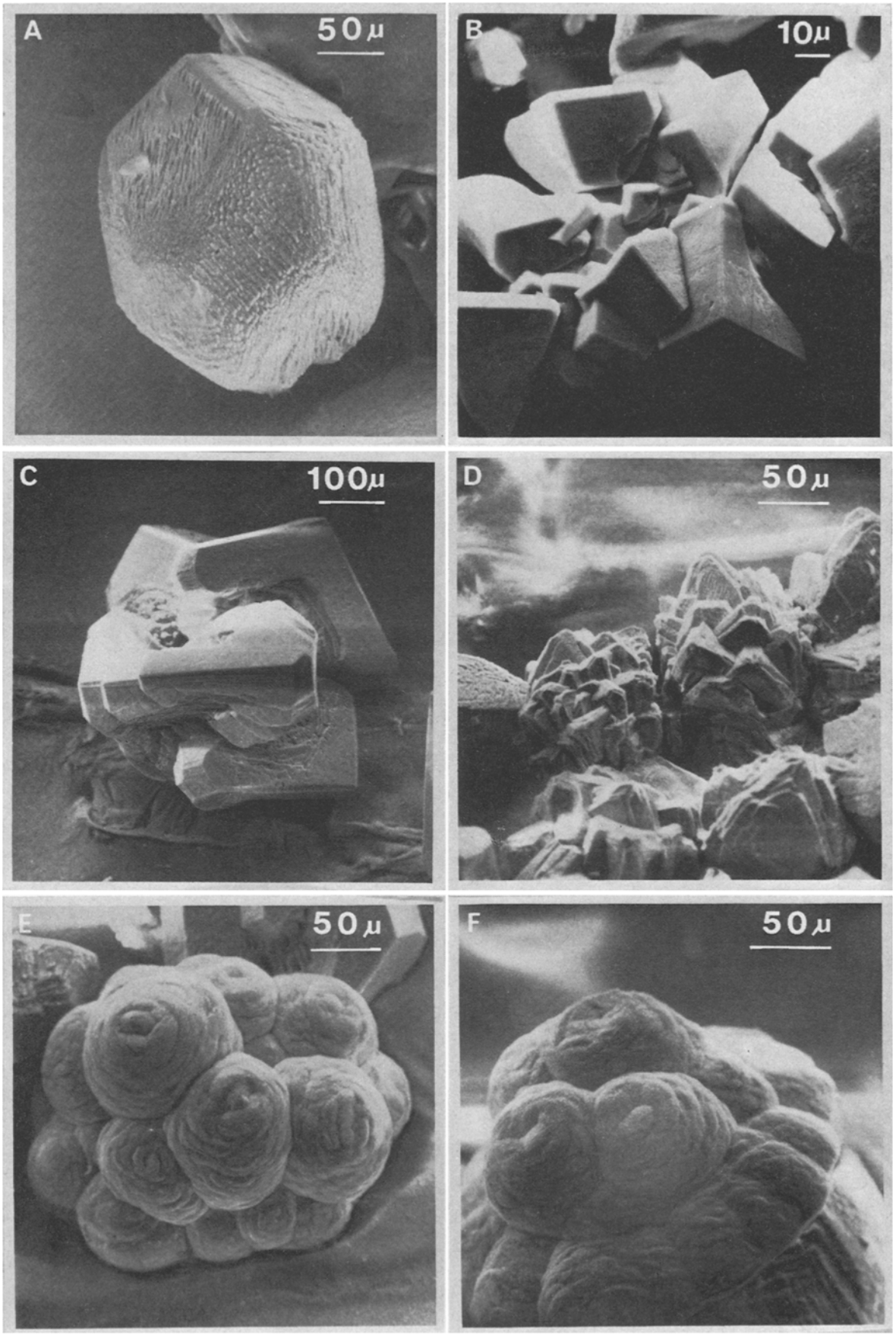

Scanning electron micrographs of calcite grown in gelatin-gel under different environmental conditions. *A* Single crystal grown in a U-tube at 25 °C. *B* Single crystals with a different morphology grown in a cylindrical tube at 45 °C. *C* Mechanically interlocked aggregate grown in a cylindrical tube with or without formaldehyde at 25 °C. *D* Polycrystalline aggregates grown in a cylindrical tube with formaldehyde at 25 °C. *E* and *F* Highly structured concretions grown in a cylindrical tube without formaldehyde at 25 °C. (See the text.)

Growth experiments were conducted in U-shaped and cylindrical tubes with a fritted disk as reported earlier[7]. 2 different temperatures (25 and 45 °C) and 2 ionic concentrations (1% ammonium carbonate, 0.65% calcium chloride, and a 5fold of these concentrations) were tested. The effect of 0.037% formaldehyde solution in the gel medium on the growth of calcite was also evaluated.

All experiments were conducted in the dark, some at 25 °C for 3 weeks and others at 45 °C for 3 days. The crystal deposits were separated from the growth medium and washed with anhydrous ethylenediamine, dried, and prepared for electron microscopic scanning. The figure shows the remarkable variation in the crystal habit and their packing characteristics under various growth conditions. The crystals were identified as calcite by infrared spectroscopy and differential thermal analysis.

With the U-tube method in which the calcium and carbonate ions diffuse against each other across a 5.5% gelatin-gel, calcium carbonate crystals grew in a well-defined zone as trigonal single crystals of calcite but only at high ionic concentrations at 25 °C (figure, *A*). However, with the cylindrical tube method involving a 20% gelatin-gel, single calcite crystals with a completely different morphology were produced using low ionic concentrations at 45 °C (figure, *B*). No polymorphic transition to the less stable aragonite or vaterite was noticed. When a 5.5% gelatin-gel at 25 °C was employed in the cylindrical tubes, the growth behavior of crystals was different from that observed in the U-tubes. For example, the crystals occurred in the upper portion of the gelatin-gel, at the interface between the gelatin-gel and the calcium chloride solution, and also within the calcium chloride solution. Unlike in the U-tube experiments, there was no well-defined crystallization zone. The appearance of different crystallization zones in the cylindrical tubes may be attributed particularly to the diffusion rate of the carbonate ions during the growth process. Obviously, different equilibrium positions with different growth rates are established.

At either low or high ionic concentrations, with or without formaldehyde, calcium carbonate precipitated as mechanically interlocking single crystals, closely packed in a repeated pattern at the gel-solution interface and inside the solution itself (figure, *C*). However, at high ionic concentrations, in the presence of formaldehyde, additional crystals were observed which were polycrystalline aggregates. These aggregates consisted of sharply terminated single crystals, packed together in a specific orientation (figure, *D*). At high ionic concentrations, but in absence of formaldehyde, highly organized structures were formed similar to natural concretions and with distinct morphological characteristics of circular ridges and reticulated surfaces, rather than sharp angles (figure, *E* and *F*).

These observations are consistent with our previous results[7] and imply that the organic gel and the environmental conditions control the evolution of the crystal habit and the pattern of aggregates.

The nucleation of calcite appears to be determined entirely by the nature of the gel substrate which provides limited sites for nucleation. Other variables, such as diffusion fluctuation depending on ionic concentrations and additives, exert a fine kinetic control on the geometry of the packing, the morphology of the building units and the macroscopic surface characteristics of the crystallites. Formaldehyde not only modifies the gel structure, but it also appears to influence the macroscopic surface of individual crystallites. By selective interfacial absorption on particular crystallographic faces, it produces polycrystalline aggregates with well-defined faces and sharp edges. On the other hand, the absence of formaldehyde apparently provides favorable conditions for the solute-solvent reaction on the crystal surface. The 2 opposite processes of dissolution and precipitation change the topography of the surface and produce structures with more rounded edges and ridged surfaces which resemble certain chambered tests of foraminifera.

The in vitro growth of organized calcite concretions simulating natural concretions may prove to be important in helping elucidate the problem of shell formation in nature. With further information on marine environment and through specially designed model experiments approximating natural conditions, distinct progress can be made in the understanding of biomineralization.

Effect of adrenalectomy and dexamethasone treatment on the monoamine oxidase activity in the thyroid gland of the rat

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Summary. Adrenalectomy increased MAO activity in the thyroid gland of rats. The administration (200 µg/100 g daily for 7 days) to adrenalectomized rats decreased MAO activity below levels of intact controls.

Monoamine oxidase (MAO, E.C. 1.4.3.4) has been found in thyroid gland[1]. This enzyme may participate in the synthesis of thyroid hormones, because one of the products of the reaction catalyzed by MAO is hydrogen peroxide. Yet H_2O_2 together with an unknown enzymatic system in the thyroid gland is needed for oxidation of iodide to a more reactive state (I^+)[2]. Parvez et al.[3] and others[4,5] have found that corticosteroids play an important role in the regulation of the activity of MAO. This effect of corticosteroids has been studied in various tissues of several animal species, and different responses to the hormone were found depending on the organs studied. In this paper the MAO activity in the thyroid gland of adult adrenalectomized rats and the effect of dexamethasone administration on this activity is reported.

Materials and methods. Wistar albino male rats weighing approximately 250 g were used. Adrenalectomy was performed under ether anesthesia, and, to these animals, drinking water was replaced by 0.9% saline. One group of adrenalectomized rats was injected with 200 µg of dexamethasone i.p. per 100 g b.wt. during the last 7 days. Completeness of adrenalectomy was verified by inspection of the abdominal cavity. Thyroids were removed, placed