ther the quantity or activity increases with the muscular differentiation. The cholinesterase disappears entirely in the larva which has undergone metamorphosis and in which the muscular system has been reabsorbed. The central nervous system does not show the presence of cholinesterase in any stage of development. The treatment with specific inhibitors demonstrates that the enzyme described corresponds to specific cholinesterase or acetylcholinesterase.

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Riassunto

Con metodo istochimico, sono state effettuate ricerche sulla presenza, la localizzazione, e il tempo di comparsa della colinesterasi nello sviluppo di *Ciona intestinalis* (Ascidie). È stato visto che: 1) l'attività colinesterasica, negativa negli stati precoci di sviluppo, si palese durante e dopo la neurulazione; 2) il territorio in cui si localizza la reazione è esclusivamente quello muscolare; 3) esiste una relazione tra la presenza dell'enzima e la capacità funzionale dell'embrione; 4) nella reazione è interessato un solo tipo di enzima e precisamente la colinesterasi specifica o acetilcolinesterasi.

Trace Element Stimulation of Keratin (Hair) Degradation by Oral Keratinolytic Microflora[1]

The proteolysis-chelation theory explains the etiology of dental caries as two interrelated reactions occurring simultaneously on enamel: (a) microbial destruction of the organic matrix which is composed largely of keratin, and (b) loss of apatite through dissolution by organic chelators some of which originate as matrix breakdown products[2]. This theory was derived in part from consideration of numerous systems throughout nature where ordinarily insoluble inorganic materials are mobilized and transported as soluble sequestration complexes[3]. It was also suggested by the Ca activation and stabilization of enzymes and other proteins, the role of Ca in proteolysis, and the ability of proteins and their derivatives to form water-soluble chelate complexes with alkaline earths[4]. The fact that $CaCO_3$ and $Ca_3(PO_4)_2$ stimulate metabolism of oral keratin-digesting microflora[2] motivated the present studies to determine whether Ca, PO_4, and other trace elements influence keratinolysis, and whether this proteolysis dissolves enamel apatite.

Experimental.—The method of cell production, conditions for Warburg respirometry, preparation of enamel and hair, and composition of the trace element supplement have already been reported[2]. Finely cut human hair was a convenient source of keratin since it is impractical to obtain purified yet undenatured enamel keratin in adequate amounts.

Results.—Both autorespiration and hair oxidation were stimulated by $CaCl_2$ (Table). The complete trace element supplement depressed autorespiration but accelerated O_2 uptake with keratin. Even without Ca and PO_4, the supplement increased O_2 consumption with hair. Thus other elements may participate in the catabolism of this material. The maximum O_2 uptake observed with the Ca-free supplement cannot mean that Ca inhibited since $CaCl_2$ enhanced the oxidation, but probably reflects trace metal unbalance or ion antagonism[5]. The enamel supplied sufficient trace elements to stimulate hair catabolism, but not enough organic matter to in-

[1] This investigation was supported in part by a research grant D-182(C) from the National Institute of Dental Research of the National Institutes of Health, United States Public Health Service.

[2] A. SCHATZ and J. J. MARTIN, N. Y. St. dent. J. *21*, 367 (1955). – A. SCHATZ, K. E. KARLSON, and J. J. MARTIN, N. Y. St. dent. J. *21*, 438 (1955). – A. SCHATZ, J. J. MARTIN, K. E. KARLSON, and V. SCHATZ, N. Y. St. dent. J. *22*, 161 (1956).

[3] A. SCHATZ, N. D. CHERONIS, V. SCHATZ, and G. S. TRELAWNY, Proc. Penn. Acad. Sci. *28*, 44 (1954). – J. J. MARTIN, H. D. ISENBERG, V. SCHATZ, G. S. TRELAWNY, and A. SCHATZ, Euclides *14*, 311 (1954). – A. SCHATZ, Umschau *24*, 746 (1955).

[4] F. R. N. GURD (editor), *Chemical Specificity in Biological Interactions* (Academic Press Inc., New York 1954).

[5] S. H. HUTNER, L. PROVASOLI, A. SCHATZ, and C. P. HASKINS, Proc. Amer. philos. Soc. *94*, 152 (1950).

Trace element stimulation of keratin (hair) degradation by oral keratinolytic microorganisms

Distilled H_2O cell suspension supplemented with	400 min Warburg experiment			
	Autorespiration (no hair present)		100·0 mg hair added to each vessel	
	μl O_2 uptake	μl O_2 stimulation over control due to trace elements	μl O_2 uptake	μl O_2 stimulation over control due to trace elements
Ca as $CaCl_2$ (5·0 mg%)	78	30	832	95
Trace element supplement	37	−11	1025	288
Trace element supplement minus Ca and phosphate	55	7	850	113
Trace element supplement minus Ca	58	10	1042	305
Human enamel as source of trace elements (50·0 mg added to each vessel)	47	− 1	950	213
No minerals added (controls)	48		737	

pH of cell suspension initially adjusted to 7·0; KOH in each center well; 30°C. Trace element supplement includes Na, K, Ca, Mg, Mn, Zn, Mo, Fe, Co, Cu, P, S, and Cl in the same concentrations employed in the basal culture medium for growing keratinolytic microorganisms[2]. The data have not been corrected by subtracting autorespiration.

crease O_2 uptake over the hair-free control. To accomplish this, at least 100·0 mg of enamel were required. Thus, in these experiments involving keratin degradation, trace elements increased O_2 consumption to a greater extent than is attributable to stimulation of autorespiration alone.

These observations agree with other experiments (to be published) in which oral proteolytic microflora catabolized pulverized Ca^{45}-rat bone, as a result of which the pH rose from around 7·0 up to 8·0 and appreciable amounts of Ca^{45} passed into solution. The most likely mechanism whereby bone and enamel minerals dissolved under the conditions in these experiments is by the release of chelating agents as degradation products of organic matrix constituents.

Conclusions.—These studies indicate that enamel minerals stimulate keratinolysis, and keratinolysis contributes to dissolution of enamel apatite. Consequently, the simultaneous disintegration of organic and inorganic components of enamel during formation of dental caries appears more likely than an independent attack on either phase alone. In terms of comparative biochemistry, the proteolysis-chelation theory views tooth decay as due to essentially the same mechanism responsible for pathological bone resorption, normal deciduous tooth root resorption, dental cavities in marine mammals, lobster and mollusc diseases where the calcareous shells demineralize, and solubilization of rock phosphate (i.e. apatite) fertilizer in neutral and alkaline soils[6]. In these systems, acid is not the sole or major factor causing solubilization or decalcification, but chelation seems to be the common denominator. Organic chelators of microbial origin which dissolve calcium phosphates, even under neutral and alkaline conditions, may be useful biological reagents with which to attack such problems as the nature of the bonding between the organic and inorganic components of calcified body structures, changes with age, and the influence of fluoride.

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Research Laboratory, National Agricultural College, Doylestown, Bucks County, Pennsylvania, March 3, 1956.

Zusammenfassung

Nach der Proteolyse-Chelationstheorie der Zahnkaries werden organische und anorganische Bestandteile des Zahnschmelzes mehr oder weniger gleichzeitig abgebaut. Die Ergebnisse dieser Untersuchungen zeigen, dass 1. mineralische Bestandteile des Schmelzes die mikrobiologische Zersetzung des Keratins fördern und dass 2. die enzymatische Zersetzung des Keratins Stoffe entwickelt, welche die Apatitauflösung mittels Chelation fördern.

[6] A. Schatz and J. J. Martin, N. Y. St. dent. J. *21*, 367 (1955). – A. Schatz, K. E. Karlson, and J. J. Martin, N. Y. St. dent. J. *21*, 438 (1955). – A. Schatz, J. J. Martin, K. E. Karlson, and V. Schatz, N. Y. St. dent. J. *22*, 161 (1956). – A. Schatz, N. D. Cheronis, V. Schatz, and G. S. Trelawny, Proc. Penn. Acad. Sci. *28*, 44 (1954). – J. J. Martin, H. D. Isenberg, V. Schatz, G. S. Trelawny, and A. Schatz, Euclides *14*, 311 (1954). – A. Schatz, Umschau *24*, 746 (1955).

The Biometry of the Cirripede, *Chthamalus stellatus* (Poli)

Data have recently been given by Tenerelli[1] on the sizes of *Chthamalus stellatus stellatus* (Poli) and *C. stellatus depressus* (Poli) together with a discussion of the status of the 2 forms. These results are of considerable interest and would seem to warrant a more detailed statistical treatment.

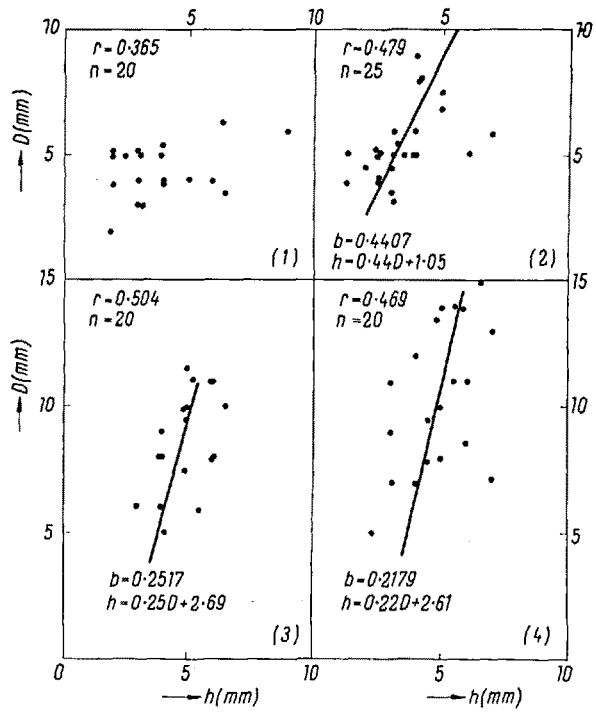

Fig. 1.–Plot of diameter (D) against height (h) for *Chthamalus stellatus stellatus* (Poli). Data from Tenerelli: (1) Zona inferiore di bassa marea. (2) Zona intercotidale, vicino alla linea superiore di alta marea. (3) 40–60 cm sulla linea di alta marea. (4) 150–180 cm sulla linea superiore di alta marea. Correlation coefficients and regression lines are also shown.

Measurements are given of the height and basal diameter of individuals of both varieties collected at several levels of the intertidal zone. Tenerelli points out that the form *depressus* reaches greater dimensions than *stellatus* but he does not distinguish between the different year classes. He further shows that the ratio of the height to the diameter is different in the 2 forms. It is more instructional to consider the relation between the heights of the individuals in respect to the diameter, at the different levels and in particular to determine whether this relation varies with the level in either of the forms; any such variation could indicate the effect of environment on the growth form of the animals.

All the data, height against diameter, have been plotted in the Figures 1 and 2. With the exception of form *stellatus* at the lowest level (zona inferiore di bassa marea) and the form *depressus* at the highest level (180–260 cm sulla linea superiore di alta marea) there is a significant correlation between the 2 dimensions. In the former case the lack of correlation may be due to the

[1] V. Tenerelli, Atti Accad. Gioenia Sci. nat. Catania [6] *9*, 92 (1952–53).