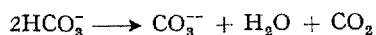


## Possible Effects of Zooxanthellae on Coral Growth

Several recent reviews on the subject of coral growth<sup>1,2</sup> have drawn attention to some problems of the calcification of the skeleton. Calcium ions are absorbed from the sea water by the tissues of the coral and calcium carbonate is precipitated on a mucoprotein matrix outside the epidermis. It seems likely that the carbonate ions involved are formed through the fixation of carbon dioxide by the zooxanthellae of the corals, i.e.



The zooxanthellae appear, however, to have other effects as their presence favours calcification even in the absence of any photosynthesis, an effect that led Goreau<sup>3</sup> to suggest that the zooxanthellae exert a 'stimulant effect on the host's metabolism mediated through a vitamin or hormone-like factor'.

In some recent work the author has used a model system involving the removal of carbon dioxide from an artificial sea water in order to precipitate calcium carbonate. It has been found that inorganic orthophosphates, pyrophosphates and organic phosphates such as glycerophosphate or adenosine triphosphate are powerful inhibitors to the precipitation of calcium carbonate. These phosphates produce their effect at concentrations within the normal physiological ranges, being effective at dilutions as great as  $10^{-6}M$ . They appear to act as crystal poisons and to this extent many of the compounds seem to behave in a similar way to the crystal poisons of bone salts<sup>4</sup>.

It was shown by YONGE and NICHOLLS<sup>5,6</sup> that corals could excrete considerable quantities of phosphates, but under normal conditions these phosphates were absorbed by the zooxanthellae and so did not reach the outside of

the animal. It is suggested here that as the coral contains enzymes capable of hydrolysing complex phosphates<sup>7</sup> and as the zooxanthellae are capable of absorbing the resulting orthophosphates this will remove these potential inhibitors of calcification. This would provide the beneficial effect upon coral growth that GOREAU discovered even in the absence of photosynthesis<sup>8</sup>.

*Zusammenfassung.* Da bekannt ist, dass Verbindungen mit Phosphationen auf Calciumcarbonat als Kristallgifte wirken, wird als besondere Wachstumseinwirkung der Zooxanthellen bei den Korallen angenommen, dass sie Hemmungen der Calcifikation beseitigen.

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- <sup>1</sup> C. M. YONGE, in *Advances in Marine Biology* (Ed. F. S. RUSSELL, 1963), vol. 1, p. 209.
- <sup>2</sup> T. GOREAU, *Endeavour* 20, 32 (1961).
- <sup>3</sup> T. GOREAU, *Biol. Bull. Woods Hole* 116, 59 (1959).
- <sup>4</sup> W. F. NEUMAN and W. M. NEUMAN, *The Chemical Dynamics of Bone Mineral* (University Press, Chicago 1958).
- <sup>5</sup> C. M. YONGE and A. G. NICHOLLS, *Scientific Reports Great Barrier Reef Expedition 1928-29* 1, 135 (1931).
- <sup>6</sup> C. M. YONGE and A. G. NICHOLLS, *Scientific Reports Great Barrier Reef Expedition 1928-29* 1, 177 (1931).
- <sup>7</sup> T. GOREAU, *Nature, Lond.* 177, 1029 (1956).
- <sup>8</sup> The experimental work mentioned in this report was performed in the Zoology Department of Duke University during the tenure of a Fulbright Travel Grant and supported by a United States P.H.S. Grant No. D.E. 017501-01 from the National Institute of Dental Health, Public Health Service.

## Leucine Aminopeptidase Activity in Muscles of Dystrophic Mice

Leucine aminopeptidase (LAP) is a hydrolytic enzyme which has been detected histochemically in various tissues. It is present in fibroblasts and is especially active in proliferative connective tissue, such as found in inflammatory or neoplastic processes<sup>1</sup>. The significance of connective tissue proliferation<sup>2</sup> and of LAP activity in dystrophic muscles is still uncertain<sup>3,4</sup>.

*Methods.* Histochemical and quantitative determinations of LAP were made in gastrocnemius muscles of strain 129 dystrophic mice and their normal littermate obtained from the Roscoe B. Jackson Memorial Laboratory. The presence of LAP was demonstrated histochemically on cryostat sections by the technique of NACHLAS et al.<sup>5</sup>, using L-leucyl- $\beta$ -naphthylamide as substrate and Fast blue B as colouring agent. Quantitative measurements were made by the direct coupling method described by GREEN et al.<sup>6</sup> and were expressed in  $\mu g$  of  $\beta$ -naphthylamine produced after 2 h of incubation. Two age groups of 8 pairs of dystrophic and normal mice each were studied; the first group was 50 to 60 days old, and the second 110 to 125 days. Since growth hormone stimulates the proliferation of connective tissue<sup>7</sup>, a few mice (4 pairs) of the younger age group were treated for 5 days with growth hormone<sup>8</sup> (250  $\mu g$ /day s.c.) before measuring the enzyme activity in muscles.

*Results and Discussion.* The histochemical reaction was virtually absent in control muscles and very weak in dystrophic muscles of the younger age group. It was more intense in numerous zones of connective tissue proliferation in the gastrocnemius of dystrophic mice aged 110 to 125 days; in some cases, the reaction was seen over degenerating fibres, thus giving the impression of a localization in the fibre itself; however, because of the diffusion of naphthylamine from the site of its production<sup>9,10</sup>, no

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- <sup>2</sup> G. H. BOURNE and M. N. GOLARZ, *Nature* 183, 1741 (1959).
- <sup>3</sup> E. BAJUSZ and G. JASMIN, *Rev. canad. Biol.* 21, 409 (1962).
- <sup>4</sup> G. RUCART, personal communication.
- <sup>5</sup> M. M. NACHLAS, D. T. CRAWFORD, and A. M. SELIGMAN, *J. Histochem. Cytochem.* 5, 264 (1957).
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- <sup>7</sup> H. SELYE, in *The Hypophyseal Growth Hormone, Nature and Actions*. An International Symposium (The Blakiston Division, McGraw-Hill Book Company Inc., New York 1955), p. 123.
- <sup>8</sup> Gift of the Endocrinology Study Section, National Institutes of Health, Bethesda (Maryland, U.S.A.).
- <sup>9</sup> M. M. NACHLAS, B. MONIS, D. ROSENBLATT, and A. M. SELIGMAN, *J. Biophys. Biochem. Cytol.* 7, 261 (1960).
- <sup>10</sup> M. M. NACHLAS, M. M. FRIEDMAN, and A. M. SELIGMAN, *J. Histochem. Cytochem.* 10, 315 (1962).