## Possible Effects of Zooxanthellae on Coral Growth

Several recent reviews on the subject of coral growth[1,2] 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.

$$2HCO_3^- \longrightarrow CO_3^{--} + H_2O + CO_2$$

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[3] 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[4].

It was shown by YONGE and NICHOLLS[5,6] 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[7] 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[8].

*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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## 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[1]. The significance of connective tissue proliferation[2] and of LAP activity in dystrophic muscles is still uncertain[3,4].

*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.[5], using L-leucyl-β-naphthylamide as substrate and Fast blue B as colouring agent. Quantitative measurements were made by the direct coupling method described by GREEN et al.[6] and were expressed in μg of β-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[7], a few mice (4 pairs) of the younger age group were treated for 5 days with growth hormone[8] (250 μg/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[9,10], no

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conclusion can be drawn from this observation. Quantitative measurements reported in the Table showed a significant increase in LAP activity only in gastrocnemius of mice of the older group.

Theoretically, growth hormone, through its influence on connective tissue[7], might have been an activator of dystrophic degeneration and have induced an increase of LAP activity. However, treatment of the animals with this hormone produced no measurable effect on LAP, and did not stimulate body growth; apparently, the growth hormone preparation used here, though growth-promoting in rats[11], was ineffective in dystrophic mice.

Leucine aminopeptidase activity in muscles of dystrophic mice[a]

| Groups | 50–60 days old | | 100–125 days old | |
|---|---|---|---|---|
| | Untreated | Growth hormone | | |
| Controls | $3.2 \pm 0.4$ | $2.9 \pm 0.3$ | $5.3 \pm 0.8$ | $p < 0.002$ |
| Dystrophic | $4.2 \pm 0.5$ | $3.8 \pm 0.8$ | $10.5 \pm 1.1$ | |

[a] Expressed in μg of naphthylamine produced per g of tissue after 2 h of incubation ± S.E.

The present quantitative results confirm previous histochemical reports on the presence of LAP activity in muscles of dystrophic mice[3,4]. They do not indicate an acceleration of the enzyme activity prior to connective tissue proliferation in dystrophic muscles but suggest that both changes occur simultaneously[12].

*Résumé.* L'activité de la leucine aminopeptidase (LAP) a été mesurée dans le muscle de la souris dystrophique. La LAP est peu active dans le muscle dystrophique de la souris jeune (50 à 60 jours). D'autre part, l'activité de l'enzyme est fortement augmentée dans le muscle de la souris plus agée (110 à 125 jours). Un traitement de 5 jours à l'hormone de croissance chez la souris jeune n'a pas modifié significativement l'activité de la LAP dans les muscles des souris dystrophiques ou témoins.

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## Inhibitory Action of γ-Aminobutyric Acid (GABA) on *Ascaris* Muscle

Piperazine, the drug most commonly used for the treatment of roundworm infections, produces a flaccid paralysis of the somatic musculature of *Ascaris lumbricoides*. A recent investigation has shown that this effect cannot be accounted for by a block or curarization of excitatory nerve-muscle synapses, as was generally assumed. New experimental evidence suggests that piperazine behaves as a pharmacological analogue of an inhibitory neuromuscular transmitter[1].

The repetitive spike potentials responsible for the contraction of *Ascaris* muscle are not transmitted from motor nerve fibres by discrete bursts of synaptic activity. Instead, they are generated by pacemakers located in a specialized region of the muscle which we have called the *syncytium*[2,3]. This is a structure formed by the terminal arborizations of the *arms*, extensions or processes, sent by each muscle cell to the nerve cord (see Figure 1).

The functional properties of the muscle syncytium resemble those of mammalian visceral muscle. On the one hand, the muscle cell arms are electrically interconnected at this level, i.e. current injected into any muscle cell flows along its arm into the syncytium where it spreads electrotonically to the surrounding arms across low impedance pathways[2]. On the other hand, the syncytial surface membrane possesses autorhythmic properties, generating spike potentials which are conducted within the syncytium itself as well as away from it along the arms, to the contractile region of the muscle cell or *spindle* (see Figure 2).

The frequency of the rhythmic spike potentials is modulated by excitatory and inhibitory synapses established between the nerve cord fibres and the muscle syncytium. The chemical transmitter liberated by the former, believed to be acetylcholine or a related choline ester, exerts a depolarizing effect on the syncytial membrane increasing the frequency of the spike activity[3]. The chemical transmitter, of unknown nature, released at the inhibitory synapses causes a hyperpolarization of the syncytium and a decrease in the frequency of the spikes, which disappear when the potential difference across the membrane increases above 40 mV.

The addition of piperazine (to a concentration of $10^{-3} M$) to the saline bathing *Ascaris* preparations causes hyperpolarization of the muscle cells and suppression of the spike activity. Electrophoretic application of this compound to different regions of the muscle cells has shown that the piperazine-receptors are located in the muscle syncytium and are probably identical with the postsynaptic receptors of the inhibitory nerve-muscle junctions. The activation of such receptors results in an increased conductance of the syncytial membrane to $Cl^-$ ions, which, moving into the cell along the existing con-

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