Table 1. INDUCTION OF TRYPTOPHAN PYRROLASE BY DIFFERENT AGENTS

Cortisone ('Cortisoneacetate', Merck) (0.01 gm./kgm.) and L-tryptophan (1 gm./kgm.) were injected intraperitoneally, and reserpine ('Serpasil', Ciba) (0.001 gm./kgm.) subcutaneously. The four equivalents of microsomes employed gave maximal stimulation

Substance injected	Hours post-injection	Tryptophan pyrrolase activity (μ moles kynurenine/gm. liver/hr.)	
		Unsupplemented cell sap	Cell sap + four equivalent microsomes
—	—	1.6	5.9
Tryptophan	1	3.8	6.0
	2	8.4	8.8
	4	13.0	15.8
Cortisone	1	2.0	5.2
	2	2.8	8.0
	4	4.8	16.0
Reserpine	2	2.6	4.4
	4	3.6	7.8

the absence of added activator) was much greater following treatment with tryptophan.

Previous work with tryptophan⁷ suggested that the activation of tryptophan pyrrolase provides the stimulus for induction (defined as increase in total obtainable activity). In the case of cortisone and reserpine, such an activation does not take place, indicating an alternative mechanism for the initiation of enzyme induction.

This work was supported in part by the National Science Foundation (grant NSF-G 11430) and the U.S. Public Health Service (grant CY 2332).

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¹ Knox, W. E., *Brit. J. Exp. Path.*, **32**, 462 (1951).

² Knox, W. E., Auerbach, V. H., and Lin, E. C. C., *Physiol. Rev.*, **36**, 164 (1956).

³ Civen, M., and Knox, W. E., *J. Biol. Chem.*, **234**, 1787 (1959).

⁴ Canal, N., and Maffei-Faccioli, A., *Naturwiss.*, **46**, 494 (1959).

⁵ Westermann, E. O., Maickel, R. P., and Brodie, B. B., *Fed. Proc.*, **19**, 269 (1960).

⁶ Greengard, O., and Feigelson, P., *Biochim. Biophys. Acta*, **39**, 191 (1960).

⁷ Feigelson, P., and Greengard, O., *J. Biol. Chem.*, **236**, 153 (1961).

⁸ Greengard, O., and Feigelson, P., *J. Biol. Chem.*, **236**, 158 (1961).

Biosynthesis of Citrulline in Plants

In 1930 citrulline was discovered in water-melons¹. Later it was shown to be an important participant in the Krebs and Henseleit ornithine cycle in animals and micro-organisms. In recent years several laboratories have worked on the ornithine cycle in plants. In this laboratory citrulline has been shown to play an important part in transporting and storing nitrogen in several families of higher plants²⁻⁴. Relatively high concentrations of citrulline in exuding sap and citrulline accumulated in artificially rooted leaves of alder trees show the root to have a determining influence in the biosynthesis of citrulline. However, excised branches of alder and hazel could also synthesize citrulline from ammonium nitrate under aseptic conditions. Though ornithine, the precursor of citrulline, is metabolized very quickly, we could not find significant amounts of it, but very high concentrations of the mono-N-acetyl-ornithine were found in all members of the family Fumariaceae, being characteristic of this family⁵. Ornithine reacting with carbamyl phosphate in the presence of carbamyl-transferase is converted into citrulline. My experiments have shown this reaction to occur under

physiological conditions even without enzyme, though converting carbamyl phosphate much more slowly to cyanate, which reacts with the amino-groups^{6,7}. It was also possible to establish the biosynthesis of citrulline from ornithine and carbamyl phosphate in higher plants⁸⁻¹¹.

To examine the possibility of activity of carbamyl phosphate in seaweeds, different green, brown, and red algae were ground in a mortar with *tris* buffer pH 8 at + 4° C. The extract, being usually weakly acid, was brought up to pH 8 with sodium bicarbonate, centrifuged (30 min. at 7,000 r.p.m. at 5° C.), and incubated at 30° C. The reaction was stopped by freezing at -20° C., and the lyophilized reaction mixture re-solved with 70 per cent ethanol immediately before determination. After separation on paper chromatograms the amino-acids were determined colorimetrically with ninhydrin by comparison with standards⁴.

The results given in Table 1 show that green and red seaweeds are able to synthesize citrulline from ornithine and carbamyl phosphate. 70-80 per cent of the ornithine was metabolized within the period of reaction. During this time some amino-acids increased also; primarily citrulline and glutamic as well as aspartic acid in small amounts, but not arginine and urea. After 30 min. incubation I found most of the initially produced ornithine was metabolized to citrulline; and there is scarcely a difference between incubation for 30 min. or 6 hr. Before incubation I found a relatively high concentration of citrulline in *Gracilaria*; whereas citrulline synthesis does not occur in *Taonia*. For the present we must leave it undecided whether this lack of citrulline synthesis is dependent on the developmental state of the plant, or whether it is specific only to *Taonia* or for all brown algae, having a special place in the biochemistry of seaweeds.

Table 1. BIOSYNTHESIS OF CITRULLINE IN SEaweeds
The complete system contained: 10 μ moles ornithine, 10 μ moles carbamyl phosphate (CP), 5 μ moles magnesium chloride, 1 ml. plant extract (= 1.5 gm. fresh weight of algae extracted and made up to 1 ml. with *tris* buffer pH 8)

	μ Moles citrulline/1 ml. plant extract			
	Complete system	Complete system	Without CP	Without ornithine
Time of incubation (hr.)	0.5	6	6	6
Green algae				
<i>Cladophora prolifera</i>	6.85	8.60	0	0
<i>Ulva lactuca</i>	3.43	4.00	0.80	0
Red algae				
<i>Neomonospora furcellata</i>	6.29	6.85	1.60	0.69
<i>Gracilaria confervoides</i>	12.00	14.85	8.60	6.58

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¹ Wada, M., *Biochem. Z.*, **224**, 420 (1930).

² Wolfgang, H., and Mothes, K., *Naturwiss.*, **40**, 605 (1953).

³ Reuter, G., and Wolfgang, H., *Flora* (Jena), **142**, 146 (1954).

⁴ Reuter, G., *Flora* (Jena), **144**, 420 (1957).

⁵ Reuter, G., *Flora* (Jena), **145**, 326 (1957).

⁶ Reuter, G., *Z. Naturforschung*, **14**, b, 475 (1959).

⁷ Reuter, G., *Tagung Gesellsch. Physiol. Chem.*, Berlin, 1959 (in the press).

⁸ Kleczkowski, K., and Reifer, I., *Bull. de l'Academie Polonaise des Sci.*, Cl. II, **7**, 53 (1959).

⁹ Kasting, R., and Delwiche, C., *Plant Physiol.*, **32**, 471 (1957).

¹⁰ Coleman, R. G., and Hegarty, M. P., *Nature*, **179**, 376 (1957).

¹¹ Bone, D. H., *Plant Physiol.*, **34**, 171 (1959).