amide of synthetic isooctanoic acid. Further proof of identity was obtained by comparison of the gas chromatograms of the methyl esters and paper chromatograms of the hydroxamates of the natural and synthetic acids.

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Uncoupling Action of Chloramphenicol as a Basis for the Inhibition of Ion Accumulation

SUTCLIFFE[1] has reported that the bacteriostat chloramphenicol at concentrations of 2 g/l. effectively stops ion accumulation by tissue slices of red beet root and carrot root. Respiration and protoplasmic streaming were unaffected. The concentration of chloramphenicol required was much higher than that needed for inhibition of protein synthesis in most bacteria (2,000 versus about 10 μg/ml.), but the necessity for high concentrations in plant and animal systems is commonly observed[2]. Sutcliffe assumed that chloramphenicol was acting as a specific inhibitor of protein synthesis, as in bacteria, and interpreted the results as evidence for a close relationship between the process of protein synthesis and salt accumulation as suggested by Steward[3]. Jacoby and Sutcliffe[4] later showed chloramphenicol to inhibit uptake of carbon-14-labelled glutamic acid, glycine and proline by carrot tissue. The incorporation of the amino-acids into protein was inhibited in short-term experiments, but glycine and proline incorporation was not affected in long-term (24 h) experiments. Net protein synthesis was inhibited.

This work of Sutcliffe's could have great theoretical importance, and we have accordingly included chloramphenicol in studies of calcium uptake by isolated mitochondria from etiolated maize shoots. The experiments were patterned somewhat after those of Brierley *et al.*[5] and Vasington and Murphy[6]. About 100 g of 3½-day shoots (*Zea mays*, *WF*9 × *M*14) were ground in a cold mortar with about 250 ml. of 0·25 M sucrose + 0·05 M potassium dihydrogen phosphate + 0·005 M ethylenediamine tetra-acetic acid (EDTA) adjusted to *p*H 7·5 with *tris*(hydroxymethyl) aminomethane (*tris*). Mitochondria were isolated by centrifugation in the cold between 2,000*g* for 6 min and 12,000*g* for 15 min, and were washed once in the grinding medium and once in 0·25 M sucrose. Final suspension was in 0·25 M sucrose. The mitochondria were incubated for 10 min at 30° C with the additives listed in Table 1. Ice-cold 0·5 M sucrose was layered below the reaction mixture, and the mitochondria centrifuged through the sucrose. The pellet was suspended in water and assayed for radioactivity. A complete report on the properties of these mitochondria in absorbing calcium is being prepared. For now it can be noted that except for some non-specific adsorption the calcium uptake is dependent on respiratory energy or ATP and is accompanied by phosphate, much as with animal mitochondria[5,6].

Chloramphenicol proves to be an inhibitor of calcium binding by mitochondria at concentrations in excess of 0·4 mg/ml. At 1·6 mg/ml., which is in the range of concentration used by Sutcliffe, the extra calcium-binding mediated by substrate is virtually eliminated (Table 1).

However, it was difficult to imagine any appreciable protein synthesis occurring in these isolated mitochondria in the absence of added amino-acids. In attempting to find an explanation we noted that the inhibitions of calcium binding were very much like those we had obtained with the uncoupling agent, 2,4-dinitrophenol. Although chloramphenicol is not considered to be an uncoupling agent, it was conceivable that at these high concentrations some uncoupling of oxidative phosphorylation might occur.

Table 1. INHIBITION OF CALCIUM-BINDING IN MAIZE MITOCHONDRIA BY CHLORAMPHENICOL

Substrate	Chloramphenicol	Total ^{45}Ca (c.p.m./mg N)	Substrate mediated binding (c.p.m./mg N)
−	−	26,400	—
+	−	65,900	39,500
+	0·8 mg/ml.	45,100	18,700
+	1·6 mg/ml.	29,000	2,600

Media contained 625 μmoles sucrose, 6·25 μmoles $MgCl_2$, 0·25 μmoles $CaCl_2$ + ^{45}Ca, 17 μmoles phosphoric acid neutralized with *tris*, 0·1 ml. ethanol, 0·6 μmoles NAD, 0·4 μmoles thiamine pyrophosphate, 0·1 μmoles coenzyme *A*, 20 μmoles succinate + 40 μmoles pyruvate (as substrate where indicated), 0·335 mg mitochondrial N, and water to a volume of 2·5 ml. The *p*H was adjusted to 7·5 with *tris*. Chloramphenicol from Parke, Davis and Co. (listed as 1,000 μg/mg) was dissolved in ethanol, and equivalent ethanol was added to controls. The ethanol is slightly inhibitory to calcium-binding. Specific activity of calcium was 172 c.p.m./mμ mole.

Table 2. UNCOUPLING OF OXIDATIVE PHOSPHORYLATION BY CHLORAMPHENICOL

Chloramphenicol	Uptake O_2 (μ atoms)	Uptake PO_4 (μmoles)	P/O
None	16·8	29·2	1·74
0·8 mg/ml.	14·8	8·8	0·59
1·6 mg/ml.	9·1	1·3	0·14

Warburg vessels contained 425 μmoles sucrose, 5 μmoles $MgCl_2$, 40 μmoles succinate, 40 μmoles pyruvate, 42 μmoles phosphate, 100 μmoles KCl, 6·5 μmoles ATP, 0·6 μmoles NAD, 0·4 μmoles thiamine pyrophosphate, 0·1 μmoles coenzyme *A*, 2·5 μmoles EDTA, 100 μmoles glucose, 25 KM units hexokinase, 0·1 ml. ethanol, 0·320 mg mitochondrial N, and water to 2·5 ml. The *p*H was adjusted to 7·5 with *tris*. Reaction was at 30° C for 15 min. Chloramphenicol was added in ethanol as in Table 1, with equivalent ethanol in all vessels. The ethanol is slightly inhibitory to phosphorylation.

Table 2 shows that uncoupling does occur with concentrations of chloramphenicol which inhibit calcium binding. Oxygen uptake is definitely depressed at 1·6 mg/ml. In some experiments 0·8 mg/ml. chloramphenicol did not affect oxygen consumption, but phosphorylation was always lowered.

Uncoupling agents such as dinitrophenol are known to inhibit salt accumulation by plant tissues[7], although the mechanism of inhibition is subject to various interpretations[8]. Uncoupling could also reduce an endergonic process such as protein synthesis. Hence the effects of high concentrations of chloramphenicol ascribed to inhibition of protein synthesis might be due to inhibition of ATP synthesis. The fact that respiration rates of tissue are not altered by chloramphenicol (or are even accelerated[1]) would be expected over a certain concentration range if the compound were uncoupling oxidative phosphorylation. The depression of mitochondrial respiration by 1·6 mg/ml. chloramphenicol (Table 2) is the result to be expected from high concentrations of uncoupler; dinitrophenol in excess of 10^{-4} M also depresses oxygen consumption of these mitochondria. Calo *et al.*[9] have reported variable effects of chloramphenicol on potato tuber tissue respiration, including an inhibition of 25 per cent.

These experiments led us to the opinion that the inhibition of salt accumulation in plant tissue by high concentrations of chloramphenicol could be due to uncoupling of oxidative phosphorylation rather than to the action of the compound as a specific inhibitor of protein synthesis.

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