The substance CCC, at a concentration which reduced elongation of seedlings by about 20%, reduced the content of chlorophyll by about 80%. The various salts of sodium and magnesium used separately at concentration of 500 ppm stimulated the elongation of seedlings and increased the chlorophyll accumulation. The maximum accumulation of chlorophyll occurred in the salts containing sulphur and chlorine as their anions. Salts of sodium and magnesium reversed the inhibitory effect of CCC on elongation growth of the seedlings. However, salts containing SO_4^{--} and Cl^- anions could also significantly reverse the inhibition of chlorophyll accumulation, the effect being independent of the concomittant reversal of elongation of seedlings. In those cases where nitrates were present as anions, very slight reversal of chlorophyll inhibition was noticed and this was possibly due to stimulated reversal of elongation growth of seedlings. It is thus evident that, of the various salts of sodium and magnesium tested, those containing sulphur and chlorine as an ingredient of their anions, could reverse the inhibition of chlorophyll synthesis by CCC.

Effects of sodium and magnesium salts alone and in combination with CCC on growth of seedlings and chlorophyll accumulation in the cotyledons of *Brassica campestris*

Treatment	Total length of seedlings (mm)	Chlorophyll a + b (µg/ml)
Distilled water	40	3.57
CCC, (1000)	32	0.79
$NaNO_3$, (500)	53	4.28
NaCl, (500)	46	4.41
Na_2SO_4, (500)	50	4.69
Na_2CO_3, (500)	48	3.98
CCC + $NaNO_3$, (1000 + 500)	44	0.80
CCC + NaCl, (1000 + 500)	35	1.28[a]
CCC + Na_2SO_4, (1000 + 500)	40	1.65[a]
CCC + Na_2CO_3, (1000 + 500)	36	0.79
$Mg(NO_3)_2$, (500)	49	3.1
$MgCl_2$, (500)	43	4.2
$MgSO_4$, (500)	46	4.7
$MgCO_3$, (500)	48	3.5
CCC + $Mg(NO_3)_2$, (1000 + 500)	40	0.81
CCC + $MgCl_2$, (1000 + 500)	35	1.26[a]
CCC + $MgSO_4$, (1000 + 500)	37	1.30[a]
CCC + $MgCO_3$, (1000 + 500)	36	0.79

Figures in parenthesis represent concentration of salts in ppm.
[a] Significant reversal of chlorophyll at P 0.01.

It has been postulated that CCC more or less selectively inhibited synthesis of proteins necessary for chlorophyll biogenesis or the conversion of proplastid to chloroplasts[4]. Evidence is available to suggest that CCC blocked conversion of β-carotene to phytol[6]. There is no report available in the literature to pinpoint the stages at which sulphur and chlorine participate in chlorophyll biosynthesis. Earlier KNYPL[4] reported that symptoms of action of CCC in chlorophyll development can he reversed if KCl is added simultaneously. On this basis he suggested that K^+ can reverse the inhibition of chlorophyll accumulation. The results of our experiments suggest that, even in the case of KCl, there is a possibility that Cl^- ions participate to reverse the effect of CCC in addition to K^+. It is evident on the basis of results presented in the Table that, wherever Cl^- and SO_4^{--} were present, the accumulation of chlorophyll did increase over the control cotyledons, irrespective of the cation used; in combination with CCC, they significantly reversed the inhibition of chlorophyll accumulation. The reversal of chlorophyll by sulphur and chlorine raises at once the question of possible participation of these elements directly or indirectly in one or more biochemical reactions linked with chlorophyll metabolism. The answer to the question must await further experimentation.

Zusammenfassung. 2-Chloroäthyltrimethylammoniumchlorid (CCC) in einer Konzentration, welche das Längenwachstum von *Brassica*-Keimlingen um etwa 20% hemmt, senkt den Chlorophyllgehalt um rund 80%. Na- und Mg-Salze (500 ppm) vermögen die Wachstumshemmung teilweise aufzuheben. Mit Cl^- und SO_4^{--} als Anionen beobachtet man zusätzlich eine signifikante Verminderung der CCC-Wirkung auf die Chlorophyllakkumulation.

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In vitro Demonstration of Peroxidase Activity in the Fish Kidney Soluble Supernatant and its Physiological Importance

A large number of reports have appeared in recent years regarding the in vitro studies on mammalian thyroid peroxidases[1-3]. BHATTACHARYA and DATTA[4] reported the presence of a peroxidase in the soluble supernatant fraction of avian thyroid. Peroxidase activity in mammalian kidney has also been demonstrated by some investigators[5,6]. However, no report is available regarding the in vitro peroxidase activity in the kidney of fishes. As thyroid is ill-developed and diffused in fishes, and as thyroid follicular structures are present in the head kidney of fishes[7-9], the presence of peroxidase activity in the head kidney of fishes is very interesting. Further, the present communication demonstrates that the *Anabas testudineus* head kidney peroxidase is active in the peroxidation of iodide to triodide (I^{-3}) which was measured at 353 nm.

Materials and methods. Each time 4 *Anabas testudineus* were sacrificed. Head kidney was carefully dissected out from each specimen and homogenized in a Potter-elvehjem homogenizer in 0.05 M sodium phosphate buffer, pH 5.5

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Table I. Peroxidase activity in sub-cellular fractions

Fractions	Total activity[a] (OD/min)	OD/min/mg protein
Cell debris	0.002	0.005
Mitochondria (5 mg protein/ml)	0.37	0.74
Boiled Mitochondria	0.03	0.05
Microsome (4 mg protein/ml)	0.128	0.32
Boiled Microsome	0.02	0.03
Soluble supernatant (2 mg protein/ml)	0.8	4.01
Boiled soluble supernatant	0.001	0.004

[a] 0.1 ml of enzyme was added in each case.

to make a 0.5% homogenate. At first the homogenates were centrifuged at $1000 \times g$ for 10 min. The residual cell debris was suspended in 5 ml buffer. The supernatant fraction was then subjected to a centrifugation of $10,000 \times g$ for 15 min and the residue (mitochondrial fraction) was suspended in a 5 ml of buffer. Finally the supernatant was centrifuged at $105,000 \times g$ for 1 h in a spinco model L preparative ultracentrifuge[10]. The residue was suspended in 5 ml of the buffer and termed as 'microsomal fraction', while the supernatant was used as 'soluble supernatant'. Peroxidase activity was measured by following the increase in optical density at 470 nm in a Carl Zeiss Spectrophotometer Model P.M. Q II, using 1 cm light path with guaiacol as hydrogen donor. The reaction mixtures contained 150 µmoles of sodium phosphate buffer pH 5.5, 1 µmole of guaiacol, H_2O_2, 1:2 µmoles and enzyme preparation of suitable volume and water to make the final volume 3.0 ml. Enzyme protein was measured according to the method of LOWRY et al.[11].

Results and discussion. Mammalian thyroid peroxidases are commonly particulate bound and either trypsin or deoxycholate treatment is necessary to get full peroxidase activity[1,2]. The result presented in Table I clearly shows that head kidney peroxidase, at least in this particular fish, is soluble in nature. Slight activity was found in the mitochondrial fraction.

The pH optimum of head kidney peroxidase was undertaken in buffers having different pH's using sodium phosphate and acetate buffers with overlapping regions and using soluble supernatant as the enzyme. Highest peroxidase activity was obtained at pH 5.5.

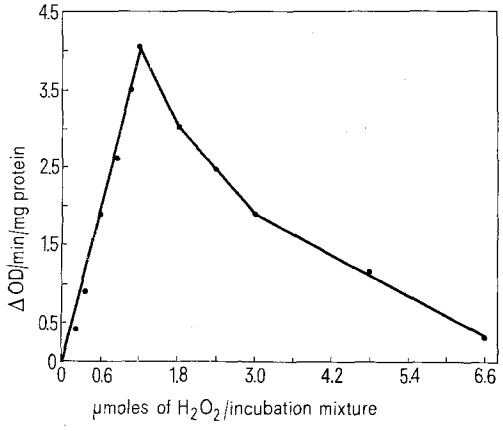

Fig. 1. Effect of varying H_2O_2 concentration on peroxidase activity. Additions and assay were same as described in the text but the concentration of H_2O_2 was varied.

Table II. Effect of some peroxidase inhibitors

Inhibitors	Concentration (M)	Inhibition (%)
Thiouracil	3×10^{-3}	61.5
Cyanide	3×10^{-3}	76
Thiourea	3×10^{-3}	—
	2×10^{-2}	48
Ascorbic acid	3×10^{-3}	35.2
Reduced glutathione	3×10^{-3}	83.3

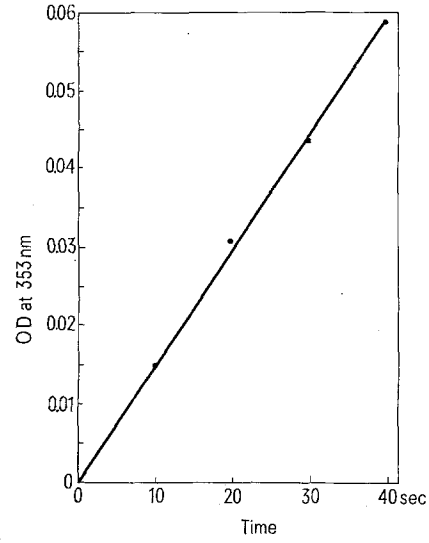

Fig. 2. Peroxidation of iodide by the peroxidase of head kidney soluble supernatant. Additions and assay were same as mentioned in the text.

Though H_2O_2 is a substrate for peroxidase enzyme, excess of H_2O_2 has an inhibitory effect[4,6,12,13]. Therefore optimum H_2O_2 concentration is a very important criterion for assaying the enzyme. Head kidney soluble supernatant peroxidase activity was linear against the concentration of H_2O_2 and a peak was obtained at 1.2 µmoles/incubation mixture, enzyme activity was then sharply inhibited (Figure 1).

Role of some known peroxidase inhibitors was tested on the soluble head kidney peroxidase and the results are depicted in Table II. Thiouracil and cyanide inhibited the enzyme at the concentration of 10^{-3} M, but in this concentration thiourea had no inhibitory effect. Thiourea inhibited the enzyme activity at 10^{-2} M concentration. Reducing agents like ascorbic acid and reduced Gluta-

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thione inhibited the peroxidase activity at 10^{-3} *M*. Over and above the reagents mentioned in the text above compounds are added in a final concentration as mentioned.

Whether these peroxidases are iodide peroxidase or not was assayed according to the method of ALEXANDER[14]. 150 µmoles of sodium phosphate buffer pH 5.5, 20 µmoles of KI, 2.4 µmoles of H_2O_2 and an appropriate amount of enzyme in a total volume of 3 ml. Optical density was then measured at 353 nm. Figure 2 shows that this head kidney soluble supernatant peroxidase significantly oxidized iodide into tri-iodide. Formation of I^{-3} at 353 nm suggest that the enzyme is active in the oxidation of iodide which means that thyroid activity may be located in the soluble supernatant portion of the head kidney. As the thyroid of this fish is very diffused and ill-developed, and as some earlier reports conclude the inactivity of thyroid gland in fishes[15,16], this iodide oxidation is very significant.

Zusammenfassung. In der Kopfniere des Teleostiers *Anabes testudineus* wurde eine besonders hohe Peroxidase-Aktivität nachgewiesen, insbesondere deren Iodid-Oxydation zu Tri-Iodid.

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Collagen Biosynthesis in vitro and the Consequences of Bacterial Contamination. Studies on Vascular Connective Tissue in Rabbits

The collagen biosynthesis in connective tissue of various organs has been studied extensively in the last decade. One of the methods used for this purpose has been the measurement of the synthesis of ^{14}C-hydroxyproline from ^{14}C-proline, as completely described for the skin by UITTO[1].

Normally, antibiotics are added to incubation systems in vitro to eliminate a possible effect of bacterial contamination. To the authors knowledge, however, no systematic studies have been published on the effect of bacteria on the analytical system. Furthermore, an effect of added antibiotics per se on the biosynthesis of collagen and elastin cannot be excluded.

The aim of the present investigation was to compare the results obtained under sterile conditions and those in the presence of bacteria.

Material and methods. Male albino rabbits, about 5 months old and weighing about 3 kg, were fed a commercial chow and sacrificed by an animal pistol. The hair of the abdominal wall was washed with 60% ethanol, whereupon the skin was cut with a sterile pair of scissors.

Sterile conditions: With a new sterile pair of scissors, the thorax and abdomen were opened and the aorta was dissected free with a third set of sterile instruments. The aorta was immediately transferred to a sterile Krebs-Ringer-Bicarbonate solution placed in a sterile 1 m^3 incubator through which a laminar air flow was led. All the following manipulations were done within this sterile incubator. The intima-media part of the aorta was isolated as described by LORENZEN[2], and then sliced parallel to the axis of the aorta in eight 0.8 mm broad strips using parallel razor blades and a manual vice.

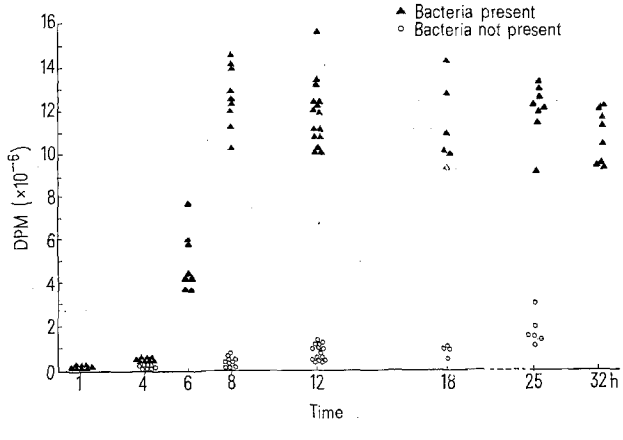

Fig. 1. DPM ^{14}C-proline incorporation in intima-media of rabbit aorta per 2000 µg hydroxyproline as function of incubation time. △, Bacteria present; ●, Bacteria not present.

The tissue-slices were transferred to sterile tubes containing 2 ml of a Krebs-Ringer-Bicarbonate medium[3], 20 m*M* N-2-hydroxy-ethylpiperazine-N^1-2-ethanesulphonic acid (Hepes) pH 7.4, 20 m*M* glucose and 2.5 µCi L-^{14}C-proline (New England Nuclear Cooperation. Radiochemical purity greater than 99.5%. Uniformly labelled).

The tubes were aseptically stoppered, placed in a waterbath and shaken at 37 °C for 12 h unless otherwise stated. The time taken from when the animal was shot until the tubes were stoppered was 4–5 min. At the end of the incubation period samples of the incubation medium were removed for bacterial examination in a bacteriological laboratory. The incubation tubes were stored at –20 °C until biochemical examination, which was performed up to 2 weeks later.

Bacterial conditions: The same procedures as described above were used except that only 1 pair of scissors was used and that the incubator was not used. No bacteria were added to the sterile incubation medium and the treatment of the tissue could best be described as 'as sterile as possible'. Bacterial examinations were done with one inoculation stick from each of the samples at the end of the incubation time, as well as from the ^{14}C-proline batch and the remaining incubation medium. All inoculation sticks were taken to a bacteriological laboratory[4] and numerous amounts of bacteria were taken as an index of contamination.

After storage at –20 °C, all samples were treated equally as follows. The samples were homogenized in 13 ml water in a VirTis '45' at high speed for 10 min. The temperature surrounding the vial was kept at –6 to –8 °C, thus keeping the temperature inside the sample at about +2 °C.

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