provide additional evidence that in mammalian cardiac muscle I_{Na} and I_{Ca} are separate current systems. Though they have similar effects on I_{Na}, La and Ca have opposite effects on I_{Ca}.

In the second series of experiments, we investigated the effect of La on the Na-Ca exchange system in left guinea-pig auricles. It has been shown that Ca efflux from cardiac muscle[5] or squid axon[15] is largely coupled to Na inward movement, presumably through a carrier-mediated transport system. Na and Ca ions compete for the carrier on both sides of the membrane. Therefore, the inwardly directed Na-concentration gradient across the membrane may provide the energy for uphill Ca transport from the interior of the cell into the extracellular space[5,15]. In squid axon La acts as an inhibitor of ^{45}Ca efflux[16]. We performed this series of experiments to assess whether La also inhibits the Na- and Ca-dependent ^{45}Ca efflux from cardiac muscle.

In the experiment shown in Figure 2, Ca efflux is expressed as fraction of ^{45}Ca lost per min from the preparation into the inactive rinsing solutions. During periods I, II and III, the rinsing solutions contained 0.2 m*M* La. As described earlier[5], Ca efflux decreased by approximately 80% when Na and Ca were removed from the solution (periods III and V) and increased promptly again after readmission of these ions (periods IV and VI). The results were the same in the absence and presence of La and irrespective of wheter La was applied during periods IV–VI instead of I–III. Therefore, we conclude that the Na-Ca-sensitive fraction of ^{45}Ca efflux from cardiac muscle is not affected by La. When La was added to the efflux media during one of the later efflux periods after period I, there was a transient increase in ^{45}Ca efflux and a decline to slightly lower steady state values. The same results were obtained with the lanthanide europium.

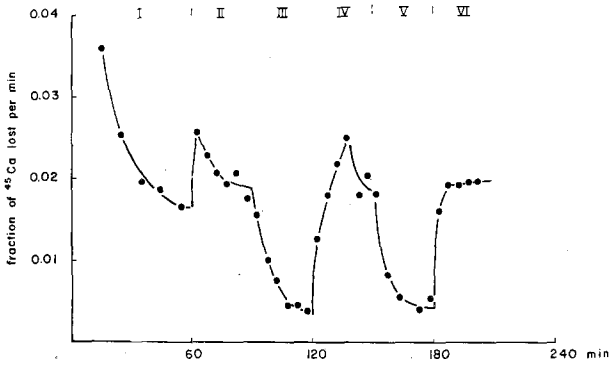

Fig. 2. Effects of changing $[Ca]_0$ and $[Na]_0$ on ^{45}Ca efflux from a guinea pig auricle in the presence and absence of 0.2 m*M*/l $LaCl_3$. Abscissa: time of tracer washout in min; ordinate: fraction of ^{45}Ca lost per min. The roman figures indicate experimental periods with different ion composition of the rinsing solutions. La was in the solutions during periods I–III. Period I: Na-containing, Ca-free solution; periods II, IV and VI: Na- and Ca- (1.8 m*M*/l) containing solution; periods III and V: Na- and Ca-free solution in which NaCl was isoosmotically replaced by choline Cl all solutions were buffered with *Tris*-HCl to pH 7.2 at 35 °C.

The lack of effect of lanthanides on the Na-Ca exchange system in mammalian cardiac muscle suggests that factors other than charge density or ionic radii determine the affinity of these carriers to ions. The electrostatic attraction should be much larger for the lanthanide ions than for Ca or Na ions which have ionic radii of similar size but much smaller charge densities[10]. The results suggest further that I_{Ca} and Na-Ca exchange in cardiac muscle are mediated by different mechanisms in the membrane since one can be inhibited by lanthanum while the other cannot[17].

Zusammenfassung. In Herzmuskelpräparaten sind zwei Systeme für den Ca-Durchtritt durch die Plasmamembranen zu unterscheiden: 1. Ein spannungs- und zeitabhängiger Ca-Einwärtsstrom, der während der Plateauphase des Aktionspotentials fliesst und durch La^{3+} gehemmt wird; 2. ein Na-Ca-Austauschsystem, das vor allem für den Auswärtstransport von Ca aus der Zelle verantwortlich ist, und durch La^{3+} nicht beeinflusst wird.

B. G. Katzung[18], H. Reuter[19] and H. Porzig

Pharmakologisches Institut der Universität Bern, Friedbühlstrasse 49, CH–3008 Bern (Switzerland),
7 May 1973.

[15] M. P. Blaustein and A. L. Hodgkin, J. Physiol. *200*, 497 (1969).
[16] C. van Breemen and P. de Weer, Nature, Lond. *226*, 760 (1970).
[17] This work was supported by the Swiss National Science Foundation grant No. 3.91.69 and NIH grant No. HE 08797-01.
[18] Present address: Department of Pharmacology, University of California, San Francisco Medical Center, San Francisco, Calif. 94122, USA.
[19] Reprints have to be asked for from: Pharmakologisches Institut der Universität, Friedbühlstrasse 49, CH–3008 Bern (Switzerland).

Reversal of the Inhibiting Effect of 2- Chloroethyltrimethyl Ammonium Chloride on Chlorophyll Synthesis by Sulphur and Chlorine

The growth-retarding chemical, 2-chloroethyltrimethyl ammonium chloride (CCC) is known to preserve the loss of chlorophyll from detached leaves[1,2] and to inhibit chlorophyll synthesis[3,4]. The elucidation, however, of the action of CCC to inhibit turnover of proteins and to stimulate turnover of RNA, suggests that this compound may inhibit selectively synthesis of proteins catalyzing both the formation and degradation of chlorophyll[3].

The seeds of *Brassica campestris* were allowed to germinate and grow on petri dishes lined with filter paper moistened with 5 ml of distilled water or an equal volume of the test solution. The dishes were transferred to germination chamber maintained at about 28 ± 2 °C and illuminated from a light bank consisting of two 40 watts cool fluorescent lamps hanging at a distance of about 1 m. After 5 days of germination the length of the seedlings and chlorophyll content of cotyledons was determined. The chlorophyll from the cotyledons was extracted in 80% acetone and was determined by using the formula of Röbbelen[5].

[1] A. H. Halevy and S. H. Wittwer, A. Bull. Mich. St. Univ agric. Exp. Stn *48*, 30 (1965).
[2] L. Beevers and F. S. Guernsey, Nature, Lond. *214*, 941 (1967).
[3] J. S. Knypl, Flora *160*, 217 (1969).
[4] J. S. Knypl, Nature, Lond. *224*, 1025 (1969).
[5] G. Röbbelen, Z. indukt. Abstamm. VererbLehre *88*, 189 (1957).

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.

S. N. SHUKLA and M. N. TEWARI

Laboratory of Tissue Culture and Biochemistry, Department of Botany, The University, Jodhpur (India), 2 August 1972.

[6] V. S. SAAKOV, Dokl. Akad. Nauk SSSR *181*, 1001 (1968).

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

[1] C. C. YIP, Biochim. biophys. Acta *128*, 262 (1966).
[2] T. HOSOYA and M. MORRISON, J. biol. Chem. *242*, 2828 (1967).
[3] R. K. BANERJE and A. G. DATTA, Endocrinology *88*, 1456 (1971).
[4] S. BHATTACHARYA and A. G. DATTA, Comp. Biochem. Physiol. *40 B*, 139 (1971).
[5] H. A. NEUFELD, A. N. LEVAY, F. V. LUCAS, A. P. MARTIN and E. STOTZ, J. biol. Chem. *233*, 209 (1958).
[6] T. FLATMARK, Nature, Lond. *196*, 894 (1962).
[7] S. K. AHUJA, Current Sci. *31*, 466 (1962).
[8] M. GUNAMANI, J. zool. Soc. India. *23*, 24 (1972).
[9] T. K. BANERJE, Anat. Anz., in press.