Relationship Between the Calcifying Power of Various Substances *in vivo* and their Solubility *in vitro*[1]

Soft-tissue calcification is generally considered a secondary phenomenon consequent to some kind of damage, or 'dystrophy'. The work on experimental calcification carried out at this Institute[2] has demonstrated that there is no correlation between the damaging effect and the calcifying potency of many compounds. It is possible to distinguish (1) direct calcifiers which induce topical calcification when placed in contact with the tissues of normal animals, and (2) calciphylactic challengers which induce calcification only when injected into animals pretreated with a systemic calcifying agent (parathyroid extract, vitamin-D compounds) that elevates the calcium-phosphorus product in blood and interstitial fluids.

To investigate the difference between the mechanism of action of direct calcifiers and calciphylactic challengers, we have tested the solubility of various compounds in two types of solution having approximately the same electrolyte content as the serum of normal and vitamin-D-pretreated rats respectively[3].

We used: Normal Tyrode composed of NaCl (0.8%), KCl (0.02%), $MgCl_2$ (0.01%), NaH_2PO_4 (0.005%), $NaHCO_3$ (0.1%), $CaCl_2$ (0.02%). The pH of this solution is 7.6. 'Reinforced Tyrode', containing $CaCl_2$ (0.03%), NaH_2PO_4 (0.06%) but otherwise identical with normal Tyrode. The pH of reinforced Tyrode is 6.2. 100 mg of each compound tested was diluted in 20 ml of normal Tyrode, 20 ml of reinforced Tyrode, or 20 ml of different aqueous solutions composed of NaCl, KCl, $MgCl_2$, NaH_2PO_4, $NaHCO_3$ and $CaCl_2$ in concentrations corresponding to both normal and reinforced Tyrode. The presence of precipitation was judged only qualitatively.

We found that in general, substances which can induce calcification in untreated animals form precipitates both in normal and in reinforced Tyrode, whereas substances requiring that animals be pretreated with vitamin D form precipitates only in reinforced Tyrode. The tests of solutions containing individual components of both Tyrodes showed that direct calcifiers and challengers precipitate in $NaHCO_3$ or NaH_2PO_4. For certain substances (ethanolamine, $InCl_3$), the precipitate is much stronger in Tyrode than in phosphate or carbonate solution. Finally, the compounds without any topical calcifying potency are all soluble in either normal or reinforced Tyrode. The Table shows the correlation between the precipitation in Tyrode and the calcifying power *in vivo* of the compounds tested.

In our experimental series there are two exceptions: potassium permanganate (a direct calcifier) and aluminium chloride (a calciphylactic challenger) do not precipitate in either normal or reinforced Tyrode. We do not know the reason for the exceptional behaviour of these two compounds, but statistical analysis confirms that the results obtained with direct calcifiers and calciphylactic challengers in normal Tyrode are different ($\chi^2 = 17.76$, $P < 0.001$). In reinforced Tyrode, the precipitation of both types of topical calcifiers is also highly significantly different from that of substances without calcifying activity ($\chi^2 = 34.05$, $P < 0.001$).

This experiment throws no light upon the mechanism of calcification *in vivo*, but perhaps an insoluble salt of the direct calcifier or challenger represents the first nucleus that elicits the accumulation of calcium phosphate. In any event we can conlude, however, that there is a relationship between the capacity of inducing a precipitate in each type of Tyrode solution and the calcifying potency of the various compounds examined[4,5].

Solubility of various compounds in normal and reinforced Tyrode

Substances inducing precipitation in:		Substances inducing no precipitation in normal or reinforced Tyrode
normal Tyrode or reinforced Tyrode	reinforced Tyrode only	
$BiCl_3$	*$AlNH_4(SO_4)_2$*	*$AlCl_3$*
$CaCl_2$	*$Al(NO_3)_3$*	$HgCl_2$
$CdCl_2$	*$CoCl_2$*	KBr
$CeCl_3$	*$CrCl_2$*	K_2CrO_4
$CuSO_4$	*$CrCl_3$*	KI
$Ga(SO_4)_3$	*Fe-gluconate*	KIO_3
$InCl_3$	*$Fe(NO_3)_3$*	**$KMnO_4$**
Pb-ac	*$SrCl_2$*	LiCl
$PbCl_2$	*$ThCl_4$*	$MgCl_2$
$ZnCl_2$	*$VOSO_4$*	$NaSO_4$
$ZnSO_4$	*$ZrOCl_2$*	$NiCl_2$
Ethanolamine oleate		Adrenaline bitartrate
		Bacitracin
		Compound 48/80
		Formalin
		Heparin
		5-HT
		Na-citrate
		Polymyxin
		TaCl
		Tannic acid
		Vitamin D_3

Bold face = compounds which induce calcification when injected into normal animals (direct calcifiers). *Italics* = compounds which induce calcification when injected into vitamin-D-pretreated animals (calciphylactic challengers). Roman = compounds which induce no calcification.

Riassunto. L'attività di varie sostanze nell'indurre calcificazioni *in vivo* è messa in relazione con la loro solubilità in soluzione Tyrode e particolarmente con la proprietà di precipitare il fosfato ed il carbonato di sodio.

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[2] H. SELYE, *Calciphylaxis* (The University of Chicago Press, Chicago 1962).

[3] P. JEAN and P. MENDELL, Rev. canad. Biol. *21*, 17 (1962).

[4] The author wishes to thank the following companies for donating compounds used in this experiment: Commercial Solvent Corp., Terre Haute, USA (Bacitracin); Pfizer, Montreal, Canada (Polymyxin); and Wander, Bern, Switzerland (vitamin D_3).

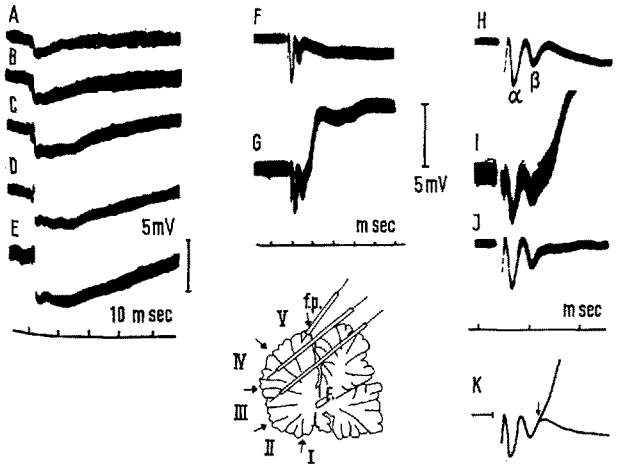
[5] The adrenaline bitartrate used was obtained from Brickman & Co., Montreal (Canada); compound 48/80 from Burroughs Wellcome & Co. (USA) Inc., Tuckahoe (USA); ethanolamine oleate from Matheson, Coleman & Bell, Cincinnati and East Rutherford (USA); heparin from Organon Inc., Montreal (Canada); and 5-HT from Nutritional Biochemicals Corp., Cleveland (USA).

The Cerebellar-Evoked Monosynaptic Inhibition of Deiters' Neurones

The nucleus of Deiters receives rather massive projection from the cerebellum, directly as well as *via* the nucleus fastigii[1]. The cerebellar influence upon Deiters' nucleus has been assumed to be dominantly inhibitory, because ablation of the cerebellum greatly enhances the vestibular-evoked motoneurone discharges which are presumed to be mediated, at least in part, by Deiters' nucleus[2]. Stimulation of the anterior vermis of the cerebellum, indeed, inhibited spontaneous unit discharges in Deiters' nucleus, though there were others facilitated by the same stimulation[3,4]. In the work to be reported, it was further revealed with the intracellular recording technique that the vermian stimulation induces inhibitory postsynaptic potential (IPSP) *monosynaptically* in Deiters' neurones.

Cats were anaesthetized with pentobarbitone sodium. Dissection technique and experimental procedure have already been described[5]. Microelectrodes were filled with solution containing 3 *M* KCl or 2 *M* NaCl. For stimulating the ipsilateral anterior vermis, concentric electrodes with outside diameter of 0.5 mm and with interpolar distance of 1 mm were inserted stereotaxically through, or under direct vision over, the posterior lobe, aiming at lobule III, IV or V (Figure, inset diagram). Square pulses of duration of 0.08 to 0.2 msec were applied between the internal and external poles of each electrode.

So far 52 cells have been impaled in the vestibular nuclei region and identified as Deiters' neurones by the antidromic invasion from the spinal cord[5]. In the majority of them (44), stimulation of lobule III, IV or V by the cathode placed at a depth of 1 to 2 mm from the cortical surface produced a hyperpolarization of the membrane potential (Figure, A–E). At relatively weak stimulation near the threshold, which was usually around 1 V, this hyperpolarization was of simple configuration (Figure, A) with a peak time of about 1 msec and half time decay of about 10 msec. With an increase of stimulus intensity, the IPSP increases not only in its amplitude but also in its duration, which often exceeded 100 msec (Figure, E). The hyperpolarization could be reversed into a depolarizing potential either when the membrane was hyperpolarized by currents applied through the microelectrode or when chloride ions were injected electrophoretically into the cell (Figure, F and G). The inhibitory nature of this hyperpolarization could readily be demonstrated by suppression of spontaneous discharges of Deiters' neurones, which often occurred due to injurious effect of penetration[5]. These observations establish that the cerebellar-evoked hyperpolarization is the IPSP of the same nature as those hitherto studied extensively in cat spinal motoneurones as well as in many other nerve cells[6]. Figure H illustrates the initial part of the potential change of Figure F at a faster sweep velocity. The two negative deflections, α and β, which occurred before the hyperpolarization developed, were not influenced by the change of the membrane potential (Figure, I) and were recorded even in the extracellular position (Figure, J). Hence, they should be field potentials produced by impulses arriving at Deiters' nucleus. In spite of this complexity of the curve, the time of onset of the IPSP can be determined accurately by superimposing the hyperpolarizing and depolarizing responses (Figure, K). The latency of the IPSP thus measured from the onset of the stimulating pulse was as short as 0.9 to 1.1 msec (mean, 1.0 msec) when lobule III was excited.



IPSPs induced in a Deiters' neurone by stimulating the lobule III of the cerebellum. Stimulating voltages, 1.5 (A), 3.3 (B), 4.9 (C), 6.7 (D) and 30 V (E). F–I, IPSPs in another Deiters' neurone. G and I were recorded under hyperpolarization by currents of $2 \cdot 10^{-8}$ and $3 \cdot 10^{-8}$ A, respectively. J, extracellularly recorded field potential. K, traces in H and I are superposed. Downward arrow indicates the time of onset of the IPSP thus determined. Inset diagram, illustrating the positions of the cerebellar electrodes. Arrows indicate fissures dividing lobules I to V; f.p., fissura prima; F, nucleus fastigii.

In cat spinal motoneurones, the latency for producing EPSP (excitatory postsynaptic potential) monosynaptically by a volley along the group Ia muscle afferent fibres is 0.5 msec and that for inducing IPSP disynaptically is 1.3 msec after the impulses enter the spinal cord[7]. The value of 1.0 msec obtained for the latency of the cerebellar-evoked IPSP in Deiters' neurones would suggest that the inhibitory pathway is monosynaptic and is formed of slowly conducting fibres; or alternatively that it is disynaptic, fast conducting fibres being relayed by a synapse at some point in their course. However, the latter possibility was excluded by the fact that, when the stimulating electrode was inserted deep towards the roof of the fourth ventricle, the latency of the IPSP decreased continuously according to the distance between the electrode and Deiters' nucleus. At the extreme it was as short as 0.73 msec, when the region near the nucleus fastigii was stimulated, and on the other hand as long as 1.3 msec when the cortex of lobule V was excited. This variation of the latency can reasonably be accounted for if the pathway is assumed to have a slow conduction velocity of 20 m/sec or even less.

In conclusion, the present results indicate that there exists, in the region including the cerebellum and the brain stem, a type of inhibitory neurones whose axons are long enough to bridge over between the cerebellar cortex and Deiters' nucleus. In consideration of the cerebellar

[1] A. Brodal, O. Pompeiano, and F. Walberg, *The Vestibular Nuclei and Their Connections* (C. C. Thomas, Springfield, Ill. 1962).
[2] E. Gernandt and S. Gilman, Exp. Neurol. *1*, 274 (1959).
[3] R. V. de Vito, A. Brusa, and A. Arduini, J. Neurophysiol. *19*, 241 (1956).
[4] O. Pompeiano and E. Coti, Arch. Sci. biol. *43*, 57 (1959), quoted from [1].
[5] M. Ito, T. Hongo, M. Yoshida, Y. Okada, and K. Obata, Exper. *20*, 295 (1964).
[6] J. C. Eccles, *The Physiology of Nerve Cells* (Johns Hopkins Press, Baltimore 1957).
[7] T. Araki, J. C. Eccles, and M. Ito, J. Physiol. *154*, 354 (1960).