Results and discussion. Table I shows the typical results of the experiments on the enzyme activities of each fraction from chicken liver. The activity of sepiapterin reductase is mostly concentrated in fraction 1 and only a little activity is detectable in fraction 2. Fraction 3, which is freed from folate reductase as reported by SILVER et al.[6], is also devoid of the activity of sepiapterin reductase. These results apparently indicate that sepiapterin reductase is distinguishable from both folate and dihydrofolate reductase. The effect of aminopterin on sepiapterin reductase further supports the above conclusion. As already known, folate and dihydrofolate reductase are completely inhibited by concentration of aminopterin as low as $1 \times 10^{-5} M$, while the inhibitory effect of the drug on sepiapterin reductase is only slight even at the concentration of $1 \times 10^{-4} M$ (Table II).

Table I. Comparison of sepiapterin-, folate- and dihydrofolate-reductase activities in enzyme fraction from chicken liver

Enzyme fraction	Sepiapterin reductase	Folate reductase	Dihydrofolate reductase
	$-\Delta E_{420}$[a]	$\mu g FAH_4$[a]	$-\Delta E_{340}$[a]
Crude extract	0.148	4.15	0.421
Fraction 1	0.320	0.47	0.050
Fraction 2	0.016	13.30	1.190
Fraction 3	0.000	0.00	0.300

[a] per mg protein per h.

Table II. Effect of aminopterin on reductase activities

Final concentration of aminopterin	Sepiapterin reductase	Folate reductase	Dihydrofolate reductase
$1 \times 10^{-5} M$	10	100	100
$1 \times 10^{-4} M$	11	100	100

Numbers in columns show % inhibition.

Other than insect tissues and chicken liver, the activity of sepiapterin reductase is widely distributed in the liver of mammals, such as rat, hog, rabbit, horse and oxen.

On the biochemical role of sepiapterin, it is interesting that the pteridine has high cofactor activity in KAUFMAN's system of phenylalanine-hydroxylation[10]. According to him, tyrosine formation in the presence of sepiapterin[10] or dihydrobiopterin[11] is dependent on dihydrofolate reductase. The present findings conflict with the idea that sepiapterin is reduced directly to tetrahydro level by dihydrofolate reductase to participate in the hydroxylation reaction of phenylalanine. The biochemical role of sepiapterin reductase is currently being investigated and will be reported elsewhere.

Résumé. Nous avons démontré dans le foie de poussin l'existence d'un ferment qui réduit spécifiquement la sépiaptérine. Il est capable de séparer le ferment de réductase d'acide folique et d'acide dihydrofolique après précipitation fractionnaire de sulfate d'ammonium. Le ferment, réductase de sépiaptérine, se trouve largement dans les foies des mammifères, tels que rat, cochon, lapin, cheval et bœuf.

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Monosynaptic Inhibition of the Intracerebellar Nuclei Induced from the Cerebellar Cortex

In the previous report[1], it has been shown that in the neurones of Deiters' nucleus of the cat the inhibitory postsynaptic potentials (IPSPs) are induced *monosynaptically* by stimulation of the cerebellar cortex at the anterior vermian part. It was then suggested that this inhibition may be carried by the long corticofugal fibres which originate from the cerebellar cortex and project directly onto Deiters' neurones. If this were so, the nature of the cerebellar Purkinje cells would be specified as inhibitory. It would follow that a similar inhibition occurs at any target neurones of the corticofugal projection either within or without the cerebellum[2]. In the work to be reported, this postulate was substantiated by the intracellular recording from the intracerebellar nuclei during stimulation of the cerebellar cortex.

Adult cats were used under anaesthesia by pentobarbitone sodium. The procedure of dissection and the experimental arrangement were the same as those employed for the intracellular recording from Deiters' neurones[1,3]. Microelectrodes were inserted into the nuclei dentatus, interpositus and fastigii by way of the pharyngeal approach[3]. As seen in Figure A, they were reached at the depth of 11 to 13 mm along tracks with lateral angle of −5 to 35 degrees (− indicates medial). In order to stimulate antidromically the nuclei interpositus and dentatus, concentric electrodes with the outside diameter of 0.5 mm and interpolar distance of 1 to 1.5 mm were inserted stereotaxically into the red nucleus (RN) and also into the nucleus ventralis lateralis of the thalamus (VL), as shown in Figure B. Concentric electrodes of the same type were inserted into the anterior lobe of the cerebellum stereotaxically[1] or into the cerebellar hemisphere under direct vision. In the latter case three electrodes were implanted at intervals of 3 to 5 mm along the crus II, and their internal poles at 1 to 1.5 mm under the cortical surface were used as cathode against their respective outside

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tubes as anode (Figure A). Square pulses of duration of 0.08 to 0.2 msec were employed for stimulation, and their strength varied from zero to 30 V.

When a microelectrode was inserted into the region of the nucleus dentatus or interpositus, negative field potentials could be obtained by stimulating VL (Figure C) or RN (D), and accordingly, when the electrode penetrated a cell, spike potentials up to 60 mV in amplitude were recorded intracellularly (Figure E, F). The so-called giant extracellular spike[4] also was often observed (Figure G). These intra- and extra-cellular spikes showed an inflection at their rising phase, though not so conspicuous as in spinal motoneurone[5] or Deiters' neurones[3]. From the small values of the latency for initiating these spikes (around 0.5 msec from RN), it is expected that they are induced antidromically by impulses firing back along axons at a conduction velocity of about 20 m/sec. The cells of the intracerebellar nuclei were sometimes found to be activated also by stimulating the cerebellar cortex (Figure H). The extremely short latency for this activation (within 0.5 msec) indicated that it occurred antidromically. This finding is consistent with the suggestion by CARREA, REISSIG, and METTLER[6] that the axon collaterals extend up to the cerebellar cortex from the intracerebellar nuclei as the climbing fibres.

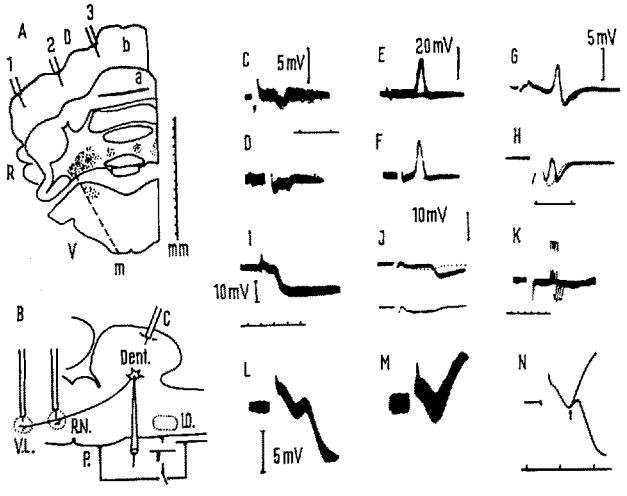

Fig. A, a, transverse section of the brain stem and the cerebellum through the dentate nucleus. m, track of the microelectrode which was left inserted in the tissue after the intracellular recording. b, another section at 1 mm rostral from a. Three concentric electrodes (1, 2, and 3) were attached at the level of b. B, showing the ways of stimulating the axons and other input sources for dentate neurones. P., pons, I.O., inferior olive. C, D, field potentials in the dentate nucleus evoked by stimulating V.L. and R.N., respectively. E, F, intracellularly recorded spike potentials in a dentate cell under the same stimulations as in C and D, respectively. G, H, extracellularly recorded spikes of a dentate neurone under stimulation of R.N. and the cerebellar cortex, respectively. The dotted line in H indicates the field potential change that was recorded in the extracellular position. I, IPSP produced in a dentate cell in the experiment of A during stimulation at No. 1 electrode. J, upper trace shows the sequence of EPSP and IPSP obtained intracellularly in a dentate cell during application of current pulses of 0.1 msec duration and of intensity of 30 V to the ventral surface of the medulla. Lower trace in J is the field potential just outside of the impaled cell which was drawn in the upper trace by a dotted line so as to show the net transmembrane potential change. K, extracellular recording from the same neurone of J during stimulation at the ventral surface of the medulla. L, the IPSP same as in I. M, same as in L but during passage of a hyperpolarizing current of $3 \cdot 10^{-9}$ A. N, superposed tracing of L and M. Explanation is in text. Time scales are all in msec and apply in common to C, D, E, F; G, H; J, K; and L, M, N, respectively. Voltage scales are common in C and D, in E and F, in G and H, in J and K, and in L, M and N, respectively.

In the cells of the intracerebellar nuclei stimulation of the cerebellar cortex at an appropriate portion induced an IPSP with a short latency of less than 1 msec (see below). This happened in the nucleus dentatus only during stimulation of the ipsilateral cerebellar hemisphere, and in those of the nuclei interpositus and fastigii under stimulation of the anterior vermis. In the case of Figure I the cell was sampled in the most lateral region of the dentate nucleus and accordingly the most lateral cerebellar electrode (No. 1 in Figure A) alone was effective in producing the IPSP, the threshold value being only 1 V, while the other two (Nos. 2 and 3 in A) failed even when the stimulating voltage was increased up to 30 V. Hence there appears to be regional correspondence between the intracerebellar nuclei and the overlying cerebellar cortex which inhibits the former, such as has been suggested histologically for the corticofugal projection[2]. Figure L illustrates the initial time course of the potential change given in I at a higher amplification and a higher sweep velocity and Figure M that during application of a hyperpolarizing current through the impaled microelectrode which reversed the IPSP into a depolarizing potential. As shown in Figure N, superposition of the traces of L and M enables us to determine accurately the starting point of the IPSP (marked by an upward arrow). The latency for the IPSP thus measured from the onset of the stimulating pulses ranged from 0.7 to 0.9 msec. Obviously these IPSPs occur monosynaptically[1]. It is emphasized that this presumed monosynaptic IPSP was found in all of forty cells of the intracerebellar nuclei so far examined, thirty from the nucleus dentatus, six from the nucleus interpositus and four from the fastigial nucleus. Since these cells receive massive projection of the short corticofugal fibres[2], the present results strongly support the view that the cerebellar Purkinje cells are inhibitory in their nature.

On the other hand, when the ventral surface of the medulla and the pons was stimulated with relatively large voltages up to 30 V (Figure B), IPSPs appeared at a latency which has never been less than 2.5 msec (Figure J). It is possible that this stimulation excited the cerebellar cortex synaptically through the cerebellar afferent fibres. Usually these delayed IPSPs were preceded by small EPSPs (Figure J) and the cells sometimes were found to fire at this EPSP phase (Figure K). These facts indicate that the cerebellar nuclei receive excitatory signals from somewhere at or through the brain stem region. It is then conceivable that the intracerebellar nuclei are reflex centres, the activities of which are controlled by inhibition from the cerebellar Purkinje cells.

Résumé. Dans les neurones des noyaux cérébelleux des enregistrements intracellulaires ont montré que la stimulation du cortex cérébelleux peut évoquer les mêmes monosynaptiques IPSP que dans le noyau de Deiters.

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