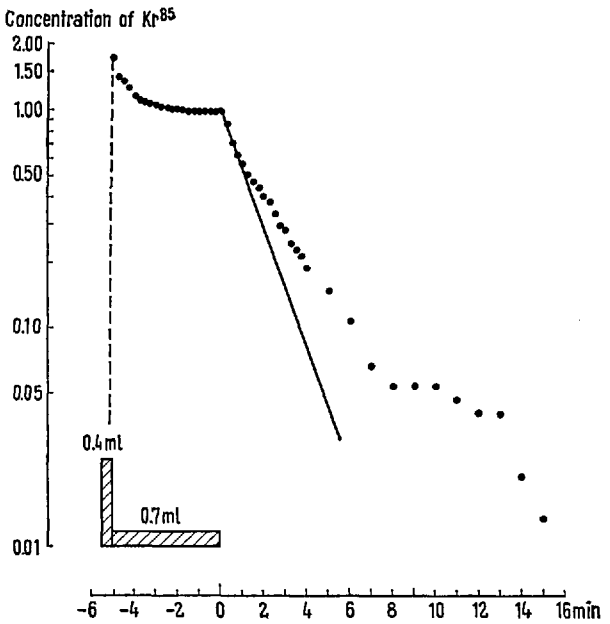
The exposed pial surface was covered with a 0.01 mm thick sheet of 'Mylar' (Dupont), a relatively gas-tight polyesther film. The sheet was fitted to the opening in the dura so that no air bubbles and only a minimal fluid layer remained between the sheet and the brain. A small end-window Geiger-Müller tube (General Electric type MD. 4. H., or Philips 18745) was mounted 0.5 mm above the 'Mylar' sheet. A lead-shield was employed so that a cortical area of a diameter of 6.5 mm was recorded from. A gentle stream of air was continuously replacing the air between the GM-tube and the 'mylar' sheet. The GM-tube was coupled to an ECKO ratemeter (type n 522. B) with the time constant set at 1 sec and the signal was recorded by an ink-writing recorder (SPEEDOMAX, time constant 1 sec).

The intracarotid injection of the saline containing Kr^{85} was made through a thin polyethylene cannula inserted into the cut lingual artery. A continuous infusion pump was used and about 0.5 ml of the saline was injected during the first $^1/_2$ min followed by about 0.5 ml in the next 5 min. Then the injection was abruptly stopped. The counting rate over the brain at the end of the injection was usually about 4000–5000 min. By interposing a layer of aluminum foil, it was calculated that about 90% of the emissions recorded originated from a tissue layer of 0.6 mm. Practically complete clearance of Kr^{85} from the cortex was obtained in 15–20 min. Injection into a peripheral vein did not cause a significant rise above cortical background radioactivity. Thus the curve recorded was only determined by the Kr^{85} taken up by the cortex during the intracarotid injection and by the subsequent outwash by arterial blood containing insignificant amounts of recirculating Kr^{85}. A semilogarithmic plot of the recorded curve was made after correction for background radioactivity. It was assumed that all the tissue compartments counted from had the same blood/brain partition coefficient of 0.9 for Kr^{85} as determined for the cortex as a whole by LASSEN[2]. Assuming also that the various tissue compartments had the same concentration of Kr^{85} at the end of the injection, it can be shown that the average blood flow of these compartments in ml/g/min is equal to the numerical value of the product of the blood/brain partition coefficient and the exponential coefficient of a monoexponential curve fitted to the initial part of the desaturation curve (Fig.).

The blood flow of the parietal cortex of the cat was found to vary considerably depending on the experimental situation studied, especially the respiration of the animal was important. Values ranging between 0.4 and 1.6 ml/g/min were recorded. This is in agreement, as to order of magnitude, with the results of KETY et al.[3]. Repeated measurements of cortical blood flow in the same animal and under the same conditions demonstrated that the coefficient of variation of the random experimental error does not exceed 8%.

The present technique reveals that even so small a cortical tissue block as that counted from in these studies consists of components differing with respect to clearance rate of Kr^{85}. This is evident from the consistent deviation of the desaturation curve from the monoexponential curve expected in a homogeneous system. This observation necessitated the use of a stepwise injection of Kr^{85} as described above in order to obtain approximately the same Kr^{85} concentration in all tissue compartments at the end of the injection. When applying the method to an experimental situation differing from that of the present studies, the duration and the relative injection speeds may have to be changed somewhat in order to approximate most closely to this condition.

With due consideration to the slope of the Kr^{85} clearance curve in a control study changes of the slope may be taken to indicate variations in perfusion. The method has also been used in a continuous fashion by continuously infusing the isotope over $^1/_2$–1 h. The level of the resulting cortical radioactivity is inversely related to blood flow, and calibration of the curve can be made by the above described discontinuous clearance method.



Krypton^{85} concentration in the cerebral cortex of a cat. The blood flow is calculated as 0.9 times the numerical value of the exponential coefficient of the monoexponential curve fitted to the initial part of the desaturation curve (the straight line on the Figure).

Zusammenfassung. Der radioaktive indifferente Luftbestandteil Krypton^{85} (in Ringer-Flüssigkeit) wird in die Arteria carotis communis injiziert und die Radioaktivität über der freigelegten Gehirnoberfläche verfolgt. Die Methode ergibt reproduzierbare quantitative *corticale* Perfusionswerte und eignet sich auch für andere Gewebe.

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Laboratory of clinical Neurophysiology, University of Lund (Sweden), October 17, 1960.

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STUDIORUM PROGRESSUS

Molecular Mechanism on the Energy Transfer and Conversion in the Photosynthetic System

This paper describes an attempt to develop a physical model for the transfer and conversion of the excitation energy in the photosynthetic system. According to the recent experimental results[1] on the system, it must be

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supposed that the parts of the chlorophyll-A molecular aggregates in the photosynthetic apparatus are to receive excitation energy from the other accessory pigments (i.e., carotenoids, phycobilins, chlorophyll-B, -C, etc.), to absorb the light energy from the external field, and to transmit the excitation energy to some reaction site and convert it into oxidation-reduction chemical energy. The main energy transfer processes consist of the transfer from the accessory pigments to chlorophyll-A and the energy migration in the aggregate system of the chlorophyll-A molecules. The former is the sensitization with different kinds of pigments for photosynthesis, the latter is a short of host sensitization.

The excitation energy transfer probability per unit time from S_1 molecule to S_2 molecule by the transitional dipole-dipole interaction is given by [2]

$$W_{S_1S_2} = \frac{3}{4\pi} \cdot \left(\frac{\hbar c}{n}\right)^4 \cdot \frac{Q_{S_2}}{\tau^0_{S_1} R^6} \cdot \int \frac{F_{S_1}(E)\, A_{S_2}(E)}{E^4}\, dE \ , \quad (1)$$

where n is the index of refraction of the medium, E the energy of photon related to the process, c the velocity of light, $\hbar$ Planck's constant, $\tau^0_{S_1}$ the optical lifetime of the excited state of S_1, R the intermolecular distance between S_1 and S_2, $F_{S_1}(E)$ the normalized function characterizing the fluorescence spectrum of S_1 and $\int F_{S_1}(E)\, dE = 1$, Q_{S_2} a constant related with the absorption cross-section $\sigma_{S_2}(E) = Q_{S_2} A_{S_2}(E)$ of S_2, and $A_{S_2}(E)$ is the normalized function characterizing the absorption spectrum of S_2 and $\int A_{S_2}(E)\, dE = 1$.

By assuming the Gaussian distribution around a peak $E_f^{S_1}$ of the fluorescence spectrum of S_1, and a peak $E_a^{S_2}$ of the absorption spectrum of S_2 for $F_{S_1}(E)$ and $A_{S_2}(E)$, we take the easy form to understand for $W_{S_1S_2}$.

$$W_{S_1S_2} = \frac{3(\lambda_f^{S_1})^3(\lambda_a^{S_2})}{4\pi^4} \cdot \frac{Q_{S_2}}{\tau^0_{S_1} R^6} \left(\frac{k}{\pi}\right)^{1/2} \cdot \exp\{-k(E_a^{S_2} - E_f^{S_1})^2\} \ , \quad (2)$$

where k is a constant determined by the widths of the fluorescence and absorption spectra of S_1 and S_2, and $\lambda_f^{S_1} \equiv \hbar c/E_f^{S_1}$, $\lambda_a^{S_2} \equiv \hbar c/E_a^{S_2}$.

Since $W_{S_1S_2}$ has the exponential dependence on the negative of the square of $(E_a^{S_2} - E_f^{S_1})$, it can be concluded that the excitation energy is transferred in a regular sequence among the accessory pigments from the first excited molecule to the molecule having the nearest value of absorption maximum to the fluorescence maximum of the first, and from the second to the third molecule having the absorption maximum nearest to the fluorescence maximum of the second, and finally to chlorophyll-A in a step-by-step way.

The maxima of the fluorescence and absorption spectra of the pigments in the photosynthetic system are shown in Figure 1. The excitation energy transferred to chlorophyll-A or absorbed by chlorophyll-A itself will be able to migrate in the chlorophyll-A aggregate system with the same mechanism beyond the nearest neighbouring intermolecular distance. This migration may be treated on the diffusion model of the 'intra-molecular exciton' introduced by FÖRSTER[3] in the first place, and discussed recently by RABINOWITCH[4].

On the assumption of a continuous body for the chlorophyll-A aggregate system, we can indicate the probability $P(\boldsymbol{R}, t)$ that an intra-molecular exciton lies in the position $\boldsymbol{R}$ in the system at a time t. Then, the probability that the exciton lies in $\boldsymbol{R}'$ at the time $t + \tau$ is obtained by means of the transition probability $W(\boldsymbol{R}', \boldsymbol{R}; \tau)$ during τ per unit volume V_0 as the following;

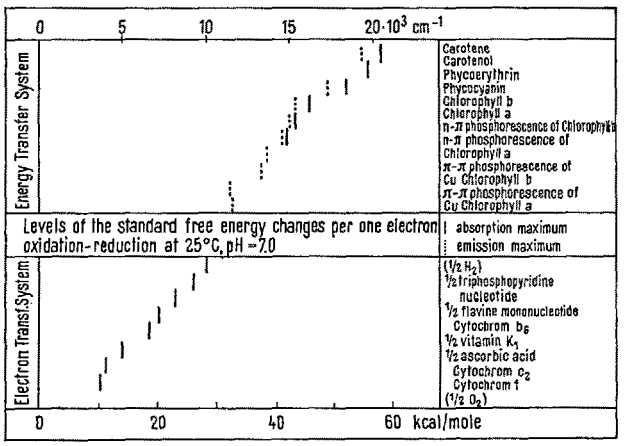

Fig. 1. Energy levels of the absorption and emission maxima of the energy transfer system, and levels of the standard free energy changes per one electron of the electron transfer system.

$$P(\boldsymbol{R}', t+\tau) = \int W(\boldsymbol{R}', \boldsymbol{R}; \tau)\ P(\boldsymbol{R}, t)\, d^3\boldsymbol{R} \ , \quad (3)$$

with $$\int W(\boldsymbol{R}', \boldsymbol{R}; \tau)\, d^3\boldsymbol{R} = 1 \ ,$$

where

$$W(\boldsymbol{R}', \boldsymbol{R}; \tau) = \frac{3}{4\pi} \cdot \left(\frac{\hbar c}{n}\right)^4 \cdot \frac{\tau}{\tau^0} \cdot \frac{\varrho Q}{|\boldsymbol{R}' - \boldsymbol{R}|^6} \cdot \int \frac{F(E)A(E)}{E^4}\, dE$$

$$W(\boldsymbol{R}', \boldsymbol{R}; \tau) = 0 \qquad \begin{matrix} \text{for } |\boldsymbol{R}' - \boldsymbol{R}| \geq r_0 \\ \text{for } |\boldsymbol{R}' - \boldsymbol{R}| < r_0 \end{matrix} \ ,$$

and the unit volume is given by $V_0 = (4\pi/3)\, r_0^3 = 1/\varrho$, ϱ is the molecular density in the system. Here τ^0, Q, $F(E)$, and $A(E)$ are the above-mentioned constants and functions of the chlorophyll-A molecule. After the Taylor expansion of both sides of the equation (3) about $r \equiv |\boldsymbol{R}' - \boldsymbol{R}|$ and t, the diffusion equation of the intramolecular exciton is found.

$$\frac{\partial P(\boldsymbol{R}, t)}{\partial t} = D_e \operatorname{div} \operatorname{grad} P(\boldsymbol{R}, t) \ . \quad (4)$$

Here we have defined $D_e = 1/6\tau \cdot \int r^2\, W(r; \tau)\, d^3r$, this is the diffusion coefficient of an exciton. The final expressions of D_e and the diffusion distance l_e of an exciton during its optical lifetime in the system are given by

$$\left.\begin{aligned} D_e &= \left(\frac{\pi}{6}\right)^{1/3} \cdot \left(\frac{\hbar c}{n}\right)^4 \cdot \frac{Q}{\tau^0} \cdot \int \frac{F(E)A(E)}{E^4}\, dE \cdot \varrho^{4/3} \ , \\ l_e &\equiv (D_e \cdot \tau^0)^{1/2} = \left(\frac{\pi}{6}\right)^{1/6} \left(\frac{\hbar c}{n}\right)^2 \cdot \left\{Q \int \frac{F(E)A(E)}{E^4}\, dE\right\}^{1/2} \cdot \varrho^{2/3} \end{aligned}\right\} \quad (5)$$

The diffusion length of an exciton is a measure of the spatial damping of the diffusional flux of the excitation energy in the system. For the chlorophyll-A aggregate system at the concentration 0.1 M/l which is the local concentration of chlorophyll-A in grana, $D_e \cong 1.1 \times 10^{-3}$ cm²/sec, and $l_e \cong 250$ Å are obtained by means of graphical integration of the overlapping areas between the fluorescence and absorption spectra. It is a remarkable thing that the excitation energy may be able to migrate to the spatially different region in the chlorophyll-A aggregate system by the diffusional motion owing to the transitional dipole-dipole interaction with the diffusion coefficient and diffusion length comparable to the aromatic hydrocarbon

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[3] TH. FÖRSTER, Ann. Phys. *2*, 55 (1948).
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crystal[5]. This work gives a molecular-physical basis for RABINOWITCH's qualitative discussion and reasonable speculation[4].

The excitation energy transmitted through the chlorophyll-A aggregate must be captured by some 'energy-sink' in the system.

The most important mechanism of the conversion of the excitation energy is the radiationless transition from the first excited singlet (S^*) state to the lowest triplet (T) state. Unfortunately the position of the T state of chlorophyll-A remains still unidentified, but $n - \pi$ T level was found in the strictly 'dry' condition free from any hydroxyllic impurity[6]. The $n - \pi$ T state is quenched in the 'wet' condition in accordance with the blue shift of the $n - \pi$ absorption band by the hydrogen bonding with the hydroxyllic molecule and the chelation between the central Mg atom and the hetero-atom of the hydroxyllic molecule. However, it was known that the transition-metal (Cu) substituted chlorophyll molecule for the central Mg showed the characteristic phosphorescence[6,7], due to the $\pi - \pi$ $T \rightarrow S$ transition being insensitive to the presence of the hydroxyllic molecules. On the other hand, the absorption spectra of the chlorophyll molecules due to the transition from the lowest T state to the higher T state can be obtained by the flash light method in the usual medium[8]. These experimental facts show that the stabilization of the $\pi - \pi$ T state due to the intra- or intermolecular perturbation effect of the paramagnetic metal complex is needed, and the $\pi - \pi$ $T \rightarrow S$ transition becomes possible for the first time as the result of the stabilization of the T state[9].

The photosynthetic system *in vivo* cannot be considered as being in the 'dry' condition even in the lipoid zone. So we cannot impose the leading part on the $n - \pi$ T state of the chlorophyll molecule.

The presence of the paramagnetic metal enzymes (e.g. catalase, cytochromes, Cu-enzymes) in the photosynthetic electron transport system may be important as the stabilizer of the $\pi - \pi$ T state.

Let us consider a molecular complex between one of the chlorophyll-A aggregate and a paramagnetic metal enzyme in the doublet electronic state. The S, S^*, and T states of the chlorophyll-A molecule will be modified to the three doublet D, D^*, and D' states with the complex formation, respectively. The unperturbed T state coming from the S^* state by the radiationless transition at the crossing point c_1 of Figure 2a has a lifetime sufficient to make the $T \rightarrow T'$ transition observable, but has insufficient stability for the occurrence of the $T \rightarrow S$ forbidden transition. The excited energy in the T state will be dissipated into the thermal vibrational energy through the crossing point c_2, and the T state returns radiationlessly to the S state at the same time.

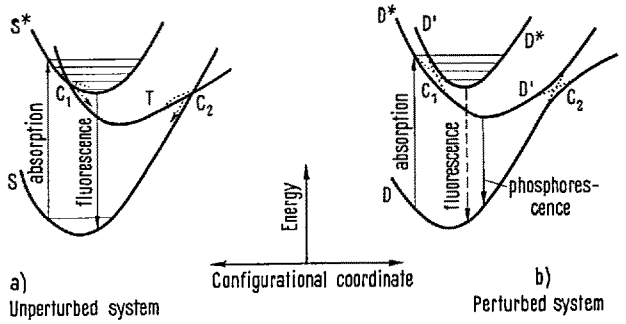

Fig. 2. Configurational diagrams of the electronic states; the stabilization of the triplet state with the perturbation of the molecule in a doublet state.

In the perturbed system all D, D^*, and D' states are the modified doublet states, and the energy separations at the crossing points become larger than the unperturbed system as shown in Figure 2b.

The larger the separation of the levels at c_1 and c_2, which is determined partly by the binding energy of the molecular complex, the greater the stabilizing effect the paramagnetic metal enzyme produces on the T state of the chlorophyll molecule. The $D^* \rightarrow D'$ radiationless transition probability increases, and the $D' \rightarrow D$ transition becomes partially allowed and observable as a phosphorescence with the shorter lifetime than normal. It may be expected that the D' state remains still 'metastable' and long-lived enough to play an active part for the successive chemical reaction.

After the capture of the excitation energy in the metastable state, an electron-hole in the ground state may be able to migrate to the ground state of the unexcited nearest neighbour molecule in the chlorophyll-A aggregate, while an electron is transferred from the nearest neighbour to a hole in the capture site. An electron-hole may be transported to a spatially different site by repeating this process during the lifetime of hole-electron recombination ($\sim$ the phosphorescence lifetime)[10].

A negative chlorophyll-A ion (reduced semiquinone form) is produced at the excited energy capture site, while a positive chlorophyll-A ion (oxidized semiquinone form) is produced at an opposite site. The former will take part in an electron donating process to the lowest vacant level of an oxidant which may be a member of the photosynthetic electron transport system; probably TPN, the latter will take part in an electron accepting process from the highest occupied level of a reductant; perhaps a HO negative ion.

The processes described above are one cycle of the sensitization in the photosynthetic primary steps, based on a proposed model.

It must be noticed from the energetical point of view that the energy level of the $\pi - \pi$ triplet state of the intramolecularly perturbed chlorophyll molecule by Cu ion ($\sim 11\,500\ cm^{-1}$) has the same magnitude as the difference of the standard free energy changes per one electron between the O_2 electrode and the H_2 electrode at 25°C, pH = 7.0 ($\sim 10\,000\ cm^{-1}$), on the other hand, the fluorescence level and the $n - \pi$ triplet level of the chlorophyll-A molecule lie in $\sim 14\,600\ cm^{-1}$ and $\sim 13\,300\ cm^{-1}$, respectively[11].

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[10] This is the mechanism of the photoconductivity in the chlorophyll molecular crystals. A. TERENIN, E. PUTZEIKO, and I. AKIMOV, Disc. Faraday Soc. *1959*, No. 27, 83.
[11] If the transition probability per unit time of an electron transfer by the electron exchange interaction is W_e, then the diffusion coefficient of a hole migration is $\sim R^2 W_e$ and the mobility is given by $\sim R^2 W_e / kT$, where R is the nearest neighbour intermolecular distance, and the diffusion length of a hole during the phosphorescence lifetime τ_{ph} is $\sim R(W_e \cdot \tau_{ph})^{1/2}$ which may be obtained to $\sim 10^3$ Å.
[12] This work was supported by grants from the Japanese Society for the Promote of Science. The author wishes to express his gratitude to Professor M. KOTANI of Tokyo University and Professor K. OOMORI and Professor G. TOMITA for their encouragement.

Zusammenfassung. Auf molekularer Basis wird die Energieübertragung und die Energieumsetzung in einem primären photosynthetischen System untersucht. Die Energieübertragung in den Pigmenten und im Chlorophyll-A wird mit der Energieübergangswahrscheinlichkeit und dem Diffusionsmodell eines intramolekularen «Exitons» mit Dipol-Dipol-Wechselwirkung behandelt. Bei einer Chlorophyllkonzentration von 0.1 M/l wird die Diffusionskonstante des «Exitons» $D_e \cong 1.1 \times 10^{-3}$ cm²/sec und seine Diffusionslänge $l_e \simeq 250$ Å. Als Energieumsetzung wird die Anregung eines $\pi - \pi$-Tripletts des Chlorophyll-A mit Hilfe eines paramagnetischen Metallenzyms vorgeschlagen und diskutiert.

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Institute of Biophysics, Faculty of Agriculture, Kyushu University, Fukuoka (Japan), July 21, 1960.

Pyramidal Activation of Interneurones of Various Spinal Reflex Arcs in the Cat

It is known that spinal reflexes can be controlled from supraspinal centres through inhibition at an interneuronal level. This effect is exerted through brain stem centres which, in the decerebrate state, tonically inhibit interneurons mediating effects from the flexion reflex afferents (group II and III muscle afferents, high threshold joint and cutaneous afferents), and from Ib afferents, but not the interneurones mediating Ia inhibition[1].

In the cat, pyramidal activation is known to facilitate flexor and extensor motoneurones through polysynaptic pathways[2]. Im addition fascilitation of three-neuron-arc reflex dischanges at internuncial levels has been described[3]. It will presently be shown that interneurones of reflex arcs are excited on stimulation of the sensory-motor cortex and that this effect is mediated by the pyramidal tract.

In this investigation, the effect of cortical stimulation on the synaptic actions by impulses in muscle, cutaneous and joint afferents has been investigated by measuring the effect of conditioning volleys on monosynaptic test reflexes and by intracellular recording from motoneurones. Figure 1 shows monosynaptic reflex discharges from gastrocnemius-soleus recorded at two sweep speeds. Record A shows the unconditioned test reflex and B the effect of a conditioning submaximal group I volley from the antagonist deep peroneal (tibialis anterior + extensor digitorum longus) nerve. In the corresponding records C and D, a train of 4 cortical stimuli was added with the result that the inhibitory effect of the deep peroneal volley increased from 5% in B to 55% in D. The inhibitory action in B and D is an example of the reciprocal Ia inhibition. In corresponding experiments with intracellular recording, cortical stimulation gave a large increase of the Ia IPSP. It is concluded that the increment in inhibition is due to a facilitation of the interneurones known to be interpolated in the Ia inhibitory pathway.

In similar experiments, it has been established that the inhibitory action exerted in extensor motoneurones by Ib muscle afferents, by group II and III muscle afferents, by cutaneous and by high threshold joint afferents are all

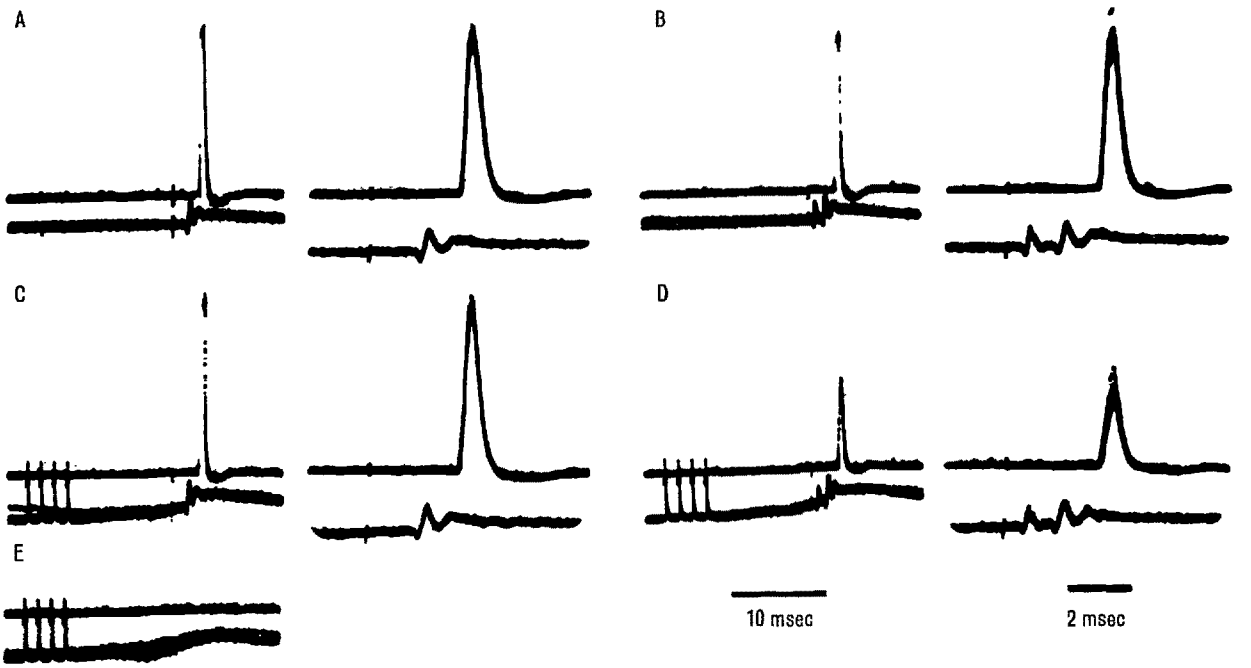

Fig. 1. The upper traces are monosynaptic reflex discharges evoked from the gastrocnemius-soleus nerve and recorded at two sweep speeds in the L7 ventral root. Lower traces were recorded from the L7 dorsal root entry zone. A shows the unconditioned test reflex and B the effect of a conditioning volley from the nerve to the antagonist muscles, extensor digitorum longus and tibialis anterior, evoked at a stimulus strength of 1.2 times threshold. In the corresponding lower records the contralateral postcruciate gyrus was stimulated in addition. E shows the effect of cortical stimulation alone.

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