The experiments were supported by the Biomagnetic Research Foundation.

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Distribution of Cyanole and Neutral Red in the Giant Axon of the Squid

Previous work on the permeability of frog muscles[1] and giant axons of Cephalopoda[2,3] to vital dyes has shown that basic and acid dyes rapidly penetrate the cell and its intracellular content may be less or greater than in the medium, depending on the outside concentration and type of the dye. For giant axons in the steady state the coefficient of distribution ($Q = \frac{C_{in}}{C_{out}}$, where C_{in} and C_{out} represent respectively intra- and extra-cellular concentration) for the acid dye (cyanole) was found to be 0·12 with the external dye concentration varying from 0·1 to 1·0 per cent (ref. 3). At the same time the coefficient of distribution of the basic dye (neutral red) changed from 5·5 to 0·65 on a corresponding increase in C_{out} from 0·004 to 0·16 per cent (ref. 2).

The purpose of the work recorded here was to investigate the Q values for axoplasm itself and compare them with the previously obtained values for the whole axons. Therefore the content of the dye was determined both in the whole axons and pipetted axoplasm.

Experiments were performed on the axons of *Loligo* sp., 200–300μ in diameter. Isolated axons were soaked in cyanole or neutral red solution for 20 min. As has already been shown[2], a period of 10–20 min is enough to reach equilibrium. Then the axons were washed up (5–10 sec) in sea-water and blotted. The amount of dye in ethanol acid extracts of axons was determined with the aid of a visual microcolorimeter. For measuring the dye content in axoplasm the stained fibres were cut; axoplasm was then extracted with a micropipette and also transferred to the ethanol acid mixture. The amount of dye was calculated per unit of the fibre or axoplasm volume.

The results obtained are given in Table 1.

Table 1

Dye	Concentration in the axon (C_{in}) (mg %)	Concentration in the axoplasm (C_{ap}) (mg %)	$\frac{C_{in}}{C_{ap}}$	$\frac{C_{ap}}{C_{out}}$
1% Cyanole	120 ± 5	82 ± 16	1·46	0·082
0·01% Neutral red	63 ± 5	35 ± 5	1·80	3·5

As can be seen, an increase as well as a decrease in the bulk dye concentration in axons compared with the medium depends on the concentration of dye in the axoplasm. In accordance with the previous studies[1–3], the observed distribution of dyes can be considered as a result of two factors: (1) decreased solubility of protoplasm for the substances with the size of molecules as big as that of dyes; (2) adsorption or chemical binding of dyes by protoplasmic components. Hence the total content of the dye in axoplasm may be expressed with the formula given by Troshin[1]: $C_{in} = K \times C_{out} + A$, where K is the solubility coefficient of the dye in protoplasm as compared with the medium; thus $K \times C_{out}$ gives the dissolved portion of the dye; A is adsorbed or chemically bound dye per unit volume.

The analysis of cyanole distribution shows[2] that, by varying C_{out}, A is practically equal to 0, whereas for neutral red the bound dye fraction is big enough[2]. This is also confirmed by special experiments made to investigate the efflux of dyes from axons. When cyanole-stained axon is put in sea-water, the dye leaves the fibre very soon, and the axon becomes completely colourless within 5 min. Similar experiments with neutral red show that the colour is retained for a much longer time. Thus, after 30 min soaking in sea-water, the neutral red concentration in axoplasm was still 14 ± 4 mg per cent, that is, 40 per cent of the dye still remained in the axon. The same slow efflux of cyanole was observed only in killed or damaged axons.

The experiments show that in the case of cyanole the dissolved fraction of the dye gave only $0{\cdot}12 \times C_{out}$ while for neutral red[2] $K = 0{\cdot}25$.

The values obtained for K, considerably less than unity, probably indicate that not the whole protoplasmic water is available for the substances with the size of molecules more than 3 Å. In any event, a steady-state distribution of dyes with the Q value less than unity cannot be explained by the Donnan effect[2,3]; analogous Q values were obtained for the distribution of non-electrolytes in muscles[1,4,5].

Thus we can conclude that the difference in the steady-state concentration of the dye in axon as compared with medium (decrease as well as increase) may be explained by the specific properties of axoplasm, and for this explanation no membrane pump hypothesis is needed.

We thank the Science Committee of the Peoples Republic of Viet Nam where the work was performed, and also Dr. Vũ Dinh Tân for assistance.

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Dispersal Rates of Several Ungulates introduced into New Zealand

Between 1851 and 1923 several species of ungulates were introduced into New Zealand. During this period there were also many liberations into unstocked areas of game animals taken from recently established populations. Dates and localities of liberations have been summarized[1,2] and the present or recent distributions of most species have been published[2–5].

Table 1 gives mean rates of dispersal over periods exceeding 50 years, taken from populations that were continuously expanding their ranges over those periods. The rate is measured as the distance between the point of liberation and the farthest known breeding colony, divided by the number of years that have elapsed.

Table 1. Rates of Dispersal

Species	Locality	Miles a year
Chamois (*Rupicapra rupicapra*)	South Island	5·4
Thar (*Hemitragus jemlaicus*)	South Island	1·1
Red deer (*Cervus elaphus*)	Nelson	1·0
	Fiordland	1·0
Sika deer (*Cervus nippon*)	North Island	1·0
Whitetail deer (*Odocoileus virginianus*)	Stewart Island	0·6
Fallow deer (*Dama dama*)	Wanganui	0·5
Rusa deer (*Rusa timorensis*)	North Island*	0·5
Sambar deer (*Rusa unicolor*)	Manawatu	0·4
Wapiti (*Cervus canadensis*)	Fiordland	0·4

There have been more than 100 liberations of red deer[6] and the populations developed from these have coalesced so that the pattern of dispersal is now completely obscured. But the distributions had been roughly mapped before this occurred[7], and a rate of dispersal can be calculated for the Nelson herd between 1851 and 1924. This is probably one of the few red deer herds the distribution of which was not strongly modified by secondary liberations near its periphery. The rate of spread in Fiordland