the corresponding liver (Table). These results suggest that the prompt degradation of polysomes in incubated liver slices is the outcome of a metabolic release from the messenger, rather than the consequence of enzymatic breakdown. The action of amino acids in the preservation of part of the polysomes can be satisfactorily explained in this context. The reason why liver slices, with a ribosomal population almost entirely reduced to monosomes, can still sustain a good and linear rate of protein synthesis for a fairly long time is not clear and will need further investigation.

Polyphenylalanine synthesis by monosomes isolated from a) liver or b) liver slices after 30 min incubation in vitro

	Time (min) 5	10	15
a) Liver	429,700	666,570	790,360
b) Liver slices	491,150	695,200	814,680

Results of a typical experiment: cpm/mg RNA.

Riassunto. Durante l'incubazione a 37°C i poliribosomi delle sezioni di fegato di ratto subiscono un processo di monomerizzazione. Il processo viene rallentato dalla presenza di forti concentrazioni di aminoacidi ed è inibito dal trattamento con cicloheximide. I monomeri che si formano durante l'incubazione in vitro rispondono al poly-U come i monosomi isolati direttamente dal fegato. Si ritiene quindi che la degradazione dei polisomi sia il risultato di un distacco metabolico dal messaggero con un rallentamento del riciclo dei monomeri piuttosto che la conseguenza di una azione enzimatica.

A. BERNELLI-ZAZZERA, F. CAJONE and LUISA SCHIAFFONATI[9]

Istituto di Patologia Generale dell'Università di Milano, Via Mangiagalli, 31, I-20133 Milano (Italy),
18 August 1971.

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The Active Form of Local Anesthetic Drugs

Most local anesthetics are secondary or tertiary amines and can therefore exist either as uncharged molecules or as ammonium ions, depending on the pK_a of the molecule and on the pH of the solution. The question whether the active form of the drug is the cationic or the undissociated one received considerable attention, but the experimental results are conflicting[1-14], because of the variety of methods employed and of the large number of parameters which are involved. Therefore the problem remains to be clarified; we therefore looked for a different experimental approach and carried out experiments on the action of tetracaine on the transfer of ions across a model of membrane, at different pH values.

Methods. The three-phases partition model system described by ROSANO and SCHULMAN[15,16] has been used because it behaves like a physiological membrane as to the different rate of transfer of various inorganic cations[17-19] and because the 2 interfacies are equivalent to monomolecular phospholipidic layers[15,16,20]. The model system allowed us to study the facilitated diffusion of ions from one water compartment to the other one, across a phospholipidic layer[21]. For this purpose we adopted a 3.5 mg/ml solution of brain cephaline (Koch and Light, Bucks.) in a diffusion blocking solvent, namely 1-pentanol+petrol ether 4:1. Salt solutions of KCl and $MgCl_2$ (both $\times 0{,}17\,M$) were allowed to exchange against NaCl and $CaCl_2$ ($0{,}17\,M$), which were placed in the opposite water compartment. The effect of local anesthetics has been studied by using 3 mM tetracaine in both water compartments. The pH value has been set at 5.0–7.3–9.3 with $0.01\,M$ piperazine buffer; in these conditions, tetracaine is dissociated >99%–90%–10%, respectively.

The ion concentration has been measured at different time intervals by atomic absorption (Atomspec, Hilger and Watts). The Figures are the average of 4 to 6 determinations.

Results. In the above-mentioned experimental conditions, a negligible passage of ions occurs by simple diffu-

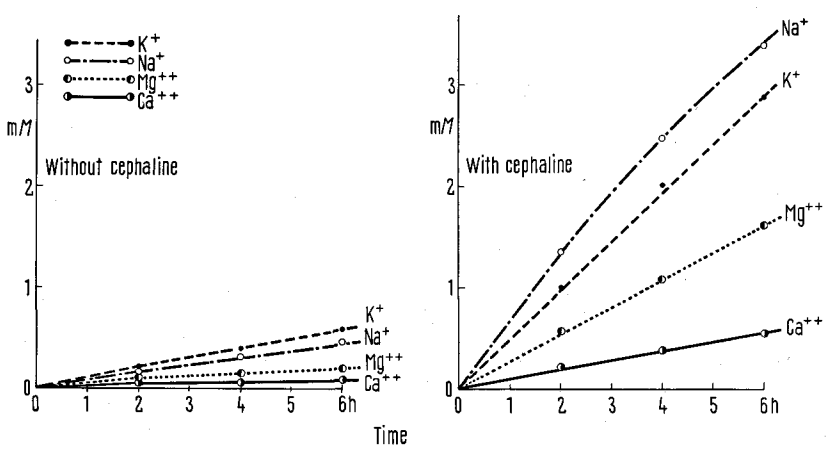

Fig. 1. Effect of cephaline (3,5 mg/ml) on the passage of Na^+, K^+, Ca^{++}, Mg^{++} accross pentanol-petrol ether 4:1. Water solutions: pH 5,0.

sion. In contrast to this (Figure 1) the addition of cephaline to the diffusion-blocking solvent system brings about a strong increase in the rate of transfer of the 4 tested cations; hence it can be assumed that brain cephaline is able to act as carrier for monovalent and divalent cations and that the experimental cell works as a facilitated diffusion model system.

As illustrated in Figure 1, transfer of Na, K, Mg, Ca takes place at a rate inversely proportional to their binding affinity for phospholipids[22]. Although the rate of transfer is not the same for the tested ions, it seems possible to assume that phospholipids can act as unspecific carriers for different cations. This view is supported also by the experiments of FRAZIER[13,14] showing that a common mechanism is responsible for the passage of K^+ and of Na^+, since both ions are blocked by anesthetics. By increasing the pH value, the dissociation of the acidic groups of the phospholipids and the rate of ion transport increase too (Figure 2), according to the view of several authors that phospholipids could act as ion exchangers with their phosphatidic groups.

Along the same lines, it is possible to consider also the mode of action of the local anesthetic drugs. The action of tetracaine has been tested at pH 5.0–7.3–9.3; as the pK_a value of the drug is 8.3, at this pH values tetracaine is dissociated >99%–90%–10%, respectively: in this way it is possible to study the relationship existing between dissociation of the molecule and inhibiting effect on the exchange of ions.

Tetracaine inhibits the transfer of the 4 tested ions approximately to the same extent; as shown in Figure 2, the inhibiting action attains the maximal level at an acidic pH value, i.e. when the drug is more dissociated; by lowering the dissociation of the molecule, as by pH 7.3, also the action of tetracaine becomes less pronounced (50% inhibition of ion transport, instead of 60% inhibition, as observed by pH 5.0). The effect is almost absent when the pH of the solution is alkaline. Since under the last condition no precipitation of tetracaine occurs, only a difference in the degree of dissociation can be responsible for the lack of activity; hence, it seems to be reasonable to assume that tetracaine is able to inhibit the transfer of ions across a phospholipidic layer only when the molecule is present in a cationic form. As it is known that the nerve transmission is dependent on the passage of ions across the membrane, it is possible to speculate that the action of tetracaine on the model-system is similar to that on the nerve transmission, namely that local anesthetic drugs act as cations and not as undissociated molecules.

Earlier experiments[1–4] showing that local anesthetics are more active when supplied in alkaline than in neutral or acidic solution could be readily explained on the assumption that the undissociated base is important for penetration of the drug into the nerves.

Finally our experiments confirm the view that phospholipids can carry cations by acting as ion exchangers, although the model used allows us to study only the transport of ions by facilitated diffusion. Tetracaine could inhibit this process by binding itself in the cationic form to the acidic groups of the phospholipids, with a higher binding affinity than inorganic monovalent and divalent cations.

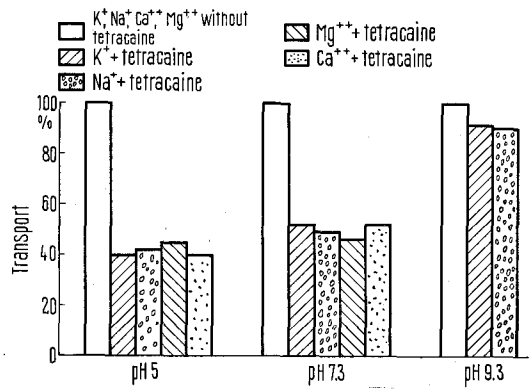

Fig. 2. Effect of Tetracaine on the transport of K^+, Na^+, Ca^{++} and Mg^{++} at different pH values. 100% values are (in mmol/h): pH 5 = Na 0.58, K 0.49, Mg 0.28, Ca 0.10; pH 7.3 = Na 0.84, K 0.66, Mg 0.35, Ca 0.14; pH 9.3 = Na 0.91, K 0.80.

Riassunto. Il problema della forma attiva degli anestetici locali é stato studiato determinando l'effetto della tetracaina sul trasporto di Na, K, Ca, Mg in un modello di membrana. Poiché l'azione dell'anestetico é massima a pH acido, é probabile che il farmaco agisca in forma cationica.

F. PICCININI, A. CHIARRA and F. VILLANI

Cattedra di Saggi e Dosaggi Farmacologici dell'Università, Via Taramelli 14, I-27100 Pavia (Italy), and Istituto di Farmacologia dell'Università, Via Vanvitelli 32, I-20129 Milano (Italy),
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