mM. La première et la dernière de ces valeurs correspondent remarquablement aux 2 efflux observés par HOPE, SIMPSON et WALKER[7] au moyen de ^{36}Cl chez *N. translucens* plongée dans une solution où $[Cl]_o = 1{,}1$ mM ($\Phi_{Cl} = 0{,}61$ pmoles cm^{-2} sec^{-1}) et dans un autre où les chlorures sont remplacés par du benzène sulphonate ($\Phi_{Cl} = 0{,}11$ pmoles cm^{-2} sec^{-1}). En outre, au retour, l'efflux des chlorures augmente à nouveau (cfr. Figure 2) alors que la cellule reste hyperpolarisée. Une diminution de l'influx de Na du type de celle observée par SMITH[8] lorsque la concentration en chlorures décroît dans le milieu extérieur est insuffisante pour rendre compte de l'hyperpolarisation observée. Nous estimons donc que le benzène sulphonate pénètre activement dans *Nitella* et que cette pompe est électrogénique. Comme l'hyperpolarisation persiste alors que la concentration externe en benzène sulphonate diminue nous devons admettre que l'efflux passif de cet ion est très faible.

L'influx actif de benzène sulphonate chez *Nitella* est à rapprocher de l'accumulation de colorants acides du type sulphonique chez de jeunes cellules de plantes supérieures (cfr. KINZEL[9]). D'autre part la Figure 3 établie d'après les valeurs expérimentales moyennes de $[Cl]_i$ (en supposant que les interactions de la paroi sont proportionnelles à $[Cl]_o$) démontre qu'il n'est pas impossible que l'influx actif de Cl^- chez *Nitella* se fasse au moyen d'un transporteur.

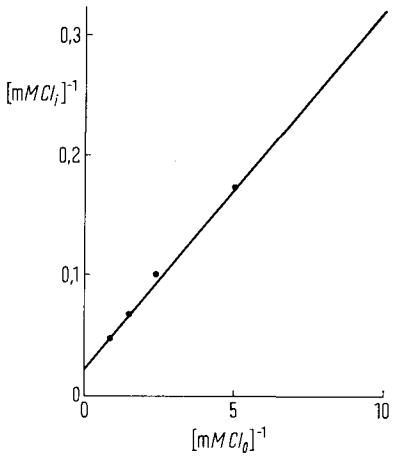

Fig. 3. Réciproque de l'accumulation des chlorures en fonction de la réciproque de la concentration externe en chlorures.

Summary. The chloride electrochemical potential difference between the inside of cells of *Nitella translucens* and some external solutions, where Cl has been progressively replaced by benzenesulphonate, has been measured by means of Ag-AgCl electrodes. It appears that benzenesulphonate moves into the cell by an electrogenic pump and that the uptake of Cl by *Nitella* is not in contradiction with a carrier hypothesis.

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[10] This work was supported by grants from the F.N.R.S. and was carried out in the Biophysics Section of the Edinburgh University. We are indebted to Dr. E. J. WILLIAMS and Dr. HOGG for having sponsored this research and kindly provided the material.

In vitro Action of Polyvinylsulfate on Acylation and Methylation of Nucleic Acids

Polyvinylsulphate (PVS) is an effective inhibitor of ribonuclease[1] and in many papers it has been used for that protective effect in enzymatic incorporations like aminoacylation of tRNA or cell free protein synthesis. Actually PVS action is not specific for ribonuclease but has other reactions: PVS combines with 70 S ribosomes to form a polysome-like structure in a reversible binding[2]; PVS gives a complete dissociation of proteins from rRNA[3]; while SHINOZAWA et al.[2] found that PVS reduces aminoacylation of tRNA, ILAN and ILAN[4] show an increased amount of aminoacylation. In the two cases concentrations of PVS are very different. This work gives the relation between different concentrations in PVS and acylation or methylation of nucleic acids in enzymatic assays: a kinetic study of methylation and the results of pre-incubated assays with PVS and enzymes or nucleic acids. Polyvinylsulfate potassium salt was obtained from General Biochemicals, Chagrin Falls (Ohio) (lot: 85284); adenosyl-L-methionine methyl ^{14}C from Schwarz, specific activity 52 mc/mM, yeast protein hydrolysate ^{14}C from Schwarz and DNA of *M. lysodeikticus* from Miles. *E. coli* tRNA for methylation assays was extracted from *E. coli* K12 W6 RC^{rel} met$^-$ and tRNA for acylation studies was extracted from *E. coli* B, both extractions were performed by the method of RAMMLER[5], using isoamyl alcohol and purifying with DEAE column chromatography and NaCl linear gradient. Crude extract enzymes from *E. coli* B are utilized after a 30–50% ammonium-sulphate precipitation.

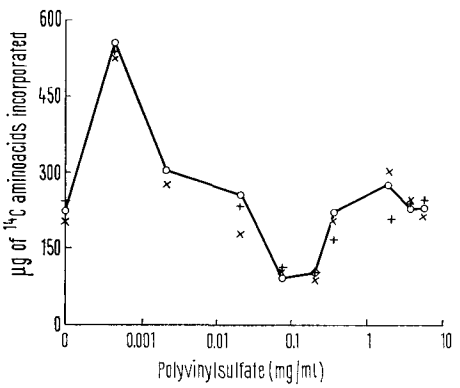

Fig. 1. Semi-log plot of action of PVS at different concentrations on the acylation of tRNA. ○—○, 30 min normal incubation; +—+, 15 min action on enzymes and normal completed incubation; ×—×, 15 min action on tRNA and normal completed incubation.

The methylation assays of tRNA were performed in a final reaction volume of 0.4 ml containing per ml: *Tris*-HCl pH 8, 100 μmoles; $MgCl_2$, 4 μmoles; CH_3 ^{14}C-S-adenosyl-L-methionine, 40 μmoles; mercaptoethanol, 4 μmoles; 2 O.D.$_{260}$ units of tRNA, enough crude methyltransferase protein to charge the tRNA and PVS at different concentrations.

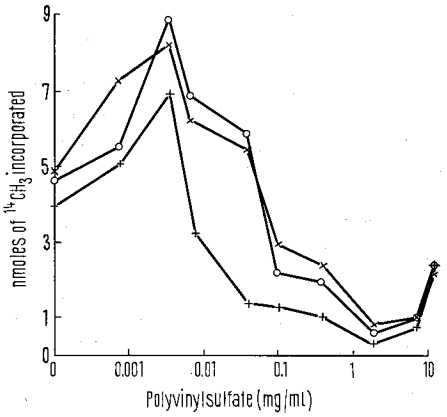

Fig. 2. Semi-log plot of action of PVS at different concentrations on the methylation of tRNA. Indication as for Figure 1.

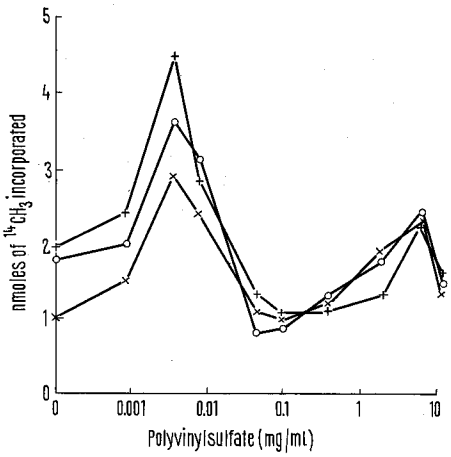

Fig. 3. Semi-log plot of action of PVS at different concentrations on the methylation of DNA. Indication as for Figure 1.

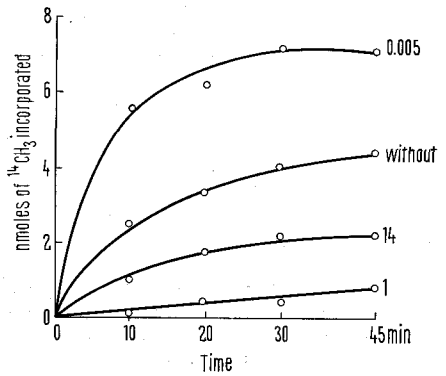

Fig. 4. Kinetics of methylation of tRNA.

The methylation assays of DNA are the same as for tRNA but with *Tris* buffer pH 7.5; 4 O.D.$_{260}$ units of DNA and 5 μg of ribonuclease. The aminoacylation assays of tRNA contained in a final volume of 0.5 ml: *Tris*-HCl pH 7.5, 100 μmoles; ATP, 4 μmoles; CTP, 4 μmoles; $MgCl_2$, 20 μmoles; KCl, 20 μmoles; mercaptoethanol, 1 μmole; ^{14}C amino acids 4 μC (10–50 mμmoles); 6 O.D.$_{260}$ units of mixed tRNA, enough crude synthetase protein to charge completely the tRNA and PVS at specific different concentrations. In Figure 1 influence of a large increased quantity of PVS action on aminoacylation of tRNA is shown. The same study is made in Figure 2 for methylation of tRNA and in Figure 3 for methylation of DNA. For each 3 assays are made: (1) normal 30 min enzymatic incubation with PVS; (2) 15 min PVS action at 37 °C on incubation mixture without nucleic acids and radioactivity donor, followed by a normal complete incubation of 30 min: (3) 15 min PVS action at 37 °C on incubation mixture without enzymes and radioactivity donor, followed by a normal complete incubation of 30 min.

The kinetics of tRNA methylation was made for 0.005 mg, 1.0 mg, 14 mg and no PVS. Profiles of action are very similar for normal and pre-incubated assays. The maximum of incorporated radioactivity is situated between 0.0005 and 0.01 mg/ml of PVS and increase of radioactivity is a real protective effect of PVS. The minimum of incorporation is more difficult to situate and this is probably an effect of the variation in nucleic acid content. But if the first peak results from protection of ribonuclease and deoxyribonuclease action, the second seems to be very difficult to explain, although this is a real one in comparison with a control. Figure 4 shows that reaction with PVS is readily obtained and differences in incorporations are very important.

It is interesting to note that the experiments of SHINOZAWA et al.[2] showing a decrease of acylation of 10% and those of ILAN and ILAN[4] showing an increase are easily comprehensible from our work. In conclusion, as for mercaptoethanol[6] which protects enzymes but acts on methylation, the PVS should be used with very great care in enzymatic incubation because it can mask or modify a real phenomenon. The complex problem of the mode of action of PVS in relation to the quantity used and their pattern of action remains.

Résumé. Le polyvinylsulfate modifie les réactions enzymatiques de méthylation des DNA et tRNA et d'aminoacylation des tRNA. En fonction de la concentration en PVS il y a soit inhibition des méthyltransférases et aminoacyl-synthétases soit inhibition des nucléases.

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