The effects of glycine on the levels of serine hydroxymethyltransferase in both the wild type and *Ser-1* mutant suggest that synthesis of this enzyme may be to some extent regulated by exogenous glycine. This effect would have physiological significance if the serine hydroxymethyltransferase reaction served as a route for serine synthesis in *Neurospora*. Evidence for involvement of this enzyme in serine synthesis is well documented for several organisms including *Saccharomyces*[23,24,15]. In the latter species glycine can serve as the precursor of the β-carbon of serine[25] and may act also as an inducer of serine hydroxymethyltransferase synthesis[15]. From the present studies it would appear that glycine and to a lesser extent serine, can regulate this key enzyme of C-1 metabolism in *N. crassa*. The origins of the C-1 unit required for synthesis of serine from glycine remain to be elucidated. If 5,10-CH_2-H_4PteGlu readily arises from glycine in the *Ser-1* mutant it is difficult to account for the lack of appreciable growth stimulation when glycine was supplied exogenously (Figure 3). On the other hand, if C-1 units for serine synthesis arise via 10-HCO-H_4PteGlu, formate together with glycine would serve as precursors of serine formed in the serine hydroxymethyltransferase reaction. These and related possibilities are currently under more detailed investigation.

Résumé. Dans un milieu minimal la glycine et la sérine, ajoutées en concentrations physiologiques, augmentent le taux de la sérine-hydroxyméthyltransférase durant la croissance logarithmique du *Neurospora crassa*. La glycine influence également la synthèse de cet enzyme chez le mutant *Ser-1* de *Neurospora*. Ainsi la glycine exogène exerce une régulation de la sérine-hydroxyméthyltransférase et cet enzyme joue un rôle possible dans la synthèse de la sérine dans cet organisme en phase logarithmique de croissance.

E. A. COSSINS[26,27], G. COMBEPINE and G. TURIAN

Laboratory of General Microbiology, University of Geneva, CH-1211 Geneva 4 (Switzerland), 9 July 1973.

[23] R. D. SAGERS and I. C. GUNSALUS, J. Bact. *81*, 541 (1961).
[24] E. A. COSSINS and S. K. SINHA, Biochem. J. *101*, 542 (1966).
[25] J. F. DEBORSO and A. O. M. STOPPANI, Biochim. biophys. Acta *148*, 48 (1967).
[26] Permanent address: Department of Botany, University of Alberta, Edmonton, Alberta, Canada. Professeur Invité, Université de Genève 1972-73.
[27] Acknowledgements. This investigation was supported by grant No. 3.540.71 from the Fond National Suisse de la Recherche Scientifique. E.A.C. was the recipient of a National Research Council of Canada travel fellowship. The authors thank Mlle MARTINE GOURJON for excellent technical assistance.

Effect of Wall-Degrading Enzymes on Protoplasts Transaminase Activity

Eliminating plant cell walls in order to obtain large amounts of protoplasts is usually achieved by using enzymes which destroy the skeletal envelope[1]. But such enzymes may alter some of the protoplast properties. It was, therefore, necessary to ascertain such a probable deterioration. Since protoplasts can be produced by slicing walls with no biochemical treatment[2], a comparative study of both kinds of protoplasts might possibly solve this question. In fact, it has been observed[3] that the transaminase activity of protoplasts enzymatically prepared from *Allium* roots was frequently lower than that of protoplasts mechanically obtained. However, such a comparison was not very helpful because the volume and the nature of the cells giving protoplasts and the size of the protoplasts themselves depend to a large extent on the two techniques adopted[4]. To decide whether wall-destroying enzymes really change the protoplasts metabolism during their enzymatical preparation, the effect of these enzymes has to be directly tested on the protoplasts obtained mechanically. The biochemical reactivity of the protoplasts will be analysed by measuring – as done previously[3] – their transaminase activity.

Roots (30 mm $\pm$ 3) of *Allium cepa* were used[5]. After removing the tip (3 mm) and first soaking (5 min) in 20% sucrose solution, they were cut into small sections which were placed immediately (85 min) in a solution of sucrose (20%) and NaCl (1.2%)[2]. On both faces, each thin root section gave protoplasts (approximately $4.5 \times 10^6 \pm 1 \times 10^6$ for 20 g of roots). The protoplasts suspension was immediately used or stored (dark, 25 °C $\pm$ 0.5) in a 20% sucrose solution with Penicillin G (Specia) at 8×10^{-4} *M*. and with or without enzymes mixture. Penicillin was found to be ineffective on cells of higher plants[6] but it stopped any bacterial contamination for at least 16 h. It has to be noted that the enzymes used were not purified and undoubtedly contained many different active species[7]. The incubation (12h), the nature (Macerozyme [pectinases]: UNWIN, Herts; Cellulase ONOZUKA P 1500: All Japan. Biochem.) and the concentration (5%) of the enzymes were chosen identically to those previously adopted for the enzymatical preparation of protoplasts[8]. Because of the optimal pH of the enzymes[9], all the solutions were buffered at pH 5.0 $\pm$ 0.3. Before extracting the enzymes, the protoplasts were washed carefully 4 times with a buffered 20% sucrose solution[5]. Isolating the transaminase[10] was adapted to the protoplasts[3], and the Aspartate-aminotransferase (EC 2.6.1.1) was analysed (incubation: 60 min; 37 °C $\pm$ 0.5) by determining spectrophotocolorimetrically (492 nm) the coloured hydrazones formed after using a pyruvate standard[11].

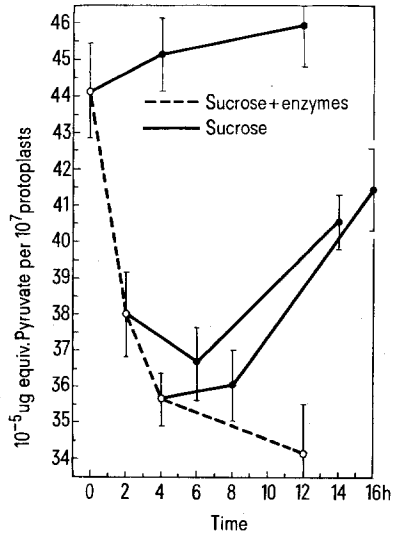
[1] E. C. COCKING, Ann. Rev. Plant. Physiol. *23*, 29 (1972).
[2] J. A. PLOWE, Protoplasma *12*, 196 (1931); R. PRAT and J. C. ROLAND, C. r. hebd. Séanc. Acad. Sci., Paris *271*, 1862 (1970).
[3] P. E. PILET, Experientia *28*, 638 (1972).
[4] P. E. PILET, in *Protoplastes et fusion de cellules somatiques végétales* (Coll. Intern. CNRS, 1973), vol. 212, p. 99.
[5] P. E. PILET, R. PRAT and J. C. ROLAND, Pl. Cell Physiol. *13*, 297 (1972).
[6] B. PARTHIER, Nature *206*, 783 (1965); M. A. VENIS, Phytochemistry *6*, 799 (1967).
[7] K. SELBY, in *Protoplastes et fusion de cellules somatiques végétales* (Coll. Intern. CNRS, 1973), vol. 212, p. 33.
[8] J. B. POWER and E. C. COCKING, J. exp. Bot. *21*, 24 (1970); T. NAGATA and I. TAKEBE, Planta *92*, 301 (1970).
[9] R. U. SCHENK and A. C. HILDEBRANDT, Phyton, Argentina *26*, 155 (1969).
[10] S. RELTMAN and S. FRANKEL, Am. J. clin. Path. *28*, 56 (1957).
[11] P. E. PILET and M. ATHANASIADES, C. r. hebd. Séanc., Acad. Sci., Paris *262*, 1090 (1966).

The comparison (Table) between the protoplasts, mechanically and enzymatically prepared, indicated that, for the first type, the volume was lower and the transaminase activity higher than for the second type; all data were found to be significant. Results (Figure) call for a few comments. 1. Keeping the mechanically obtained protoplasts for 12 h in the sucrose solution did not change their transaminase activity significantly. 2. When incubating these protoplasts in the cell-wall-degrading enzymes mixture, a significant decrease of the transaminase activity was observed. Such effect seemed to be very rapid since the depressed transaminase activity remained the same after 4 and 12 h of enzyme incubation. 3. When protoplasts were replaced in the sucrose solution, after 2 or 4 h in the enzymes mixture, their transaminase activity was partly restored. 4. The difference in the transaminase activity between the control and the treated protoplasts was found to be much higher than that observed between the protoplasts mechanically and enzymatically prepared (see Table). It may be noticed that, in the first case, the direct action of the enzymes on the protoplasm transaminases was obviously very much stronger than in the assays with the cells still enveloped by their walls.

Comparative volume and transaminase activity of protoplasts mechanically (M) and enzymatically (E) prepared from *Allium* roots (30 mm) previously decapitated at 3 mm

	Protoplasts	
	M	E
Volume[a] in μ^3 per protoplast	2370 ± 304	9109 ± 832
Transaminase activity[b] per 10^7 protoplasts	44,27 ± 1,34	40,31 ± 1,52

[a] The diameter of respectively 212 and 201 protoplasts was measured.
[b] In µg equiv. ($\times 10^{-5}$) of pyruvate formed.



Changes with time in transaminase activity (in pyruvate formed) of the root (*Allium*) protoplasts mechanically prepared. Continuous lines: assays with protoplasts kept in 20% sucrose solution. Broken lines: protoplasts incubated in the enzyme mixture. Vertical lines extending on one side of the points show the standard error of the mean.

In conclusion, the cell wall-degrading enzymes (or the impurities present in the active mixture) – used for preparing enzymatically the protoplasts – change some of the properties of the protoplasts obtained mechanically. Consequently, it can be supposed that the enzymes preparation may act on the protoplasts produced enzymatically. It is clear that such enzymes mixture depress the transaminase activity with an optimum action after 4 h incubation. From these results, it is not possible to determine at what level of the protoplasts, transaminase is lost, and to decide whether the enzymes act on the formation, the degradation or the activation of transaminase. But it is not excluded that some transaminase co-factors may be destroyed during the enzymatical preparation of protoplasts. Such stimulating agents may be progressively reformed when the protoplasts were replaced in the stored medium.

Résumé. Les enzymes employées pour l'obtention enzymatique des protoplastes de racines (*Allium*) altèrent les transaminases (EC. 2.6.1.1.) de protoplastes mécaniquement préparés. Cette inhibition transaminasique est partiellement levée lorsque les protoplastes ne sont plus en contact avec ces enzymes. Les effets des enzymes dégradant les parois cellulaires sont discutés relativement aux propriétés biochimiques des protoplastes.

P.-E. Pilet

Institut de Biologie et de Physiologie végétales de l'Université de Lausanne, CH–1005 Lausanne (Switzerland), 15 June 1973.

Can the C_4-Dicarboxylate Transporter be Specifically Labelled?

Although the use of affinity labelling substrate analogues has not proved especially useful for studies on membrane systems[1], the non-specific alkylating reagent, N-ethylmaleimide (NEM), has been used, in conjunction with substrate protection, to specifically label the lactose permease of *E. coli*[2–4] and the adenosine triphosphatase (ATPase) of sarcoplasmic reticulum[5].

A transport system for the uptake of C^4-dicarboxylic acids in *Escherichia coli* has been described and characterized[6]. We have recently tried to label some of the membrane component(s) comprising this uptake system.

Materials and methods. E. coli, wild type strain AT 2752 were obtained from Professor H. Kornberg and grown and induced for C_4-uptake as previously de-

[1] S. I. Chavin, FEBS Lett. *14*, 269 (1971).
[2] C. F. Fox and E. P. Kennedy, Proc. natn. Acad. Sci. *54*, 891 (1965).
[3] J. R. Carter, C. F. Fox and E. P. Kennedy, Proc. natn. Acad. Sci. *60*, 725 (1968).
[4] T. H. D. Jones and E. P. Kennedy, J. biol. Chem. *244*, 5981 (1969).
[5] R. Panet and Z. Selinger, Eur. J. Biochem. *14*, 440 (1970).
[6] W. W. Kay and H. L. Kornberg, Eur. J. Biochem. *18*, 274 (1971).