Planta 143, 285–289 (1978)

Planta

Localization of Adenosine 5′-phosphosulfate Sulfotransferase in Spinach Leaves*

Heinz Fankhauser[1] and Christian Brunold[2]

Pflanzenphysiologisches Institut der Universität Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

Abstract. Roots of spinach (*Spinacia oleracea* L.) seedlings contained only a very low activity of adenosine 5′-phosphosulfate sulfotransferase compared to the cotyledons. Adenosine 5′-phosphosulfate sulfotransferase activity increased about tenfold in cotyledons during greening. Preparation of organelle fractions from spinach leaves by a combination of differential and isopycnic density gradient centrifugation showed that adenosine 5′-phosphosulfate sulfotransferase banded with NADP-glyceraldehyde-3-phosphate dehydrogenase, a marker enzyme for intact chloroplasts. In the fractions of peroxisomes, mitochondria and broken chloroplasts virtually no adenosine 5′-phosphosulfate sulfotransferase activity was measured. Comparison with the chloroplast enzyme NADP-glyceraldehyde-3-phosphate dehydrogenase indicates that in spinach, adenosine 5′-phosphosulfate sulfotransferase is localized almost exclusively in the chloroplasts.

Key words: Adenosine 5′-phosphosulfate sulfotransferase – Chloroplasts – *Spinacia* sulfate reduction.

Introduction

Adenosine 5′-phosphosulfate is the sulfonyl donor in assimilatory sulfate reduction of higher plants (Schmidt, 1975), algae (Tsang and Schiff, 1975) and the phototrophic bacterium *Rhodospirillum rubrum* (Schmidt, 1977). Adenosine 5′-phosphosulfotransferase catalyses the transfer of the sulfonyl group of APS to a carrier-protein (Car-SH) to form Car-S-SO_3^-. The enzyme has been partially purified from *Chlorella* (Goldschmidt et al., 1975) and spinach (Schmidt, 1976a). Isolated chloroplasts are capable of reducing sulfate, suggesting that they contain the entire sequence of enzymes needed for sulfate reduction (Trebst and Schmidt, 1969; Burnell and Anderson, 1973; Schwenn et al., 1976). Schmidt (1976a) found high APSSTase activity in chloroplasts isolated by differential centrifugation. Experiments on the light-induced chloroplast development in *Euglena* indicated however, that in this organism APSSTase is neither predominantly nor exclusively localized in the chloroplasts (Brunold and Schiff, 1976). Furthermore, studies on the localization of O-acetyl-L-serine sulfhydrylase, another enzyme of assimilatory sulfate reduction, showed that only about 20% of the total enzyme activity was associated with chloroplasts from spinach leaves (Fankhauser et al., 1976).

These findings taken together prompted us to study the cellular distribution of APSSTase in spinach.

In this paper we present evidence suggesting that APSSTase is localized almost exclusively in the chloroplasts of spinach.

Materials and Methods

Spinacia oleracea L., cv. Nobel, was cultivated either under field conditions or aseptically in agar cultures. For the agar cultures, spinach seeds were sterilized for 1 h with 13–14% sodium hypochlorite. After washing 10 times with sterile H_2O, 4 seeds were transferred aseptically into sterile cultivation tubes (3 × 20 cm, with metal caps) each containing 50 ml nutrient solution (1/4 concentrated E-NO_3 nutrient solution, Erismann and Finger, 1968, containing 0.83 mmol l^{-1} NH_4Cl and 0.1% w/v sucrose) solidified with agar (1% w/v). The cultures were illuminated 10 h per day with 5000 lx at 25° to 27° C. During the dark period the temperature was 20° C.

* The results presented in this paper are taken from the Ph. D. thesis of H.F.

[1] *Present address:* Institute for Photobiology of Cells and Organelles, Brandeis University, Waltham, MA 02154, USA

[2] Author to whom all correspondence should be addressed

Abbreviations: APS = Adenosine 5′-phosphosulfate; APSSTase = Adenosine 5′-phosphosulfate sulfotransferase; BSA = Bovine serum albumin; BRIJ58 = Polyethylene glycolmonostearylether; DTE = Dithioerythritol; DTT = Dithiothreitol; EDTA = Ethylenediaminetetraacetic acid; ME = 2-Mercaptoethanol; NADP-GPD = NADP-linked glyceraldehyde-3-phosphate dehydrogenase; PAPS = Adenosine 3′-phosphate 5′-phosphosulfate; POPOP = 1,4 Di [2-(5-phenyloxazolyl)] – benzene; PPO = 2,5 – Diphenyloxazol

0032-0935/78/0143/0285/$01.00

Extracts were prepared in a glass homogenizer at 0–4° C in 0.1 mol l^{-1} tris-HCl, pH 8.0 containing 0.1 mol l^{-1} KCl, 0.02 mol l^{-1} $MgCl_2$ and 5 mmol l^{-1} DTE. 2 ml of buffer were used per g fresh weight of plant material. After centrifugation at 4500 *g* for 10 min, the supernatant fluids were used immediately for the enzyme assays.

For the preparation of cell organelles, 50 g of fresh spinach leaves cultivated under field conditions were stored overnight at 0–4° C prior to isolation. Isolation and purification of cellular organelles were performed according to Rocha and Ting (1970). APSSTase was measured by the production of sulfite^{35}S assayed as acid-volatile radioactivity from AP^{35}S, using DTT as acceptor (Hodson and Schiff, 1971). The reaction mixture contained (in a total volume of 400 µl):

AP^{35}S (67 ct/min/nmol for gradient fractions; 900–1000 ct/min/nmol in the other assays), 78 nmol; tris-HCl (pH 9.25), 50 µmol; DTT or DTE, 4 µmol; Na_2SO_4, 400 µmol; enzyme extract 50 µl. The incubation time was 30 min under N_2 at 37° C.

Radioactivity was measured with a Picker Nuclear Liquimat 220 Liquid Scintillation Spectrometer. The counting fluid was toluene-triton X-100 (2:1, v/v) with 4 g/l PPO and 120 mg/l POPOP (Shimshi, 1969, modified).

Catalase activity was assayed using the method of Lueck (1962) and cytochrome c oxidase activity by the method of Rocha and Ting, 1970. NADP-GPD was measured according to Frosch et al. (1973).

Chlorophyll was determined by its absorption at 652 nm (Arnon, 1949), using the proceeding of Tolbert et al. (1968).

Protein was determined turbidimetrically (Fankhauser, 1978) using BSA as a standard. AP^{35}S was prepared according to Tsang et al., 1976. $^{35}SO_4^{2-}$ was purchased from EIR, Würenlingen, Switzerland.

Results

The results presented in Table 1 show the APSSTase activity in extracts from different organs of spinach. Whereas in dry seeds no activity was detected, extracts from inbibed seeds had low levels of extractable APSSTase. In roots of 10 day old seedlings, APSSTase activity was about 10 times lower than in cotyledons,

Table 1. Activities of adenosine 5′-phosphosulfate sulfotransferase (APSSTase) in extracts from seeds, roots, cotyledons and leaves of spinach. Mean values ± standard errors from 6 individual measurements are presented

	APSSTase activity (nmol sulfite · mg fresh weight^{-1} · h^{-1})
Dry seeds	not detected
Imbibed seeds[a]	0.013 ± 0.003
Roots (10 mm long) of 5 day old seedlings[a]	0.046 ± 0.008
Root (60 mm long) of 10 day old seedlings[a]	0.086 ± 0.013
Cotyledons of 10 day old seedlings[a]	0.978 ± 0.146
8–10 week old leaves of spinach[b]	1.309 ± 0.177

[a] Plant material from sterile agar culture
[b] Plant material from spinach cultured under field conditions

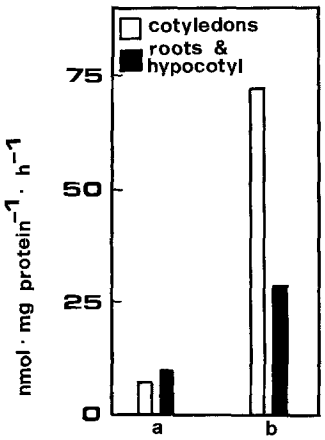

Fig. 1. Activity of adenosine 5′-phosphosulfate sulfotransferase in extracts of cotyledons and of roots and hypocotyls from seedling of *Spinacea oleracea* L. cultivated under aseptic conditions. a = 5 day old seedlings with white cotyledons. b = 11 day old seedlings with green cotyledons

Table 2. Distribution of adenosine 5′-phosphosulfate sulfotransferase (APSSTase) and NADP-linked glyceraldehyde-3-phosphate dehydrogenase (NADP-GPD) in cellular fractions from spinach leaf homogenate and recovery of both enzyme activities from fractions of sucrose density gradients

Fraction	APSSTase		NADP-GPD	
	Total activity (µmol h^{-1})	Percentage distribution	Total activity (µmol min^{-1})	Percentage distribution
250 *g* supernatant of homogenate	58.15	100	267.69	100
1000 *g* pellet	7.89	13.6	28.31	10.6
1000 *g* pellet fractionated[a]	6.90	11.9	29.06	10.9
3000 *g* pellet	2.69	4.6	4.89	1.8
3000 *g* pellet fractionated[a]	2.67	4.6	5.33	2.0
3000 *g* supernatant	44.37	76.3	201.25	75.2

[a] Total enzyme activity in gradient fractions obtained after centrifugation on sucrose density gradients in either the 1000 *g* or 3000 *g* pellet of a homogenate

but there was no significant difference, on a fresh weight basis, between the level of APSSTase activity in extracts from cotyledons and that obtained from leaves of fully developed plants.

Figure 1 shows that during chloroplast development in the cotyledons specific APSSTase activity increased 9.8 fold. In the same period of time, APSSTase activity increased by a factor of 2.9 in roots and hypocotyls. There is no significant difference be-

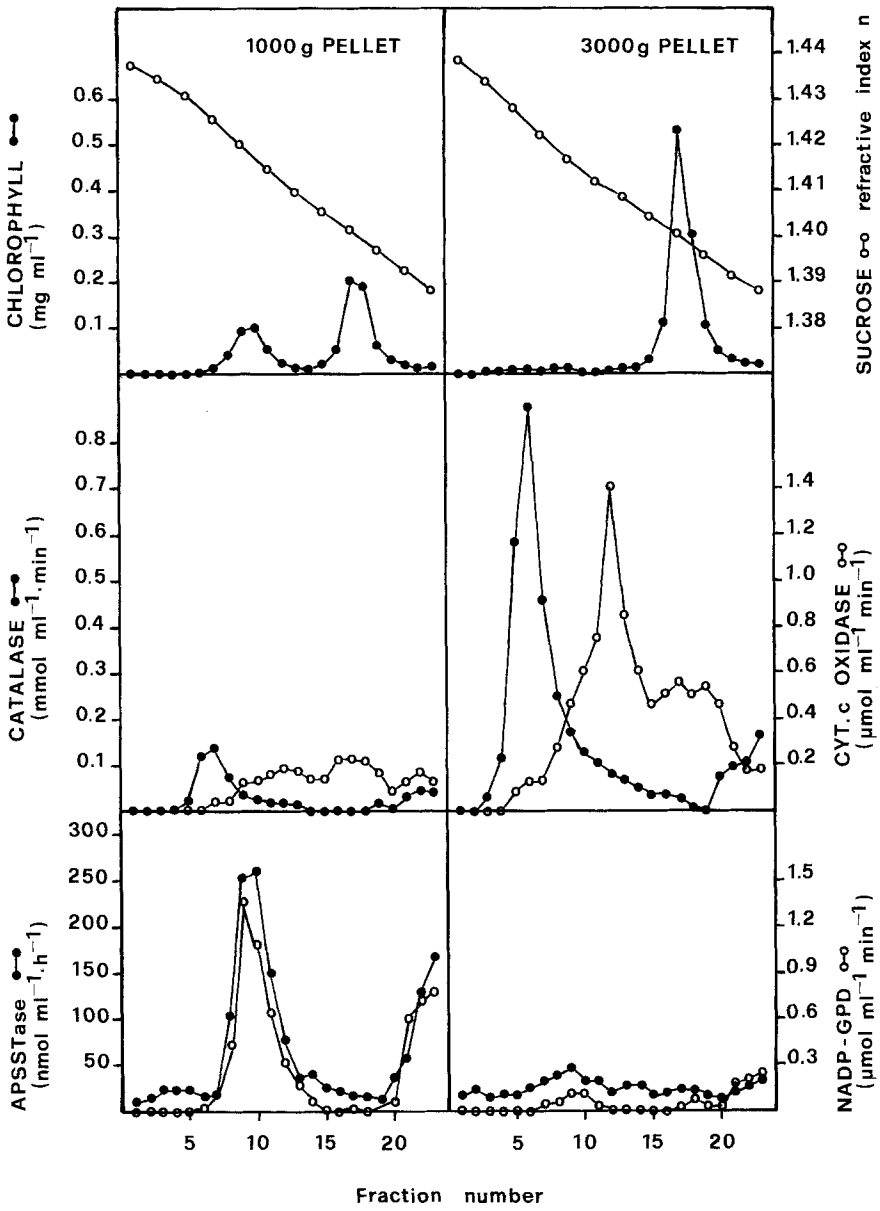

Fig. 2. Distribution of adenosine 5′-phosphosulfate sulfotransferase (APSSTase), chlorophyll and the marker enzymes NADP-linked glyceraldehyde-3-phosphate dehydrogenase (NADP-GPD), catalase and cytochrome c oxidase on sucrose density gradients of the 1000 *g* and the 3000 *g* pellets obtained from homogenate of spinach leaves

tween specific APSSTase levels in white cotyledons and in roots plus hypocotyls.

The distribution of activities of APSSTase and the chloroplast enzyme NADP-GPD in fractions obtained by differential centrifugation is given in Table 2. The 1000 *g* pellet contains intact and broken chloroplasts, the 3000 *g* pellet contains broken chloroplasts, mitochondria and peroxisomes. The distribution of NADP-GPD and APSSTase in the different fractions is very similar, indicating a similar subcellular distribution. It is clear from Table 2 that essentially, the total APSSTase activity of the 1000 *g* and the 3000 *g* pellet could be recovered in the fractions of sucrose density gradients.

A typical distribution of APSSTase, marker enzymes and chlorophyll on the sucrose density gradients is represented in Figure 2. On the gradient of the 1000 *g* pellet two bands of chlorophyll, attributable to intact and broken chloroplasts, were detected. The intact chloroplasts, localized on the gradients with NADP-GPD, contained APSSTase activity, whereas in mitochondria and peroxisomes, located by cytochrome c oxidase and catalase, respectively, no appreciable APSSTase activity was detected. Furthermore Figure 2 shows that APSSTase did not band with broken chloroplasts in either the 1000 *g* or 3000 *g* pellet. A minor peak of APSSTase activity was found in the sucrose density gradient of the 3000 *g* pellet.

Table 3. Chlorophyll levels and activities of adenosine 5′-phosphosulfate sulfotransferase (APSSTase) and NADP-linked glyceraldehyde-3-phosphate dehydrogenase (NADP-GPD) in the 250 *g* supernatant of homogenate and in the extract of intact chloroplasts from spinach leaves. Intact chloroplasts were taken from fraction 9 of the sucrose density gradient obtained with the 1000 *g* pellet (Fig. 2)

	250 *g* supernatant of homogenate[a]	Intact chloroplasts[a]
Chlorophyll (mg ml^{-1})	0.147	0.093
APSSTase (nmol ml^{-1} h^{-1})	534.328	254.900
NADP-GPD (μmol ml^{-1} min^{-1})	2.677	1.355
APSSTase/Chlorophyll (nmol mg $chlorophyll^{-1}$ h^{-1})	3634.9 (100%)	2740.9 (75.4%)
NADP-GPD/Chlorophyll (μmol mg $chlorophyll^{-1}$ min^{-1})	18.211 (100%)	14.570 (80.0%)

[a] APSSTase assay started 6 h 30 min after homogenization

This peak coincided with NADP-GPD activity, indicating the presence of a small amount of intact chloroplasts in the 3000 *g* pellet.

The percentage of APSSTase and NADP-GPD associated with intact chloroplasts relative to the total activity present in the 250 *g* supernatant of a homogenate of spinach leaves is calculated on a chlorophyll basis as shown in Table 3. Whereas about 75% of the total APSSTase activity can be attributed to intact chloroplasts, the value for NADP-GPD is 80%.

These calculations are based on the assumption that both APSSTase- and NADP-GPD-activity are constant in homogenates. Indeed, no change in NADP-GPD activity was detected in homogenates and in resuspended chloroplasts (data not shown).

There is a loss, however, in APSSTase activity. In the experiment shown in Table 3, about 30% of the activity initially present in both the 250 *g* supernatant and the resuspended chloroplasts is lost during the 6 h 30 min following homogenisation. These losses in both fractions were similar in three repetitions of the experiment. Therefore, no correction for the losses was made.

Discussion

In our experiments with spinach, APSSTase showed typical behaviour associated with a chloroplastic enzyme, e.g., the enzyme activity increased about 10 fold during chloroplast development in the cotyledons and the enzyme activity was very low in hypocotyls and roots. As the spinach seedlings were cultivated aseptically, possible effects of substances excreted by contaminating microorganisms on APSSTase activity can be excluded in our experiments. Similar low levels of extractable APSSTase activities in roots were reported from sunflower seedlings (Schmidt, 1976b).

The results of the chloroplast development experiments with spinach are consistent with the distribution of APSSTase activity on sucrose density gradients: APSSTase banded with intact chloroplasts. The fact that no appreciable APSSTase activity was measured in the fractions of broken chloroplasts indicates that we were dealing with a soluble stromal enzyme.

A further indication of the localization of APSSTase in the chloroplast was the very similar distribution of APSSTase and NADP-GPD among subcellular fractions. NADP-GPD is an enzyme which is localized exclusively in the chloroplasts (Latzko and Gibbs, 1968; McGowan and Gibbs, 1974). The fact that we only found 80% of NADP-GPD in the chloroplasts may be the result of 1) rupture and self-sealing following the loss of stromal protein, as proposed by Lilley and Walker, 1975; and 2) the synthesis of the enzyme in the cytoplasm and its subsequent transport into the chloroplasts (Ireland and Bradbeer, 1971). The same interpretations can be applied to APSSTase which may be synthesized in the cytoplasm on 80S ribosomes in *Euglena* (Brunold and Schiff, 1976) and in *Lemna minor* (Brunold and Schmidt, 1976).

Our results indicate then that in spinach APSSTase is localized almost exclusively in the chloroplasts. This is consistent with the report of an elevated specific APSSTase activity in chloroplast extracts relative to crude extracts (Schmidt, 1976a). Our results differ however, from those obtained with *Euglena* (Brunold and Schiff, 1976).

We thank Dr. J.A. Schiff for a gift of Chlorella, Dr. A. Schmidt for a gift of $PAP^{35}S$ and $AP^{35}S$, Dr. R. Thomas for improving the style of our manuscript and A. Berchtold for performing preliminary experiments.

The work was supported by the Swiss National Science Foundation grant Nr. 3.610.75

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. **24**, 1–15 (1949)

Brunold, C., Schiff, J.A.: Studies of sulfate utilization by algae. 15. Enzymes of assimilatory sulfate reduction in *Euglena* and their cellular localization. Plant Physiol. **57**, 430–436 (1976)

Brunold, C., Schmidt, A.: Regulation of Adenosine-5′-Phosphosulfate Sulfotransferase Activity by H_2S in *Lemna minor* L. Planta **133**, 85–88 (1976)

Burnell, J.N., Anderson, S.W.: Adenosine 5′-Sulphatophosphate kinase activity in spinach leaf tissue. Biochem. J. **134**, 565–579 (1973)

Erismann, K.H., Finger, A.: Lemnaceen in kontinuierlicher Kultur. Ber. Schweiz. Bot. Ges. **78**, 5–15 (1968)

Fankhauser, H., Brunold, C., Erismann, K.H.: Subcellular localization of O-acetylserine sulfhydrylase in spinach leaves. Experienta **32**, 1494–1496 (1976)

Fankhauser, H.: Untersuchungen zur Lokalisierung der assimilatorischen Sulfatreduktion bei Spinat (*Spinacia oleracea* L.) Dissertation, Universität Bern Switzerland (1978)

Frosch, S., Wagner, E., Cunning, B.G.: Endogenous rhythmicity and energy transduction. I. Rhythmicity in adenylate kinase, NAD- and NADP-linked glyceraldehyde-3-phosphate dehydrogenase in *Chenopodium rubrum*. Can. J. Bot. **51**, 1355–1367 (1973)

Goldschmidt, E.E., Tsang, M.L.-S., Schiff, J.A.: Studies of sulfate utilitation by algae. 13. Adenosine 5′-phosphosulfate (APS) as an intermediate in the conversion of adenosine 3′-phosphate 5′-phosphosulfate (PAPS) to acid-volatile radioactivity. Plant Sci. Lett. **4**, 293–299 (1975)

Hodson, R.C., Schiff, J.A.: Studies of sulfate utilitation by algae. 9. Fractionation of a cell-free system from *Chlorella* into two activities necessary for the reduction of adenosine 3′-phosphate 5′-phosphosulfate to acid-volatile radioactivity. Plant Physiol. **47**, 300–305 (1971)

Ireland, H.M.M., Bradbeer, J.W.: Plastid development in primary leaves of *Phaseolus vulgaris*. Planta **96**, 254–261 (1971)

Latzko, E., Gibbs, M.: Distribution and activity of enzymes of the reductive pentose phosphate cycle in spinach leaves and in chloroplasts isolated by different methods. Z. Pflanzenphysiol. **59**, 184–194 (1968)

Lilley, R. McC., Walker, D.A.: Carbon dioxide assimilation by leaves, isolated chloroplasts, and ribulose bisphosphate carboxylase from spinach. Plant Physiol. **55**, 1087–1092 (1975)

Lueck, H.: Katalase. In: Bergmeyer, H.-H.: Methoden der enzymatischen Analysen, 1. Aufl. p. 885–894. Weinheim/Bergstrasse: Chemie 1962

McGowan, R.E., Gibbs, M.: Comparative enzymology of the Glyceraldehyde 3-phosphate Dehydrogenase from *Pisum sativum*. Plant Physiol. **54**, 312–319 (1974)

Rocha, V., Ting, I.P.: Preparation of cellular plant organelles from spinach leaves. Arch. Biochem. Biophys. **140**, 398–407 (1970)

Schmidt, A.: Distribution of APS-sulfotransferase activity among higher plants. Plant Sci. Lett. **5**, 407–415 (1975)

Schmidt, A.: The Adenosine-5′-phophosulfate sulfotransferase from spinach (*Spinacea oleracea* L.). Stabilization, partial purification and properties. Planta **130**, 257–263 (1976a)

Schmidt, A.: Development of the adenosine-5′-phosphosulfate sulfotransferase in sunflower (*Helianthus annuus* L.) Z. Pflanzenphysiol. **78**, 164–168 (1976b)

Schmidt, A.: Adenosine-5′-phosphosulfate (APS) as sulfate donor for assimilatory sulfate reduction in *Rhodospirillum rubrum*. Arch. Microbiol. **112**, 263–270 (1977)

Schwenn, J.D., Depka, B., Hennies, H.-H.: Assimilatory sulfate reduction in chloroplasts: evidence for the participation of both stromal and membrane-bound enzymes. Plant & Cell Physiol. **17**, 165–176 (1976)

Shimshi, D.: A rapid field method for measuring photosynthesis with labelled carbon dioxide. J. exp. Bot. **20**, 381 (1969)

Tolbert, N.E., Oeser, A., Kisaki, T., Hageman, R.H., Yamazaki, R.K.: Peroxisomes from spinach leaves containing enzymes related to glycolate metabolism. J. Biol. Chem. **243**, 5179–5184 (1968)

Trebst, A., Schmidt, A.: Photosynthetic sulfate and sulfite reduction by chloroplasts. In: Metzner, H.: Progress in Photosynthesis Res., Vol. 3; W. Junk. The Hague: 1969

Tsang, M.L.-S., Schiff, J.A.: Studies of sulfate utilization by algae. 14. Distribution of Adenosine-3′-phosphate-5′-phosphosulfate (PAPS) and Adenosine-5′-phosphosulfate (APS) Sulfotransferases in assimilatory sulfate reducers. Plant Sci. Lett. **4**, 301–307 (1975)

Tsang, M.L.-S., Lemieux, J., Schiff, J.A. and Bojarski, T.B.: Preparation of Adenosine 5′-Phosphosulfate (APS) from Adenosine 3′-Phosphate 5′-Phosphosulfate (PAPS) Prepared by an Improved Procedure. Anal. Biochem. **74**, 623–626 (1976)

Received 1 June; accepted 15 June 1978