monolayer cultures of TuWi cell line, and in sections and imprints of the solid tumor derived from this line. All cultured tumor cells showed a high degree of enzyme activity, with extensive deposits of reaction product occurring in cells with mitosis and in areas of dense cell growth in late cultures (Figure 2a). The solid tumor provided a massive amount of uniform tissue rich in GGTP (Figure 2b) and it was therefore easy to extract from the tumor homogenate sufficient amounts, approximately 65% of total enzyme activity, into a supernatant fraction (Table).

The proposed role of this widely distributed, membrane-associated enzyme as a translocase in amino acid transport[16] and its accumulation in various tissue, notably the kidneys, which are actively involved in this process, undescore its possible significance in nephroblastoma. Fetal and adult kidneys show high concentrations of GGTP in the brush borders of proximal convoluted tubules[14]. The presence of this enzyme in several renal tumors, as reported by ALBERT et al.[17], and shortly thereafter also described in Wilm's tumor (nephroblastoma)[18], has been advanced as evidence for their origin in cells of the proximal tubules.

As GGTP has been shown to express tissue-specific isoenzyme forms[19], and to be present in the serum elevated levels during certain stages of some malignant disorders[20–22], we also made attempts in these two directions. Repeated examinations using cellogel strip electrophoresis technique showed that enzyme extracted from the nephroblastoma had a mobility comparable to that GGTP derived from normal fetal and adult human kidney, but slower than that of the enzyme from fetal liver (Figure 3). As a control, kidney and pancreas tissue extracts from the host of the tumor (the tumorbearing mouse) were used. Here again the tumor tissue extract showed mobility identical with that of the kidney and different from that of the pancreatic enzyme. Adult mouse liver did not show any GGTP activity[14, 23].

Preliminary examinations of GGTP activity in the serum of nephroblastoma-bearing mice showed activity 4 days before surgical removal of the 3-week-old tumor and on the day of removal an average of 15 IU/l. 3 and 7 days after tumor resection either no enzyme activity or traces only could be found in the serum. Since the serum level of GGTP in tumor-bearing mice was quite remarkably elevated over that found in control mice before the tumor implantation, and initial experiments showed that these values returned to normal following surgical resection, this suggested that serum GGTP originated in the tumor. While the tumor is encapsulated and not invasive, its extensive peripheral vascularization may permit ready access of tumor-derived substances into the circulation. It remains unclear whether the enzyme is actively secreted or is released upon necrotic destruction of tumor tissue.

[16] M. ORLOWSKI and A. MEISTER, Proc. natn. Acad. Sci., USA *67*, 1248 (1970).
[17] Z. ALBERT, J. ORLOWSKA and M. ORLOWSKI, Acta histochem. *18*, 90 (1964).
[18] Z. ALBERT, Nature, Lond. *205*, 407 (1965).
[19] K. JACYSZYN and T. LAURSEN, Clin. chim. Acta *19*, 345 (1968).
[20] E. H. COOPER, Br. J. Cancer *30*, 190 (1974).
[21] G. LUM and S. R. GAMBINO, Clin. Chem. *18*, 358 (1972).
[22] S. FIALA and E. S. FIALA, J. natn. Cancer Inst. *51*, 151 (1973).
[23] E. MÜLLER, J.-P. COLOMBO, E. PEHEIM and J. BIRCHER, Experientia *30*, 1128 (1974).

Sulfur Dioxide as a Sulfur Source in Duckweeds (*Lemna minor* L.)[1]

CHR. BRUNOLD[2] and K. H. ERISMANN

Pflanzenphysiologisches Institut der Universität, Altenbergrain 21, CH-3013 Bern (Switzerland), 19 September 1975.

Summary. Isotope competition experiments with *Lemna minor* L. indicate that SO_2-sulfur enters the sulfur amino acids of the proteins and the sulfoquinovose of the sulfolipids following oxidation to SO_4^{2-} and subsequent reduction.

Plants take up a great amount of sulfur dioxide, an atmospheric pollutant which is mainly produced by burning of fossil fuels[3]. Thus, plants can play a significant role in reduction of the concentration of a toxic gas; at the same time they can satisfy at least part of their sulfur requirements, unless the concentration of SO_2 is so high that they are damaged or killed[4]. In plants, the absorbed SO_2 occurs predominantly in the form of SO_3^{2-}[5]. In vitro studies with plant extracts have demonstrated that SO_3^{2-} can either be oxidized to SO_4^{2-}[5,6] or reduced to H_2S[7–9]. If, in vivo, the absorbed SO_2 is oxidized, the sulfur is metabolized as sulfate, the normal sulfur source of plants[10]. If the SO_2 is reduced, the sulfide could be used directly for formation of cysteine[11] in a reaction which is quantitatively the most important step for sulfur incorporation into organic compounds[10]. Another important reaction, in which SO_3^{2-} could be used without prior reduction or oxidation, is the formation of 6-sulfoquinovose found in the sulfolipids of plants[12].

The results reported here show to what extent the systems for reduction and oxidation of SO_3^{2-}, which have been demonstrated in vitro, may be significant in vivo in the duckweed *Lemna minor* L. and how far SO_2 is used directly for the formation of sulfolipids.

In the Figure results of an isotope competition experiment are presented in which duckweed was cultivated in an atmosphere with 0.3 ppm SO_2 on a nutrient solution containing $^{35}SO_4^{2-}$. The specific activities of the sulfur in the sulfate, the proteins, and the sulfolipids, reached

[1] This work was supported by the Swiss National Foundation (SNF).
[2] C.B. thanks Dr. J. M. EGAN JR., Department of Biology, Brandeis University, Waltham, Mass. 02154, USA, for correcting the manuscript.
[3] W. W. KELLOG, R. D. CADLE, E. R. ALLEN, A. L. LAZARUS and E. A. MARTELL, Science *175*, 787 (1972).
[4] K. GARBER, *Luftverunreinigungen und ihre Wirkungen* (Bornträger, Berlin/Nikolassee 1967).
[5] W. LIBERA, H. ZIEGLER and I. ZIEGLER, Planta *109*, 269 (1973).
[6] K. ASADA and K. KUNIAKI, Eur. J. Biochem. *33*, 253 (1973).
[7] K. ASADA, G. TAMURA and R.S. BANDURSKI, J. biol. Chem. *244*, 4904 (1969).
[8] A. TREBST and A. SCHMIDT, Progr. photosynth. Res. *3*, 1510 (1969).
[9] G. TAMURA and S. ITOH, Agric. Biol. Chem. *38*, 225 (1974).
[10] J. F. THOMPSON, A. Rev. Plant Physiol. *18*, 59 (1967).
[11] L. K. SMITH and J. F. THOMPSON, Biochem. biophys. Res. Commun. *35*, 939 (1969).
[12] J. A. SCHIFF and R. C. HUDSON, A. Rev. Plant Physiol. *24*, 381 (1973).

Table I. Specific growth rate, sulfate content, and specific activities of the sulfur in sulfate, proteins, and sulfolipids of *Lemna minor* after 10 days with 0,6 ppm SO_2 and different $^{35}SO_4^{2-}$-concentrations in the nutrient solution

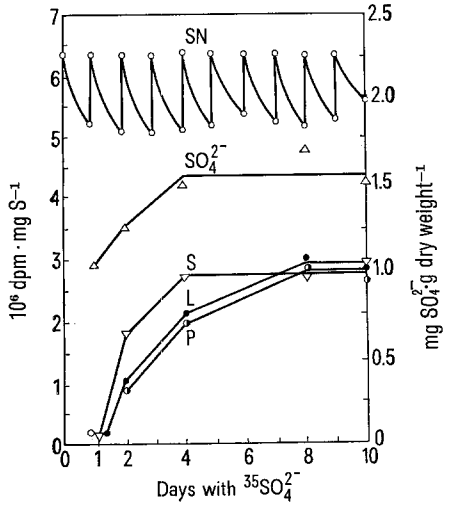
SO_4^{2-} in nutrient solution (M)	Specific growth rate[a]	Sulfate content (μg/mg dry weight of tissue)[b]	Specific activity(dpm/μgS)		
			SO_4^{2-}[b]	Proteins[b]	Sulfolipids[b]
7.5×10^{-6}	0.40	4.1 ± 0.38	2,916 ± 145	2,724 ± 203	3,030 ± 204
10^{-5}	0.42	4.4 ± 0.29	4,175 ± 250	3,914 ± 162	4,258 ± 242
2.5×10^{-5}	0.42	4.6 ± 0.40	1,858 ± 182	1,683 ± 94	2,064 ± 143
5×10^{-5}	0.41	4.0 ± 0.31	2,565 ± 165	2,666 ± 177	2,703 ± 201
2.5×10^{-4}	0.40	5.8 ± 0.42	723 ± 38	853 ± 59	775 ± 48

[a] Growth was measured by counting the total number of fronds on the 4th, and the 7th day of the experiment and the specific growth rate μ in fronds per day was calculated. [b] Average and standard error of 4 estimations.

Table II. Sulfite reducing system extracted from *Lemna minor*

Reaction mixture	$H_2{}^{35}S$ produced (nmoles · min^{-1} · mg $protein^{-1}$)[b]
Complete[a]	0.55
Complete minus extract from *Lemna minor*	0.03
Complete with boiled extract	0.02
Complete with SO_4^{2-} instead of SO_3^{2-}	not detectable

[a] The complete reaction mixture contained in a total volume of 3 ml: *tris*-HCl, pH 8.0, 250 μmoles; $MgCl_2$, 10 μmoles; ferredoxin from spinach, 0.041 μmoles; $^{35}SO_3^{2-}$, 0.5 μmoles (336,000 cpm); NADP, 0.2 μmoles; glucose-6-phosphate, 5 μmoles; glucose-6-phosphate dehydrogenase, 0.5 units; ferredoxin-NADP-reductase, 0.4 mg; extract from *Lemna minor* with 1.3 mg proteins according to ELLIS[16]. [b] Determined according to SCHMIDT[17].



Specific activity of ^{35}S in sulfate of nutrient solution (SN) and in sulfate (S), proteins (P), and sulfolipids (L) in *Lemna minor* cultivated with 8.8×10^{-4} M $^{35}SO_4^{2-}$ in ENO_3[13] and 0.3 ppm SO_2.
The sulfur was determined according to JOHNSON and NISHITA[14]. The sulfolipids were extracted using FOLCH's[15] method. The SO_2-concentration was produced with a permeation tube (Metronics Associates Inc.). (SO_4^{2-} = sulfate content in mg · g dry weight^{-1} of tissue).

the same constant value. Thus, we conclude that SO_2-sulfur enters the sulfur amino acids of the proteins and the sulfoquinovose of the sulfolipids following oxidation to SO_4^{2-} and subsequent reduction; for if SO_2-sulfur is incorporated directly to a significant extent, the specific activities of the sulfolipids and the proteins would be lower than that of sulfate.

The specific activity of the sulfate in the plant material was about 50% of that of the sulfate of the nutrient solution, indicating that oxidized SO_2 diluted the cell sulfate pool. The fluctuations of the specific activity of the nutrient solution is due to the absorption of SO_2, its rapid oxidation to SO_4^{2-} and the daily renewal.

Due to the uptake of SO_2 and its oxidation, the sulfate content in the plant material increased to a constant value after about 4 days.

The results in Table I, taken from a similar isotope competition experiment, confirm those of the Figure; after 10 days with $^{35}SO_4^{2-}$ and SO_2 there was again no significant difference in the specific activities of the sulfur of sulfate, proteins and sulfolipids, although the SO_2-concentration was raised from 0.3 to 0.6 ppm and various sulfate concentrations in the nutrient solution were used. So, as previously shown, only an insignificant amount, if any, of SO_2 was directly assimilated as sulfite. The specific growth rates were not lower than those of duckweed cultivated without SO_2[18]; indicating that 0.6 ppm SO_2 apparently does not affect important metabolic processes in *Lemna minor*.

The inability for the direct incorporation of significant amounts of atmospheric SO_2 into sulfur amino acids could be due to a lack of sulfite reductase in duckweed. Table II shows that there is a system which can reduce SO_3^{2-} to sulfide. But the results presented in Table I and in the Figure suggest that this system probably does not play a significant role in vivo along with the sulfite oxidizing systems. These systems, which oxidize SO_2 very effectively, could be the reason for the resistance of duckweed to relatively high concentrations of SO_2.

[13] K. H. ERISMANN and A. FINGER, Ber. Schweiz. bot. Ges. *78*, 5 (1968).
[14] C. M. JOHNSON and H. NISHITA, Analyt. Chem. *24*, 736 (1952).
[15] J. FOLCH, M. LEE and G. H. SLOANE STANLEY, J. biol. Chem. *226*, 497 (1957).
[16] R. J. ELLIS, Planta *88*, 34 (1969).
[17] A. SCHMIDT, Thesis, Göttingen (1968).
[18] K. H. ERISMANN and C. BRUNOLD, Ber. Schweiz. bot. Ges. *83*, 213 (1973).