The most important low molecular weight complexes formed by Cu(II), Fe(III), Mn(II) and Zn(II) in plasma, as found by computer simulation are listed in Table 1. The computer model that calculated these percentages included almost 5000 species that could be produced in the presence of seven metal ions and 40 ligands at $-\log [H^+] = 7.4$. Although the details of this work are to be published elsewhere (manuscript in preparation[17]), the present purpose is served by noting that the percentage distribution recorded is independent of the free metal ion concentrations chosen for the model. This is valid to a precision of one percent when computed for concentrations within the ranges shown in Table 2.

The similarity between the metal ion distributions published here and those reported previously[13–15] deserves some comment. Although the agreement is certainly not perfect, it is surprisingly good in view of the disparities that exist with regard to the number of kinds of metal ion and the number of complexes the models contain. This is also a consequence of the fact that the percentages depend so strongly on the total concentration and on the protonation constants of the ligands in the system. The formation of complexes by one transition metal has essentially no influence on the percentage distributions of other metal ions. Thus, it is possible to obtain a reliable distribution for one transition metal without including all the others in the model.

Of course, models are only as good as the parameters upon which they depend. As many of the formation constants, especially for the ternary complexes, remain to be determined, this type of investigation is subject to continuous improvement. However, it is true that with only a few exceptions, the formation constants of the species appearing in Table 1 have been measured. Moreover, the vitally important ligand pK_as have all been determined, mostly under physiological conditions of temperature and ionic strength. Hence, whilst the actual percentages recorded may be changed in the future and whilst it is possible that other complexes may turn out to be significant, in our opinion, the broad picture will remain. In particular, this applies to the ratio between the concentrations of complexes whose formation constants have been adequately established. Of even greater importance, the idea that information about transition metal ion complexes in plasma can be obtained from simulations which omit protein equilibria provides encouragement both to measure the formation constants still outstanding and to expand existing models of blood and other biofluids.

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Subcellular Localization of *O*-Acetylserine Sulfhydrylase in Spinach Leaves[1]

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Summary. A combination of differential centrifugation and isopycnic sucrose density gradient centrifugation of extracts from spinach leaves (*Spinacia oleracea* L.) shows that about 20% of the O-Acetylserine sulfhydrylase are associated with chloroplasts. No appreciable amounts of O-Acetylserine sulfhydrylase band with mitochondrial and peroxisomal marker enzymes.

From the quantitative standpoint, the most important function of sulfate reduction is to produce cysteine. There is evidence from different organisms that L-cysteine is produced by sulfhydrylation of *O*-acetyl-L-serine (OAS) with sulfide: *O*-acetyl-L-serine + sulfide → L-cysteine + acetate. The reaction is catalyzed by *O*-acetylserine sulfhydrylase, abbr. OAS-S (*O*-acetyl-L-serine acetate lyase [adding hydrogensulfide], EC. 4.2.99.8). The enzyme has been reported in bacteria[2], fungi[3,4] and several higher plants[4–7].

Results of different authors[7–9] indicate that in higher plants the enzyme is localized in the cytoplasm. This is in contrast to reports that isolated chloroplasts can form cysteine[10–12,15]. It seemed desirable, therefore, to clarify whether the enzyme might be associated, at least in parts, with organelles such as chloroplasts, mitochondria and peroxisomes.

The extraction and isolation of organelles from spinach leaves (*Spinacia oleracea* L., var. Nobel) was achieved according to ROCHA and TING[13] by means of a sucrose based extraction medium and a combination of differential centrifugation and isopycnic sucrose density gradient centrifugation. The method consists of 3 differential centrifugation steps: 250 g for 90 sec (250 g crude ex-

Table 1. Percent organelle cross contamination of spinach leaf organelles in a 40–75% (w/v) linear sucrose density gradient

Organelle fraction	Isopycnic density (g/cm³)	Contaminating organelle			
		Peroxisomes %	Mitochondria %	Intact chloroplasts %	Broken chloroplasts %
Peroxisomes	1.255–1.26	–	1.5	< 1	< 1
Mitochondria	1.20	3	–	1.5	2.5
Intact chloroplasts	1.226	2.5	5	–	
Broken chloroplasts	1.178	2	19.5	< 1	–

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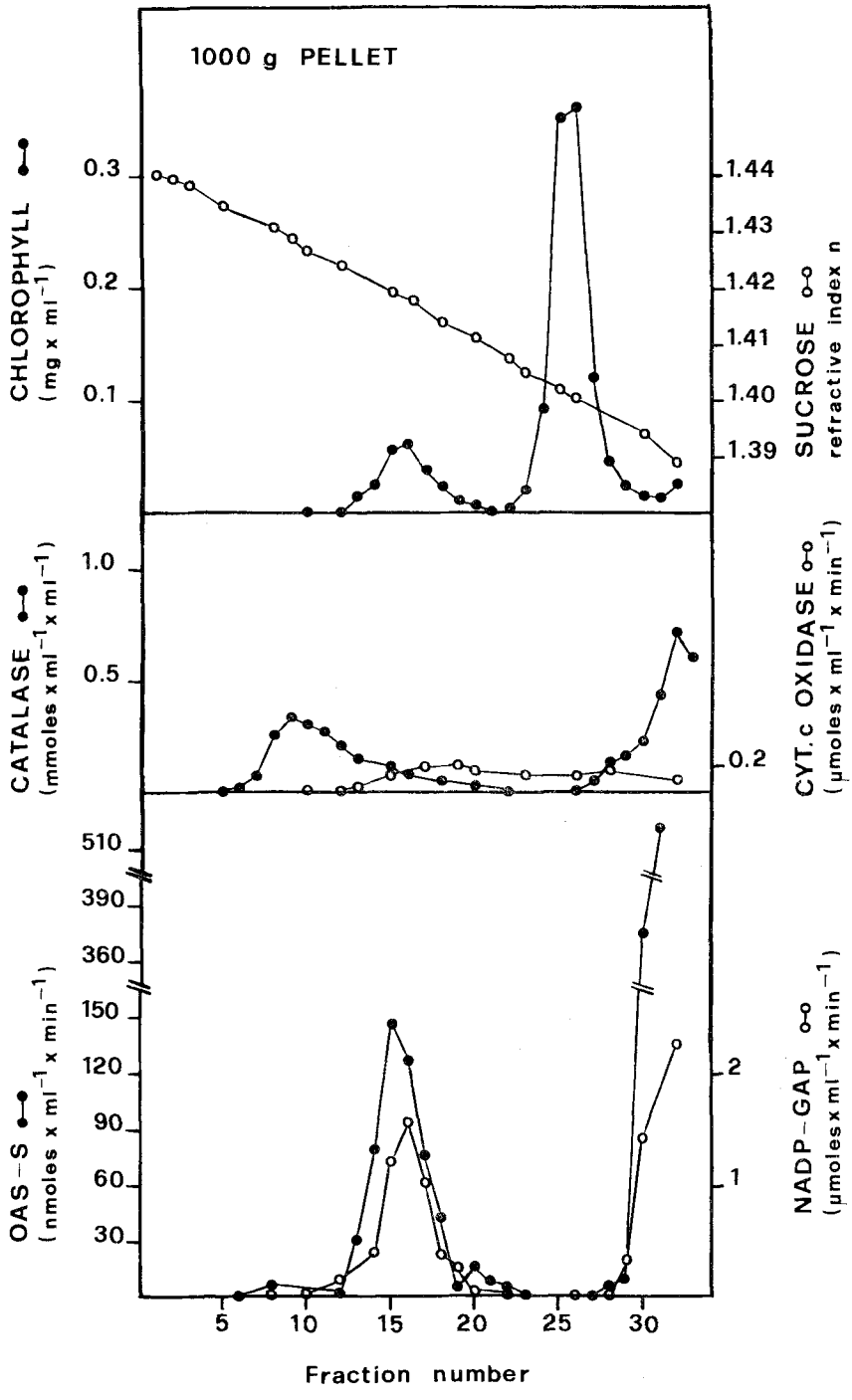

Fig. 1. Distribution of *O*-Acetylserine sulfhydrylase on a sucrose density gradient from a 1000 g spinach leaf pellet. Chlorophyll was determined according to Arnon[16]. Enzyme assays: Catalase according to Lueck[17], Cytochrome c oxidase (Cyt. c oxidase) according to Rocha and Ting[13], NADP-glyceraldehyde-3-phosphate dehydrogenase (NADP-GAP) according to Heber et al.[18]; *O*-acetylserine sulfhydrylase (OAS-S) according to Becker et al.[19].

Table 2. *O*-Acetylserine sulfhydrylase (OAS-S) and chlorophyll in the 250 g crude extract and in intact chloroplasts from spinach leaves

Fractions	Chlorophyll (total amounts in mg)	OAS-S (total activities in μmoles cysteine min^{-1})	OAS-S/chlorophyll (μmoles cysteine · mg $chlorophyll^{-1}$ min^{-1})
250 g crude extract	10.7316 (100%)	108.0160 (100%)	10.065 (100%)
Intact chloroplasts	0.2488 (2.318%)	0.5106 (0.473%)	2.052 (20.39%)

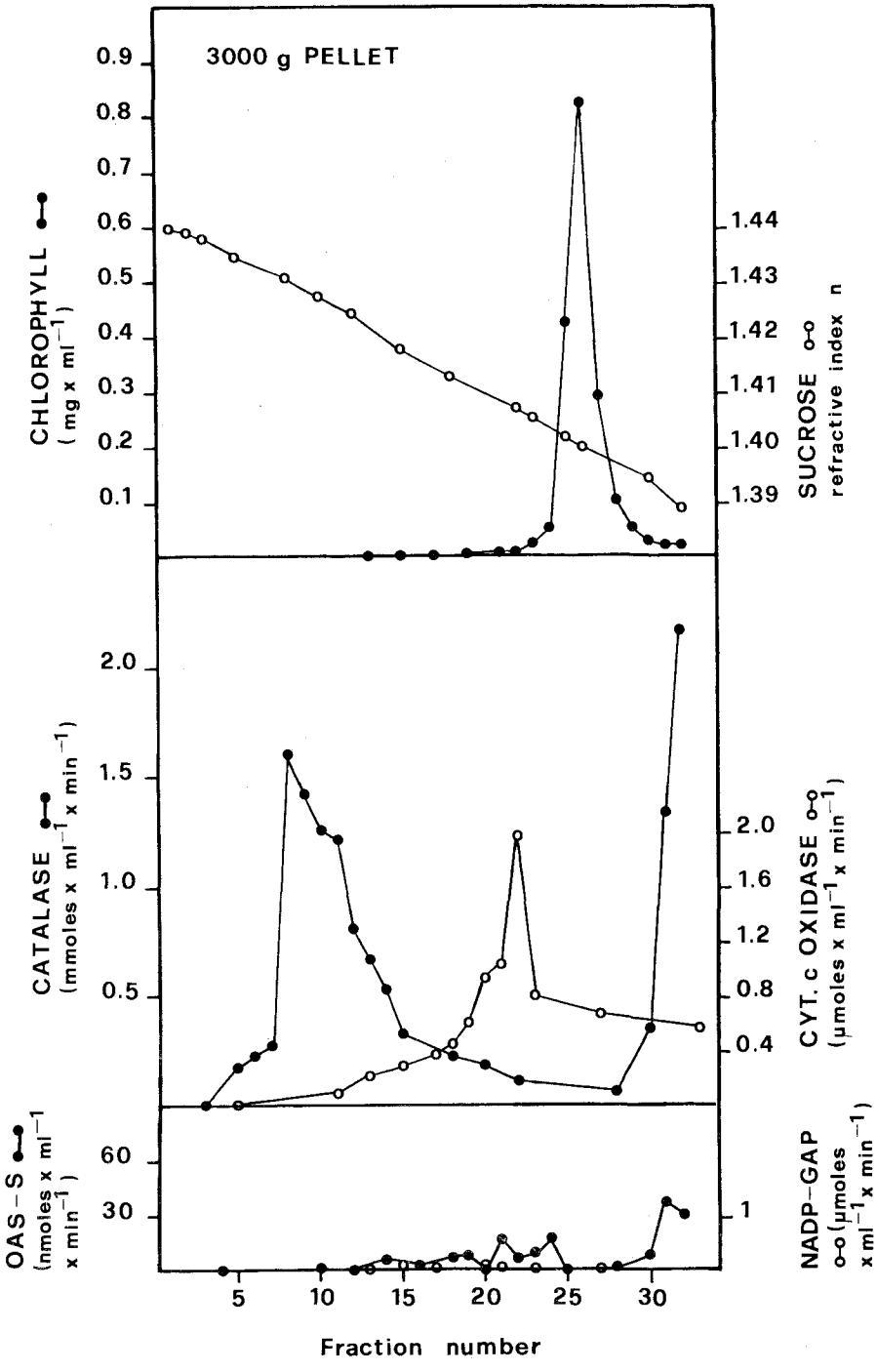

Fig. 2. Distribution of *O*-acetylserine sulfhydrylase on a sucrose density gradient from a 3000 g spinach leaf pellet. The assays were carried out as described in Figure 1.

tract), 1000 g for 5 min and 3000 g for 15 min. Both the 1000 g and 3000 g resuspended pellets were layered on 40–75% (w/v) sucrose gradients, which were centrifuged for 3 h in a Beckman SW-27 swinging bucket rotor at 25000 rpm in a Spinco L2-65 B preparative ultracentrifuge.

Table 1 shows the contamination of the different organelle fractions and their isopycnic densities. The estimation of the percent organelle cross contamination is based on chlorophyll contents and the activity of organelle marker enzymes (NADP-glyceraldehyde-3-phosphate dehydrogenase for intact chloroplasts, cytochrome c oxidase for mitochondria and catalase for peroxisomes). The isopycnic densities are comparable to those found by other workers[14].

Figure 1 shows a peak of OAS-S activity in the fractions of intact chloroplasts. It contains about 25% of the enzyme activity of the 1000 g pellet. The OAS-S activity found in the supernatant of the gradient may be explained as consequence of chloroplast breakage during preparation. Figure 2 shows a gradient of the 3000 g pellet. There is no appreciable OAS-S activity banding with the mitochondrial and peroxisomal marker enzymes.

In the gradients both of the 1000 g and 3000 g pellets, no OAS-S activity bands with the broken chloroplasts. Thus we assume that the OAS-S localized in the chloroplasts is not a thylakoid-bound, but a soluble, stromal enzyme.

The values of Table 2, taken from a typical experiment, show that on a chlorophyll basis 20.39% of the total OAS-S activity present in the 250 g crude extract are associated with the intact chloroplasts. The rest of the activity is found in a non-particulate form and may be attributed to the cytoplasm.

Our results are consistent: 1. with the reported formation of cysteine in chloroplasts[10–12, 15]; 2. the proposed localization of the OAS-S in the cytoplasm[7–9] and 3. the results from light-induced chloroplast development experiments with *Euglena* which indicate that OAS-S is not exclusively or predominantly in the chloroplasts[20]. In contrast to *Euglena* however, Spinach leaves have not appreciable amounts of OAS-S activity banding with mitochondria[20].

The reason for the exclusive cytoplasmic localization of OAS-S by other workers[7–9] may be due to difficulties in the estimation of the OAS-S contents of chloroplasts.

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Partial Sparing of Dietary Methionine by Lanthionine in *Argyrotaenia velutinana* Larvae

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Summary. Lanthionine was able to partially spare the dietary methionine requirement of *Argyrotaenia velutinana* (Walker) larvae but the sparing of methionine by lanthionine was not as efficient as cysteine. Partial sparing of dietary methionine by lanthionine, a non-member of the cystathionine pathway, indicated the possibility of sulphur amino acid metabolism by routes other than the cystathionine pathway.

The sulphur amino acid methionine is required in the diet of almost all insects studied and cannot usually be replaced by other sulphur amino acids[3]. Nutritional investigations on *Argyrotaenia velutinana* (Walker) have shown that methionine is essential[4] but that approximately 75% of methionine requirement could be spared by cyst(e)ine[5] or other members of the cystathionine pathway (cystathionine and homocysteine)[6]. Lanthionine, a non-member of the cystathionine pathway has been found to spare the dietary cyst(eine) requirement of rats[7]. Lanthionine has been isolated from the acid hydrolysate of locust wing muscle[8], and the haemolymph of *Bombyx mori*[9, 10] and *Antheraea pernyi*[9]. The present investigation was undertaken to investigate the possibility of partial sparing of dietary methionine by lanthionine, a non-member of cystathionine pathway in *A. velutinana* larvae.

The composition of the control diet containing 17 amino acids, in which methionine at 100 mg/100 g diet was the only sulphur amino acid, was similar to that described by Sharma et al.[6]. In the cysteine supplemented diet 241 mg/100 g diet of cysteine was provided to supplement approximately 75% dietary methionine requirement. The sulphur level was kept constant in all diets, and the amount of supplemented lanthionine provided the quantity of sulphur present in 241 mg cysteine. The non-availability of L-isomer necessitated the use of DL-

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