

treatments than that in *N. muscorum*. The inhibitory effect of DNase treatment is more severe than that of RNase; with RNase only a 5% inhibition is noted in *N. muscorum*. It may be due to the difference in penetration of nucleases. The distribution of ^{14}C into the photosynthetic products in both the organisms is however affected by either of the nucleases in varying degrees. Sucrose synthesis appears to be the most affected. Considerable radioactivity was detected in 'the nucleotide area' of the chromatograms of *N. muscorum* and RNase treatment has been found to result in a marked accumulation of the products involved, probably at the expense of sucrose synthesis. ^{14}C incorporation into aspartic and glutamic acids is stimulated by both the nucleases in *N. muscorum*, particularly by RNase. In *C. pyrenoidosa* DNase markedly retards aspartic acid synthesis but glutamic acid synthesis is severely affected by RNase. Nucleases have also an inhibitory effect on glycine-serine synthesis in both the organisms. Alanine synthesis in *N. muscorum* is totally abolished by the application of RNase in *C. pyrenoidosa* on the other hand there is a slight stimulation. DNase has some inhibitory effect on alanine synthesis in *C. pyrenoidosa*, but not at all in *N. muscorum*. No incorporation of ^{14}C into malate or citrate is observed in nucleases treated *C. pyrenoidosa*. In *N. muscorum* malate or citrate had only traces of radioactivity and no conclusion can be made regarding the effect of nucleases.

The effect of nuclease treatment on the products of $^{14}\text{CO}_2$ fixation in *Chlorella pyrenoidosa* and *Nostoc muscorum*
(Data expressed as % variation from control for each compound)

Compound	<i>C. pyrenoidosa</i>		<i>N. muscorum</i>	
	RNase ^a	DNase ^b	RNase ^c	DNase ^d
Nucleotide area ^e ^f	...	+227	-12
Sugar phosphates . .	-44	-50
Sucrose	-96	-100	-76	-63
Aspartic acid	-2	-43	+255	+8
Glutamic acid	-55	-18	+109	+11
Glycine-Serine	-50	-69	-100	-100
Alanine	+11	-21	-100	0
Citric acid	-100	-100
Malic acid	-100	-100
Total ^{14}C incorporation	-49	-76	-5	-29

^a Packed cell volume 0.8 ml, suspended in 10 ml phosphate buffer of pH 7.0 containing 7 mg RNase. Incubation at 37°C for 2.5 h. After incubation washed, centrifuged, and resuspended in 5 ml phosphate buffer, cells allowed to metabolise 50 μC $\text{NaH}^{14}\text{CO}_3$ for 2 h in light from two 1000 W photoflood lamps 18" away from the incubation mixture, one on either side.

^b Same as above except that 4.3 mg DNase was added.

^c 20 ml cell suspension; 5.5 mg RNase, incubation period 2 h after washing and centrifugation resuspended in 5 ml phosphate buffer and incubated with 50 μC $\text{NaH}^{14}\text{CO}_3$ for 2 h otherwise same as above.

^d 10 ml suspension, 5 mg DNase, resuspended in 6.5 ml phosphate buffer and 100 μC bicarbonate added, otherwise same as above.

^e The radioactive area near the origin of chromatograms containing nucleotides etc.

^f Low counts.

From the observations recorded here it appears that both RNase and DNase affect the synthesis and metabolism of some of the CO_2 fixation products in photosynthesis, particularly sucrose. The response to pretreatment with nucleases for other compounds is varied and the effects are apparently complicated. The metabolic

interrelationship among the products of photosynthesis except the very early ones is not well understood and it is not possible at present to suggest which of the reactions involved are nucleic acid controlled. It should, however, be borne in mind that the effects observed may not be direct effects of nuclease treatments since whole cells were used and the question of permeability is there though it is reported that ribonucleases of molecular weights of about 13000 can penetrate living onion root tip cells^{5,6}.

The experiments with isolated chloroplasts and uses of inhibitors in nucleic acid syntheses will further elucidate the point.

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Zusammenfassung

Es scheint, dass DNase und RNase bei der photosynthetischen Fixierung des CO_2 verschiedene und komplizierte Wirkung ausüben. Die Saccharose-Synthese wird durch die Behandlung mit DNase und RNase deutlich vermindert.

⁵ J. BRACHET, Nature 174, 876 (1954).

⁶ B. P. KAUFMAN and N. K. DAS, Proc. nat. Acad. Sci., Wash. 40, 1052 (1954); Chromosoma 7, 19 (1955).

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Cystine Monosulfoxide

Several pathways for the metabolism of cystine to taurine are still under consideration¹. Among the more probable intermediates in the metabolism of cystine or cystine, only the cystine monosulfoxide has been neither isolated nor synthesized. Its preparation was unsuccessfully attempted by TOENNIES and LAVINE²⁻⁴. Cystine monosulfoxide is of growing theoretical interest since simple thiosulfinic esters (monosulfoxides of alkyl disulfides) studied by WEISBERGER and PENSKY⁵ were shown to possess cancer blocking activity, which these authors derived from the inhibiting effect of allicin on sulphydryl (-SH) containing enzymes. Allicine (allylthiosulfinic allyl ester) is an enzymic metabolite of alliin, the active principle of garlic (*Allium sativum*)⁶⁻⁸.

L-Cystine monosulfoxide has now been prepared by reduction with hydriodic acid in the cold of cystine disulfoxide. The latter had been synthesized in nonaqueous solvent by TOENNIES and LAVINE³.

In contrast to the disulfoxide, the monosulfoxide is stable in the presence of 0.5 N NaOH. It darkens and slowly decomposes upon heating above 175°C, $[\alpha]_D^{25} = -111^\circ$ ($\pm 3^\circ$), $c = 0.70\%$ in N HCl. The ninhydrin spot appears immediately and is deeper colored than the one given by

¹ L. YOUNG and G. A. MAW, *The Metabolism of Sulphur Compounds*, Chapter V (Methuen Co. Ltd., London 1958), p. 97.

² G. TOENNIES, J. Amer. chem. Soc. 56, 2198 (1934).

³ G. TOENNIES and T. F. LAVINE, J. biol. Chem. 113, 576 (1936).

⁴ T. F. LAVINE, J. biol. Chem. 113, 584 (1936).

⁵ A. S. WEISBERGER and J. PENSKY, Cancer, Res. 18, 1301 (1958).

⁶ C. J. CAVALLITO, J. BUCK, and C. SUTER, J. Amer. chem. Soc. 66, 1952 (1944).

⁷ A. SOLL and E. SEEBECK, Helv. chim. Acta 31, 189 (1948).

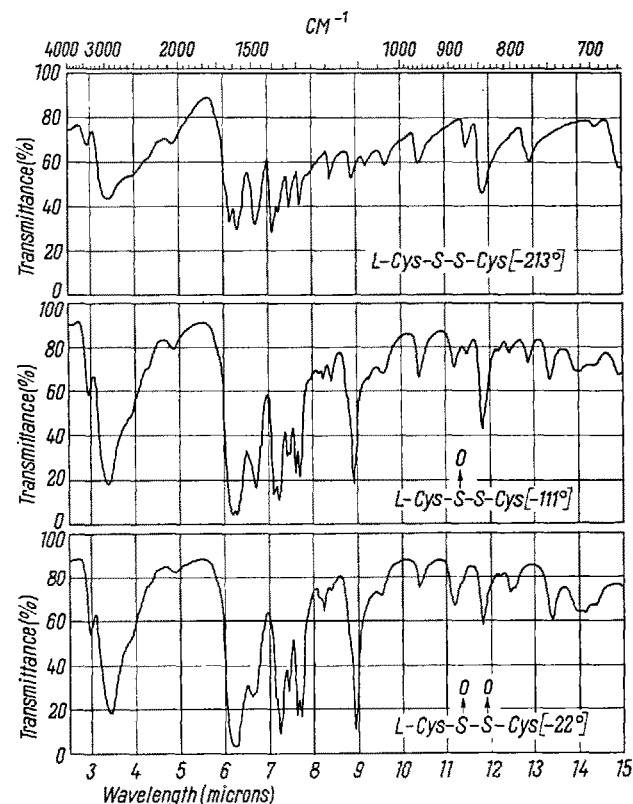
⁸ E. D. WILLS, Biochem. J. 63, 514 (1956).

cystine. Contrary to the conclusion drawn by TOENNIES and LAVINE³ from oxidation mixtures, the monosulfoxide of cystine does respond to the cyanide nitro prusside test⁹ although slowly (3–5 min at room temperature). This indicates that the sulfur of the sulfoxide group is still liable to nucleophilic attack by the cyanide ion¹⁰.

The monosulfoxide slowly reduces dichloro-indophenol *in vacuo* at room temperature but has no effect on methylene blue in either its leuco or oxidized form.

The I. R. spectrum (Fig.) of the monosulfoxide is distinguished from the corresponding cystine spectrum by a sharp peak at 8.93 μ which is assigned to the sulfoxide group¹¹. A needle shaped peak at 7.49 μ allows the discrimination from the disulfoxide, which lacks this peak but shows the usual sulfoxide peak slightly shifted to 8.95 μ .

Cystine, as well as its sulfoxides, is relatively immobile with a variety of chromatographic solvents, except in the presence of coal gas and ammonia. Under these conditions, the presence of mercaptan permits the existence and chromatographic movement of monosulfur derivatives of the disulfides and their sulfoxides. These would recombine in the presence of air. We could confirm the ratio of the Rf values reported by WINEGARD *et al.*¹² for cystine (0.25) and its disulfoxide (0.21), but found the mobility in agreement with CONSDEN *et al.*¹³ 0.13 and 0.11, respectively. The cystine monosulfoxide could not be discriminated chromatographically from the disulfoxide. Its Rf value in phenol/H₂O 4:1 in coal gas/NH₃ on Whatman paper No. 3 is 0.11.



I. R. spectra of cystine, cystine monosulfoxide, and cystine disulfoxide* in KBr 1:400 measured on a Perkin Elmer Spectrophotometer, Modell 21, with Slave Recorder

* The structure of 'cystine disulfoxide' is not yet established beyond any doubt. The other alternative is the thiosulfonic ester.

Experimental. Crude cystine disulfoxide was prepared by the general method of LAVINE⁴. Due to the presence of acetic anhydride in the oxidizing medium, this product contained mono-N-acetyl-cystine disulfoxide. The N-acetyl impurity precipitated first on neutralizing with ammonium hydroxide a solution of the mixed crystals in N HCl. The mother liquor contained further cystine monosulfoxide.

The thus purified L-disulfoxide showed $[\alpha]_D^{25} = -22^\circ$ ($\pm 2^\circ$), $c = 1.38\%$ in 1 N HCl. Lit.³ $[\alpha]_D^{25} = -30.2^\circ$ in 1 N HCl. L-cystine disulfoxide (2.00 g, 7.5 mmoles) was suspended in 40 ml of water and dissolved with 6 N HCl (6 ml 18 mmoles). This solution was cooled in ice and placed in the dark. Five molar KI (3 ml, 15 mmoles) was added and stirred occasionally during $\frac{1}{2}$ h. The solution was then transferred to a cooled separatory funnel and extracted rapidly 12 times with 30-ml portions of chloroform until the chloroform and the aqueous solution were colorless. The aqueous solution was then neutralized in the cold with 8 N NH₄OH. The precipitate was quickly filtered and washed with alcohol followed by acetone. Yield: 1.38 g (75.2%). Dec. above 175°C. It was stored in an evacuated dessicator.

The automatic titration recorder gave 56 mg = 4.38×10^{-4} mol (2.19 cm^3 0.2 N NaOH); eq. wt. 128 (calculated 128.12).

For analysis the compound was heated 1 h to 100°C in a 1 mm Hg vacuum.

$\text{C}_6\text{H}_{12}\text{N}_2\text{OS}_2$ 256.24

Calculated C	28.10	H 4.72	N 10.93	S 25.02%
Found	28.34	4.93	10.66	25.17%

This work is part of an investigation of the 'iodine trapping syndrome' described by SCOTT *et al.*¹⁴ and supported by Cancer Research funds of the University of California. It was aided by facilities of the School of Pharmacy of the University of California.

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Zusammenfassung

Es wurden die Herstellung und die Eigenschaften von Cystin-monosulfoxid beschrieben und die IR.-Spektren von Cystin, Cystin-monosulfoxid und Cystin-disulfoxid wiedergegeben.

⁹ G. TOENNIES and T. F. LAVINE, J. biol. Chem. 105, 115 (1934).

¹⁰ A review on the scission of the disulfide bond was recently published by A. J. PARKER and N. KHARASCH, Chem. Rev. 59, 584 (1959).

¹¹ H. M. RAUEN'S Biochemisches Taschenbuch (Springer-Verlag, Berlin-Göttingen-Heidelberg 1956), lists strong IR peaks from 7.5 μ to 9.5 μ .

¹² H. M. WINEGARD, G. TOENNIES, and R. J. BLOCK, Science 108, 506 (1948).

¹³ R. CONSDEN, A. H. GORDON, and A. J. P. MARTIN, Biochem. J. 40, 580 (1946).

¹⁴ K. S. SCOTT and C. PENG, Univ. Calif. Publ. Pharm. 2, 345 (1955).