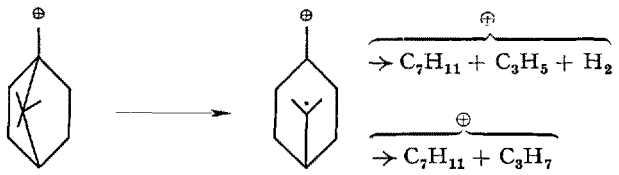
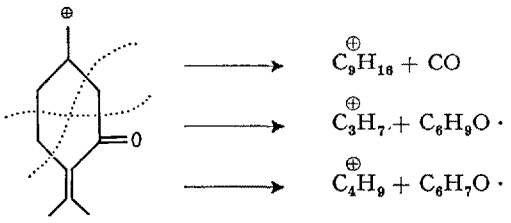
system does not seem to do so in these systems. This is, however, observed in the break-down of the camphane molecular ion



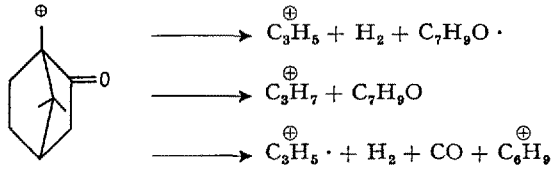
where the ions of masses 95, 43, and 41 are very abundant. Ring-strain seems an important factor in all these systems as the presence of the *gem*-dimethyl bridge system gives a conformational rigidity to the molecule. This is supported by the fact that the elimination of 43 units from 1-methyl-4-*iso*propylcyclohexane while marked is not as intense as in the systems discussed[2].

Other prominent fragments which occur in certain of the spectra, namely mass 68 in dipentene, and 75 in Δ^3 carene may readily be interpreted as allylic-bond fissions. The prominent peak of mass 79 in the α- and δ-fenchenes is not readily explained on this basis without postulating hydrogen migrations also and this system is to be further examined.

These studies have been extended to pulegone and to camphor to allow of comparison with the hydrocarbons. In accordance with previous observations[4,5] the predominant process seems to be the elimination of carbon monoxide. The remaining fragments may be obtained as follows



giving fragment ions of 57,43, and 28. In a similar way camphor yields fragments of masses 41,43, and 81.



The other significant fragment ion at low energies, 55, is not so readily interpreted.

It is concluded, therefore, that the principal fragment ions obtained in this series of compounds may, with two exceptions, be obtained directly from the carbocyclic skeletons. They do not at least at the low energies used require re-arrangements of this skeletal structure for their interpretation.

We acknowledge with gratitude the provision of a sample of pulegone by Dr. G. BUCHANAN, The University of Glasgow, of Δ^3 carene by Dr. L. N. OWEN of the Imperial College of Science and Technology, London, and part of the mass-spectrum of camphor by Mr. W. SNEDDEN of The University of Glasgow.

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and R. I. REED

Chemistry Department, The University of Glasgow, July 27, 1959.

Résumé

Les auteurs ont trouvé quelques corrélations entre la formation des ions les plus abondants et la disposition des liaisons-éthyléniques dans les ions moléculaires de quelques terpènes.

[4] J. H. BEYNON, personal communication.
[5] J. H. BEYNON, R. G. LESTER, and A. E. WILLIAMS, *A.S.T.M. E-14 Committee on Mass Spectrometry*, May 1959.

The Effects of Nucleases on Photosynthetic CO_2 Fixation

The occurrence of nucleic acids in chloroplasts has been recognised for quite sometime[1] but very little is known about their function, except possibly an involvement in the propagation of chloroplasts. It has been indicated that the activity of RNA[2] could be linked to the synthesis of specific proteins i.e. enzymes involved in photosynthesis[3]. In view of some recent observations on nucleic acid control of protein and polysaccharide syntheses it was considered of interest to study whether and to what extent the CO_2 fixation reactions in photosynthesis are nucleic acid dependent processes.

Young cultures of *Chlorella pyrenoidosa* and *Nostoc muscorum* were used. The cells were centrifuged in the cold in an MSE centrifuge, suspended in phosphate buffer of pH 7·0 and incubated with deoxyribo- or ribonucleases for 2–2·5 h at 37°C. The cells were then centrifuged again, resuspended in phosphate buffer allowed to metabolise $NaH^{14}CO_3$ (obtained from the Radiochemical Centre, Amersham, England) for a further period of 2 h at 25°C. The cells were extracted in hot 80% ethanol, filtered and the residue washed in 80% ethanol. An aliquot of the filtrate was transferred to stainless steel planchets for counting with an end window β-counter and another chromatographed two dimentionally on Whatman No. 1 filter paper with phenol-water butanol acetic water (4:1:1) as the developing solvent[4]. The radioactive areas on the chromatograms were located by exposure to X-Ray films and counts were taken directly on paper. The DNase (1 × cryst) used was obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A. and RNase (5 × cryst.) was supplied by Sigma Chemical Co., St. Louis, Missouri, U.S.A.

Nuclease treatment resulted in slight inhibition (less than 10%) of the incorporation of ^{14}C into the 80% ethanol-insoluble fraction. Examination of the ethanol soluble fraction however revealed rather severe effects on the synthesis and metabolism of $^{14}CO_2$ fixation products. The effect of nuclease treatment on the total incorporation of ^{14}C into the alcohol-soluble fraction and the more important products of $^{14}CO_2$ fixation during a 2 h photosynthesis is shown in the Table. It appears that the CO_2 fixation process in *C. pyrenoidosa* is more susceptible to nuclease

[1] E. I. RABINOWITCH, *Photosynthesis and related processes*, Vol. 2, Part 2 (Interscience Publishers, Inc., New York 1956).
[2] The following abbreviations have been used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; DNase deoxyribonuclease; RNase, ribonuclease.
[3] G. BRAWERMAN and E. CHARGAFF, Biochim. biophys. Acta *31*, 164 (1959).
[4] B. B. BISWAS and S. P. SEN, Nature *181*, 1219 (1958).

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 $^{14}CO_2$ fixation in *Chlorella pyrenoidosa* and *Nostoc muscorum*
(Data expressed as % variation from control for each compound)

Compound	*C. pyrenoidosa*		*N. muscorum*	
	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 $NaH^{14}CO_3$ 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 $NaH^{14}CO_3$ 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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Radiochemical Laboratory, Bose Research Institute, Calcutta (India), June 5, 1959.

Zusammenfassung

Es scheint, dass DNase und RNase bei der photosynthetischen Fixierung des CO_2 verschiedene und komplizierte Wirkung ausüben. Die Saccharaose-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 consideration[1]. Among the more probable intermediates in the metabolism of cysteine or cystine, only the cystine monosulfoxide has been neither isolated nor synthesized. Its preparation was unsuccessfully attempted by TOENNIES and LAVINE[2–4]. Cystine monosulfoxide is of growing theoretical interest since simple thiosulfinic esters (monosulfoxides of alkyl disulfides) studied by WEISBERGER and PENSKY[5] were shown to possess cancer blocking activity, which these authors derived from the inhibiting effect of allicin on sulfhydryl (-SH) containing enzymes. Allicine (allylthiosulfinic allyl ester) is an enzymic metabolite of alliine, 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 LAVINE[3].

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, $[\alpha]_D^{24} = -111°$ (± 3°), 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).

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[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. STOLL and E. SEEBECK, Helv. chim. Acta *31*, 189 (1948).

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