

Table III. 1-Methyladenine-containing sequences (from 41)

Base residues per tRNA chain	Methylation in vitro		Endogenous 1-Methyladenine	
	<i>E. coli</i> tRNA 0.9	Yeast tRNA 0.2	Yeast tRNA 0.55	Liver tRNA 0.90
Sequences				
Py-A-1MA-U	—	4%	0.05	0.05
Py-G-1MA-U	—	35%	0.20	0.20
Py-G-1MA-C	—	8%	0.05	0.10
Py-A-1MA-A-U	10%	20%	0.10	0.30
Py-G-1MA-A-U	90%	23%	0.10	0.20
Py-G-1MA-A-C	—	10%	0.05	0.05

the problem why some tRNAs are methylated and others are not. Of the known yeast tRNAs, for instance, tyrosine-, valine-, isoleucine- and phenylalanine-tRNA contain m¹A, whereas the three others don't, although they can be methylated by rat liver methylase. Thus as yet no clear picture as to the reason for the different extent of methylation emerges. From our knowledge of the specificity of tRNA methylases and from the known sequences it appears only that the question to be raised should not be anymore: why have some nucleotides been modified, but rather: why has the

modification not occurred in all tRNAs, since there are apparently enzymes capable of modifying the parent residues in these positions?

Zusammenfassung. Aus einem Vergleich der beschriebenen Sequenzen verschiedener Transferribonukleinsäuren lassen sich Schlüsse über die allgemeine Struktur dieser Moleküle ziehen. Sämtliche Sequenzen lassen Basenpaarungen zu, die sich zweidimensional in einer Kleeblattform darstellen lassen. Die bisher bekannten Transferribonukleinsäuren lassen sich in 3 Klassen aufteilen, je nachdem, ob sie 3–4 Basenpaare im DHU- und einen kurzen oder langen Extra-Arm besitzen. Die Verteilung der seltenen Nukleotide weist eine auffallende Regelmässigkeit auf. Methylierte Basen kommen meistens nur an einer bestimmten Stelle des Moleküls vor, nur vier wurden an zwei Stellen beschrieben. Dihydrouridin und Pseudouridin wurden an 6 bzw. 9 Stellen gefunden. Dies dürfte darauf hinweisen, dass die Enzyme, die Nukleotide in der Transferribonukleinsäure modifizieren, nicht spezifische Transferribonukleinsäuren erkennen, sondern spezifische Stellen in den verschiedensten Molekülen. Anhand der serinspezifischen Transferribonukleinsäuren aus Rattenleber wird gezeigt, dass multiple Species vorliegen, die zum Teil das gleiche Codon erkennen können, sich aber in der Primärsequenz unterscheiden.

SPECIALIA

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Modifications of Lichen Substances and Morphology Induced by Mechanical Shock in *Cladonia pacifica*

Microchemical and crystalline tests of thalli of shocked *Cladonia pacifica* revealed additional substances not known to occur in this lichen and which did not occur in the controls. The only morphological alteration after shock was the color of the thallus. Control thalli were white, whereas shocked thalli were tan to khaki in color.

Lichens are considered to be hardy plants because of their wide geographic distribution and their adaptation to xeric habitats, being very resistant to extremes of temperature and drying. They are, however, highly responsive to environmental factors^{1–6}. The present study examines the gross morphology of lichen thalli and lichen substances after exposing the lichens to mechanical shock, where shock is defined as a fast-rising pressure pulse of several seconds duration. The present work stems from an interest in the possibilities for using plants as experimental bio-indicators of underground shock⁷.

Since lichens are such slow growing plants, only obvious changes in their appearance and obvious micro-

chemical changes were observed in the present study in which unshocked plants were used as controls. Because the lichen acids are mostly phenolic derivatives, 2 post-shock periods were selected to allow for accumulation of any newly formed acids or degradation products of the existing acids.

¹ D. N. RAO and F. LeBLANC, *Bryologist* 69, 69 (1965).

² A. W. HERRE, *Am. Midl. Nat.* 28, 752 (1942).

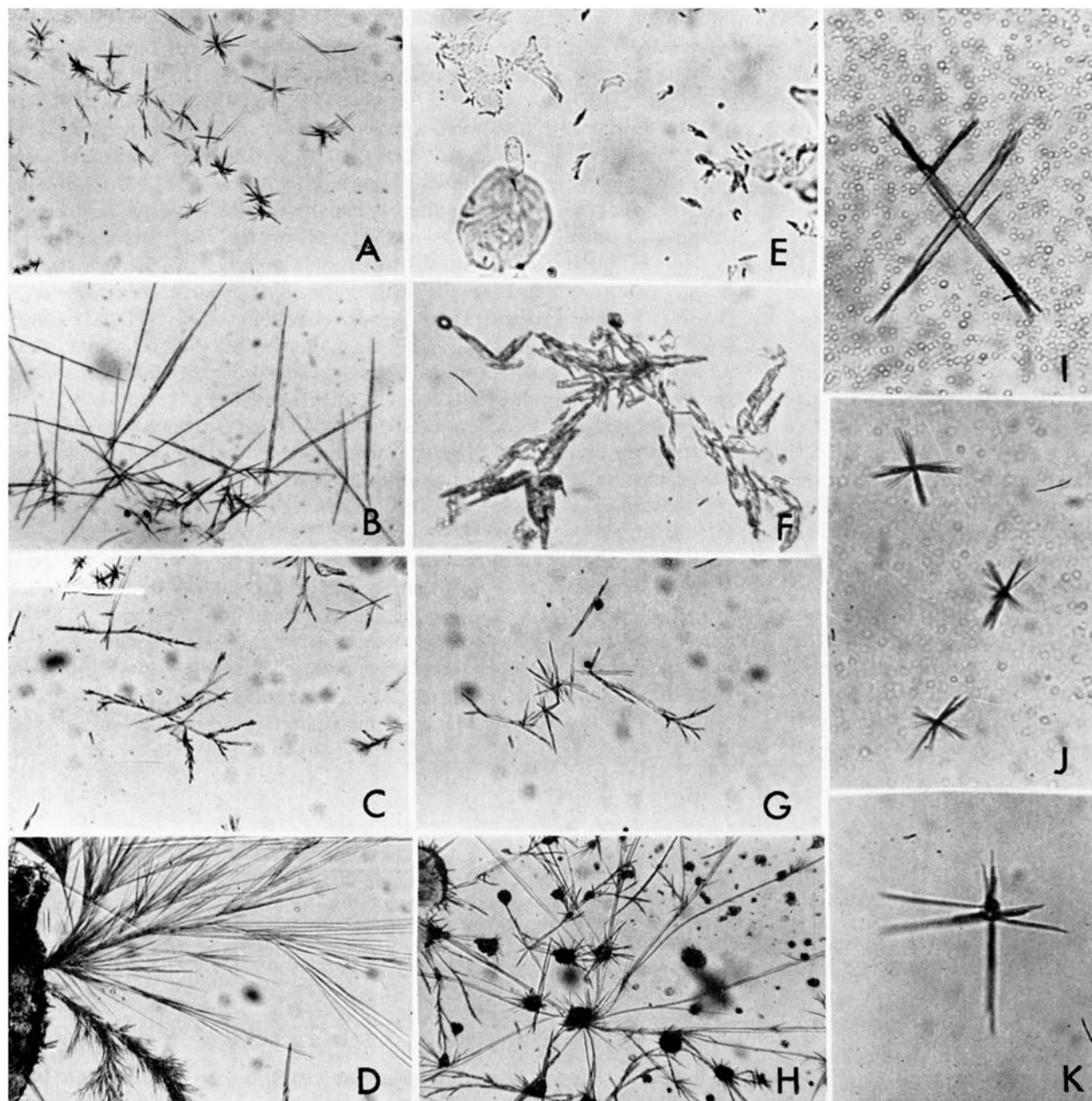
³ W. A. WEBER, *Svensk. bot. Tidskr.* 56, 293 (1962).

⁴ D. C. SMITH, *Ann. Bot.* 24, 52, 172, 186 (1960).

⁵ P. F. SCHLOLANDER, W. FLAGG, V. WALTERS and L. IRVING, *Am. J. Bot.* 39, 707 (1952).

⁶ P. W. RUNDELL, *Bryologist* 72, 40 (1969).

⁷ C. L. NEWCOMBE, *An Experimental Study of Shock Effects on Surface and Subsurface Organisms* (U.S. Naval Radiological Defense Laboratory, San Francisco, Calif. USNRDL-TRC-69-23, 1969).



Results of the microcrystal tests in *Cladonia pacifica* for control thalli (A, E, and I) and thalli shocked at 50 Ψ 2 weeks after shock (B, F, and J) and 5 weeks after shock (C, D, G, H, and K), where A–D were treated with GE, E–H with GAW, and I–K with GAA. (A) and (B), usnic acid; (C) and (E) unidentified substance; (D), evernic acid; (F), novochlorophaeic acid; (G), lobaric or didymic acid; (H), atranorine or cryptochlorophaeic acid; (I), atranorine; (J) and (K), usnic acid.

Materials and methods. An Alaskan lichen from Amchitka was collected by Dr. H. T. SHACKLETTE and identified by Dr. W. A. WEBER as *Cladonia pacifica*. THOMSON⁸ treated this taxon as a synonym of *C. impexa* Harm. but AHTI⁹ retained it as separate species. The living lichens were cultured at room temperature on moistened soil mix and under 12 h of low-level (about 10 foot candles) indirect light each day. Several weeks after culturing, the cortical areoles of the thalli in many plants took on a greenish hue; however, in most plants they remained white. The lichens were allowed to acclimatize to laboratory conditions for about 6 weeks, then they were subjected to shock treatment. The culture was sub-

divided into a control and 2 test fractions. The test fractions were shocked at 50 pounds per square inch (ψ ; 3.52 kg/cm²) in an air loader¹⁰, the second test being 3 weeks after the first test. The pressure duration was approximately 4–6 sec.

⁸ J. W. THOMSON, *The Lichen Genus Cladonia in North America* (University of Toronto Press, Toronto 1968).

⁹ T. AHTI, *Bryologist* 71, 292 (1968).

¹⁰ S. A. MURRAY, *Experientia* 26, 319 (1970).

The techniques for the thalline color test and crystal tests as outlined by HALE¹¹ were used. The lichen substances were extracted from thallus tips with warm acetone. 2 or 3 extractions of each shock group and the control were prepared for each reagent used in the microcrystal tests. All of the tests for each culture group were done in time sequence. The slides were observed immediately after the reaction and at periodical intervals up to 6 months time. The reactions were immediate and stable, and no new substances formed during this period.

Results and discussion. Thalli of the shocked lichens appeared tan to khaki in color compared to the white color of the control lichens. The noted color change was observed several days after shock. No other morphological changes were observed in the shocked plants.

The thalline tests were performed on specimens consisting of half a lichen thallus and were observed under low magnifications. The results of the thallus tests agree with those listed by THOMSON⁸ for *C. impexa*. There were no changes in the color tests after shock.

The results of the microcrystal tests were compared with pictures and descriptions of crystalline acids as given by ASAHINA¹², EVANS¹³, HALE^{11,14} and THOMSON⁸. 3 reagents, namely, glycerin-acetic acid, 1:1 (GE), glycerin-95% alcohol-water, 1:1:1 (GAW), and glycerin-alcohol-aniline 2:2:1 (GAAn) gave positive crystal tests for all groups. No crystals were observed with glycerin-alcohol-*o*-toluidine, 2:2:1 (GAo-T), or glycerin-alcohol-quinoline, 2:2:1 (GAQ). The results of crystal tests are indicated in the Figure. Row 1 (A, E, I) represents crystals occurring in the control lichens, row 2 (B, F, J) 2 weeks post shock, and rows 3 and 4 (C, D, G, H, K) 5 weeks post shock. All those in column 1 were treated identically with GE reagent, whereas those in column 2 were treated with GAW and column 3 with GAAn.

ASAHINA's microcrystal tests¹² are useful in determining which substances occur in the thallus. An attempt was made to standardize this technique in the present study by holding the slides one inch above the flame and to remove them as soon as a bubble formed. The slides were air cooled.

Anthraquinone in Plant Surface Waxes

The most abundant constituents of the surface waxes of plants are aliphatic compounds, for example hydrocarbons, esters, aldehydes, ketones, acids and alcohols¹. Although terpenoid and steroidal compounds² are present in some waxes, few waxes are known to contain aromatic structures. We wish to report the occurrence of anthraquinone in a cuticular wax [*Lolium perenne* L. (perennial rye grass)].

Lolium perenne L. was grown in seed boxes under varying conditions of light and temperature, harvested and extracted with chloroform to remove the surface wax (0.1–0.2% by weight of the freshly harvested grass). Separation of the surface wax by thin layer chromatography (TLC) on silica gel with a solvent of 5% diethyl ether: light petroleum (40–60°C) revealed a small spot on visualization with dichlorofluorescein, of R_f 0.16. This fraction was isolated by preparative TLC, and GLC analysis gave a component of retention Index (I) = 2150 on 5% Apiezon L at 235°; this component had I = 2430 on 3% OV-17 at 235°. GLC analyses at higher temperatures showed components of greater retention indices

The predominant acids in the control were usnic and atranorine. It is possible that the concentration of these acids was depressed after shock, thereby permitting the detection of other substances. Perhaps in the present study, various reactions, such as oxidations, recombinations and other reactions¹⁵ were initiated by shock. However, such substances which were observed after shock treatment were not observed in the controls and are not typically found in *C. impexa* or presumably *C. pacifica*. *C. pacifica* must contain the same substance as *C. impexa* since apparently there is no contradictory information on this lichen. Therefore, the modification in the crystals are attributed to shock treatment¹⁶.

Résumé. Une augmentation rapide de la pression produisant comme une rafale d'air dans un tube de choc fit apparaître des substances additionnelles et des modifications de couleur dans les talles du lichen *Cladonia pacifica*.

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¹¹ M. E. HALE, *Lichen Handbook* (Smithsonian Inst., Wash. D.C. 1961), Publ. No. 4434.

¹² Y. ASAHINA, *Jap. J. Bot.* 12–16 (1936–1940).

¹³ A. W. EVANS, *Bull. Torrey bot. Club.* 70, 139 (1943).

¹⁴ M. E. HALE, *The Biology of Lichens* (Edward Arnold, Publishers Ltd., London 1967).

¹⁵ S. NEELAKANTAN, in: *Advancing Frontiers in the Chemistry of Natural Products* (Hindustani Publ. 1965), p. 34.

¹⁶ This work was supported in part by funds from Advanced Research Projects Agency, Department of Defense, through the U.S. Geological Survey, under A.R.P.A. Order No. 938. Special thanks are expressed to Dr. C. L. NEWCOMBE of San Francisco State College, and Dr. H. T. SHACKLETTE of the Geological Survey for making this study possible. I also thank Dr. J. A. ERDMAN of USGS for helpful suggestions and Mrs. DORIS E. BALZO of the Lichen Herbarium at the University of California, Berkeley, for critically reading the manuscript.

present in this fraction, and these are still under investigation.

GC/MS determination of the peak with retention Index = 2150 on Apiezon L on an AEI/MS 902 gave a parent ion at 208 *m/e* and fragments at 180, 152 and 76 *m/e*, which suggested anthraquinone. A sample of authentic anthraquinone was found to have similar chromatographic characteristics to those quoted above, and the UV-spectrum (λ_{max} 252.5 nm, 264 nm, 273 nm, 324 nm) of the compound from *Lolium perenne* L. was identical to that of anthraquinone. The extinction coefficients showed that anthraquinone accounts for 0.2% of the total wax.

In addition, the IR-spectrum of the total fraction, run as a potassium bromide disc, showed absorption at

¹ J. E. ALLEBONE, R. J. HAMILTON, B. A. KNIGHTS, B. S. MIDDLEDITCH and D. M. POWER, *Chem. Phys. Lipids* 4, 37 (1970).

² G. EGLINTON and R. J. HAMILTON, *Science* 156, 1322 (1967).