Reports

Apollo 12 Lunar Material: Effects on Lipid Levels of Tobacco Tissue Cultures

Abstract. *Tobacco tissue cultures grown in contact with lunar material from Apollo 12, for a 12-week period, resulted in fluctuations of both the relative and absolute concentrations of endogenous sterols and fatty acids. The experimental tissues contained higher concentrations of sterols than the controls did. The ratio of campesterol to stigmasterol was greater than 1 in control tissues, but less than 1 in the experimental tissues after 3 weeks. High relative concentrations (17.1 to 22.2 percent) of an unidentified compound or compounds were found only in control tissues that were 3 to 9 weeks of age.*

Certain plant species in germ-free cultures responded favorably when grown in contact with lunar material during quarantine periods of Apollo 11 and Apollo 12 (*1*). In our initial studies on the nature of this response, we reported that tobacco tissue grown in contact with the lunar material contained significantly more chlorophyllous pigmentation than the control tissues (*2*). We now report the results of fatty acid and sterol analyses from tobacco tissue cultures grown in contact with Apollo 12 lunar material for a 12-week period.

Tissue cultures of tobacco (*Nicotiana tabacum*) were grown in contact with 0.22 g of lunar material and treated as described (*1*). Control cultures were treated in an identical manner but without the addition of lunar material. All lipids were extracted from tissues at 3-week growth intervals, first with acetone, and then with a mixture of chloroform and methanol (3 : 1). Nonlipid components were removed by partitioning them three times between distilled water and *n*-hexane. Both phases were evaporated to dryness and stored at −4°C. The total sterols (free and esterified) and the fatty acids were isolated from the alkaline hydrolyzate of one-half of the total lipid extract. The sterols and fatty acids were then analyzed by gas chromatography alone and by the combination of gas chromatography and mass spectrometry (*3–6*). Fatty acids were separated in the form of their methyl esters, on Chromosorb Q coated with 3 percent diethylene glycol succinate and packed in a stainless steel column (1.8 m by 3 mm). The sterol components were analyzed in their free form and also as the acetate and trimethylsilyl ether derivatives on Chromosorb Q coated with 1 percent OV-17 and packed in a glass column (1.8 m by 3 mm). Column temperatures were maintained at 190° and 265°C for the fatty acid and sterol analyses, respectively (*4*).

Differences in the viable-cell ratio of the cultures were present (*7*). No differences were noted in the total mass or the growth rates of the experimental and control cultures. The relatively slow growth rate of these tissues was attributed to the small size of the initial culture inoculum.

Variations in experimental and control cultures became obvious when they were compared at the molecular level. The concentrations of total sterols in experimental tissues were higher than in the controls throughout the experiment. From the 3- to 9-week period of the experiment, these concentrations in the experimental cultures ranged from 128.2 to 220.1 μg per gram (fresh weight) with an approximate ninefold increase during the 9- to 12-week period. However, the amount of sterol in the control group ranged from 58.4 to 128.8 μg/g (fresh weight) and remained below those of the experimental groups for each age. An approximate fivefold increase was noted during the 9- to 12-week period. Stigmasterol, campesterol, and β-sitosterol were the major sterols identified in the tissues studied, and their presence is consistent with previous reports of sterols found in tissues of tobacco origin (*8–14*). However, a notable exception was the absence of cholesterol in the tissues used in our study. These tissues contained a mixture of two compounds with molecular weights of 320 and 322 (No. 3, Table 1) that had, in gas liquid chromatography (GLC), the identical retention time of cholesterol. Cholesterol was later detected, however, when larger quantities of tissue were analyzed.

Additional sterol or triterpenoid components were noted in low relative concentrations in tissues like those in our study and had molecular weights in the 412 and 426 range. These molecular weights correspond to the 4,4-dimethyl- and 4α-methylsterols previously reported for large quantities (200 g, dry weight) of tobacco tissue cultures (*9*). The remaining compounds were in such low concentrations in the quantities of tissues examined that reliable molecular weights could not be determined for

Table 1. Changes in the relative concentration of sterols (percent of total) of experimental (containing Apollo 12 lunar material) and control (without particulate material) tobacco tissue cultures; E and C are experimental (containing 0.22 g of lunar material) and control cultures, respectively.

Compound	Sterols (%) in cultures at week:								
	1	3		6		9		12	
	E and C*	E	C	E	C	E	C	E	C
Unidentified 1	0.9	0.8	Tr†	0.1	Tr	Tr	Tr		
2	1.4	0.6	Tr	0.3	0.3	0.2	0.1	0.3	Tr
3‡	2.6	1.1	1.8	2.3	1.7	3.0	1.7	2.7	1.6
Campesterol	9.3	22.7	6.4	19.2	8.1	21.6	5.8	11.8	7.2
Stigmasterol	11.3	27.5	8.7	13.9	15.1	18.1	13.7	10.6	8.8
β-Sitosterol	71.0	44.5	60.9	63.2	42.7	55.5	47.1	73.5	81.0
Unidentified 4	Tr	Tr		Tr	Tr	Tr			Tr
5	0.7	2.0	7.4	1.3	9.3	0.5	12.4	0.3	0.8
6	1.9	2.6	17.1	1.0	22.2	0.3	19.0	0.3	2.5
7	0.7	0.1	Tr	0.3	Tr	0.9	Tr		Tr
8	Tr	2.4	Tr	Tr	0.7		Tr		
Campesterol/ stigmasterol	1.21	1.21	1.35	0.72	1.82	0.84	2.36	0.89	1.22

* Relative percent distribution of the sterols of tissues used for inoculum. † Trace. ‡ Mixture of two compounds with molecular weights of 320 and 322, and with GLC retention times similar to that of cholesterol.

the compounds by mass spectrometry. There was a difference in the relative distribution of the major sterol components (Table 1) of the experimental and control tissues throughout the 12 weeks of growth.

The reciprocal relation between campesterol and stigmasterol in the experimental and control tissues (Table 1) is of particular significance. In controls the ratio of campesterol to stigmasterol (C/S) was greater than 1 throughout the 12 weeks of growth, whereas in the experimental tissues the ratios were less than 1 during the 6- to 12-week periods. The C/S ratio in the 3-week-old experimental tissues was identical to that in the tissues at the outset of this experiment. Campesterol and stigmasterol appear to have no direct precursor-product relationship (*11*). Another major difference in the relative sterol composition of these tissues was the increased relative concentration of compound No. 6 (Table 1) of 17.1, 22.2, and 19.0 percent in the 3-, 6-, and 9-week-old control cultures, respectively, and the decrease to 2.5 percent in the 12-week cultures. A corresponding increase at 3, 6, and 9 weeks of this component did not occur in the experimental tissues. Upon preliminary examination of 12-week-old tobacco tissue cultures grown in contact with basalt materials found on Earth, no differences in the relative or absolute sterol concentrations of experimental and control tissues were noted.

When examined, the fatty acid constitutents were identical to those previously reported for tobacco tissue cultures (*15*), and no qualitative differences were found between the experimental and control tissues. However, there were quantitative differences in relative and absolute fatty acid concentrations in the experimental and control tissues over the 12-week growth period.

In summary, greater total sterol concentrations were present in tobacco tissue cultures grown in contact with lunar material than in those grown without particulate material. The relative concentrations of phytosterols and their potential precursors were also significantly different. Absolute and relative differences in fatty-acid concentration also were found. No gross differences were apparent in the size or mass of the culture. These results are consistent with previous studies which show higher concentrations of pigment in similarly treated experimental tissues. The findings of increased growth or pigment production for an algal species grown in contact with the lunar material returned by Apollo 11 (*16*) supports previous observations and the results presented here. The metabolic significance of the differences in relative and absolute sterol concentrations of the experimental and control tissues could not be explained from these data. The above data do, however, reflect the metabolic response by a sensitive plant system whose environment has been altered, presumably by available nutrients (*2*, *16*) provided by lunar material.

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Aragonite Crystals within Codiacean Algae: Distinctive Morphology and Sedimentary Implications

Abstract. *Morphologic studies of single crystals of aragonite within Codiacean algae reveal characteristic crystal forms produced by two distinctly different modes of calcification. Diagnostic serrated crystals (1 micrometer in length) of aragonite originating within the extracellular sheaths of capitular filaments are incorporated into modern lime sediments and may serve as effective tracers for particles of algal origin. Intracellular calcification within* Penicillus dumetosus, *previously unreported, is represented by doubly terminated aragonite crystals ranging in size from 48 to 160 micrometers.*

The origin of both recent and ancient lime mud sediments has perplexed geologists for decades. Much attention has been focused on the origin of modern aragonitic lime mud inasmuch as it is believed to be the precursor of calcitic mud matrices found in ancient carbonate rocks. Physical breakage and abrasion of skeletal debris in agitated waters provides one mechanism for lime mud production (*1*). Extensive lime mud deposits found in relatively quiet sheltered waters, such as Florida Bay and the Great Bahama Bank, however, pose a different problem. These muds are characterized by an abundance of elongate single crystals of aragonite that are generally less than 15 μm.

Some workers (*2*, *3*) believed this aragonite to be a nonskeletal, physicochemical precipitate; others (*4*) suggested the possibility of direct biochemical precipitation through the metabolic activity and decay of bacteria. An alternate hypothesis was proposed by Lowenstam (*5*) who reported the precipitation of prismatic aragonite "needles," 2 to 10 μm in length, as an integral skeletal component in several green algae and suggested algal disintegration products as a source of lime mud. Although some workers (*3*, *6*) doubted the capability of algae to produce large quantities of lime mud, their potential as prolific producers of lime mud has been demonstrated (*7*) in the shallow marine waters of south Florida.

Within the 2- to 10-μm size fraction, it is virtually impossible to distinguish morphologically an aragonite "needle"