prove effective, but 1000 volts d-c gave negative results with *C. cinctus* larvae at −12°C when a 3/8-in. air gap and exposures up to 1 minute were used.

The necessity of having the sample close to an electrode or even forming an extension of it suggests that the conditions used in these experiments were near-minimal. The incomplete response by *E. solidaginis* larvae also supports such a view. However, if the field is strengthened by decreasing the gap, discharges will occur. Although nucleation was induced under these conditions, the side effects of the sparks, such as possible heating, were not investigated.

These results represent a preliminary excursion into a field that is new, biologically at least, and it is quite likely that some aspects that will later assume importance have been neglected or overlooked at this time. It has been demonstrated, however, that supercooled water and certain insects can be induced to freeze at higher temperatures than otherwise when placed in an electrostatic field. The effect becomes more certain and occurs earlier as the amount of supercooling is increased.

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1 August 1960

Lipids of Ankistrodesmus braunii

Abstract. Ankistrodesmus braunii was grown to stationary phase on a chemically defined medium and its cellular lipids were analyzed. The lipid content was found to vary from 18 to 73 percent of dry weight for cultures of different age and method of analysis. The pigments of the nonsaponifiable fraction were separated by adsorption chromatography and counter-current extraction and tentatively identified. The fatty acid fraction was converted to the corresponding methyl esters and analyzed by gas chromatography. The principal fatty acids present were: palmitic, oleic, and linolenic acids. Traces were detected of caprylic, capric, lauric, and palmitoleic acids.

Previous microscopic studies of *Ankistrodesmus braunii* during stationary phase indicated that the quantity of lipids synthesized by this green alga might be equal to or greater than that made by *Chlorella* spp. and related organisms (*1*). Potential use of algal metabolic products both as human nutritional supplements and in the closed ecosystems of space travel invited further investigation of the lipids of *A. braunii*.

Cultures of *A. braunii* strain 750 (Indiana University algal culture collection = McMillan clone 245-St 1.1) were grown in large erlenmeyer flasks to stationary phase in GFS medium previously described (*1*). The cells were maintained at 25° to 27°C with continuous daylight fluorescent illumination of 400 to 500 ft-ca for 4 to 8 weeks.

Cells were harvested by centrifugation and washed once with water. They were then suspended in 95 percent ethanol and placed in a boiling-water bath for 15 minutes to inactivate enzymes. The suspension was cooled, an equal volume of hexane was added, and the system was treated for 30 minutes in a 9 kcy/sec Raytheon sonic oscillator at maximum frequency. The suspension was transferred to a flask, most of the hexane was removed under vacuum, and the volume was made up with alcohol. A large aliquot was transferred to a boiling flask, potassium hydroxide was added, and the solution was saponified under reflux for 2 hours. The alkaline saponification mixture was extracted with hexane, then it was acidified and extracted with ether by the usual technique. All fractions were made up to volume and aliquots were removed for compositional analysis.

Pigments were separated on columns of powdered sugar and of anhydrous calcium carbonate by the procedure of Cowgill and Pardee (*2*). The hexane-soluble pigments were also subjected to countercurrent extraction on a 100-plate Craig apparatus in a solvent system consisting of 1.8 parts of petroleum ether and 1.0 part of 99 percent methanol.

Methyl esters of the algal fatty acids were prepared by the method of Clinton and Laskowski (*3*). Gas chromatographic separations were achieved on the Beckman GC-2 gas chromatograph with an 18-foot column of Resoflex R-446. Identification was based on standard samples.

Varying results were obtained in the determination of lipid content of *A. braunii* in stationary phase, depending on the age of the culture and method of analysis. Total lipids ranged from 18.6 to 33.7 percent for cultures from 4 to 7 weeks of age respectively. This is comparable to the findings of Collyer and Fogg (*4*) for a number of Chlorophyceae. Of the total lipids, 85.5 percent were recovered in the following distribution: nonsaponifiable, 17.1 percent; saponifiable, 61.5 percent; and glycerol, 6.8 percent. In one experiment where lipids were extracted from vacuum-dried cells, a lipid content of 72.8 percent was obtained, a value which would appear quite unreasonable were it not for similar observations of extremely high lipid content in cells subjected to vacuum drying while still living (*5*).

Adsorption chromatography and countercurrent distribution led to the separation of four principal pigments which were detectable by both techniques. Identification was based on known carotenoid spectra and is tentative: beta carotene, astaxanthin, lutein, and possibly a derivative of neoxanthin. The correct spectra and solubility were obtained for the first three. All of these pigments have previously been detected in algae (*6*).

The characteristic fatty acid composition of *A. braunii* in stationary phase is shown in Fig. 1. The principal fatty acids were found to be palmitic, oleic, and linolenic acids with traces of caprylic, capric, lauric, and palmitoleic acids. One sample showed a trace of linoleic acid. By the peak area method, the relative amounts of the three principal acids were linolenic acid, 13 percent; oleic acid, 54 percent; and palmitic acid, 33 percent. This algal fatty acid fraction is generally similar in composition to other vegetable oils, although

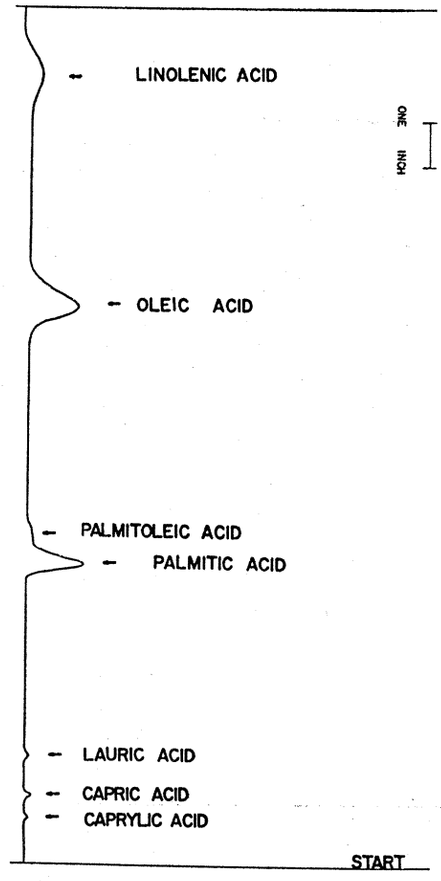

Fig. 1. Gas chromatogram of methyl esters of fatty acids isolated from *A. braunii*. Column: 18-foot Resoflex R-446; helium flow rate, 140 cm³/min; filament current, 320 ma; temperature, 240°C; chart speed, 0.4 in./min.

the predominance of linolenic acid over linoleic is somewhat unusual (*7*). Like the vegetable oils, algal oil would be digestible and nutritious for human beings.

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21 September 1960

Appearance of a Spring Maximum in Nuclear Test Debris in 1960

Abstract. The existence of a spring maximum in fallout deposition due to the disruption of the tropopause in middle latitudes has been debated for the past several years. Evidence obtained from air filters indicates elevated levels of radioactivity from nuclear tests in surface air during April through July 1960.

Increased concentrations of fission product radioactivity, primarily Sr^{90} and Cs^{137}, observed in spring rains led Stewart *et al.* (*1*) to postulate a seasonal variation in the rate of release of stratospheric debris. Earlier Brewer (*2*) and Dobson (*3*) attributed the springtime increase in levels of stratosphere-produced ozone found in surface air to a similar seasonal variation. Martell (*4*) on the other hand has pointed out that such maxima in fission activity have coincided with, or followed shortly after, major test series, particularly those tests conducted in more northern latitudes. Inherent in Martell's supposition is the assumption that debris from more northerly sites has a materially shorter stratospheric residence time than does that coming from equatorial tests. This assumption seems to be justified (*5*), and, indeed, tests were carried out at northern latitudes during the springs of 1957 and 1958. The spring maximum seen in 1959 may also fall into this category since a fair portion of the activity observed originated in the Soviet October 1958 series (*6*).

Due to the cessation of nuclear tests in October 1958, and to the degree of mixing in the upper atmosphere which took place by early 1960, any maximum observed should be the result of meteorological mechanisms rather than recent testing. Some caution is still in order, however, since two nuclear detonations (albeit relatively small) were carried out by France in North Africa in February and April 1960. Therefore a rise in gross fission activity may be subject to misinterpretation, although analysis for specific radionuclides would avoid this difficulty.

Surface air-borne activity was collected from approximately 5×10^5 cubic meters of air per month by means of HV-70 (Hollingsworth-Vose) filters placed in air pumps located on the Argonne site and adjacent area (*7*). Measurements of radioactivity were made on ashed filters by means of sodium iodide scintillation spectrometry (*8*). The monthly sums of activity (gross gamma) due to Ce^{144}, Ru^{106}, Cs^{137}, and Zr^{95}-Nb^{95} in curies per cubic meter are shown in Fig. 1 for the time interval of January 1958 through July 1960. Maxima in the springs of 1958 and 1959, as well as a secondary maximum in the fall of 1958, are clearly evident. Those in 1958 coincide fairly well with the immediately preceding test series as indicated. Similar behavior is evidenced by Cs^{137} from January 1957 through July 1960.

In the spring of 1960 the gross gamma activity shows a rise during April through June, with a somewhat broader peak for Cs^{137} than for the mixture. A portion of the gross gamma activity in February and March was due to Ce^{141} and Ru^{103}, thus causing the relative increase in April to be somewhat less pronounced than in the case of Cs^{137}. Short-lived fission products were also present in precipitation at Argonne during this time, and were observed in the air as far north as Great Britain (*9*). A fivefold increase in Cs^{137} was seen in the spring during 1957 through 1959, whereas only a twofold increase was observed in 1960. This may reflect the presence of higher relative concentrations of debris in the polar stratosphere during past years than prevailed during the spring of 1960. In any event there appears to be a definite spring maximum in fission product radioactivity in 1960 which is not attributable to recent testing.

It should be mentioned that a portion of the total Cs^{137} activity present in the spring of 1960 (as much as 10 percent) was associated with debris from high-altitude missile detonations (August 1958) as indicated by increased con-

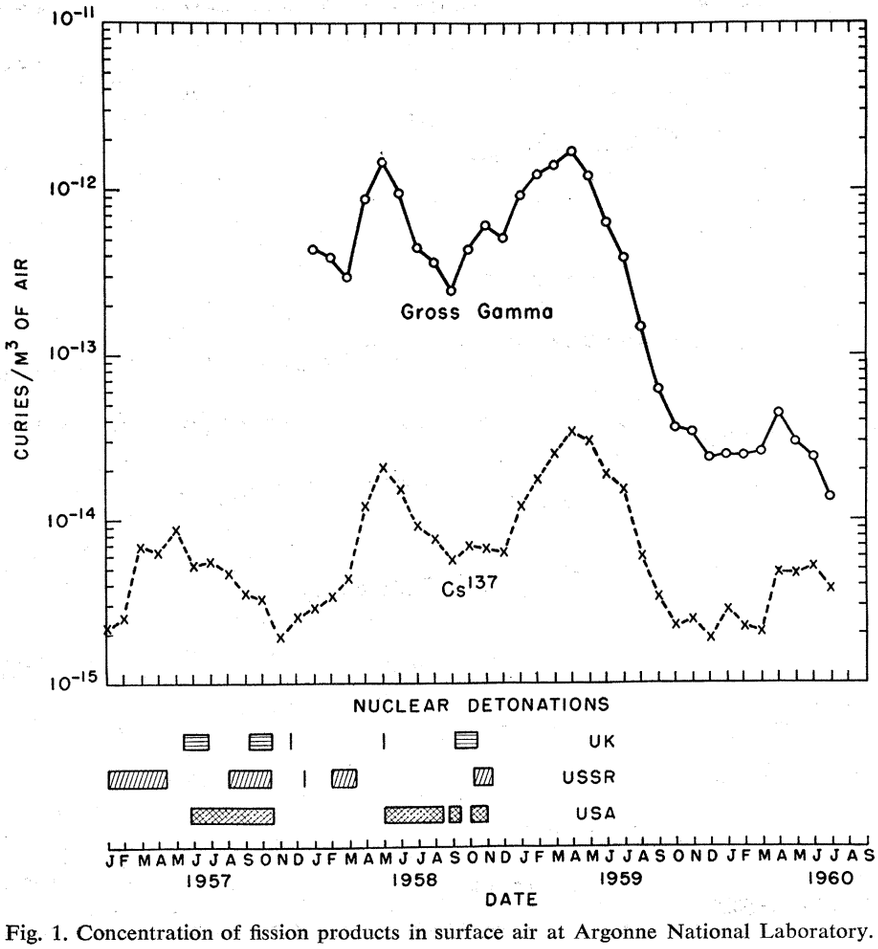

Fig. 1. Concentration of fission products in surface air at Argonne National Laboratory.