

messenger synthesis may result from catabolite repression (8) and is manifest before significant inhibition of protein formation has occurred. These data with puromycin are in accord with the findings of Paigen (9), who reported that chloramphenicol suppressed induced enzyme formation while protein synthesis was unaffected.

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Fatty Acids in Blue-Green

Algal Mat Communities

Abstract. The concentrations of the major fatty acids were determined for the varied layers of an algal mat community. The living mat contains substantial amounts of the unsaturated acids, while the underlying layers become progressively depleted in unsaturated molecules. A similar progressive increase in the ratio of saturated to unsaturated acids was detected in a sediment core from a hypersaline lagoon.

The fatty acids are major components of all living organisms. As such they may be an important source material for petroleum and perhaps for the ubiquitous kerogen. Fatty acids have been found in recent and ancient sediments by Cooper and Bray (1) and by Abelson *et al.* (2). However, the chemical procedure of Cooper and Bray excluded unsaturated acids. The algal mats and their associated sediments provide a natural laboratory in which to test the relative survivability of different fatty acids.

Extensive algal mat communities in

the Texas lagoons and mud flats develop in water from 1 to 50 centimeters deep. Many organisms are present in the mat but the biomass is largely made up of the filamentous blue-green algae, *Lyngbya confervoids* (3). Well-developed communities have a structure which is of use to the geochemist. The living mat covers the mud bottom like a sheet of leather. Directly under the living mat is a layer of black mud at times a few centimeters thick. The remains of a former mat are under the first mud layer, and they too have a mud layer under them. This pattern of mat and mud repeats itself. In the samples taken for study, the living mat, two former mats, and two mud layers were distinct. All samples were frozen at the time of collection and kept frozen until used.

The analytical procedure was designed to detect fatty acids containing 12 to 20 carbon atoms. Care was taken to minimize decomposition of the unsaturated acids. The frozen sample was treated with dilute HCl to remove carbonate. After filtration the moist sample was treated with methanol for 15 minutes while being stirred and subjected to ultrasonic vibrations. The methanol was recovered by filtration. The sample was treated with chloroform and again stirred and subjected to ultrasonic vibrations. The recovered chloroform was combined with the methanol, and the mixture almost completely dried with a rotating evaporator. This residue was taken up in chloroform and washed with 1N HCl to remove inorganic salts. The chloroform was dried under a stream of dry nitrogen. After saponification with potassium hydroxide, impurities were removed by extracting the basic alkaline solution with chloroform. Finally the alkaline solution was acidified, and the fatty acids were extracted into chloroform. The methyl esters were prepared with BF_3 (4).

The methyl esters were identified and measured by gas chromatography on a diethyleneglycol succinate column (5). Either an Aerograph 202 or an F & M model 400 instrument was used. The chemical yields for the procedure were good, 75 to 95 percent as determined with the use of carbon-14-labeled palmitic acid. The organic carbon content of the sediments was determined by combustion with CuO on a vacuum line, freezing out the CO_2 , and allowing it to expand against a calibrated manometer.

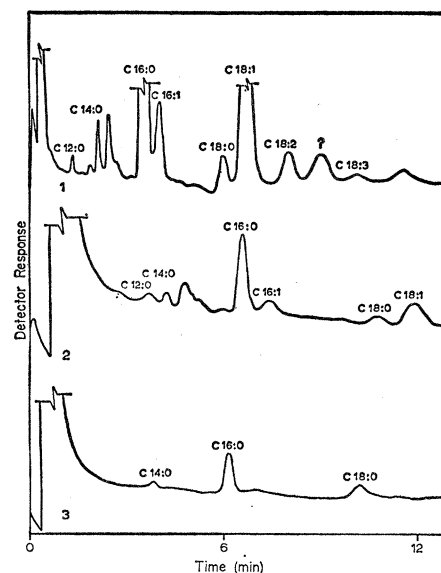


Fig. 1. Gas chromatogram of methyl esters of fatty acids from algal mat: 1, the living mat; 2, first mud layer; 3, second mud layer.

All recent sediments examined thus far are rich in fatty acids (Table 1). All the molecules identified are those which have even numbers of carbon atoms, and which are usually found in organisms. A few unidentified peaks have been encountered, but they do not correspond to any straight-chain fatty acids with even or odd carbon numbers. In the algal mat acids the most striking observation is the systematic change in the ratio of saturated to unsaturated acids (Fig. 1).

The living mat is rich in the unsaturated acids, especially oleic acid. The relative survivability of the different acids is proportional to the degree of unsaturation. This suggests that chemical interactions, such as polymerization, among the organic com-

Table 1. Concentration of the major fatty acids in recent sediments expressed as parts of fatty acid per million parts of organic carbon of each sediment. n.d., not detected.

Location	Organic carbon (%)	C ₁₆ (0)*	C ₁₆ (1)	C ₁₈ (0)	C ₁₈ (1)
<i>Harbor Island algal mat</i>					
Living mat †	32	3100	560	130	1200
1st mud layer	1.1	1200	330	180	330
2nd mud layer	0.84	200	23	97	n.d.
<i>Baffin Bay core</i>					
0-10 cm	2.0	149	53	53	89
37-41	0.85	192	35	35	50
60-64	1.1	154	6	58	20

* Numbers in parentheses indicate the number of double bonds. † The living mat contained 296 ppm C₁₈(2) and 31 ppm C₁₈(3).

ponents are important in the early modification of biochemicals. Nevertheless, biological activity probably determines the overall organic content in the sediments.

The same pattern was found in a second algal mat from the middle Laguna Madre. This mat was much larger in area than the first, but the layered structure was not as well developed. Preliminary data from a sediment core show the early disappearance of highly unsaturated acids (Table 1). However, oleic acid has not fallen to the very low level of the algal mat, although the sediment is much older. This may be due to the very different type of environment.

A detailed study of the geochemistry of fatty acids in recent sediments is being carried out in this laboratory. A more complete report will be published later.

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Circulating DNA as a Possible Factor in Oncogenesis

Abstract. *Infectious DNA from the tumor-inducing polyoma virus and pneumococcal-transforming DNA can be recovered from the blood of mice in biologically active form after intraperitoneal injection. Polyoma DNA appeared to undergo less inactivation than did transforming DNA. In light of these observations, the metastatic spread of cancer may possibly be favored by circulation of tumorigenic DNA in the blood stream.*

Tumors frequently develop at places remote from the site of inoculation when infectious DNA (1) isolated from the tumor-inducing polyoma virus (2) is injected into newborn and adult hamsters (3). This suggests that tumorigenic DNA may be transported by way

of the blood or the lymphatic system and later penetrate cells of various tissues. Mammalian cells in vitro (4), and possibly in vivo (5), can take up DNA. However, it is so far unknown whether biologically active DNA can appear and persist in the blood stream after its injection elsewhere in the body. Accordingly, DNA prepared from polyoma virus, pneumococcus, L1210 leukemia cells, or Ehrlich ascites cells, was administered to mice, and its appearance in the blood was studied.

Infectious DNA was isolated by a trichloroacetate modification (6) of the phenol procedure (1) from a stock of polyoma virus prepared from infected mouse kidney cells (7). The infectious titer of the DNA, determined by plaque assay (6, 8) in the presence of mouse plasma, was about 2.8×10^4 plaque-forming units (PFU) per milliliter. Pneumococcal-transforming DNA (9) was prepared according to the procedure of Hotchkiss (9). Transformation assays for the streptomycin marker were performed with the R36A receptor strain of pneumococcus with antiserum to the rough strain of pneumococcus (10). Tritium-labeled DNA (specific radioactivity, $0.5 \mu\text{C}/\text{mg}$) was isolated by the detergent technique from L1210 ascites leukemia cells grown in BDF₁ mice which had received thymidine-³H intraperitoneally (Schwarz Bio-Research, Inc.) (11). DNA labeled with ³²P was similarly isolated from Ehrlich ascites carcinoma cells (5 days old) grown in mice of the A-strain, 24 hours after an injection of $20 \mu\text{C Na}_2\text{H}^{32}\text{PO}_4$ (Commissariat de l'Energie Atomique, France).

Male Swiss mice (about 25 g) were injected with DNA either intraperitoneally or intravenously (Tables 1 to 3). Blood, collected by heart puncture with syringes containing saline-citrate, was immediately centrifuged to obtain plasma. After being washed, the separated blood cells were resuspended in a volume of saline equal to that of the blood withdrawn.

To learn the extent of its appearance and possible breakdown in the blood, DNA-³²P was injected intraperitoneally into mice, and the fractions of the plasma soluble and insoluble in perchloric acid (12) were examined. The relative radioactivities of these fractions over a 2-hour period (Fig. 1) indicate that there was a rapid and considerable transfer of radioactivity from the peritoneal cavity to the blood stream. The DNA did not break down

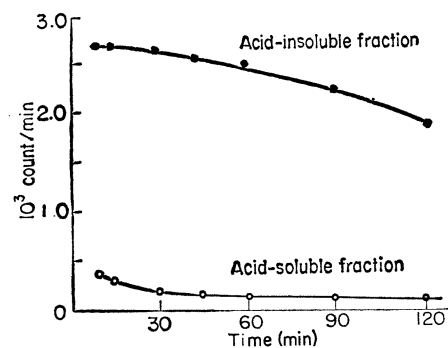


Fig. 1. Changes in plasma of mice after intraperitoneal injection with 0.2 mg of DNA-³²P (5×10^4 count/min mg^{-1}) isolated from mouse ascites cells. Each point, which represents the average of data for eight mice, is calculated for the total blood plasma which is taken as 1.3 ml for a mouse weighing 30 g.

extensively in the blood since only small amounts of acid-soluble material appeared in the plasma during a 2-hour period in which the bulk of the radioactivity remained in an acid-insoluble form, and at an almost constant level.

To estimate the relative distribution of DNA, or fragments thereof, between the blood cells and plasma, DNA-³H (from L1210 ascites leukemia cells) was injected, and radioactivities of these two blood fractions were determined over a 45-minute period (Table 1). When the DNA was given intravenously, about half of its radioactivity could be demonstrated in the blood; roughly twice as much was associated with the cellular elements as was present in the plasma. On the other hand, roughly one-fourth of the radioactivity of DNA injected intraperitoneally was found in the blood, and the amount of label associated with the cells was about three times that present in the plasma.

Although the form or forms in which the intraperitoneally injected DNA-³H circulated in the blood plasma are not yet known precisely, it was considered, as a result of CsCl density-gradient centrifugation, to be macromolecular, 25 percent of the material having the buoyant density of the native and the remainder that of heat-denatured nucleic acid (13). The amount of radioactive substance in the blood remained constant for 45 minutes (Table 1) after the injection of DNA-³H by either route.

The radioactivity remained at the same absolute concentration in the blood even when the amount of DNA-³H injected intraperitoneally was varied over a 20-fold range (Table 2). The