

Table 1. Cesium-137 body burdens (nanocuries) in various age categories of Anaktuvuk Pass, Alaska residents during July and August 1965.

Group	Age (yr)	July			August		
		No. persons	Body burden	Per kg body wt.	No. persons	Body burden	Per kg body wt.
Adults	>21	37	920 ± 49*	15.1 ± 0.8	23	920 ± 58*	15.7 ± 1.0
Minors	15-20	8	560 ± 58	9.7 ± .9	5	490 ± 42	9.5 ± 1.0
Children	3-14	36	200 ± 18	7.8 ± .4	22	170 ± 17	6.2 ± 0.3
Controls	>21	23	870 ± 88	14.7 ± .9	16	900 ± 60	16.0 ± 1.3

* Mean ± one standard error.

Table 2. Cesium-137 concentrations in caribou flesh obtained from family food caches and human body burdens at Anaktuvuk Pass, Alaska during the summers of 1962 to 1965.

Caribou flesh		Adult humans	
No. samples	¹³⁷ Cs/kg dry wt. (nc)*	No. persons	¹³⁷ Cs/kg body wt. (nc)
<i>Summer 1962</i>			
4	43 ± 11†	38	7.3 ± 0.4
<i>Summer 1963</i>			
3	60 ± 17	42	10.3 ± .5
<i>Summer 1964</i>			
8	200 ± 19	41	21.0 ± 1.0
<i>Summer 1965</i>			
15	140 ± 22	38	15.1 ± .8

* Standard dry weight. † Mean ± one standard error.

cent lower than the 1964 value. The maximum value during the summer of 1965 was found in the same man who usually had had the highest burden. On 24 July his body contained 1710 nc ¹³⁷Cs, on 24 August 1740 nc, and on 1 September 1740 nc. These values were 30 percent less than during the summer of 1964.

The occurrence of maximum ¹³⁷Cs body burdens in these Eskimos during the summer months was due to the custom of stockpiling caribou during the animal's northward migration through the Anaktuvuk Pass region in the spring (May), and again during the southward migration in the fall (October). Because of the caribou's winter diet of lichens, the flesh of caribou killed in the spring contains three to six times more ¹³⁷Cs than that of caribou killed in the fall. The seasonal cycle of ¹³⁷Cs in Alaskan arctic ecosystems was discussed in earlier reports (5).

The values obtained in the summer of 1965 are consistent with the gradual increase of ¹³⁷Cs in lichens, caribou, and people at Anaktuvuk Pass since the study was begun in 1962. The unexpectedly high values in caribou flesh, and consequently in the people who ate it during the summer of 1964, were inconsistent with this pattern and

were probably the result of an unusual wintering and migration pattern in caribou during the 1963 to 1964 winter months. At that time, the animals wintered farther south of Anaktuvuk Pass than usual, and the spring kill by the Eskimos evidently included animals that had acquired greater ¹³⁷Cs concentrations in flesh than usually associated with Anaktuvuk Pass caribou during this period.

Standards applicable to the radiation exposure of these people have recently been issued by the Federal Radiation Council and discussed before the Joint Committee on Atomic Energy of the U.S. Congress. The Federal Radiation Council Radiation Protection Guide recommended for this group of people corresponds to an annual average body burden of 3000 nc of ¹³⁷Cs in individual adults (6). It is the general consensus that current amounts of fall-out radionuclides in the diet and bodies of northern Alaskan natives do not constitute a radiological health hazard (7).

WAYNE C. HANSON
Biology Department, Battelle Memorial
Institute, Pacific Northwest
Laboratory, Richland, Washington

References and Notes

1. H. E. Palmer, W. C. Hanson, B. I. Griffin, W. C. Roesch, *Science* **142**, 64 (1963); H. E. Palmer, W. C. Hanson, B. I. Griffin, D. M. Fleming, *ibid.* **144**, 859 (1964); H. E. Palmer, W. C. Hanson, B. I. Griffin, L. A. Braby, *ibid.* **147**, 620 (1965).
2. W. C. Hanson, in preparation.
3. —, H. E. Palmer, B. I. Griffin, *Health Phys.* **10**, 421 (1964).
4. W. C. Hanson and H. E. Palmer, *Trans. North Amer. Wildlife Natural Resources Conf.* **29**, 215 (1964).
5. W. C. Hanson and H. E. Palmer, *Health Physics* **11**, 1401 (1965); W. C. Hanson, *Amer. J. Vet. Res.* **27**, 359 (1966).
6. Federal Radiation Council, *Staff Report No. 7* (U.S. Government Printing Office, Washington, D.C., 1965).
7. Joint Committee on Atomic Energy, *Federal Radiation Council Radiation Protection Guides, Hearings, 89th Congress* (U.S. Government Printing Office, Washington, D.C., 1965).
8. I thank the people of Anaktuvuk Pass, Alaska, for cooperation and hospitality, and Dr. Max C. Brewer, director of the Arctic Research Laboratory, for support and use of field facilities. This work was performed under U.S. AEC contract AT(45-1)-1830.

19 May 1966

Synthetic Detergents: Their Influence upon Iron-Binding Complexes of Natural Waters

Abstract. Organic compounds extracted from Michigan lakes and streams and added to algal cultures increase the growth rate of the green alga (*Chlamydomonas reinhardtii*) when iron is present. Two-dimensional paper chromatography has shown that the iron is complexed by organic fractions containing an amine group. When isolated from natural waters containing a concentration of over 0.3 part per million alkyl benzene sulfonate, these compounds do not show an iron-binding capacity. Separation of this sulfonate from the amine complexes restores the iron-binding capability. These findings suggest that detergents may influence the mobility of iron by reducing the number of binding sites, and in this way may have an important secondary effect upon the primary production of lakes and streams.

The binding of iron by organic compounds has been shown to have an important influence upon cycling of iron in lake ecosystems (1) and secondarily upon the utilization of iron in photosynthesis (2). Amines isolated from lake waters increase the growth rate of algae (3) by forming iron complexes (2). We now report upon the influence of synthetic detergents on the iron-binding capability of naturally occurring amines and amino acids.

A large series of samples of naturally occurring organic compounds was collected from Michigan lakes and streams during the past 3 years by filtering 3000 to 3800 liters of water through a portable underwater filtering system using activated coconut charcoal (6-14 mesh) as an absorbent. We eluted organic compounds from the charcoal with a modified Soxhlet apparatus and by employing the solubility differentials of petroleum ether, methanol, and chloroform. Solvents were concentrated by rotary vacuum evaporation and a Kuderna-Danish evaporative concentrator. We combined the residues and extracted with ether. We extracted the ether solution with dilute HCl (5 percent) and made the HCl extract strongly basic with NaOH. This basic solution was then extracted with ether to remove amines. We removed water-soluble amino acids from the basic solution by acidifying with HCl to a pH of 1.5 and extracting with *n*-butanol.

We identified amines by paper chromatography, with a ninhydrin spray with a solvent system of *n*-butanol, glacial acetic acid, and water (4:1:4). To test for iron binding we subjected amine residues to two-dimensional chromatography. The solvent system described and ninhydrin were used for the first phase. For the second phase a solvent system consisting of *t*-amyl alcohol, water, formic acid, and ethyl acetate (30:20:30:20) was used. An iron fraction separated from the amine fraction during the second phase of chromatography. Iron was identified by spraying with a solution of 10 mg of diphenylthiocarbazone in 50 cm³ of chloroform.

Identification of organic compounds was achieved by the use of infrared and combustion analysis. For infrared analysis we used a Perkin-Elmer model 237B double-beam spectrophotometer employing both a NaCl cell with Nujol and a compressed potassium bromide crystal. Bands between 1700 and 1600 cm⁻¹ were interpreted as indicating NH deformation, and carboxylate ion was indicated by bands between 1600 and 1500 cm⁻¹. Most significant, however, was absorption between 2800 and 2300 cm⁻¹, which is indicative of an acid or protonated amine (4). Combustion analysis was undertaken with a F&M model 180 carbon, hydrogen, nitrogen (CHN) analyzer. A column temperature of 137°C and furnace temperature of 760°C were used. Helium was used as a carrier gas at a flow rate of 9 cm³/sec.

We prepared extracts of amines from 14 Michigan lakes and streams in the manner described. All extracts except those from marl lakes have exhibited iron-binding capability and have brought about a significant increase in growth rate of *Chlamydomonas reinhardtii* when added to laboratory cultures of this species. Four ninhydrin-positive areas have consistently appeared in paper chromatography of these extracts. Mobility data have indicated histidine, tyrosine, and aspartic and glutamic acids. We confirmed the identity of the latter two compounds, which were most abundant in these extracts, by infrared and combustion analysis.

Samples collected from the Huron River, Washtenaw County, Michigan, on 19 April 1965, failed to show the iron-binding and growth-response characteristics, although we noted these properties in samples collected earlier

Table 1. The effect of the alkyl benzene sulfonate (ABS) concentration of Huron River water upon the iron-binding capability of amino acids extracted from river water and added to (i) algal culture media, and (ii) culture media containing *Chlamydomonas reinhardtii*. Data given are means and their 95-percent confidence intervals. Numbers in parentheses indicate number of flasks read for percent transmittance values. Percent transmittance of media without algae was set at 100 percent. Percent transmittance of cultures without iron or amino acid was 91.1 ± 3.6 percent.

ABS concentration	(i) Culture media with iron		(ii) Media and <i>Chlamydomonas</i> with iron: Percent transmittance of culture after 10 days' incubation	
	Iron stain intensity*	Amino acid staining intensity†	With amino acid extracts	Without amino acid extracts
0.08	73.6 ± 2.6 (3)	82.2 ± 3.6 (3)	Not tested	Not tested
0.16	71.4 ± 7.6 (3)	78.6 ± 1.8 (3)	65.6 ± 7.8 (6)	79.3 ± 2.2 (6)
0.31	21.6 ± 5.0 (3)	34.2 ± 4.2 (3)	(Not tested)	(Not tested)
0.41	0.0 (3)	29.4 ± 4.8 (3)	81.3 ± 1.2 (6)	82.6 ± 3.1 (6)
After ABS removal				
(0.31)	37.0 ± 3.8 (3)	51.1 ± 6.5 (3)	Not tested	Not tested
(0.41)	35.2 ± 6.1 (3)	43.3 ± 5.3 (3)	71.1 ± 3.1 (6)	81.3 ± 1.9 (6)

* Percentage of light absorbed by photometer scan after two-dimensional chromatography and staining with diphenylthiocarbazone. † Percentage of light absorbed by photometer scan after one-dimensional chromatography and staining with ninhydrin spray.

from this location. Analyses of river water on 19 April indicated that anionic detergents were present at a concentration of 0.54 mg/liter (5). Since binding of anionic surfactants to protein molecules has been noted previously (6), we then investigated the possibility that detergents were bound to these amino acids.

We analyzed fractions that failed to complex iron, by infrared spectrophotometry. These analyses indicated that alkyl benzene sulfonates were bound to the amino acids (7). We separated the alkyl benzene sulfonate (ABS) group from the amino acids by chloroform extraction. A binding capability was established when we reintroduced these amino acids into media containing iron (Table 1).

Further analysis of Huron River water indicated that detergent concentration varied considerably and that iron binding did not occur either in culture media containing algae or the media alone when the detergent concentration was greater than 0.31 part per million (Table 1). Although removal of the ABS group from the amino acids restored the ability to complex iron, this removal did not restore the amino acid and iron stain intensity to the level found in waters with low concentrations of ABS (Table 1). This may have been owing to incomplete recovery of amino acids or incomplete ABS removal. Slight alteration of the amino acid by the ABS may also have been possible. Evidence that iron binding decreases at ABS levels above 0.3 mg/liter but is not

affected in a concentration below 0.1 mg/liter suggests that ABS competes with iron for binding sites.

The addition of various levels of ABS to laboratory cultures containing amino acids from the Huron River confirmed the finding that 0.3 mg/liter is a critical concentration as regards iron binding. Iron staining intensity dropped from 69 percent at ABS concentrations of 0.2 mg/liter to 12.3 percent at 0.3 mg/liter. We could not detect iron binding at 0.4 mg/liter of ABS.

Alkyl benzene sulfonate concentrations frequently exceed 0.3 mg/liter in lakes and streams receiving domestic drainage and sewage effluents. Our findings suggest that primary production of these waters may be reduced by the interference, of detergents, with transport of iron and other metals required in photosynthesis.

FRED KENT

FRANK F. HOOPER

*Institute for Fisheries Research,
University Museums Annex,
Ann Arbor, Michigan 48104*

References and Notes

1. C. L. Schelske, F. F. Hooper, E. J. Haertl, *Ecology* **43**, 645 (1962).
2. F. Kent and F. F. Hooper, *Papers Mich. Acad. Sci.* **50**(1964), 3-10 (1965).
3. G. E. Fogg and D. F. Westlake, *Proc. Intern. Assoc. Limnol.* **12**, 219 (1955).
4. L. J. Bellamy, *The Infrared Spectra of Complex Molecules* (Wiley, New York, ed. 2, 1958), pp. 234-247.
5. O. E. McGuire and F. Kent, *J. Amer. Water Works Assoc.* **54**, 665 (1962).
6. E. Gould, *Anal. Chem.* **34**, 567 (1962).
7. E. M. Sallee, *ibid.* **28**, 1822 (1956).
8. Contribution from Dingell-Johnson Project F-27-R-4, Work Plan 11, Job 7, Michigan.

31 May 1966