Composition of Combustible Concretions of the Alewife, Alosa pseudoharengus

Abstract. *Alewives,* Alosa pseudoharengus, *wash ashore from Onondaga Lake, N.Y., in the form of combustible concretions in which the muscles are replaced by calcium salts of fatty acids. In both distribution pattern and total concentration of fatty acids the concretions differ strikingly from normal carcasses. Carbon-13: carbon-12 ratios indicate that the concretions may have formed from lipids of terrestrial or freshwater organisms or from organic pollutants of nonmarine origin, or from lipids and pollutants.*

The alewife, *Alosa pseudoharengus* (Wilson) (*Pomolobus pseudoharengus*), a marine species, normally invades fresh water only in coastal regions in order to spawn. However, the species also occurs in lakes of upstate New York where it frequently makes its presence known by the many dead specimens that wash ashore. Dead alewives from Onondaga Lake, a small, polluted lake near Syracuse, wash up in the form of characteristic concretions (Fig. 1, *1*) composed of light-colored, crumbly, chalk-like material that has a very low density and that burns when ignited. The head and tail portions and the vertebral columns are always missing, but the ventral scutes and often some scales, ribs, and pelvic fins remain (*1*). The muscles (Fig. 1) are replaced by the chalk-like material, while practically all the bone tissue and the internal organs have disappeared. The unusual characteristics of these concretions prompted investigation of their chemical composition.

In elemental composition the concretions differ strikingly from normal alewife carcasses collected from Lake Ontario (Table 1). The low nitrogen and phosphorus contents of the concretions point to the presence of only traces of proteins and bony tissues. On the other hand, their high calcium content indicates the presence of other calcium salts. Direct Soxhlet extraction yielded only negligible quantities of ether-soluble material. Shaking the concretions with a mixture of 0.5*N* aqueous hydrochloric acid and ether yielded an average of 82 percent of ether-soluble material from pooled samples; conventional analyses showed these substances to be fatty acids whose concentration was high enough to bind all the calcium. Various chemical treatments and gas-liquid chromatography failed to prove unequivocally the presence of neutral lipids, steroids, or hydrocarbons in the concretions. Treatment of normal alewife carcasses with typical fat solvents yielded 7 to 8 percent of extractables (dry weight), regardless of the presence or absence of 0.5*N* hydrochloric acid.

The fatty acids present in the alewife concretions and normal carcasses were identified by gas-liquid chromatography. The ether-soluble material from the carcasses was saponified, and the resulting fatty acids and those obtained from the concretions were converted to the methyl esters with diazomethane. The methyl esters were gas-chromatographed (*2*); because retention data on a single stationary phase can be misleading, 1.82-m columns of two chemically distinct types of stationary phases, Apiezon-L and diethylene glycol succinate, were used to define the chain length and structure of the acids. The results (Table 2) show great differences in distribution pattern between the concretions and normal carcasses: in concretions, unsaturated fatty acids account for 9.4 percent of the total; in carcasses, 32.3 percent. Myristic and palmitic acids make up 79 percent of the total fatty acids present in concretions but only 35 percent in carcasses. The molecular weight distribution also differs: concretions have more low-molecular-weight components and fewer high-molecular-weight acids than do carcasses. The biggest differences with regard to unsaturated acids were found with oleic acid, normal carcasses being approximately four times richer in terms of percentage than concretions.

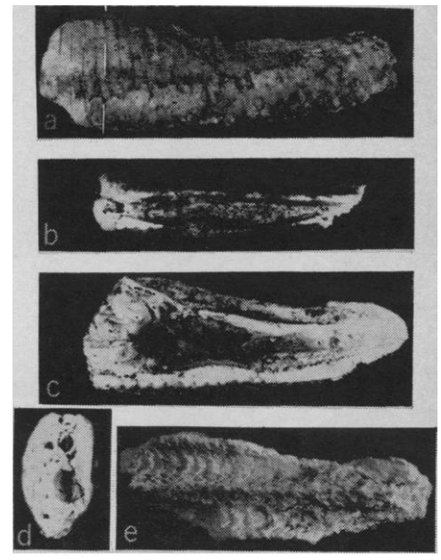

Fig. 1. Alewife concretions. *a*, Lateral view; ventral side down and head portion on the left. *b*, Dorsal view; cavity represents missing spinal column. *c*, Sagittal section, with ventral side down and head portion on the left. *d*, Transverse section, with ventral side down. *e*, Concretion of alewife back, showing cast of spinal column.

If one estimates the total amount of fatty acids present on a per-fish basis, the fatty acid content of the concretions is more than 30 times that of the carcasses. Thus it seems most probable that most of the lipid material found in the concretions was deposited from an external source.

Straight-forward interpretation of the age of concretions, based on radiocarbon dating (*3*), indicates that these samples are 1350 years old—the difference between the apparent age of the concretions (1735 ± 90 years) and the apparent age of live fish (385 ± 205 years) collected from Onondaga Lake in 1963 (*4*). However, since $C^{14}:C^{12}$ ratios in freshwater lakes are subject to much greater fluctuation than similar ratios in the atmosphere, these data cannot be accepted at face value (*5*). We are concerned with two opposing effects: on the one hand, ancient carbon from limestone and other rocks reaches the lake system as carbonates and tends to increase the apparent age of organisms, while fallout-C^{14} causes apparent decrease in the age of samples.

The relative abundances of the stable carbon isotopes C^{13} and C^{12} provide information concerning the possible origin of the carbonaceous matter in the concretions. Because of selective

Table 1. Elemental composition of alewife concretions and carcasses. Samples of concretions were dried to constant weight over phosphorus pentoxide under partial vacuum at room temperature. Carcasses, showing minimum decomposition, were collected from Lake Ontario at Selkirk, N.Y., in 1963; heads, tails, and viscera were not analyzed.

Form	Content (%)								
	C	H	N	P	Ca	Mg	K	Cl	O
Concretion	66.3	10.5	0.3	0.1	6.0	0.07	0.03	0	15.9
Carcass	42.5	6.7	11.3	2.8	2.0	.27	.30	0	

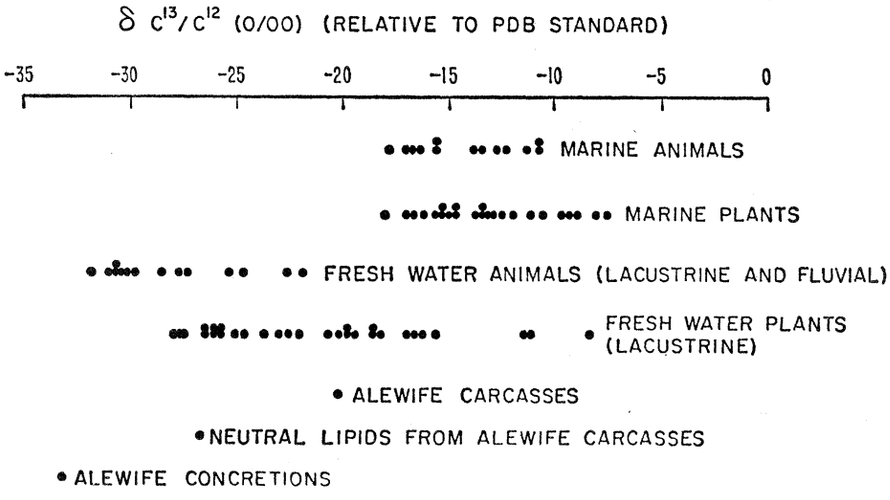

Fig. 2. Carbon-13:carbon-12 ratios of samples of alewife and other aquatic organisms.

utilization of C^{12} in preference to C^{13} in photosynthesis, the $C^{13}:C^{12}$ ratios of sedimentary-rock carbonates are about 2 to 3 percent higher than those of biologically derived organic substances (*6*). Furthermore, the lipid components of organisms have consistently lower $C^{13}:C^{12}$ ratios than those of the nonlipid organic components. Ratios of $C^{13}:C^{12}$ from marine-environment organisms are approximately 1-percent higher than ratios of terrestrial or freshwater organisms (*7*). These general relations were used in evaluating the significance of the carbon-isotopic compositions of the alewife concretions.

The $C^{13}:C^{12}$ ratios were determined mass spectrometrically and expressed as δ-values, that is, as deviations from the $C^{13}:C^{12}$ ratio of an arbitrary standard (PDB-1) according to the formula:

$$\delta\ C^{13}/C^{12}\text{ in per mille} = \{[(C^{13}/C^{12})_{sa} - (C^{13}/C^{12})_{st}]/(C^{13}/C^{12})_{st}\}\ 1000$$

the subscripts being sample and standard, respectively. The $\delta\ C^{13}:C^{12}$ values of the alewife samples are compared with values of other marine and freshwater organisms in Fig. 2; the compilation is from the data of Craig (*8*), Broecker and Walton (*5*), Oana and Deevey (*9*), Wickman (*10*), and others unpublished. These data include (*5*) the δ-value of an unidentified species of freshwater fish (−21.9 per mille).

The δ-value of the Lake Ontario carcasses is of the same order of magnitude as those of other freshwater organisms. The $C^{13}:C^{12}$ ratio of the neutral lipid components is 6.7 per mille lower than that of the whole carcass, which relation compares favorably with previously noted differences between whole organisms and their lipid fractions (*7*). The δ-values of the whole concretions (−33.1 per mille) show a marked enrichment of C^{12}; these values fall within the range noted for lipid fractions of terrestrial and freshwater organisms, and of fossil, nonmarine, and organic matter (*7*).

The literature contains many reports on adipocere, a lipid material obtained from cadavers that have lain under water or in soggy ground. However, the concretions that we have described seem to differ from typical adipocere in several respects. According to Ruttan and Marshall (*11*), adipocere is the residue of the preexisting fats of animals and contains a large amount of hydroxystearic acid derived from the oleic acid in the original fat. No hydroxy acids were detected in the alewife concretions and most of the fatty acids present are not believed to derive from preexisting lipids. A specimen that seems more closely related to the alewife concretions was described by Faber and Krejci-Graf (*12*); it was found at the Barlewitzer Sea, formerly in West Prussia, and derived from an eel; it contained 48.4 percent lipid, calculated as calcium stearate, and was apparently composed of white, sliceable, talc-like, crumbly material.

Table 2. Distribution patterns of fatty acids in alewife concretions and carcasses.

Acid	Concentration (% by wt)	
	Concretions	Carcasses
Caprylic	Trace	
Nonanoic	0.1	
Capric	.4	
Undecanoic	.1	
Lauric	3.0	0.2
Tridecanoic	0.3	
Myristic	23.6	2.1
Pentadecanoic	1.7	0.7
Palmitic	55.4	32.6
Heptadecanoic	1.6	2.3
Stearic	4.3	15.3
Heneicosanoic		Trace
Docosanoic		5.5
*Unknown 1**		1.7
Pentacosanoic		7.3
Dodecenoic	0.1	
Tridecenoic	Trace	0.6
Tetradecenoic	1.2	.2
Pentadecenoic	0.2	.2
Hexadecenoic	2.5	4.7
Heptadecenoic	0.3	1.0
Octadecenoic	4.5	19.4
Octadecadienoic	0.6	3.1
Octadecatrienoic		2.0
Unknown 2†		0.5
Heneicosenoic		.6

* Assumed molecular weight, 354. † Assumed molecular weight, 278.

Although we cannot present a definitive scheme for the mode of formation of the concretions, let us discuss certain possibilities. The fact that many concretions wash ashore from Onondaga Lake annually makes it seem likely either that they are even now forming or that some past event caused formation of the concretions which are now being gradually released from the lake bottom. Conversion of carcasses to concretions is believed to occur rapidly, while the spinal column and the internal organs are still present. Lack of structural support and the granular, crumbly character of the calcium–fatty acid salts may lead to disintegration of the concreted internal organs, yielding a concretion that in a sagittal view still shows their cavities (Fig. 1*c*). The concreted backbone is believed to break off and usually disintegrates, yet one can find pieces that are still recognizable as from the spinal columns of alewives (Fig. 1*e*). No conclusions can be reached concerning the possible participation of bacteria, fungi, or algae in the conversion process; microscopic examination, staining, and attempts to grow organisms from the concretions provided no evidence of the presence of microorganisms. The fact that concretions have been found in Onondaga Lake but not generally in other lakes probably reflects the lake's abnormal richness in soluble calcium ions and the high concentration of organic compounds resulting from pollution (*13*). The $C^{13}:C^{12}$ ratio and the radiocarbon age of the concretions can be reconciled if one assumes that the lipids of the concretions derive from organic contaminants present in this highly polluted lake. A fortuitous mixture of lipids from terrestrial and freshwater organisms and nonmarine organic pollutants could thus yield the experimentally observed distribution pattern of carbon isotopes; but there is no evidence for this hypothesis. The alewife decomposes more slowly than other

species of fish and this fact may explain why concretions are so numerous. Nevertheless, conversion to a concreted form is not peculiar to this species: a few concretions of the gizzard shad (*Dorosoma cepedianum*) and of chicken or duck bones also are found.

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References and Notes

1. W. A. Dence, *Copeia* **3**, 155 (1956).
2. Barber Colman model 10; beta ray–argon detector.
3. Radiocarbon determinations by Geochron Laboratories, Inc., Cambridge, Mass.
4. Because living alewives from Lake Onondaga were unavailable, other small fish from this lake were used.
5. W. S. Broecker and A. Walton, *Geochim. Cosmochim. Acta* **16**, 15 (1959).
6. A. O. Nier and E. A. Gulbransen, *J. Amer. Chem. Soc.* **61**, 697 (1939).
7. S. R. Silverman, in *Isotopic and Cosmic Chemistry*, H. Craig, S. L. Miller, G. J. Wasserburg, Eds. (North-Holland, Amsterdam, 1964), p. 92.
8. H. Craig, *Geochim. Cosmochim. Acta* **3**, 53 (1953).
9. S. Oana and E. S. Deevey, *Amer. J. Sci.* **258-A** (Bradley volume), 253 (1960).
10. F. E. Wickman, *Geochim. Cosmochim. Acta* **2**, 243 (1952).
11. R. F. Ruttan and M. J. Marshall, *J. Biol. Chem.* **29**, 319 (1917).
12. W. Faber and K. Krejci-Graf, *Mineral. Petro. Mitt.* **48**, 305 (1936).
13. According to State of New York Bureau of Fish Research reports and a private communication from F. W. Montanari, the calcium content of Onondaga Lake varied in 1947 from 479 to 628 parts per million, with only 27 to 65 ppm as alkalinity; the remainder was presumed to occur as chlorides. Lake Ontario water has a calcium content of 36 ppm and alkalinity of approximately 90 ppm; thus it is doubtful whether calcium salts not contributing to alkalinity would be significant.

13 December 1965

Isolation of St. Louis Encephalitis Virus from Bats (Tadarida b. mexicana) in Texas

Abstract. *A strain of St. Louis encephalitis virus has been isolated from Mexican free-tailed bats* (Tadarida b. mexicana) *collected at the time of an outbreak of encephalitis in Texas in 1964.*

An epidemic of St. Louis encephalitis in Houston, Texas, during the summer of 1964 provided us with an opportunity to explore, under field conditions, a hypothesis which has been under study in our laboratory for several years, namely, that Chiroptera may be involved in the epidemiology of certain arbovirus infections. Initial studies on experimental rabies infection in bats demonstrated viral invasion and multiplication in interscapular brown adipose tissue. This result suggested that brown fat could serve as a storage site for virus particles in the latently infected bat, thereby contributing to the ability of these animals to store rabies virus in nature (*1*). Together with reports of others on the susceptibility of bats to experimental infection with certain arboviruses (*2*), our observations (*1*) prompted studies to determine whether Chiroptera could serve as reservoir hosts for these viruses in a manner similar to that described for rabies virus. Results of studies concerned with the tissues involved in experimental Japanese B encephalitis (JBE) and St. Louis encephalitis (SLE) virus infections in bats—with gravid bats in which transplacental transmission of these agents was demonstrated, with the influence of environmental temperature on the course of experimental arbovirus infections in bats, and with the immune response of bats to experimental arbovirus infection—have indeed indicated that these animals would be ideal reservoir hosts for these agents (*3*). Furthermore, it would appear that both hibernating and migrating species, by virtue of various aspects of the physiology and ecology of the mammalian order Chiroptera, may be capable of filling certain gaps in the year-round transmission cycles of the arboviruses, and allow overwintering or reintroduction of a viral agent into a particular area.

The outbreak in Houston and environs began in the early summer of 1964 and was clearly established as St. Louis encephalitis (*4*). While efforts were being made to locate bat populations in metropolitan Houston in the vicinity of the epidemic focus, collections were made in outlying areas. Two species of bats, the Mexican free-tailed bat (*Tadarida b. mexicana*) and the evening bat (*Nycticeius humeralis*), comprised a collection made on 26 August 1964, in Angleton, Texas, 64 km (40 miles) south of Houston. Whole blood (0.1 to 0.2 ml) was obtained by cardiac puncture and placed immediately into 0.9-ml portions of chilled 10 percent rabbit serum-saline diluent. Bats were exsanguinated before tissues were removed for virus assay. Interscapular brown adipose tissue, brain, spleen, and kidneys were removed in the order indicated, with separate sets of instruments to avoid any cross-contamination, and were stored in compartmentalized containers at −76°C. Immediately prior to assay, tissues were ground in a chilled mortar with alundum and enough chilled diluent to make an approximately 10-percent suspension. All specimens were assayed by the intracerebral inoculation of 2- to 3-day old suckling white Swiss mice.

Blood specimens from 137 bats, collected at a time subsequently shown as the peak of the Houston epidemic, yielded several viral isolates. Two of these isolates, both from *Tadarida b. mexicana*, were chosen for further identification: one, designated HA-119, has been identified as a strain of Rio Bravo virus and the other, designated HA-73, proved to be a strain of SLE virus. We now report the initial isolation and identification of SLE virus from bats.

Five of nine suckling mice, each of which received 0.02 ml of a 10^{-1} dilution of blood from bat No. HA-73 intracerebrally (ic) had died or developed symptoms of central nervous system disease (CNS) 4 to 6 days after inoculation. Brains from mice which developed CNS on the 6th day were subinoculated into a litter of six suckling mice, all of which died or developed CNS 3 days later. A suspension of brain from this second suckling-mouse-brain passage (SMB_2) had a titer of $10^{-9.0}$ per 0.02 ml (ic) in suckling mice, and treatment with ether and with sodium deoxycholate reduced this titer by 3 log units. Agent HA-73 was not infective for guinea pigs or rabbits, and hyperimmune antiserum to this agent was prepared in guinea pigs. A 20-percent suspension of HA-73-SMB_3 was infective for 3-week-old weanling mice, and the second weanling-mouse-brain passage (WMB_2) had a titer of $10^{-7.3}$/0.03 ml (i.c.). Third weanling-mouse-brain passage material was infective for hamster-kidney tissue cultures, producing a cytopathic effect within 48 hours, with a titer of $10^{-7.5}$/1.0 ml. Reisolation of agent HA-73 from the original blood specimen was accomplished 2 months and, again, 6 months after the initial isolation. In addition to blood, other tissues of bat No. 73 were assayed, and virus was recovered from the interscapular brown fat and the kidneys. Prior to the isolation of agent HA-73 and the initiation of tests for its identifi-