lantic and Nigerian coasts have sunk during that period. The difference between the apparent rates (8 in contrast to 16 m per thousand years) and between the depths of the shelf-breaks (110 in contrast to 140 m) are large enough to demand explanation. Relative subsidence of the New England coast with respect to areas farther south is also shown by Redfield's (1) studies of salt-marsh dates of the past 4000 years. For both studies the subsidence is nearly twice the rate of eustatic rise of sea level, if the southern areas having lower rates of relative rise of sea level are assumed to be tectonically stable.

If subsidence of the New England (Atlantic) shelf and coast with respect to other areas did occur, perhaps it can be attributed to landward flow of subcrustal material required for the known uplift of the land areas after melting of their load of glacial ice. However, we believe that further speculation is useless in attempts to arrive at the cause of the difference between the relative rise of sea level on the Texas and the Atlantic shelves and that the existing data are inadequate even for determining which area was the more mobile. For the present, we merely wish to inject a note of caution against the acceptance of measurements of past relative sea levels on any single continental shelf as an indication of absolute changes of sea levels throughout the world ocean.

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- 6 April 1967

Postglacial Change in Sea Level in the Western North Atlantic Ocean

Abstract. Radioactive carbon determinations of the age of peat indicate that at Bermuda, southern Florida, North Carolina, and Louisiana the relative sea level has risen at approximately the same rate, 2.5 imes 10⁻³ foot per year (0.76 \times 10⁻³ meter per year), during the past 4000 years. It is proposed tentatively that this is the rate of eustatic change in sea level. The rise in sea level along the northeastern coast of the United States has been at a rate much greater than this, indicating local subsidence of the land. Between Cape Cod and northern Virginia, coastal subsidence of 13 feet appears to have occurred between 4000 and 2000 years ago and has continued at a rate of about 1×10^{-3} foot per year since then. On the northeastern coast of Massachusetts, subsidence of 6 feet occurred between 4000 and 3000 years ago; since then sea level has risen at about the eustatic rate. Between 12,000 and 4000 years ago, sea level rose at an average of about 11×10^{-3} foot per year. The part played by local subsidence or temporary departures from the average rate during this period is uncertain.

The trend of changing sea level during postglacial times, based on radiocarbon determination of the age of submerged organic material, has been reviewed by Shepard (1). Data indicate a continuous rise in level, amounting to about 100 feet during the past 10,000 years, at a rate which decreased markedly about 5000 years ago. Individual measurements scatter rather widely from the curve describing the general trend, and it is not possible to distinguish effects due to the general eustatic change in sea level from those arising from local tectonic changes in the elevation of the land, from errors in measurement, or from the displacement of the materials (shells, tree stumps, and so forth) since their formation at the dated time.

This study is an attempt to distinguish between these effects and to determine more precisely the recent eustatic rise in sea level. Consideration has been given only to data based on analysis of peat, because it is less subject to displacement since formation than logs, shells, and so forth are. Since some error is inherent in the interpretation of measurements on peat, only those data are considered that are consistent with others secured at the same locality, and only those localities are included from which the selected measurements are supported by similar measurements from other places.

During the past decade a number of determinations of the age of peat, recovered from various depths at localities on the eastern coast of the United States, have been published. Additional data from Bermuda, North Carolina, and the New England coast are reported herein.

Each investigator secures samples of peat from a series of depths measured from some local datum near which it is believed peat is now forming. By plotting the ages of the samples against these depths, curves have been drawn that are considered to show the time course of the rise in sea level at the locality in question. In comparing data from different locations, difficulty arises if a different local datum has been employed in different places. Local datum has been taken as the surface of the marsh or from some measured tide level. In either case, the relation of datum to depth, relative to mean sea

Table 1. Summary of data in this report (conversion factor: 1 foot = 0.3048 meter).

Location	Reference	No. of	No. of samples	
Location	Reference	Used	Omitted	(feet)
	Southern group			
Bermuda	Table 2	12	2	9.0
Southwest Florida	Scholl (4)	12	1	6.7
South central Louisiana	Coleman and Smith (7)	13	0	10.0
Southport, N.C.	Table 2	4	0	9.5
	Cape Cod to Virginia	!		
Barnstable, Mass.	Redfield and Rubin (2)	16	2	25
Nantucket, Mass.	Table 2	2	0	25
Eastham, Mass.	Table 2	2	0	25
Riverhead, N.Y.	Table 2	1	3	25
Brigantine, N.J.	Stuiver and Daddario (15)	4	0	25
Wachapreague, Va.	Newman and Rusnak (16)	3	1	25
~ ~ /	Massachusetts, east coa	ist		
Plum Island	McIntire and Morgan (17)	7	0	14
Neponset River	Table 2	6	0	14

* D4000 is depth of 4000-year datum below the local datum employed by the authors.

level at which the dated material was actually formed, cannot be precisely determined.

In this study, a procedure has been introduced that reduces the uncertainty. In each locality measurements are corrected to a datum defined by the depth of peat having the same age. Differences in the depth of peat having other ages thus represent differences in sea level that have occurred in the intervening time. This provides estimates of the rate of change in sea level during definite intervals which are independent of any assumptions about the local datum, since effects due to these assumptions are canceled out when any two measurements at a given locality are compared.

An age of 4000 years has been used to define the datum employed in this study, that being the greatest age at which the corresponding depths below the local datum can be determined from available measurements. Elevation of the local datum above the 4000-year datum is determined graphically by the measured depth at which curves describing the measurements cross the 4000-year coordinate.

The procedure is illustrated in Fig. 1. For each of four locations, a line having a slope of 2.5×10^{-3} foot/year has been drawn through the points. The intercepts of these lines with the 4000-year coordinate gives the depth of the 4000-year datum below the local datum. Subtracting this value from the

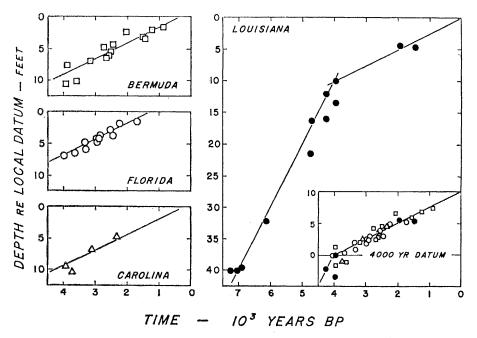


Fig. 1. Age of peat from Bermuda, Florida, North Carolina, and Louisiana at depths relative to the local datum, and (inset) at depths relative to the 4000-year datum.

depth below the local datum adjusts the data so that all points fall about a single line (see inset, Fig. 1). The cluster of points about this line indicates that since 4000 years ago sea level has risen at approximately the same rate at each of this group of locations.

The procedure of referring measurements to the 4000-year datum does not eliminate errors in the data, which are evident in the scatter of individual points about the lines describing each location. These departures can be attributed only in small part to probable error (of 100 to 200 years) in the radiocarbon dates.

Measurement of the depth of samples below the local datum is inexact because of small variations in elevation of the marsh surface, its possible slope, and the like. Compaction of peat is also a possible source of error, but this error has been reduced in most cases by securing samples from immediately above the substratum. This practice does not eliminate the effects of compaction, if any, of the substratum itself. About 10 percent of recorded data have been disregarded in this study because they depart widely from the general trend of data from the same location. Those samples that appear to be too old are from freshwater deposits that may be suspected of having been formed in freshwater swamps at an elevation above the contemporary sea level, as discussed by Redfield and Rubin (2). Those samples that are too young may have been contaminated by material from lesser depth in sampling or by the reworking of the sediments subsequent to their original deposition.

Table 1 summarizes data employed in this study, the number of samples rejected because of nonconformity, and depth of the 4000-year datum below the local datum used at each location; Table 2 presents previously unpublished data.

Change in sea level relative to land is attributed to the combined effects of factors, such as deglaciation, that have changed sea level in every part of the ocean equally (the custatic component) and the local uplift or subsidence of the earth's surface that has altered it locally (the tectonic component). Separation of these components is essential if the actual change in the ocean's level is to be discovered.

Change in sea level relative to land may be attributed to eustatic effects

only in regions that have been stable during the period in question. Shepard (3) pointed out that actually no area has been entirely stable in the past and that all one can do is to avoid areas where there has been upwarping due to isostatic recovery from ice loads, where deltas like the Mississippi are known to be sinking, where there has been recent mountain-building, or where earthquakes indicate instability.

Data from the four positions (Bermuda, Florida, North Carolina, and Louisiana) shown in Fig. 1 indicate essentially the same rate of change in relative sea level during the past 4000 years. These positions lie in an area that extends about 1400 miles (2300 km) in longitude and 300 miles in latitude. On the assumption that this is a stable area, it is tentatively concluded that the rise in sea level at these positions is eustatic and has been at the rate of about 2.5×10^{-3} foot/year.

The assumption that these stations lie in a stable area is supported by geological evidence cited by Scholl (4) that data from Florida were secured in a stable area. At Bermuda, the volcanism that produced the island terminated in the Late Tertiary (5, 6). Since Bermuda is some 600 miles from the coast of North America, it should be uninfluenced by continental tectonism. On the other hand, the conclusion that Louisiana data were obtained in a stable area is contrary to the general belief that the Mississippi delta is sinking at a very appreciable rate (3).

Coleman and Smith (7) interpret date from Louisiana to indicate that there has been no eustatic rise in sea level since about 4000 years before the present, the change in relative sea level being attributed to subsidence due to compaction of recent deltaic deposits. They attribute the rise in relative sea level prior to that time to the eustatic component. Gould and McFarlan (8) concluded that in southwestern Louisiana there has been no change in relative sea level from either eustatic or other effects during the past 3000 years. If these views are correct, it must be concluded that Bermuda and the coasts of Florida and North Carolina have subsided about equally (about 10 feet since 4000 B.P.).

If my interpretation that the recent eustatic rise in sea level has been 2.5 $\times 10^{-3}$ foot/year is correct, it would follow that the coast of southwestern Louisiana has risen 10 feet in the past 4000 years; this elevation would bal-11 AUGUST 1967 ance the assumed eustatic rise and result in the stillstand of relative sea level on that coast, as claimed by Gould and McFarlan (8) and by Mc-Farlan (9).

There appears to be no logical way to separate between these or other possibilities by the mere comparison of curves for different localities, although differences in such curves indicate real differences in tectonic changes in the elevation of land. The choice of which curve most closely represents the eustatic component must depend upon other geological considerations, such as Scholl (4) presents for the stability of southern Florida. His evidence, taken together with the agreement of data from positions over a wide area, is the basis for my tentative conclusion regarding the recent eustatic change in sea level.

Since the eustatic component is the same in all parts of the ocean, differences in relative change in sea level at different locations indicate local differences in the tectonic component. The data indicate that the relative rise in sea level from localities on the northeastern coast of the United States has been much greater during the past 4000 years than at the group of southern positions to which eustatic effects alone have been ascribed. The difference is interpreted as indicating that this coast has undergone substantial subsidence.

These coastal positions may be separated into two groups in each of which the change in relative sea level has

Table 2. Previously unpublished data on peat samples from Bermuda, North Carolina, Long Island, N.Y., and Massachusetts. MSL, mean sea level.

Site	Lab. No.	Age (years B.P.)	Mean depth be- low local datum (feet)	Depth of basement below core (feet)	Character
Bermuda (18) 32°N, 65°W Long Bay, Somerset Local datum MSL	I-1685 I-1969 I-1765 I-1764 I-1970 I-1971 I-1973 I-1974 I-1975 I-1976 I-1762 I-1686 I-1763	$\begin{array}{c} 880 \pm 120 \\ 1210 \pm 95 \\ 1440 \pm 110 \\ 1510 \pm 110 \\ 2050 \pm 105 \\ 2440 \pm 110 \\ 2530 \pm 100 \\ 2590 \pm 100 \\ 2690 \pm 90 \\ 2760 \pm 100 \\ 3170 \pm 120 \\ 3900 \pm 120 \\ 3930 \pm 120 \end{array}$	$\begin{array}{c} 1.5 \pm 0.3 \\ 2.0 \pm .2 \\ 3.4 \pm .2 \\ 3.1 \pm .3 \\ 2.4 \pm .2 \\ 4.2 \pm .2 \\ 4.2 \pm .2 \\ 6.0 \pm .2 \\ 6.0 \pm .2 \\ 6.4 \pm .2 \\ 4.6 \pm .2 \\ 10.1 \pm .2 \\ 7.5 \pm .3 \\ 10.5 \pm .3 \end{array}$	6.4 1.1 0.2 .9 .7 1.6 0.8 .2 1.7 1.2 1.2 1.2 0.9 .4 .3	Peat Peat Clay Peat Sand Peat Clay Peaty clay Sand Clay Peat Peat Peaty clay Peaty clay
Shelly Bay Local datum MSL	I-1684 I-1683	1820 ± 120 1850 ± 110	$7.6 \pm .3$ $5.6 \pm .2$	1.0 0.2	Peat Peat
Harrington Sound Local datum MSL	ML-186	9145 ± 150	68.0		Peat
Southport, N.C. (19) 33°58.5'N, 77°58.8'W Local datum, marsh surface	I-1576 I-1577 I-1579 I-1578	2310 ± 130 3100 ± 120 3720 ± 140 3920 ± 140	$\begin{array}{rrrr} 4.7 \pm & .2 \\ 6.7 \pm & .2 \\ 10.7 \pm & .2 \\ 9.5 \pm & .2 \end{array}$.1 .6 .1 .7	Peat Peat Peat Peat
Riverhead, N.Y. (20, 21) 40°54'N, 72°37'W Local datum, marsh surface	L-863A I-2076 I-2077 L-863D	930 ± 150 3900 ± 105 8070 ± 130 $10,950 \pm 300$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	~.2 ~.2 ~.2 ~.2	Saltwater peat Saltwater peat Freshwater peat Freshwater peat
Nantucket, Mass. (22) 41°17.7'N, 70°2.5'W Local datum, marsh surface	I-1441 I-1442	$1305 \pm 120 \\ 1695 \pm 110$	$4.7 \pm .2$ $6.6 \pm .2$.2 .2	Peat Peat
Eastham, Mass. (23) 41°50.7'N, 69°58.2'W Local datum, marsh surface	I-1967 I-1968	$2300 \pm 105 \\ 3460 \pm 100$	$10.6 \pm .2$ $17.4 \pm .2$.3 .1	Peat
Neponset River (20, 24) 42°16'N, 70°3.1'W Local datum, marsh surface	I-2215 I-2216 I-2217 W-1451 W-1452 W-1453	$\begin{array}{rrrr} 1310 \pm 95 \\ 1360 \pm 105 \\ 1860 \pm 100 \\ 2100 \pm 200 \\ 2790 \pm 200 \\ 3110 \pm 200 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$.5 .5 .5 .1 .1 0	Peat Peat Peat Peat Silty peat Peat

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been essentially the same (see Fig. 2). Sea level has risen much more at the positions between Virginia and Cape Cod than it has on the eastern coast of Massachusetts (at Plum Island and Neponset). A break in the rate of rise also occurred earlier in the latter region.

In Fig. 3 the entire set of measurements has been plotted relative to the 4000-year datum to show changes in sea level that have occurred in the three groups of positions. Curve A represents the southern group of positions, which have been assumed to indicate the eustatic rise in sea level. The difference between this curve and the others represents the tectonic change to be assigned, on this assumption, to the two other groups. These differences during the past 4000 years are shown in the inset in Fig. 3.

The eastern coast of Massachusetts appears to have subsided about 6 feet between 4000 and 3000 years ago; since then sea level has risen at about the eustatic rate. Along the coast between Cape Cod and Virginia, a subsidence of about 13 feet appears to have occurred between 4000 and 2000 years ago and has continued at a rate of about 1×10^{-3} foot/year up to the present.

For the period prior to 4000 years ago, the available data for peat from the western North Atlantic region are shown in Fig. 3, the depths being referred to the 4000-year datum. Two samples from New York (10), corrected to the 4000-year datum by assuming a subsidence since 4000 B.P. similar to that for the coast between Cape Cod and Virginia, are included. A sample of peat from La Jolla, California, which Shepard (3) thought indicated stability at that position, is shown, uncorrected for subsidence. It agrees closely with the sample from Bermuda, thus supporting his conclusion.

The line drawn by eye through these points passes through the sample from Bermuda. It may tentatively be considered to represent the eustatic rise in sea level between 12,000 and 4000 years ago, at an average rate of about 11×10^{-3} foot/year. Departures of the individual points from this line are not greater than about 10 feet in depth and may be attributed to local tectonic effects, to short-term variation in the rate of eustatic rise, and to errors in measurement.

It is tentatively concluded that the eustatic rise in sea level has been at a rate of about 2.5×10^{-3} foot/year

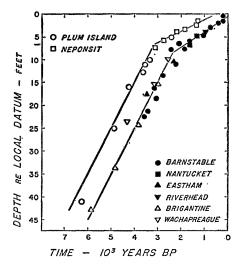


Fig. 2. Age of peat from the eastern coast of Massachusetts (Plum Island and Neponset) and from positions between Cape Cod and Wachapreague, Virginia, at depths relative to the local datum.

during the past 4000 years and at a rate of about 11×10^{-3} foot/year between 12,000 and 4000 years ago. The corresponding change in elevation of the sea relative to the present during the total period is indicated by the scale on the right side of Fig. 3. These estimates are approximations. Data do not preclude short-term departures from the general trend.

These conclusions are based on a selection of data necessary to obtain a consistent picture. About 10 percent of the measurements made at the positions included have been discarded because they do not conform to the general trend of most of the data. This is justified by the uncertainty that the samples have had the assumed origin. The findings of several investigators have also been disregarded and should be commented upon.

Kaye and Barghoorn (11) have described a number of cores obtained from excavations of filled marshland in the city of Boston. The data are difficult to interpret because of the effects of compression by overlying fill and therefore have not been included in this study. Although they conclude that relative sea level has changed less during the past 3000 years than is indicated by data from Neponset and Plum Island, their conclusion regarding the general trend since 6000 B.P. are not in serious conflict with that drawn from those positions.

Bloom and Stuiver (12) have reported determination of age by the radiocarbon method from a number of samples of wood and sedge peat from the

Connecticut coast, and conclude that the submergence of this coast was at a rate of 6×10^{-3} foot/year between 7000 and 3000 years ago. Since then the rate has been about half as great. The latter conclusion accords with the present inferences regarding the rise in sea level on the eastern coast of Massachusetts-that a period of subsidence terminated 3000 years ago and that since then sea level rose at the eustatic rate of about 2.5 \times 10⁻³ foot/year. The rate of rise in relative sea level attributed to the Connecticut coast prior to 3000 B.P. is substantially less than that shown at the other positions. There is, of course, no reason why the subsidence of the Connecticut coast should conform exactly with the other areas. Because many of the deeper samples required correction for compression and because of their freshwater origin, the 4000-year datum cannot be fixed with assurance, and, for this reason, the data have been omitted from the present study.

Lyon and Harrison (13) concluded, on the basis of radiocarbon dates for submerged tree stumps, that submergence of the land at Minas Basin, at the head of the Bay of Fundy, has been more rapid than on the coast of New Hampshire. A review of available data for tree stumps from the coasts of New Hampshire and western Maine by Redfield and Rubin (2) confirmed this conclusion and indicated a rate of recent change in relative sea level very similar to that shown by the data for peat from the eastern coast of Massachusetts. After correcting the data of Lyon and Harrison to the highwater datum, Redfield and Rubin found that the relative rise in sea level in the Minas Basin agreed approximately with data from Barnstable on Cape Cod. These observations suggest that the extent of the area of recent subsidence found in eastern Massachusetts extends into New Hampshire and eastern Maine and that a greater subsidence, similar to that which appears to have occurred between northern Virginia and Cape Cod, occurred also at the head of the Bay of Fundy.

Several investigators have been impressed by the abrupt change in relative rise in sea level that the measurements in their respective locations have indicated. Their observations have been variously interpreted as the interaction of eustatic effects and those due to subsidence. Since the abrupt change in rate occurs at various times—from 4000 to 2000 B.P.-at different places, its occurrence is obviously controlled by the different local subsidences. My interpretation is that there was an abrupt change in the rate of eustatic rise in sea level about 4000 years ago and that the more rapid rise due to eustatic effects prior to that time was prolonged by subsequent subsidence along the northeastern coast. It may be noted that the time when the abrupt change

in eustatic rise appears to have occurred coincides with the termination of the episode of maximum warmth and drought inferred from pollen studies. and so forth (14).

It has been customary to associate the postglacial changes in sea level along the New England coast with isostatic readjustment following deglaciation. Uniformity in the change in sea level along the outer coast as far south

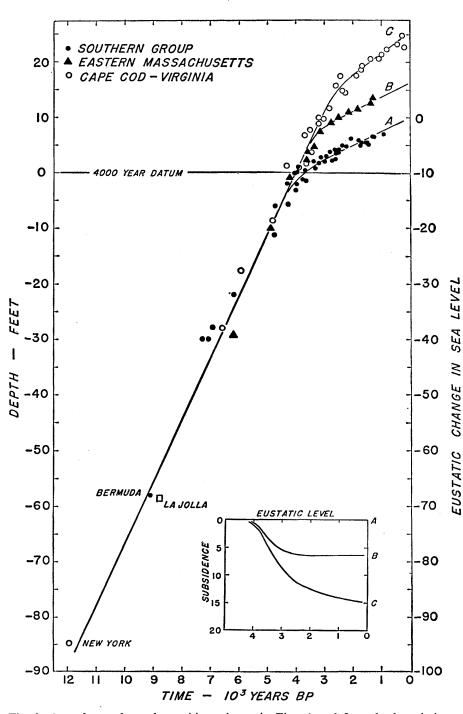


Fig. 3. Age of peat from the positions shown in Figs. 1 and 2 at depths relative to the 4000-year datum. Scale at right shows assumed eustatic rise in sea level. (Inset) Curve B, subsidence of coast of eastern Massachusetts; curve C, subsidence from Cape Cod to Virginia. Curves A, B, and C correspond to similarly designated curves in main part of figure.

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as Virginia raises a question as to whether the recent subsidence indicated by the age of peats is not due to some more general influence on the continental margin.

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- 18. Long Bay cores were from a marsh covered by 1 foot of rubble separated from the lagoon by a beach of coarse shelly sand. Large, scattered, red and black mangrove trees suggest an earlier mangrove swamp. Ground water has salinity of 1 to 2 per mil. Basement water has salinity of 1 to 2 per mil. Basement is of calcareous rock overlain by 1 foot of calcareous clay or sand, and this is overlain by uniform dark reddish peat. Samples were taken alternately from above and below clay-peat transition. Water table, from which depths were measured, was 0.95 foot above mean sale loyal as determined by grapherapute mean sea level, as determined by synchronous measurements of levels of water table, the sea off Long Bay, and a tide gauge at Ferry Reach. Cores from Shelly Bay have been omitted from consideration because their relation to the 4000-year datum cannot be determined.
- Southport cores were from a small indenta-tion of the upland on the north side of the salt marsh bordering Walden Creek, 300 feet west of the road that crosses the creek. 19. Surface vegetation is Distichlis spicata with scattered patches of Spartina alterniflora. The scattered patches of Spartina alterniflora. The marsh is bordered by Juncus romerianus. Peat deposit consisted of a 2-foot layer of fibrous peat above a dark red "granular" peat attributed to Juncus. Peat deposits ap-peared to be undisturbed in contrast to those in the main valley of Walden Creek, which consisted of uniform silt containing vegetable debris, below a thin layer of Spartina peat. D. W. Johnson, The New England-Acadian Shoreline (Wiley, New York, 1925). Riverhead cores were from salt marsh border-ing Reeves Bay at a point about 200 feet north of a parking area on the west side of the bay. Surface vegetation is Spartina patens. Saltwater peat is underlain by fresh-water deposits (20). The transition between freshwater and saltwater deposits is about 20 feet between the works with the set of the same sub-
- 21. Reshwater and saltwater deposits is about 8 feet below the marsh surface, indicating that the freshwater swamp was flooded about

4000 years ago. Sample I-2077 from 7.3 to 7.7 feet appeared to be saltwater peat, but its age-depth relation does not conform to the general relation observed between Cape Cod and Virginia. This sample and those from greater depths have been omitted from consideration.

- 22. Nantucket cores were secured from the southeast extremity of Quaise Marsh on the southcast side of Nantucket Harbor. The site was at the southeast end of the marsh about 100 feet north of Polpis Road. Surface vegetation was *Spartina patens*.
- 23. Eastham cores were from a small marsh at the north end of Nauset Bay, immediately west of the Nauset Coast Guard Station. Surface vegetation is Spartina patens and dwarf S. alterniflora. The peat is fibrous over a substratum of dark brown clay.
- 24. Neponset River cores were from a salt marsh bordering the Neponset River, which is described by Johnson (20). Since his study, large areas have been destroyed by filling and by highway construction. Samples were secured in an undisturbed area at the southern margin of the marsh one-quarter mile west of Johnson's section A-B, in the town of Milton, Massachusetts. Surface vegetation was *Spartina patens*. The peat was underlain by 0.5 to 1.0 foot of olive-colored clay above a bottem of day or south and and graval
- 0.5 to 1.0 foot of olive-colored clay above a bottom of clay or sand and gravel.
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Virus of the California Encephalitis Complex: Isolation from Culiseta inornata

Abstract. A virus of the California encephalitis complex was isolated from two pools of the mosquito Culiseta inornata, collected in mammalian burrows of Alberta during the summer of 1965. This is the first recorded isolation of California encephalitis virus from mosquitoes in Canada.

The history of the isolation of viruses of the California encephalitis (CE) complex has been reviewed in detail by Hammon and Sather (1). This virus was originally isolated by Hammon and Reeves in 1943 and 1944 from wildcaught mosquitoes (Aedes melanimon and Culex tarsalis) collected in Kern County, California (2, 3). Small wild mammals (rabbits and squirrels) have repeatedly been shown to be infected with CE virus, and they are probably responsible for the maintenance of the virus in nature through the cycle mosquito→mammal→mosquito (2, 4-6).The agent was not called California encephalitis virus until 1952, when it was proved to be etiologically related to three clinical cases of human encephalitis that had occurred in Califor-

agent of a case of encephalitis in Florida (7). Increasing evidence has been given, through isolation and serology, that CE virus may play a greater role in human pathology than was previously suspected (8). In Canada, only serological evidence of CE virus infection in small wild mammals was available (9) prior to isolation of the agent from indicator rabbits in the field in Ontario, as reported by McKiel in 1966 (10). We report here the isolation of two viruses of the CE complex from two pools of Culiseta inornata collected from mammalian burrows of Alberta. Using a procedure described by Shemanchuk (11), we collected 5571 mosquitoes from mammalian burrows, divided them into 153 pools, and tested

nia in 1945 (5). Almost 10 years after

the first-reported human cases, interest

in this virus as a human pathogen was

renewed in the United States in 1963

when it was shown to be the causative

them for arboviruses. The species of mosquitoes and the number examined were: Culex tarsalis, 1273 (39 pools); Culiseta inornata, 3659 (85 pools); and Aedes earlei, 639 (29 pools). All mosquitoes collected for virus investigation were kept alive until identification of the species was completed. They were identified on the day of collection and divided into pools of 30 to 50 mosquitoes according to species, feeding conditions, and date and areas of collection. Each pool was placed in a 7-ml (1/4-ounce) bottle which was carefully sealed; the mosquitoes were then quickly frozen on dry ice and shipped in a frozen state to the virus laboratory. The procedures employed in the isolation of arboviruses were basically those described by Chamberlain (12). Each mosquito pool was ground, in a cold mortar. in 2 ml of a mixture of rabbit serum and buffered water (pH 7.6 to 7.8) containing antibiotics and was centrifuged at 10,000 rev/min for 1 hour in a refrigerated centrifuge. The supernatant fluids were inoculated intracerebrally in 0.02 ml amounts into litters of eight suckling white Swiss mice 1 to 2 days old. The fluid remaining after mice were inoculated was kept in a freezer at -70°C for reisolation attempts. All mice were examined for 14 days and only sick mice were selected for passage. No blind passages were made. Reisolation of the strains from the original mosquito specimens was attempted in all instances. Suspensions of mosquitoes from which isolations of virus were not confirmed in reisolation attempts were regarded as

Table 1. Results of neutralization tests (NT) with mouse immune serums prepared with California encephalitis (CE) virus and western encephalitis (WE) virus. Mouse passage 3 in Swiss mice.

Immune serum (type and place prepared)	Titer of virus (log LD ₅₀)	NT index $(\log LD_{50})$
Virus strain	L.276 from Bi	rooks
CE (Atlanta)	5.8	5.8
CE (Edmonton)	6.3	4.5
WE (Edmonton)	6.3	0.5
Virus strain L.2	67 from Hays-	Vauxhall
CE (Atlanta)	4.22	4.22
CE (Edmonton)	4.48	4.48
WE (Edmonton)	4.32	0.5

negative. Isolates were identified serologically by neutralization tests in suckling mice. A crude 20 percent suspension of mouse brain was used as antigen. Three serums were used for each isolate: (i) hyperimmune-mouse serum prepared in our laboratory with the California encephalitis virus, strain Snowshoe Hare (13); (ii) immune mouse ascites fluid, prepared with California encephalitis virus, strain BFS-283 (14); and (iii) immune mouse serum prepared in our laboratory with the western encephalitis (WE) virus, strain Fleming (T.F.) (15). Mice 2 to 4 days old were inoculated intraperitoneally (0.05 ml per mouse) with mixtures containing constant amounts of serum but varying dilutions of virus. Results of the neutralization tests were expressed in terms of neutralization index; the LD₅₀ (lethal dose, 50 percent effective) end points for this purpose were calculated by the Reed-Muench formula (16). Precautions were always taken to eliminate conditions of cross-contamination. Experimental work with known viruses was never carried out concurrently with the testing of field specimens. Serological work was performed in widely separated quarters of the laboratory and only after all the virus work on isolation passages and virus identification procedures were completed.

Two viral agents were isolated from two pools of *C. inornata*, collected on 18 August. One pool, of 49 mosquitoes, came from Brooks and the other one, of 34 mosquitoes, from Hays-Vauxhall, both areas located in the south of Alberta. The mosquitoes of both pools were recently engorged, but the animal on which the mosquitoes fed was not investigated. Both viral agents caused illness and death in 5 days after inoculation of the mosquito suspension.