about osmium fumes, or the expense involved in perfusing large animals. With the method to be described, which simplifies the one outlined earlier (*6*), the perfusion fluid may enter the aorta approximately 1 minute after the chest has been opened. The need for artificial respiration to prevent the effects of anoxia is therefore eliminated. Large volumes of fixative can be used freely so that the need for isolating parts of the animal in order to conserve fixative is avoided. Finally, the formaldehyde perfusion appears to be reliable, so that the risk of losing valuable experimental material due to a poor perfusion is greatly reduced, and fixation of white matter and peripheral structures is always superior to fixation with osmium perfusion.

Our general procedure was to perfuse a 20-percent formaldehyde mixture similar to that described by Richardson (*9*), and which consisted of the following components: distilled water, 700 ml; Hanks solution, concentrated 10 ×, 100 ml (or Ringer's solution); sucrose, 93 g; chloral hydrate, 1 g; formalin (reagent grade), 200 ml. The *p*H is adjusted to 7.3 with NaOH.

This solution was perfused directly without prior use of saline to wash out the vessels. Under Nembutal anesthesia, the chest is quickly opened by splitting the sternum and spreading the opening with a self-retaining retractor. The entire pericardium should be exposed, and quickly opened with a longitudinal incision with scissors. The right ventricle is clamped with a coarse hemostat, which is held in the left hand to steady the heart. The left ventricle is incised with sharp scissors, and a cannula of appropriate size, leading from the perfusion bottle, is inserted into the aorta. It is held in place with the left hand, along with the right ventricular clamp. The right atrium is opened widely with scissors and the perfusion started. After perfusion of half the volume at room temperature, the remainder of the perfusate, prechilled at 4°C, is placed in the perfusion bottle carefully, to avoid air bubbles in the perfusion tube, and the perfusion is continued at a reduced rate until a total of 300 to 600 ml/kg has been perfused. A ligature around the aorta may be used to hold the cannula in place, but valuable time is saved if this step is omitted and the cannula is held in place by hand until the perfusion is well under way. Later, the perfusion tube may be clamped or tied to the wall of the left ventricle.

The perfusion apparatus consists of a perfusion bottle (usually of 2 to 4 liters in capacity), a rubber tube about 40 inches long, and a cannula of proper size to fill the aortic bulb and with a slight flare near the tip. A narrow neck on the cannula should be avoided. A clamp is placed on the tube near the cannula, and all air bubbles should be removed from the line before the perfusion is begun. The perfusion bottle is placed on a shelf about 80 cm above the animal, since higher pressure is not necessary to wash out blood from vessels of the central nervous system.

After removal of brain, spinal cord, or other tissues, fragments of tissue to be ultimately embedded in plastic are usually dissected out immediately and placed in 1-percent buffered osmium tetroxide solution, for 1 to 2 hours, for completion of the fixation. Subsequent dehydration and embedding is carried out in the usual way.

Small mammals, such as mouse or hamster, are readily perfused by injecting the fixative into the left ventricle with syringe and needle, after opening the right atrium to allow return of the perfusate. The tip of the needle may be held in place by clamping the ventricle around it with a hemostatic clamp (*12*).

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14 December 1962

Submergence of the Connecticut Coast

Abstract. *Radiocarbon-dated samples show that the Connecticut coast has submerged about 9 feet in the last 3000 years and about 33 feet in the last 7000 years. The rate of submergence is similar to rates reported from other coasts. The finding strengthens the hypothesis that a worldwide postglacial rise of sea level is the cause.*

Age determinations of 16 samples (Table 1) by the radiocarbon method have provided data from which an estimate can be made of the rate of submergence of the Connecticut coast. The samples used were mainly sedge peat formed from fragments of *Scirpus, Typha,* and *Phragmites,* as well as twigs and leaves of shrubs. This peat is similar to that now accumulating at the landward edges of salt marshes and in estuaries upstream from the high-tide limit of salt water.

Dated peat and wood samples were overlain by various thicknesses of bay mud and salt-marsh peat. Most depth ranges indicated in Table 1 include an uncertainty factor of about ± 0.2 foot as a result of incomplete core recovery; in every range another ± 0.2 foot has been included to allow for the surface relief of the tidal marshes. Datum is mean high water; the additional factor has been used because limited plane table surveys indicate that the surfaces of the tidal marshes are within ± 0.2 foot of the datum. Samples were collected with a ¾-inch-diameter Davis–U.S. Geological Survey piston corer, except for samples Y-840, Y-843, Y-855, Y-1054, and Y-1077, which were collected from wave-cut cliffs in the intertidal zone or from pits dug below tidal-marsh level.

The radiocarbon dating method employed was CO_2 gas counting in proportional quartz counters. The customary C^{14} half life of 5568 years was used in the calculation of dates. Use of the 5568-year value follows the recommendations of the 5th Radiocarbon Dating Conference (Cambridge, 1962). The reference year of zero age is A.D. 1950.

From the environment of modern sedge-peat accumulation it can be inferred that the dated deposits formed

at or slightly above mean high water. Their present depth below mean high water can therefore be used as a measure of the relative rise of mean sea level, assuming that the tidal range has not changed.

Most of the samples were collected from the Hammock River tidal marsh in Clinton, where the sedge-peat bed was traced continuously from a depth of nearly 38 feet to a depth of less than 9 feet over a horizontal distance of 4000 feet. At this locality a presubmergence subaerial valley was submerged by the transgressing sea. As the basal peat bed formed it was progressively overlain by estuarine mud.

The submergence of the Connecticut coast is shown graphically in Fig. 1. The positions of dated samples are shown by rectangles, the heights of which represent depth uncertainty factors, and the lengths of which are the statistical errors inherent in the radiocarbon dating technique. The depths recorded in Table 1 refer to former high-tide level with reference to present high-tide level, but depths plotted in Fig. 1 are the equivalent positions of former mean sea level with reference to present mean sea level. The solid line, representing the position of mean sea level against the coast of Connecticut during the last 7000 years, is drawn through seven samples whose vertical range does not require correction because of compaction. These seven samples were either the basal few inches of sedge peat at the contact with underlying glacial drift, or wood lying on or rooted in the substratum. All seven samples were underlain by permeable sand or gravel that had a surface slope of at least 1 percent. For these reasons, the sedge peat accumulation and the death of the dated trees are believed to have been controlled by a rising water table closely associated with rising high-tide level. Undoubtedly, high spring tides and abnormal storm tides also killed fresh-water vegetation and contributed to the accumulation of organic trash near the high-water mark, in the same manner they do today.

Sample Y-1178 was a composite sample from two borings 50 feet apart. The basal peat was very sandy, and the merging of samples was necessary to get sufficient carbon for analysis. The composite sample needs no correction for compaction, but its interpretation is nevertheless uncertain. Toward the sea, the buried valley beneath the Hammock River tidal marsh at Clinton flattens out

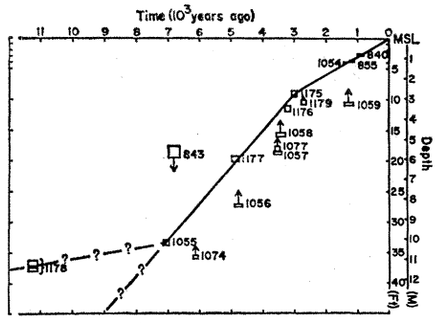

Fig. 1. Submergence of the Connecticut coast. The line is the locus of a point now at mean sea level. The numbers represent the Yale Geochronometric Laboratory numbers. Arrows indicate sample depths that are maximal or minimal only.

at a depth of 37 to 38 feet. The peat of sample Y-1178 may have accumulated in a wet area on the flood plain of the postglacial river valley, not under the direct influence of rising sea level. Thus it is uncertain whether the curve of submergence should be extended through the position of sample Y-1178, or whether it should be continued downward through Y-1055. At least, sample Y-1178 establishes a minimum limit for the submergence of the last 11,240 years. No deeper samples were discovered along the entire Connecticut coast.

Sample Y-843 was a log buried in sandy alluvium (*1*). The alluvium was deposited during a postglacial episode of stream aggradation at an indeterminate height above the sea level of the time.

The plotted position of the remaining samples probably is too low as a result of compaction. Sample Y-1179 was collected from the base of a buried peat bed, 10.4 feet below the present salt-marsh surface. However, the locality is on a golf course, and the marsh has been extensively filled, ditched and diked, so that the marsh surface probably has been lowered a foot or more. If the estimated upward correction were made for the marsh surface, and therefore for the plotted sample depth, the sample would be close to or on the plotted curve. Sample Y-1077 was collected from the base of a peat bed, 18 feet below marsh surface in the side of a commercial clay pit. An earth dike had compressed the overlying peat to between one-half and one-third of its original thickness, and had also caused the underlying clay to be displaced vertically along faults paralleling the pit face. Two feet of downthrow could be measured, but the figure is a minimum. The age of the sample probably relates to less than 16 feet of submergence.

Samples Y-1056, Y-1057, Y-1058, Y-1059, and Y-1074 were collected from the top few inches of the buried sedge-peat bed at Clinton. The thickness of the peat at these localities ranges from 1.2 to 3 feet. The samples from the top of the buried peat bed have been lowered by an amount that is a function of the original peat thickness and the degree of compaction. Their plotted positions cannot be used to define the curve of submergence, but must lie below the curve. However, having established the curve of submergence from the depths and ages of uncompacted samples, it is possible to calculate the degree of compaction of the buried peat bed by comparing the displacement of each compressed sample below the curve to the present thickness of the peat at that locality. Such

Table 1. Radiocarbon-dated samples from coastal Connecticut.

Laboratory No.	Locality	Sample	Depth (ft)	Age (years before present)
Y-840*	Branford	Cedar root	2.7 ± 0.2	910 ± 120
Y-843	North Haven	Log	18.5 ± 1.0	6810 ± 170
Y-855*	Guilford	Oak log	3.8 ± 0.2	1180 ± 80
Y-1054*	East Norwalk	Tree root	4.0 ± 0.2	1400 ± 70
Y-1055*	Clinton	Peaty sand	33.3 ± 0.4	7060 ± 100
Y-1056	Clinton	Sedge peat	27.2 ± 0.3	4780 ± 130
Y-1057	Clinton	Sedge peat	18.6 ± 0.3	3540 ± 130
Y-1058	Clinton	Sedge peat	15.6 ± 0.3	3450 ± 160
Y-1059	Clinton	Sedge peat	10.7 ± 0.3	1280 ± 150
Y-1074	Clinton	Sedge peat	35.7 ± 0.4	6130 ± 90
Y-1077	North Haven	Log	18.0 ± 0.5	3560 ± 80
Y-1175*	Clinton	Sedge peat	9.1 ± 0.6	3020 ± 90
Y-1176*	Clinton	Sedge peat	11.4 ± 0.5	3220 ± 90
Y-1177*	Clinton	Wood and bark	19.6 ± 0.5	4880 ± 120
Y-1178*	Clinton	Sedge peat (combined)	36.6 ± 0.5 37.7 ± 0.3	11,240 ± 160
Y-1179	Westport	Sedge peat	10.4 ± 0.4	2710 ± 90

* Samples whose depth range does not require correction because of compaction.

calculation indicates that the peat bed has been compressed to between one-half and one-seventh of its original thickness, with the deeper and older samples showing greater compaction.

The details of the evaluation of compressed samples will be considered elsewhere, but it may be stated that their depths and ages do not contradict the evidence from the uncompacted samples. The radiocarbon dates and general stratigraphic relationships demonstrate that submergence has been continuous on the Connecticut coast for at least 7000 years and probably for over 11,000 years, with no evidence of pauses or reversals in the submergent trend. From 7000 to 3000 years ago submergence was at the rate of 0.6 foot per century; during the last 3000 years the rate has been only half as great. It is significant that not one of several hundred borings in Connecticut tidal marshes showed more than 10 feet of salt-marsh peat overlying bay mud. Apparently, sediment accumulation and salt-marsh growth have been able to keep pace with submergence only during the last 3000 years; prior to that time the more rapid submergence maintained open, although shallow, estuaries and lagoons on the sites of the present salt marshes.

It must be emphasized that Fig. 1 shows submergence, the relative movement of land and sea level. The cause of the submergence remains to be evaluated. However, the close correspondence of the curve in Fig. 1 with similar curves published for the northwestern Gulf of Mexico (*2*) and for the Netherlands (*3*) supports the hypothesis that the submergence of the last 7000 years is the result of a worldwide, or eustatic, rise of sea level. Contradictory evidence of a stillstand of the sea for the last 3000 years in southwestern Louisiana (*4*) and for the last 5000 years in southern Louisiana (*5*) may be due to unrecognized variables such as compaction, local tectonic deformation, and reworking of old shells into more recent beach ridges (*6*).

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Biochemical Tests Indicative of Reaction of Castor Bean to Botrytis

Abstract. *Pectic and cellulolytic enzymes caused browning and maceration of capsules of susceptible varieties of castor bean but not of resistant varieties. Results of tests in which pericarp tissue of the capsule was treated with vanillin and H_2SO_4 and with an aqueous solution of $FeCl_3$ and $K_3Fe(CN)_6$ showed that resistance to the hydrolytic enzymes was correlated with both a low concentration of flavanols and related compounds and the presence of oxidized compounds in injured tissue. Resistance appeared to be due to inactivation of pectic, cellulolytic, and other hydrolytic enzymes by oxidation products of phenolic compounds. The biochemical tests made rapid measurement of resistance possible.*

Histological studies of castor bean capsules (*1*) failed to show stomatal or cuticular penetration by germ tubes of spores of *Botryotinia* (*Botrytis*) *ricini* (Godfrey) Whet. prior to maceration of the tissue. The pectic enzyme action of *Botrytis* has been recognized as important in pathogenesis (*2*), and the enzymatic activity of isolates from castor bean has been demonstrated (*1*). Since pathogenesis and symptom development in *Botrytis* capsule mold of castor bean appeared due to pectic or cellulolytic enzymes produced by the causal fungus on the capsule surface, we studied the reaction of capsules to these hydrolytic enzymes in relation to varietal susceptibility. The development of a biochemical test for resistance was suggested by work on Panama disease of banana (*3*).

Fully developed green capsules of resistant, moderately susceptible, and highly susceptible varieties of castor bean were sprayed with a 25-percent solution of Pectinol 59L (Rohm and Haas) in distilled water containing a wetting agent. The sprayed capsules were incubated at a relative humidity of 100 percent for 16 hours at 33°C. In six tests, each with ten or more capsules of each variety, capsules of resistant varieties remained firm and green; capsules of moderately susceptible varieties turned brown (a characteristic of oxidation and polymerization of phenolic compounds); and capsules of highly susceptible varieties turned brown and the tissue became macerated. Surface-disinfested capsules reacted similarly. The enzyme solution was effective also after being sterilized by filtration. Similar results were obtained with a 12-percent solution of Cellulase 36 (Rohm and Haas) in phosphate buffer, *p*H 4.7, with a wetting agent. Temperature, time, *p*H, and enzyme concentration were critical factors in the results. The commercial enzyme preparations were shown to contain pectin methylesterase, polygalacturonase, cellulases that hydrolyze carboxymethylcellulose solution and that degrade insoluble cellulose, and enzymes that rapidly macerate carrot tissue. Intact capsules, whether or not they had been surface-disinfested, and mechanically injured capsules of resistant or susceptible varieties remained firm and green when sprayed with water or with heat-inactivated enzyme solution.

Filtrates obtained from cultures of the causal fungus grown on sterilized capsule pericarp contained enzymes that rapidly macerated carrot tissue and that hydrolyzed 1.5-percent citrus pectin solution and 1.25-percent carboxymethylcellulose solution. These filtrates had no effect on insoluble cellulose or on castor bean capsules. This possibly was due to the formation of insufficient amounts of the hydrolytic enzymes. By growing the fungus on a synthetic medium with insoluble cellulose as the sole source of carbon we obtained a cellulase capable of degrading insoluble cellulose. This enzyme produced reactions in the capsules similar to those caused by the commercial preparations.

Among the polyphenols shown to be effective as enzyme inactivators are compounds related to flavanol (*4*). The presence of flavanols and related compounds in fresh pericarp tissue of the capsule was determined by gently crushing small pieces of pericarp in six drops of a 10-percent solution of va-