proximately 80 percent the intensity of that coming from above.

The filters in the two duplicate experiments were placed with their planes of polarization perpendicular to each other to unmask any unforeseen influences, such as light reflected within, or leaking into, the experimental box.

When the test cultures that were illuminated with polarized light were inspected 3 days after fertilization, one saw not only a profusion of bipolar forms, but also a striking tendency of the rhizoids to develop horizontally and in the plane of vibration (PP, Fig. 1) of the electric vector. A portion of a shadowgraph of one of the test cultures is shown in the bottom section of Fig. 1. Portions of shadowgraphs of the controls are shown in the top and middle sections of Fig. 1.

A quantitative measurement was then made of the orientation with respect to PP of all the 171 rhizoids developed by 124 embryos that were selected as a representative sample of the two test cultures. The distribution of the angles between these rhizoids and PP showed a single sharp maximum at 0°. Of these 171 rhizoids, 118, or 69 percent, lay within 10° of PP; 167, or 98 percent, within 45° of PP; and none between 80° and 90° of PP. (In a randomly oriented population, 11 percent would lie within 10° of PP, 50 percent within 45°, and 11 percent between 80° and 90°.)

This experiment, in which two cultures were exposed to polarized light, one to unpolarized light, and one to no light, was twice repeated. It was apparent upon inspection that the rhizoids in the two confirmatory experiments showed the same marked tendency to develop in the plane of polarization.

The percentage of bipolar forms was measured in all the cultures. In the six exposed to polarized light, between 27 and 53 percent of the embryos were bipolar; in the three exposed to unpolarized light, between 4 and 12 percent were bipolar; in the three exposed to no light, between 1.3 and 3.2 percent were bipolar.

It would be premature to discuss the relative roles of the light's intensity, spatial pattern, and polarization in effecting the development of these bipolar forms. Nevertheless, the fact that up to 50 percent of these bipolar forms can be produced by some type of temporally constant illumination strongly implies that the polarity of the Fucus egg arises epigenetically rather than being determined by the rotation of a preformed asymmetric structure such as the nucleus. These phenomena are being investigated further.

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### **References** and Notes

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# Catheptic Activity in Tissues of Tumor-Bearing Rats

If cathepsins play a catabolic role in vivo, one might expect in tumor-bearing animals an elevated cathepsin concentration in such tissues as muscle which are undergoing proteolysis, while no increases would be expected in tissues that are enlarging (liver, spleen, and tumor). On the contrary, catheptic activity was found to be increased in the livers of many cancerous rats (1). However, these cathepsin assays were conducted at the customary but nonphysiological pH of 3.5. In the present study (2), cathepsin concentrations of several tissues in normal rats and in rats bearing Walker-carcinoma-256 implants have been measured at both pH3.5 and 7.5.

The cathepsin assay technique employed was essentially the Snoke-and-Neurath (3) modification of Anson's (4)method. The tissues were homogenized in a glass homogenizer with 5 volumes of 2 percent potassium chloride. (Minced muscle was dispersed in a Waring blendor.) After centrifugation, 2 ml of the resulting extract was added to 5 ml of substrate. The hemoglobin substrates at pH 3.5 and 7.5 were Anson's (4) substrates for cathepsin and trypsin, respectively. Two-milliliter samples of the digestion mixture were pipetted into 5 ml of 5-percent trichloroacetic acid at 0 and 20 minutes. After the samples had been centrifuged the difference in optical density at 280 mµ of the supernatant solutions of the 0- and 20-minute samples

Table 1. Cathepsin concentrations of various tissues of normal and tumor-bearing rats. Concentrations are in Anson's cathepsin units  $\times 10^3$  per gram of tissue. There were six rats in each group.

| Tissue | Assayed at<br>pH 3.5 |       | Assayed at<br>pH 7.5 |       |
|--------|----------------------|-------|----------------------|-------|
|        | Nor-<br>mal          | Tumor | Nor-<br>mal          | Tumor |
| Liver  | 0.74                 | 0.89  | 1.32                 | 1.30  |
| Kidney | 0.80                 | 1.36  | 1.28                 | 1.31  |
| Spleen | 2.21                 | 1.78  | 2.30                 | 1.85  |
| Muscle | 0.23                 | 0.26  | 0.24                 | 0.27  |
| Tumor  |                      | 0.75  |                      | 0.76  |

was used as the measure of proteolysis.

Table 1 shows the average cathepsin concentrations of several tissues of normal and tumor-bearing rats. The values found at pH 3.5 for liver, kidney, and spleen were similar to those of Maver, Dunn, and Greco (1) with the exception of an increase in kidney cathepsin in the tumor-bearing rats. Muscle cathepsin of the tumor-bearing rats did not reflect the rapid proteolysis that, presumably, was occurring in this tissue. Surprisingly, the cathepsin levels at pH 7.5 were fully as high as those obtained at pH 3.5 At this pH, there were no differences between the two groups of rats. The cathepsin level in the tumors was found to be intermediate. Recently, catheptic activity was found to increase markedly in spontaneously regressing Flexner-Jobling carcinomas (5). This evidence would favor a catabolic role for cathepsins in vivo. ARTHUR L. BABSON\*

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## Rate of Postglacial Rise of Sea Level

The results of careful studies of peat and shell material from Velsen in North Holland reported by van Straaten (1), and radiocarbon measurements on this material by deVries and Barendsen (2)have led to a fairly adequate knowledge of the approximate sea stand as a function of time for the Dutch coast over the past 8000 years. These authors represent their results by a figure showing radiocarbon age of shell and peat versus depth of the sample horizon relative to the present strand line. The same is shown in this report (3) in Fig. 1; we have added measurements from other localities for comparison. Most of the added measurements were made by the Magnolia Petroleum Laboratory, Houston, Tex., on material that was obtained from bays, barrier islands, and the continental shelf of the Texas and Louisiana coasts (4). The sources of other dates are indicated in the figure caption.

With the exception of several dates that were determined by the Lamont Geological Observatory, the measurements appear to show approximately equal rates in the rise of the sea level at

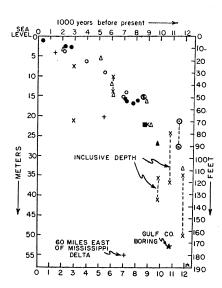


Fig. 1. Radiocarbon ages relative to depths below present sea level. Samples of wood and shells are believed to have been deposited essentially at sea level. No correction has been made for compaction or subsidence from other causes.  $\blacksquare$ , wood (Suess, U.S. Geological Survey, Australia, 9);  $\bigcirc$ , shells, and  $\bigcirc$ , peat (deVries and Barendsen, Holland);  $\triangle$ , shells, and  $\blacktriangle$ , wood (Magnolia Petroleum Laboratory, Texas Gulf); +, shells, and  $\times$ , wood (Kulp, Lamont Geological Observatory, Mississippi Delta); O, wood (Kulp, Bermuda); 🖈, shells (Humble Oil Company, Texas Shelf).

the various localities. This may indicate that the observations reflect the actual eustatic change that accompanied the melting of the last ice sheets rather than the effects of local tectonic movements or compaction. It is obvious, however, that many more measurements from a variety of locations must be made before it will be possible to distinguish conclusively between eustatism, tectonic movements, and compaction.

A possible temporary halt in the rise of the sea level some 7000 or 8000 years ago seems to be indicated by samples from both North Holland and the Gulf Coast. Such a halt may conceivably correspond to the Cochrane halt in the retreat of the ice (5).

The picture becomes confused prior to the last 10,000 years, which may be due to imperfection of the data, or, possibly, to an oscillation of the sea stand connected with the Mankato readvance of the ice sheets around the Great Lakes (6). Evidence for or against changes in the rate of eustatic rise in correlation with oscillations of the ice sheets might prove helpful in determining whether or not glacial readvances were synchronous on different continents and in the two hemispheres.

Perhaps the most interesting question connected with these observations is that

of the supposed postglacial higher sea stand during the "climatic optimum" (7). Neither the samples from the Gulf Coast nor those from North Holland give any indication of a postglacial sea level higher than that of the present. As yet no evidence from carbon-14 measurements has been produced that would prove such a high sea level, and a sample (W-185) of shells from one of the low terraces from Western Australia (+10 to 12 ft), which was expected to date this sea stand, gave an age of more than 30,000 years.

Any assistance in securing samples for radiocarbon determinations that would date a sea level as accurately and as conclusively as possible and hence give a bearing on the afore-mentioned problems would be greatly appreciated.

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### Lactose in the Sapotaceae

Lactose has been reported (1) to occur in the tropical fruit Achras sapota, but the supporting evidence is minimal. In order to confirm this report, a preliminary investigation (2) has been made in this laboratory on the fruits of the aforementioned species as well as on the related species Mimusops roxburghiana and Ponteria campechiana (3).

Samples of 30 to 40 g (wet weight) of the fresh fruit were extracted with 50percent ethanol until extraction of sugar was complete. The total amount of sugar present was determined as glucose by the anthrone method (4). The bulk of the sugar present (glucose) was removed by fermentation with washed Saccharomyces cerevisiae. Extracts of all three fruits, when examined chromatographically (butanol, pyridine, and water, 6 to 4 to 3), showed evidence of a compound

Table 1. Lactose content of fruit of Achras sapota, Ponteria campechiana, and Mimusops roxburghiana.

| Plant           | Dry<br>wt.<br>(% of<br>wet<br>wt.) | Total<br>sugar<br>(% of<br>dry<br>wt.) | Lactose<br>(% of<br>total<br>sugar) |
|-----------------|------------------------------------|--|-------------------------------------|
| A. sapota       | 25                                 | 74                                     | 0.023                               |
| P. campechiana  | 33                                 | 69                                     | 0.043                               |
| M. roxburghiana | 41                                 | 38                                     | 0.028                               |

having the same  $R_f$  as a lactose standard. In order to remove interfering compounds, the extracts were adsorbed on charcoal-celite columns (5), which were then washed with water to remove salts and monosaccharides. Lactose and small amounts of higher sugars were eluted with 50-percent ethanol.

In order to confirm the presence of lactose qualitatively and to effect a quantitative estimation, we employed a purified preparation of Escherichia coli betagalactosidase (6). This enzyme was devoid of alpha-glycosidase activity. The glucose that was split from the lactose by enzyme action was estimated spectrophotometrically with a Beckmann model DU instrument. Into a silica cuvette was pipetted 0.8 ml of buffer [0.1M tris(hydroxymethyl) aminomethane at pH 7.2 and 10<sup>-3</sup>M'MgCl<sub>2</sub>], 30 µlit of Zwischenferment (5 mg/ml), 20 µlit of TPN (8 mg/ml), 10 µlit of ATP (50 mg/ml), and 50 to 100 µlit of the sample. At this stage, any trace of glucose in the sample was detected by the increase in optical density at 340 mµ. The beta-galactosidase was then added (20 µlit) by micropipette, and the changes in optical density were followed until the rate became negligible. In this procedure, 36 µg of lactose caused a rise in density of 0.560 in 15 minutes. The theoretical value of 0.620 could be obtained by using more enzyme and a longer observation time. Only a sugar containing glucose bound in a betaglycosidic linkage will be assayed by this procedure.

It is evident (Table 1) that lactose is present only in small quantity but is readily determinable. About 10 to 20 mg would be present in a whole ripe fruit. It is our intention to examine fruits of other members of the Sapotaceae and to inquire into the mode of lactose formation in them.

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- This work was performed under contract No. AT(45-1)-507 between the U.S. Atomic Energy Commission and the University of Oregon.
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