was examined on a standard blood-counting chamber. In addition, three slides were smeared with plasma, fixed with heat, and stained respectively with Wright's blood stain, methylene blue, and bromphenol blue. No cells could be found. While the possibility of the presence of some cells or cell fragments in the plasma cannot be ruled out categorically, it seems reasonable to assume that it was cell-free.

All rabbits were given, in about 10 minutes, a whole-body dose of x-rays of 1000 r measured in air at the center of the body (210 kv constant potential, 1 mm Cu half-value layer). The animal was irradiated in a lucite box 12 in. by 6 in., with the center of the body 50 cm directly below the x-ray-tube target. The plasma was injected as soon as practicable after the irradiation. In the exploratory part of the experiment, plasma was injected intravenously in some animals and intraperitoneally in others. Since intravenous injections seemed to be ineffective, they were not used in the second series. It is important to note that the rabbits were obtained in small groups as nearly identical in age and weight as possible and that a control animal in each group received x-rays only. In many cases, the animals were litter mates. The results are shown in Table 1.

Statistical analysis of the first series of 68 rabbits shows that intraperitoneal injection of A plasma gave a statistically significant (at the 0.05 level) increase in number of days surviving as compared with the controls and also as compared with the control and B-plasma animals combined. The effect of B plasma was not statistically significant. In the second series of 75 rabbits, A plasma gave a statistically significant (at the 0.01 level) increase in number of days surviving as compared with controls. All plasma A and B animals, taken together, showed a significant (at the 0.05 level) increase in number of days surviving as compared with the controls.

Thus, the protective action of freshly drawn cell-free splenic plasma (sample A) injected intraperitoneally into chinchilla rabbits soon after exposure to 1000 r of x-rays is very definite. The protective action of B plasma is questionable but perhaps not zero. This indicates a greater concentration of some protective agent discharged into the splenic blood during the first part of the bleeding period. It is of interest to note in this connection that, after 10 to 25 cm^3 of blood has been collected, the spleen collapses and remains quite flat thereafter.

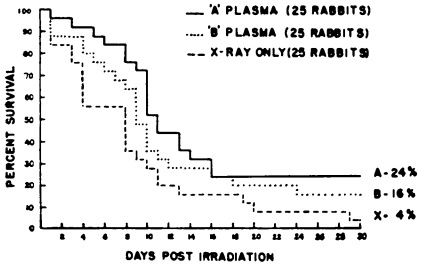

Fig. 1. Percentage survival of rabbits used in second series during 30-day test period.

It will be noted that the x-ray effect was greater in the second series than in the first, although the experimental procedure was the same. However, the rabbits of the first series were housed outdoors (mild weather) in the Loch Awe Rabbitry, from which all the animals were obtained, whereas those of the second series were kept in the animal care facilities of the College of Physicians and Surgeons, Columbia University. The survival curves of the 75 animals used in this series are given in Fig. 1.

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1. L. O. Jacobson, *Cancer Research* 12, 315 (1952).

2. This article is based on work performed under contract AT-30-1-Gen-70 for the U.S. Atomic Energy Commission. A description of the experimental procedure is in preparation. We wish to thank A. E. Brandt, New York Operations Office, U.S. Atomic Energy Commission, for the statistical analysis and for helpful suggestions in the course of this work. We also wish to thank G. Failla, Columbia University, for his interest and guidance.

27 January 1956

Effect of Polarized Light on Polarity of Fucus

In 1941 Child (*1*) noted that, when *Fucus furcatus* zygotes were exposed to unilateral light the direction of which was reversed at hourly intervals, there arose an unspecified percentage of bipolar embryos, forms with rhizoids originating at opposite poles. I have confirmed and widely extended Child's report. In particular, it was observed that bipolar forms were effected just as frequently by oppositely directed light beams when they acted simultaneously as when they acted alternately. It was also noted that the rhizoids of the bipolar forms tended to develop perpendicularly to the light beams. However, the orientation and position of the bipolar forms in the culture vessels suggested that polarization of the light by reflection might have played a determining role.

Accordingly, I investigated the results of exposing *F. furcatus* eggs to two vertical beams of deliberately polarized light, one coming from a lamp above and the other from a mirror below the eggs. Recently fertilized eggs were thoroughly washed with and cultured in artificial sea water (*2*) at 11°C in four glass petri dishes at concentrations of approximately 2 eggs/mm^2. One of these was cultured in the dark and three were cultured in light for 22 hours (starting at 2 hours after fertilization) and subsequently in the dark. The light source was a General Electric standard, cool, white, 20-w fluorescent lamp placed above the culture dishes. In each case, the light was collimated into a beam that was directed downward and held within ± 3.1° of the vertical, and that was of an intensity of about 1 ft-ca. In two cases, the beam was then filtered through a Polaroid laboratory J-filter placed just above the culture vessel. In the third case, the beam entered the dish directly. A back-surface-silvered mirror was placed just below each illuminated dish, thus reflecting upward into each a second beam of ap-

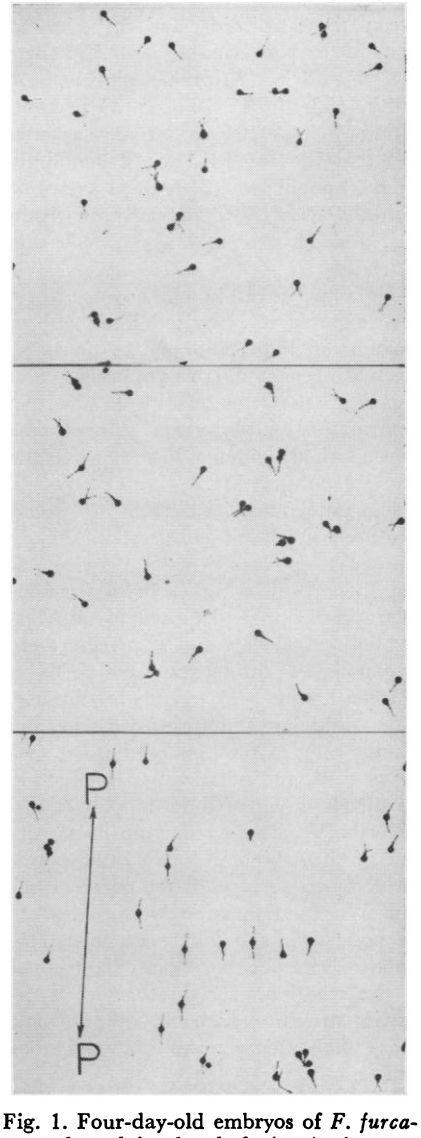

Fig. 1. Four-day-old embryos of *F. furcatus* cultured in the dark (top); in nonpolarized white light (middle); in plane polarized light (bottom). (×4).

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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2. Prepared from reagent-grade salts of Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, SO_4^{--}, and HCO_3^- dissolved in glass-distilled water in proportions taken from H. U. Sverdrup *et al., The Oceans* (Prentice-Hall, New York, 1942), Table 35, p. 173.
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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 *p*H 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 *p*H 3.5 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 *p*H 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 was used as the measure of proteolysis.

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 *p*H 3.5		Assayed at *p*H 7.5	
	Normal	Tumor	Normal	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

Table 1 shows the average cathepsin concentrations of several tissues of normal and tumor-bearing rats. The values found at *p*H 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 *p*H 7.5 were fully as high as those obtained at *p*H 3.5 At this *p*H, 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.*

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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