but rather linked to phenol- and quinone-containing heteropolymers. The seemingly great stability of the brine amino acids (occurrence in Paleozoic formations) was attributed to such linkages.

The following experiments were based on the assumption that the long-term effects of polyphenolic compounds on the thermal stability of threonine could be approximated by "artificial aging" experiments at elevated temperatures.

The polyphenolic material used was "fraction B" of *Fucus vesiculosus,* a reddish-brown extracellular product (yellow in dilute solution) isolated from sea water in which healthy *F. vesiculosus* had been maintained for several hours (*9*). "Fraction B" has several properties in common with the yellow substance (Gelbstoff) of sea water described by Kalle (*10*), and for that reason was used as a convenient source of its probable marine equivalent.

Sealed, oxygen-free tubes of 0.01*M* threonine alone, 0.01*M* threonine in the presence of 0.01*M* pyrocatechol, and 0.01*M* threonine plus 3.0 mg of "fraction B" of *F. vesiculosus* per milliliter were heated side by side for varying times between 154°C and 199°C as described (*4*). The weight ratio of "fraction B" to threonine was approximately 3:1. After thermal treatment, 0.5-ml portions were mixed with equal volumes of 6*N* HCl and hydrolyzed for 3 hours at 100°C in capped centrifuge tubes without loss of volume. The brown precipitates that formed in the tubes containing "fraction B" were removed by centrifugation. Portions, 0.5 ml in volume, were then evaporated to dryness to remove HCl and analyzed on an amino acid analyzer (Spinco 120). In order to obtain values for ammonia produced from threonine during pyrolysis the small initial levels of ammonia in hydrolyzates of unheated samples were subtracted from all experimental results. No significant destruction of threonine was observed during hydrolysis, either alone or in the presence of "fraction B." As shown, glycine appears as a pyrolytic product of threonine under these conditions (*4*).

The concentrations of threonine, glycine, and ammonia present after varying times of pyrolysis are listed in Table 1 together with *p*H values for the tests at 174°C and 199°C. The low recoveries of nitrogen in the form of ninhydrin-reactive amino compounds in the 199°C series with "fraction B" (thr + F) presumably reflect some conversion of threonine-nitrogen into unhydrolyzable or ninhydrin-unreactive compounds. At 174°C the initial *p*H of the thr + F tubes was raised to 6.60 by addition of NaOH, and comparison was made to the thr + pyrocatechol tubes of lower *p*H in order to show the effect of reversing the usual *p*H relationship. In all cases tested the decomposition rate of threonine was not affected by the presence of pyrocatechol.

Threonine was invariably less stable in the presence of "fraction B" than in its absence, this relationship persisting in the 174°C series in spite of the *p*H reversal. In the "thr + F" series at 154°C and 199°C there was consistently less glycine in relation to the amount of threonine decomposed than in the series with threonine alone (Table 1). This suggests that the stability of glycine was also decreased in the presence of "fraction B."

These experiments do not differentiate between direct and indirect effects of the polyphenol (the latter mediated by its pyrolytic products) nor between effects of phenolic as contrasted with other groupings in the polymer. They do show, however, that the stability of threonine in 0.01*M* solution is decreased rather than increased by the addition of one complex phenol-containing polymer from the marine environment, a fact that may be of significance in interpreting data on amino acids in fossil brines.

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DNA Content of a Chromosome of Trillium erectum: Effect of Cold Treatment

Abstract. *The DNA (Feulgen) contents of a specific cold-treated (3°C) chromosome and of a control (25°C) were measured by means of photographic-plate microdensitometry. Despite marked morphological alterations in the cold-treated B chromosome of* Trillium erectum, *its DNA content was unchanged from that of the control.*

It is generally agreed that root-tip chromosomes of *Trillium erectum* react to cold treatment by developing segments of reduced thickness and reduced susceptibility to Feulgen staining at specific locations that are thought to be heterochromatic. There is no such agreement concerning the quantitative effects of cold treatment on the DNA of *Trillium* chromosomes. Considerable reduction has been reported (*1*) in amounts of DNA after growth of roots of *T. erectum* at low temperatures. Yet Woodard and Swift (*2*), using the same species, found no change in content of DNA after cold treatment and concluded that the cold segments resulted from local despiralization. Since these studies were based on microphotometric measurements of whole nuclei, minute changes in amounts of DNA after cold treatment would be undetectable. Determinations of DNA in a specific control and in a cold-treated chromosome showing extensive cold segments might permit detection of such minute differences, if they exist. A procedure has been developed for measuring the DNA content of single Feulgen-stained chromosomes.

The amount of light-absorbent material in minute and nonhomogeneous biologic objects may be measured by means of the highly accurate method of photographic-plate microdensitome-

try (*3*). In essence the method consists in scanning the negative photographic image not only of the object to be measured but also of a rotating disc (*4*) so fashioned as to give a step sector with graded extinctions from 0.10 to 1.0 at 0.10 intervals. The relative extinctions recorded for the biologic object may be expressed in terms of absolute extinctions by reference to the known extinctions of the step sector.

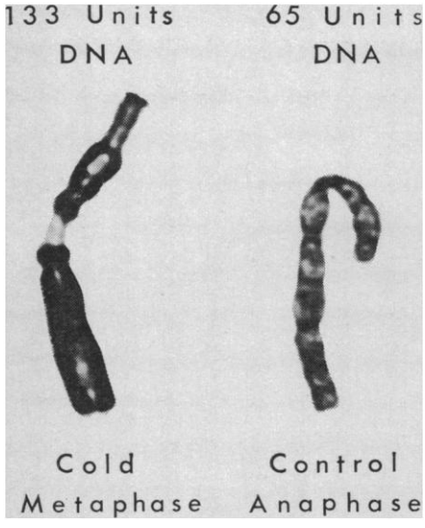

Fig. 1. Typical B chromosomes, cold-treated and control. Despite striking morphological differences between the two, each chromatid of the metaphase chromosome has the same amount of DNA (66.5 units) as in the anaphase chromosome (about × 3300).

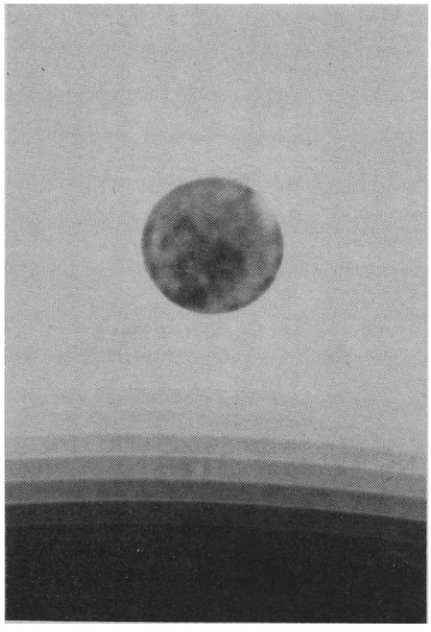

Fig. 2. Test macronucleus of *Tetrahymena* and image of the rotating disc. The micronucleus, after incubation in deoxyribonuclease, is invisible, being azure-B negative. It can be revealed by mounting in a medium of widely different refractive index (about × 2600).

In preliminary studies (Table 1) we attempted to assess the percentage of systematic error by making replicate measurements of the same object. Isolated macronuclei of *Tetrahymena* (*5*) were highly favorable test objects because of the homogeneously distributed chromophore and the roughly discoid shape. RNA of these macronuclei was stained with azure B (*6*) after incubation in deoxyribonuclease. Five photographs of the test macronucleus were taken at a wavelength of 6120 Å on Kodak M plates and developed for 4 minutes in D-19. The negative images of the macronucleus and the rotating disc were scanned with a Joyce-Loebl III-B recording microdensitometer (*7*). Tracings were taken at approximately 0.5-μ intervals across the nucleus. The total amount of RNA in arbitrary units was considered to be the sum of either: (i) areas of the tracings estimated by planimetry, or (ii) weights of cut-outs of the tracings. The weight method was favored because it gave lower standard errors than the planimetry method. The standard error was remarkably low at 0.76 percent of the mean (Table 1). Greater variability of course may be expected in material less favorable for measurement, such as the cold-treated chromosome of *Trillium*, in which irregularities of shape and inhomogeneity contributed to the higher standard error of 3.7 percent of the mean (Table 2).

For our studies of cold-treated and control chromosomes of *Trillium* we selected the B chromosome (*8*) because it responds strongly to growth in the cold by producing two large cold segments (Fig. 1). Both cold-treated and control roots were fixed in a mixture of ethanol and acetic acid (3:1), stained with Feulgen, and squashed on the same slide. All conditions of scanning and calculation of quantities of DNA were identical with those used on the test macronucleus. Exposure time (14 minutes) and development time (4 minutes) were manipulated so as best to utilize the linear part of the H and D curve of the emulsion.

Table 2 shows amounts of DNA and standard errors for both cold-treated and control chromosomes. It is obvious that growth in the cold did not alter the amount of DNA in the B chromosome although it induced formation of cold segments. We think that, rather than being an area of disrupted metabolism of DNA, the cold segment may be a region of modified metabolism of RNA and protein. Our original conclusion (*2*) that heterochromatic DNA is not "cold labile" and that the cold segment may be considered a product of local uncoiling is fortified by these data.

Table 1. RNA values of one macronucleus of *Tetrahymena* incubated in deoxyribonuclease, taken from five replicate negatives. Microdensitometer tracings were cut out and weighed. Mean ($\bar{x}$), 528; S.E. of mean ($s_{\bar{x}}$), 4.02; $s_{\bar{x}}/\bar{x}$, 0.76 percent.

Negative	RNA (arbitrary units)
1	536
2	519
3	525
4	521
5	539

Table 2. Summary of measurements of DNA in the B chromosome of *Trillium erectum*. Cold-treated chromosomes were grown for 4 days at 3°C; control chromosomes were grown at 25°C. DNA contents of anaphase chromosomes were doubled to make them comparable with metaphase chromosomes. The wavelength selected (6120 Å) gave a maximum extinction of about 0.70.

DNA (arbitrary units)	
Cold-treated	Control
148	143
147	136
135	135
133	129
121	127
$\bar{x}$ (mean), 137	$\bar{x}$, 134
$s_{\bar{x}}$ (S.E. of mean), 5.0	$s_{\bar{x}}$, 2.82
$s_{\bar{x}}/\bar{x}$, 3.6 percent	$s_{\bar{x}}/\bar{x}$, 2.1 percent

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