animals in group 2 had small lipid vacuoles in the cytoplasm of about 30 percent of the granular pneumocytes; group 3 guinea pigs had numerous lipid vacuoles in most of the granular pneumocytes; and in animals of groups 4 and 5 the granular pneumocytes contained many large lipid vacuoles which stained with Sudan IV. However, the lamellar bodies stained only faintly. In tissue fixed in osmic acid, the lipid vacuoles were preserved but were stained only lightly with toluidine blue. The osmiophilic lamellar bodies of animals in groups 4 and 5 were smaller and fewer in number than those of the controls kept at sea level. These observations show that the fatty change of the granular pneumocyte depends on the severity of hypoxia. In hypoxia, lipid vacuoles of the granular pneumocyte are similar in morphological appearance to those of normal adipose tissue (Fig. 2). Liver and kidney sections from animals of groups 4 and 5 showed similar fat vacuoles in the hepatocytes and proximal convoluted tubule cells. These vacuoles resemble fat cysts described in cases of toxic lesions of the liver (*6*).

Monge and Singh (*7*) have described cases of high-altitude disease in which severe respiratory failure occurs and which may be related to alterations of pneumocytes. The hypoxia-induced fatty change in the granular pneumocyte indicates that this cell accumulates lipid material which may be a precursor of surfactant. Surfactant alterations have not been studied in hypoxic adult individuals. The accumulation of lipid material in the pneumocyte must be considered a vacuole or inclusion. These vacuoles may be replacing the lamellar bodies and do not resemble degenerative cellular vacuoles, cytolysosomes, cytosomes, or lysosomes. For unlike cytolysosomes and lysosomes, which contain residual membranes and myelin figures, hypoxic lipid inclusions contain a homogeneous material.

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8. Supported by PHS grants H6523 and Gm. 09387, American Thoracic Society, and a NASA grant to the University of Wisconsin Graduate School. Our gratitude to June Shada, Clara Richter, Lorraine Riskey, and Mark Blaedel for technical assistance and to Jane Roberts for reviewing the manuscript.

23 November 1965

Thermal Stability of Threonine in the Presence of a Marine Polyphenolic Material

Abstract. *The rate of decomposition of threonine in 0.01*M *aqueous solution is unaffected by an equimolar concentration of pyrocatechol, but is increased by the addition of an extracellular polyphenolic material (3 mg/ml) produced by* Fucus vesiculosus. *Glycine, a pyrolytic product of threonine, behaves similarly.*

Some analyses of fossil amino acids in hydrolyzates of carbonate exoskeletons, bone, and shale have yielded evidence of a selective diagenetic destruction of amino acids; for example, a more rapid destruction of serine, threonine, and phenylalanine than of glycine, alanine, valine, leucine, and isoleucine (*1–3*). On the basis of these data and experimental studies on the thermal stability of amino acids in dilute aqueous solution a general parallel between geological and thermal stabilities of amino acids has been proposed (*1, 3, 4*).

In other cases, however, all of the amino acids found in the proteins of living organisms have been recovered from fossil materials comparable in kind and age to those mentioned above, with little evidence of a fractional destruction (*5*). It has also been reported that the thermal stability of amino acids is greater when the acids are complexed with clay minerals than when they are present in dilute aqueous solution (*6*), and that the stability sequence obtained by the pyrolysis of natural shales differs from that based on aqueous solutions (*7*). In the latter case some of the least stable amino acids in aqueous solution (for example, serine, threonine, and ornithine) have been found to be the most stable of the amino acids naturally present in shales.

Degens *et al.* (*8*) have recently documented the occurrence of amino acids in petroleum brines. Although their analyses were largely based on methods for the determination of free amino acids, they suggested that the amino acids were not present in the free state,

Table 1. Recovery of 0.01*M* threonine (thr) and its ninhydrin-reactive pyrolytic products when heated alone, in the presence of 0.01*M* pyrocatechol (P), and in the presence of extracellular polyphenolic material (3.0 mg/ml) from *Fucus vesiculosus* (F). Concentrations in moles per 100 initial moles of threonine.

Time (min)	Contents	*p*H	Threonine (moles)	Glycine (moles)	Ammonia (moles)	N-recovery (%)
			154°C			
0	thr		100	0	0	100
0	thr + F		100	0	0	100
45	thr		100	Trace	0	100
45	thr + F		85	1	6	92
390	thr		95	3	0	98
390	thr + F		79	5	18	102
1660	thr		80	16	5	101
1660	thr + F		62	20	20	102
			174°C			
0	thr + P		100			
0	thr + F	6.60	100			
60	thr + P	5.91	92			
60	thr + F	6.39	64			
180	thr + P	5.95	79			
180	thr + F	6.62	53			
			199°C			
0	thr	6.54	100	0	0	100
0	thr + F	6.04	100	0	0	100
30	thr	6.92	54	44	4	102
30	thr + F	6.15	27	19	31	77
60	thr	7.22	28	65	2	95
60	thr + F	6.44	15	50	21	86

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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11. Supported by grant GB-1058 from the National Science Foundation. Analyses performed by Mrs. Patricia Mahool.

27 September 1965

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-