spectively, in the initial planting and no control in the delayed planting.

Applications of 4-*n*-butyl-1,2,4-triazole to wheat foliage also provided a high degree of leaf rust control in both laboratory and field experiments (Table 1). By growing the treated plants for 7 days prior to infection and then noting disease control on the new growth, the systemic fungicidal activity was demonstrated. The activity provided by foliar applications was more apparent under field conditions where a severe epidemic developed in untreated plots as a result of continuous infections (Table 1).

A narrow spectrum of fungicidal action by bioassays in vivo was noted. In fact, possibly the most selective fungicidal effect known among systemic fungicides was exhibited by 4-*n*-butyl-1,2,4-triazole, since among several rusts studied only wheat leaf rust was controlled. In foliar spray tests which lacked weathering as a factor determining activity, bean rust, crown rust of oats, and wheat stem rust were not affected by concentrations of 1200 ppm (Table 2). Similar results were noted in root uptake experiments at 10 ppm. The possibility of fungicidal selectivity due to lack of translocation was excluded by the negative result on wheat stem rust since the same wheat variety, Pennoll, was used to demonstrate leaf rust control.

Compound 4-*n*-butyl-1,2,4-triazole has thus appeared unique in two respects. Complete disease control at 0.56 kg/ha for a period exceeding 30 days demonstrated a degree of control of wheat leaf rust previously unknown. The spectrum of control within the genus *Puccinia* was limited to wheat leaf rust, thus making 4-*n*-butyl-1,2,4-triazole a remarkably selective fungicide.

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Catalytic Activities of Thermally Prepared Poly-α-Amino Acids: Effect of Aging

Abstract. *Thermally prepared poly-α-amino acids were tested after being stored in the dry state for 5 to 10 years. Polymers effective in catalyzing the hydrolysis of* p*-nitrophenyl acetate showed the same levels of activity as observed 10 years earlier. Polymers effective in catalyzing the decarboxylation of oxaloacetic acid had in 5 years become insoluble in assay medium; their activity, however, had increased by 32 to 145 percent. The results suggest that particular primitive enzyme molecules could have been stable enough to have contributed to evolutionary processes long after they had been produced.*

A model for prebiotic protein, as developed in recent years (*1*), provides an experimental basis for theoretical concepts (*2*) concerning the origin of life. Polyamino acids are formed by the simple heating together of proper proportions of dry amino acids under postulated geological conditions. The resulting polymers can range in complexity from homopolymers or few-component polymers to polymers that contain some proportion of each of the 18 common proteinogenous amino acids (proteinoids). The latter resemble present-day protein in many of their properties (see *1*).

Catalytic activity is one property of thermally prepared polyamino acids that has been investigated in considerable detail, as recently reviewed (*3*). These polymers accelerate the chemical conversion of at least 15 different substrates in four major kinds of reactions. These are hydrolyses, decarboxylations (including photo-promoted ones), aminations, and deaminations. Although generally weak in comparison to that of contemporary enzymes, the activity of the thermal polymers is in all cases greater than that of the equivalent amount of unpolymerized amino acids, which in some cases is measured as zero. Differential action, as would be necessary for metabolism (*3*), has also been shown. These and other findings have been interpreted in a context of abiotic origins of enzymes and metabolism (*3*).

The long-term stability of the catalytic properties of thermal polyamino acids is of interest, since they are regarded as models for primitive enzymes (*3*). Conceptually, a primitive catalyst would need to be stable for relatively long periods of time if it were to be available over a long period to contribute to processes of molecular evolution. An approach was made in this study toward estimating the stability of prebiotic enzymes, by retesting the thermal polymers (in the capacity of model compounds) first shown to be catalytically active 5 to 10 years ago.

The catalyzed reactions studied were

Table 1. Activities on *p*-nitrophenyl acetate before and after heating buffered solutions of thermal polyamino acids. The polymers were prepared and first assayed during the period 1960–63 (*4–6*). Assays were at *p*H 6.8, as described in the text; average of six determinations (five for polymer 0-2.8, heated) in the current study. See (*6*) for the meaning of the polymer code.

Polymer	Current			Previous		
	Activity ± S.D.* (10^{-2} μmole min^{-1} g^{-1})		Inactivation† (%)	Activity (10^{-2} μmole min^{-1} g^{-1})		Inactivation† (%)
	Unheated	Heated		Unheated	Heated	
			Proteinoids			
E-1.3	98 ± 6	9 ± 8	91	60	5	91
I-2.8-b	60 ± 6	23 ± 5	62	70	22	69
K-2.8-b	34 ± 9	17 ± 3	50	39	12	69
E-8.0	125 ± 8	67 ± 7	46	88	70	20
B-8.0	85 ± 16	48 ± 6	44	72	42	40
B-13.5	169 ± 12	109 ± 8	36	110	98	11
K-3.4	62 ± 13	41 ± 3	34	54	34	37
			Copolymers of aspartic acid and histidine			
0-2.8	50 ± 11	31 ± 5	38	48	18	62
0-12.3	214 ± 15	159 ± 8	26	232	173	25

* Standard deviation (*12*). † Percentage of inactivation is $\left[1 - \frac{\text{activity (heated)}}{\text{activity (unheated)}}\right] \times 100$.

the hydrolysis of *p*-nitrophenyl acetate (NPA) and the decarboxylation of oxaloacetic acid (OAA). The former reaction was shown a decade ago (*4–6*) to be catalyzed by acidic proteinoids [proteinoids that contain a large proportion of dicarboxylic amino acids (*1*)] and by simpler copolymers of aspartic acid and histidine. Some polymers were over ten times as active as the equivalent amount of histidine; these and other polymers could be largely or partially inactivated by heating their buffered solutions. In the current study, specific activities before and after heat inactivation were compared with earlier (*6*) results.

The decarboxylation of OAA (*7, 8*) was shown 5 years ago to be catalyzed by lysine-rich proteinoids and by samples of thermally prepared polylysine. In the current study (*9*), apparent second-order rate constants provide the main basis for comparison with the earlier report (*7*).

p-Nitrophenyl acetate (K & K Laboratories) was purified (m.p. 76° to 77.5°C, uncorrected) by precipitating from ethyl ether with petroleum ether (b.p. 37° to 59°C). Oxaloacetic acid and L-lysine hydrochloride were used as supplied (California Biochemical).

The thermal polyamino acids were those used previously (*6, 7*). They had been stored dry, without other special precautions, in screw-cap vials or bottles. Acidic proteinoids and copolymers of aspartic acid and histidine were used for the hydrolysis of NPA. Polymers K-2.8-b and K-3.4 appeared damp; they were dissolved in water and were lyophilized before retesting for activity. The other acidic polymers appeared unchanged. The acidic polymers (0.4 mg/ml) were assayed spectrophotometrically on $10^{-3}M$ NPA, as described (*6*) (0.067*M* phosphate buffer, 4 percent dioxane, *p*H 6.8, 30°C). Each polymer was also tested (at 1.0 mg/ml) in 0.167*M* buffer, a procedure that reduced the relative contribution to observed activity of the rate of spontaneous hydrolysis. (Specific activities under both conditions were in close accord in most cases; values obtained at the higher polymer concentration are reported in the few exceptional cases.) Activity values [termed k'/c in reference (*6*)] are corrected for the rate of spontaneous hydrolysis (0.8 ± 0.06 and 1.45 ± 0.08 μmole of product per minute per liter, respectively, in 0.067 and 0.167*M* buffer), the units being micromoles of *p*-nitrophenol produced per minute per gram of polymer. Partial inactivation was mediated by heating buffered solutions of acidic polymers in sealed tubes for 15 minutes in a boiling water bath. The maximum change in *p*H observed on heating—attributable to the hydrolysis of aspartoylimide linkages (*6*)—was less than 0.03 *p*H unit.

Table 2. Activities of thermal polyamino acids for the decarboxylation of oxaloacetic acid. The polymers were prepared (some under aseptic conditions) and first assayed in 1964–65 (*7*). Assays were at *p*H 5.0, as described in the text. Lysine was assayed as the hydrochloride salt, but its activities are expressed per weight of free base. The rate of spontaneous decarboxylation has units of min^{-1}. The sample designations are those used in reference (*7*).

Sample	Current		Previous	
	Activity ± S.D.* (10^{-3} liter g^{-1} min^{-1})	*n*†	Activity ± S.D.* (10^{-3} liter g^{-1} min^{-1})	*n*†
	Lysine proteinoids			
F	15.8 ± 1.6	8	11.9 ± 1.5	21
A (aseptic)	18.7 ± 1.0	5	11.4 ± 0.3	4
C-1	19.4 ± 1.2	5	11.5 ± 0.5	4
C-2	18.2 ± 1.4	5	11.5 ± 1.0	4
C-3	16.7 ± 1.6	6	12.2 ± 1.3	4
C-4	17.0 ± 1.1	5	11.0 ± 1.6	4
F-acetylated	2.9 ± 0.5	3	1.0, 1.0	2
F-hydrolyzate	4.4 ± 0.2	3	3.5, 4.0	2
	Polylysine			
F	120 ± 27	11	49 ± 3.5	22
A (aseptic)	114 ± 5.7	6	55, 53	2
C-1	97 ± 11.8	6	62 ± 6.1	4
C-2	96 ± 5.9	5	54 ± 3.0	4
C-3	90 ± 10.6	5	52 ± 6.3	4
C-4	111 ± 7.7	5	55 ± 5.3	4
F-acetylated	0.8 ± 3.0	3	1.0, 1.0	2
F-hydrolyzate	6.6 ± 0.3	3	3.5, 4.0	2
	Others			
Lysine	3.8 ± 0.5	7	4.2 ± 0.9‡	12
Spontaneous decarboxylation	3.0 ± 0.6	6	3.0 ± 0.7	

* Standard deviation (*12*). † Number of determinations. ‡ Unpublished observations, 1965.

Lysine-rich proteinoids and samples of thermal polylysine were used for the decarboxylation of OAA. Although originally (*7*) soluble in buffer, these polymers were now largely insoluble in water, assay buffer, 1*N* NaOH, 1*N* HCl, and concentrated NaCl. The manometric procedure (*7*) was used with OAA (0.2*M* acetate buffer, *p*H 5.0, 30°C ~ $4.8 \times 10^{-3}M$ OAA). The polymers, however, required special treatment because of their insolubility. They were suspended in buffer, usually at 2.0 mg/ml for lysine-rich proteinoid and 1.0 mg/ml for thermal polylysine, and homogenized with 15 passes of a Potter-Elvehjem Teflon-glass homogenizer. The homogenized polymers adhered tenaciously to glass; some material was always lost during transfer with pipettes. (Such loss was made relatively uniform by always drawing the suspension into the pipette three times before transferring to Warburg vessels.) Observed apparent first-order rate constants were corrected for the rate of spontaneous decarboxylation (0.003 min^{-1}), and were converted to unit-weight activity (based on weight before any losses during pipetting). Reported values have units of liters per gram per minute.

Polymers B-8.0 and polylysine 1-F were tested for possible microbial contamination. They were dissolved or suspended in sterile water and were transferred aseptically to broths and agar plates containing (Difco) proteose-peptone, tryptone-glucose-yeast extract, peptone-beef extract, trypticase-soy, or peptone (two concentrations). After several days at room temperature, no growth was found with the lysine polymer. Very limited—if any real (*10*)—contamination of the acidic polymer was indicated by the finding of only four microbial colonies (from several milligrams of polymer) on ten agar plates.

Values of specific activity and standard deviations with NPA are shown in Table 1; previous values (*6*) are reiterated to facilitate comparisons. After 10 years of storage, proteinoids and copolymers of aspartic acid and histidine are still active on NPA and are again partially inactivated (26 to 91 percent) by the heating of their buffered solutions. In most cases, the average values

do not vary by more than 15 percent from those noted previously, either before or after heat inactivation. Similarly, the percentages of inactivation are comparable in most cases.

A precipitate formed when polymers B-8.0 and E-1.3 were heated in buffer. Residual activity after heating was found only in the soluble fraction. The precipitate constituted about one-quarter of the total sample.

That activity and heat inactivatibility are not attributable to microbial contamination is indicated, as before (*6*), by the absence of microbial growth from a tested polymer and by the fact that activity and heat inactivatibility were noted with fractions (from a Sephadex G-50 column) of low molecular weight [less than 10,000 (*11*)].

Because the insolubility of the polymers used with OAA caused some loss of polymer, the reported apparent second-order rate constants would represent minimum rates. However, Table 2 shows that in every case the polymers are now more active than reported previously (*7*). Percentages of increase in activity for lysine-rich proteinoids are 32 and 88 at the extremes, with an average of 58. For thermal polylysine, the respective values are 73 to 145, with an average of 98. These increases, which for each polymer are statistically significant (*12*) at the 99 percent confidence level, are likely not due to (unknown) differences in assay conditions because unpolymerized lysine and also controls for spontaneous decarboxylation gave values in the current study comparable to those obtained previously. The absence of microbial growth when a representative polymer was tested on six kinds of growth media indicates that the increase in rate is not due to possible microbial contaminants. Reasons for the increase are unresolved.

The activity of both types of lysine-rich polymer was largely associated with the insoluble portions, as judged by assays on resuspended pellets; the supernatants contained less than 10 percent of the total activity. As before, mineral acid hydrolyzates of polymers gave a low level of activity, indicating the importance for activity of polymeric form (*7*, *8*). The importance of free amino groups is indicated by the low activity of acetylated polymers (*7*). The acetylated polymers, incidentally, were insoluble in assay medium; the low level of activity of these polymers suggests that the increase in activity of the parent polymers is not due to surface-adsorption phenomena.

The foregoing study has shown that individual thermal polyamino acids retain their catalytic activity for at least 5 to 10 years. The interim between testing the activity is minute in terms of geological time; also, the conditions of storage of these polymers would represent only one of many possible geological conditions. However, the current results do support the concept that primitive enzyme-like polyamino acids would have been stable enough to be available for long periods of time to contribute to processes of molecular evolution.

The feature of longevity of enzymic action, as suggested by this study, could have offered obvious evolutionary advantages to developing and competing primitive systems (*3*, *13*). The limited requirements in such systems for continued de novo synthesis of enzyme molecules could have been particularly important before template-directed mechanisms for duplication of enzymes originated.

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P-Chlorophenylalanine Methyl Ester: An Aphrodisiac?

Abstract. p-*Chlorophenylalanine methyl ester and the ester plus pargyline have been reported to facilitate sexual mounting behavior in animals, but these studies have shown only a facilitation of homosexual mounting. The present study indicates that these agents do not enhance the probability or frequency of heterosexual interactions in rats.*

The methyl ester of *p*-chlorophenylalanine (PCPA), an agent which depletes brain serotonin (*1*), has been reported to induce hypersexuality in both rats and cats (*2*, *3*). The studies reported that male animals given long-term treatment with PCPA or with PCPA plus pargyline exhibited facilitated mounting behavior. In one sense the studies may be considered as an extension of the work of Meyerson (*4*), who has implicated a serotonergic inhibitory

Table 1. Effects of *p*-chlorophenylalanine methyl ester (PCPA) and PCPA plus pargyline on the heterosexual behavior of male rats. Numbers indicate the mean ± standard error of the number of mounting responses and intromissions which preceded each ejaculation and the total number of mounts, intromissions, and ejaculations which preceded sexual satiation.

Group	No. per ejaculation		Satiation		
	Mounts	Intromissions	Mounts (No.)	Intromissions (No.)	Ejaculations (No.)
Control: before PCPA	11.2 ± 1.5	7.1 ± 1.3	72.6 ± 7.3	46.0 ± 5.2	7.0 ± 0.9
PCPA*	11.3 ± 4.0	6.1 ± 1.0	55.2 ± 17.6	29.3 ± 3.8	5.0 ± 0.4
Control: after PCPA	9.0 ± 1.5	7.2 ± 1.0	51.6 ± 7.6	43.7 ± 5.6	6.1 ± 0.5
PCPA plus pargyline*	16.0 ± 5.3	6.8 ± 1.3	63.0 ± 12.6	34.8 ± 8.7	5.0 ± 0.7

* Scores for these tests do not include scores for an individual animal which failed to mate during the first 30 minutes of the test.