Chara but the change in resistance is not as large.

This effect is consistent with the concept that electric resistance is largely determined by passive fluxes of K^+ ions in *Avena* cells. Evidence for the dependence of the potential difference on K^+ has been found (*2*, *8*). The effect of increased Ca^{++} might supposedly cause a decrease in permeability to K^+ ions and hence an increase in *R*. However, high concentration of Ca^{++} causes hyperpolarization in *Avena* cells (*8*) to about 150 mv, corresponding to no reasonable ratio of activity. It appears that the well-known enhancement of ion accumulation by Ca^{++} (*9*) may be a result of an effect on an active transport mechanism. Briggs (*10*) has suggested several ways by which an active transport system might modify the potential difference compared with that resulting in purely passive fluxes.

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Guanine: Formation during the Thermal Polymerization of Amino Acids

Abstract. *The action of heat on a mixture of amino acids was studied as a possible abiological pathway for the synthesis of purines and pyrimidines. Guanine was detected. This result is significant in the context of chemical evolution.*

The thermal polymerization of amino acids has been extensively investigated by Fox and his co-workers (*1*). The striking results that have been obtained seem to indicate that heat may have been a possible source of energy for the synthesis of protein under abiological conditions.

The heating of amino acids ordinarily results in tars and other pyrolytic matter (*2*). In the presence of large proportions of aspartic acid or glutamic acid, however, copolymeric peptides are formed. Anhydropolymers, consisting of the 18 amino acids usually present in proteins, can be obtained. The properties of these preparations are similar to those of protein (*3*).

The main purpose of our investigation was to see whether some of the nucleic acid constituents could be formed during the thermal polymerization of amino acids. Such a result is, perhaps, not to be altogether unexpected, since amino acids are intermediates in the biosynthetic pathways leading to the formation of purines and pyrimidines. Guanine, which occurs both in DNA and RNA, was identified in the reaction products obtained by heating the amino acids together.

The proteinoid was prepared according to the method of Fox. Two parts of glutamic acid, two parts of aspartic acid, and one part of an equimolar mixture of the remaining 16 amino acids were heated together in a stream of nitrogen for 6 hours at 180° to 200°C. The resulting mixture was then hydrolyzed with 6*N* HCl at 105°C immediately after its preparation. The hydrolyzate was evaporated under vacuum to a very small volume and neutralized with ammonium hydroxide. Thin-layer chromatography and paper chromatography were used for the analysis.

The technique of thin-layer chromatography is illustrated in Fig. 1. The supporting medium was a layer of silica gel G, 0.5 mm thick (*4*). The solvent was a mixture containing water-saturated butanol (99 percent) and ammonium hydroxide (1 percent). A portion of the reaction mixture was streaked along the origin of the plate. A mixture containing approximately 25 μg of each of the bases, adenine, guanine, cytosine, and uracil, was applied along the origin. In a typical run, the solvent ascended 15 cm along the plate in 4 hours. The bulk of the free amino acids was separated from the bases in this manner.

The thin-layer chromatography plates were then dried and the right marginal area in which the bases would appear was sprayed with 2,7-dichlorofluorescein. In ultraviolet light the bases were located as dark absorbing areas against a green fluorescent background (*5*).

A strip of silica gel corresponding to each of the standards was scraped off the chromatography plate and was eluted with 10 ml of 0.01*N* formic acid. The eluted material was evaporated to a small volume (200 μl) and a two-dimensional chromatograph on Whatman No. 4 paper was made. The solvents were a mixture of propanol, ammonia, and water (6:3:1 by volume), and butanol, propionic acid, and water (14:9:10 by volume). Shadowgrams of these chromatograms were prepared by laying the chromatograms over Kodagraph contact paper and shining an ultraviolet light above the chromatogram (*6*). The ultraviolet

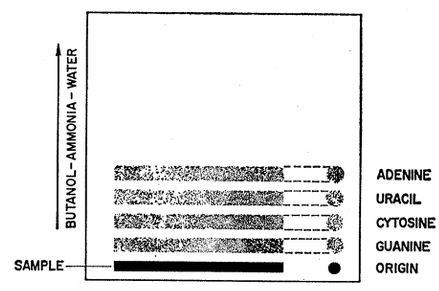

Fig. 1. Technique of thin-layer chromatography.

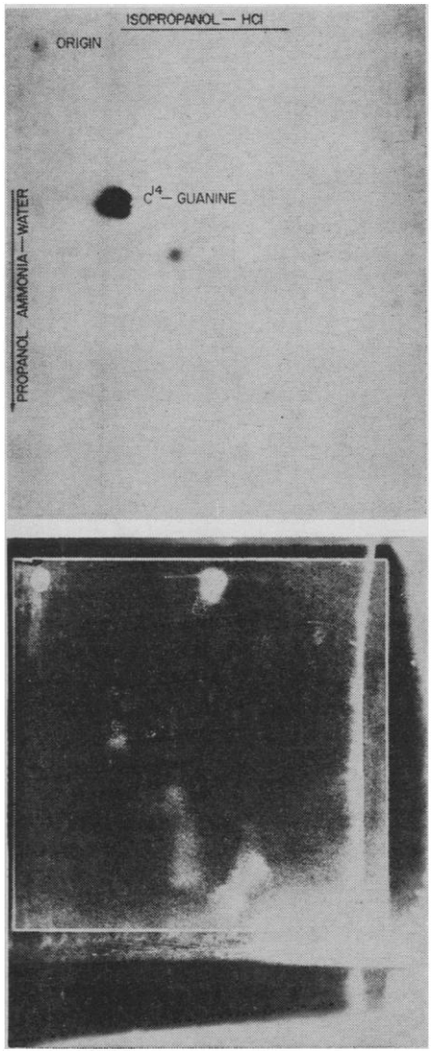

Fig. 2. The coincidence of the C^{14}-guanine spot on the autoradiograph (top) and the ultraviolet-absorbing spot on the shadowgram (bottom) illustrates the formation of guanine during the thermal polymerization of amino acids. The large ultraviolet absorbing spot to the right of the origin is probably due to a free amino acid.

absorbing areas on the chromatogram appeared as white spots on a dark background. By this method the smallest amount of purine or pyrimidine that could be detected was 0.03 μg. The paper chromatogram of the material eluted from the area corresponding to the guanine standard on the thin-layer chromatogram showed an ultraviolet absorbing spot having the R_F values of guanine in the two solvents used.

In a second experiment the eluted material was chromatographed again with a trace of C^{14}-labeled guanine. The amount of guanine-C^{14} used was large enough to darken an x-ray film after an exposure of 10 days, but too small to show any ultraviolet absorption on a shadowgram. The darkening of the x-ray film corresponded in shape and position to the ultraviolet-absorbing area of the shadowgram (Fig. 2). This coincidence was obtained in four different solvent systems: propanol, ammonia, and water (60:30:10 by volume); isopropanol and 2*N* HCl (65:35 by volume); butanol, anhydrous formic acid, and water (77:10:13 by volume); and butanol and water (86:14 by volume). Guanine was the only base detected by this technique. The ultroviolet spectrum at *p*H 1 of the material eluted showed maxima at 2750 Å and 2480 Å confirming the presence of guanine.

In a control experiment the identical mixture of amino acids was subjected to the entire experimental procedure except the polymerization by heat. No guanine was detected. In the experiment in which guanine was detected the conditions—heating to 200°C, hydrolysis with 6*N* HCl, extraction with 2*N* NH_4OH—were sufficiently rigorous to preclude any significant microbial contamination during the process itself.

The amount of material formed is extremely small. In six different runs 5 g of proteinoid gave an average yield of 0.6 μmole of guanine. During the hydrolysis of the proteinoid by 6*N* HCl some of the guanine must have been lost by conversion into xanthine. None of the other bases—adenine, cytosine, uracil, or thymine—appear to be formed.

In order to investigate the possibility of these bases being formed in quantities less than 0.03 μg, which was the smallest amount detectable by the technique used, further experiments were performed incorporating chromatographically pure aspartic and glutamic acids uniformly labeled with C^{14} into the mixture of amino acids. Radioactive guanine was detected in this experiment and confirmed our previous finding. The experiment did not, however, reveal the presence of any of the other bases.

The mechanism of the synthesis is obscure. Hydrogen cyanide, which is known to be an intermediate in purine synthesis (*7*), may be produced by the pyrolysis of amino acids. The results of the experiments with labeled material show that both aspartic acid and glutamic acid may contribute to the synthesis of guanine. It is not clear to what extent the other amino acids which were used in the proteinoid synthesis took part in the formation of guanine. It is suggested that the formation of guanine during the thermal polymerization is relevant to a discussion on chemical evolution. The yield of guanine in this process, although small, may be considered significant in the context of a time scale of the order of several hundred million years.

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X-ray Diffraction Study of a DNA Which Contains Uracil

Abstract. *Three-dimensional structure of DNA from PBS2 bacteriophage, which contains uracil in place of the more usual thymine, is the same as that of "normal" DNA. The difference in secondary structure between DNA and RNA thus appears to be due to the 2'-hydroxyl group alone.*

The DNA of the *Bacillus subtilis* transducing bacteriophage PBS2 has the unusual property of containing uracil in place of thymine (*1*). Since all DNA samples studied so far appear to have essentially effectively identical three-dimensional structures (*2*) which differ from RNA (*3*), we were interested in finding the effect on the DNA structure of a base