On Biochemical Origins and Optical Activity

Sidney W. Fox, Joseph E. Johnson, Allen Vegotsky

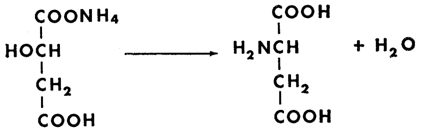
Attempts to explain the origin of the biochemical world often rest on such reactions as those of formaldehyde and ammonia (*1*), the high-energy radiation of aqueous carbon dioxide (*2*), electric discharge through presumably primitive gases (*3*), reactions of cyano compounds (*4*), and thermal reactions (*5*) that were unintentionally uncovered in experiments designed to explain the primordial origin of protein (*6*).

Simple heat in the range of 100° to 200°C has been shown to be sufficient to imitate a number of early steps in the pathways of biosynthesis. Ammonium hydrogen malate is converted by purely thermal means into aspartic acid (*5*), α-alanine (*5*), β-alanine (*7*), and polymers from which such amino acids may be recovered after hydrolysis (*5*). Preliminary indications, such as positive biuret tests, have been obtained for the presence of peptides in the polymers and of thermal conversion of the identified amino acids to yet others. Evidence that polymers formed by thermal treatment of unsubstituted aspartic acid are, at least in part, linear peptides has also been provided (*8*).

The presumption that the thermal reactions imitate prebiological chemistry is supported by the deduction that the most primitive algae are thermophilic (*9*) (the fossils of the most primitive organisms being algal, *10*), by the inference that the current biochemical world is not greatly different from the primordial biochemical world (*11*), and by additional evidence from thermal experiments, as described here. The thermal emphasis is in accord with the estimates of terrestrial temperature of billions of years ago (*12*). Furthermore, a temperature above the boiling point of water (varying, of course, with the prevailing pressure) might make it possible to explain the overcoming of the thermodynamic infeasibility (*13*) of forming the first, or any other, peptide bonds by removal of water.

$$\mathrm{H_2NCHRCOOH + H_2NCHR'COOH \rightarrow H_2NCHRCONHCHR'COOH + H_2O}\uparrow$$

These considerations, in fact, led to the first thermal experiments in this program (*14*). This paper describes the finding of ureidosuccinic acid as a product f reaction and also presents new considerations of the origin of optical activity. The conversion of ammonium hydrogen malate to aspartic acid has been studied



$$\begin{array}{l}\mathrm{COONH_4}\\ |\\ \mathrm{HOCH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{COOH}\end{array} \longrightarrow \begin{array}{l}\mathrm{COOH}\\ |\\ \mathrm{H_2NCH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{COOH}\end{array} + \mathrm{H_2O}$$

for a range of time and temperature (Table 1).

The results indicate a ceiling yield of aspartic acid of approximately 40 percent in a range of 140° to 200°C for 1 to 4 hours. The ammonium hydrogen malate was prepared from L-malic acid and concentrated ammonium hydroxide in 95-percent ethanol, from which it precipitated immediately (melting point, 159° to 160°C) (*15*). The salt was heated in an open test tube in an oil bath and cooled; the viscous product was dissolved in excess 6*N* hydrochloric acid, and the mixture was hydrolyzed at 15 lb of steam pressure in an autoclave for 16 hours. The product was treated with sodium acetate and converted to copper aspartate and thence to aspartic acid (*16*), which was examined for optical activity. Except for a small proportion of optical activity found in a preliminary experiment, the products were devoid of optical activity. Racemization had, therefore, occurred under these conditions.

Accordingly, it became of interest to determine whether the conversion of malate to aspartate could be effected through a reaction other than that requiring the ammonium salt. A faster mode of reaction might enable the conversion to proceed at a temperature sufficiently lower to preclude racemization. For this purpose, urea in admixture with malic acid was employed instead of ammonium hydrogen malate. A more compelling reason for the use of urea was the fact that it represents the urea cycle (*17*), which appears to function in autotrophic organisms as a mode of synthesis of arginine (*18*) and perhaps of other biosynthetic intermediates as well. The simultaneous employment of intermediates from the tricarboxylic acid cycle and the urea cycle in a thermal experiment might thus be expected more closely to imitate prebiological chemistry. Results of a number of experiments indicated that aspartic acid or its derivatives or both were indeed formed at temperatures such as 100° and 120°C in appreciable amounts when urea was a reactant with malic acid. Melting point and chromatographic evidence from paper and Dowex-1 columns, however, suggested that ureidosuccinic acid was also formed. Although this result was not consistently reproduced, the finding led to experiments in producing ureidosuccinic acid under conditions which enter the purview of this project.

Formation of Ureidosuccinic Acid

Ureidosuccinic acid has been prepared by Lippich (*19*) by heating aspartic acid in the presence of a mixture of urea and barium hydroxide in water. It was now of interest to ascertain whether this synthesis could be accomplished with calcium hydroxide instead of barium hydroxide. Many of the "primitive" blue-green algae are known to thrive under calcareous conditions (*9*). Any comprehensive theory of biochemical origins must therefore explain the introduction of calcium, which is found abundantly throughout phylogeny. On the basis that the ultimate biological ancestors were thermal aquatic types (*9*), it is also pertinent to study reactions simulating biochemistry in a hot aqueous environment. If some of prebiological chemistry was essentially nonaqueous and, therefore, favored peptide-bond formation, modulation to an aqueous system must also be understood.

Magnesium oxide was also tested on the basis that magnesium ion is a cofactor in ureidosuccinic acid metabolism (*20*). Experiments were also performed with potassium hydroxide. The results with these bases are presented in Table 2. The *p*H ranges for calcium and magnesium overlapped the range in which some thermal blue-green algae are known to thrive—namely 2.8 to 9.1 (*9*).

Because of the greater reproducibility of synthesis in aqueous systems it was also of interest to study the effect of variation in the proportion of water. The results are presented in Fig. 1.

The synthesis of ureidosuccinic acid from aspartic acid was far more readily reproducible in aqueous solution than in dry heating of the mixture of malic acid and urea. Table 2 reveals a much lower yield in the absence of base than in its

Dr. Fox and Dr. Vegotsky are with the Oceanographic Institute, Florida State University, Tallahassee, and Dr. Johnson is at the Massachusetts Institute of Technology, Cambridge.

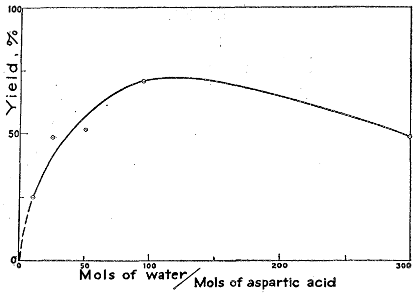


Fig. 1. Variation in yield of ureidosuccinic acid with variation in proportion of water.

presence. This is undoubtedly the main cause of the difference.

Optical Activity in Nature

The origin of the large degree of configurational uniqueness in hydroxy acids, carbohydrates, amino acids (*21*), and other substances in nature might be better understood as arising from a single molecular structure which happened to be of one optical type. In this manner, L-malate might be expected to yield L-aspartate, and other amino acids produced from a single L form by thermal conversion could be expected also to be of the L configuration. In the conditions studied so far in the experiments reported, racemization was the principal result, and it is appropriate therefore to consider other possibilities. These evaluations rely particularly on the concept of spontaneous resolution. A type of spontaneous resolution is the chance mechanical separation of a crystal of one optical type to a key intermediate (*22*).

The phenomenon of spontaneous crystalization favoring one optical form was reported by Pasteur (*23*) with the compound of prime interest in the present work, ammonium hydrogen malate. This observation of Pasteur was confirmed by van't Hoff and Dawson (*24*), and a similar behavior from melted ammonium hydrogen malate was disclosed by Kenrick (*15*). These and other cases are discussed by Greenstein (*25*). Partial resolution by seeding has also recently been reported for threonine (*26;* see also Havinga, *27*).

The biological facts indicate that some microorganisms that are relatively low on the phylogenetic scale are rich in D amino acids (*28*), whereas the D form is all but unknown in higher organisms. In addition, the puzzling occurrence of D-amino acid oxidase in mammals (*29*) can be explained as a chemical evolutionary vestige (*30*), inasmuch as the lower forms do contain D-amino acid residues. In the early stages of evolution, the spontaneous appearance of one key intermediate in a single configuration should trigger configurational one-sidedness through an entire biosynthetic chain. For example, ammonium hydrogen D-malate (*22, 23*) might spontaneously crystallize from a primitive, natural nutrient medium, and the L form left in solution would then yield L conversion products such as L-aspartic acid and L-alanine (*31*). Enzymes composed of L-amino acid residues would be expected to have a selective advantage (*32, 33*). The theoretical expectation that any statistical number of racemic molecules will contain at least a few of one enantiomorph in excess of the other (*27*) could also lead to configurational unity by Darwinian selection through the uncounted generations of evolution (*32*).

Whether a preponderance of one enantiomorph of any key biochemical substance may have arisen gradually or suddenly, it would be unnecessary for this phenomenon to have occurred in the prebiological era, and it would be more probable, for the reasons given, that it appeared in the biological era.

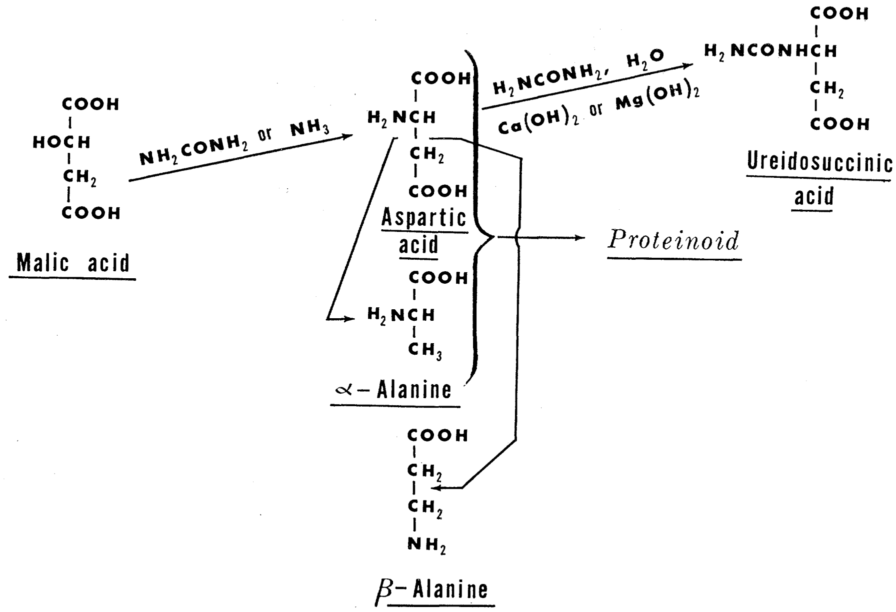


Fig. 2. Pathway of thermal synthesis (biosynthesis).

Table 1. Weights (g) of aspartic acid from 5.0-g lots of ammonium hydrogen L-malate over a range of time and temperature. The aspartic acid was obtained by heating ammonium hydrogen malate after hydrolysis of the product.

Temperature (°C)	Time (hr)		
	1	2	4
140			0.7
160	0.6	1.3	1.7
180	2.1	2.1	1.7
200	2.2	2.2	2.1

Table 2. Yields of ureidosuccinic acid from aspartic acid and urea with each of four bases in hot water.

Base	Yield (%)	Melting point (°C)	Initial *p*H	Final *p*H
$Ba(OH)_2 \cdot 8H_2O$	57	178–1/2–180	12.9	9.7
$Ca(OH)_2$	47	181	9.0	9.8
MgO	46	178	8.2	9.8
KOH	80	178	12.8	10.1
None	5	164–6		

Conclusions

The finding of ureidosuccinic acid as a principal product of the thermal reaction of malic acid and urea under hot-springs conditions is pertinent to the origins of biological chemistry in that ureidosuccinic acid has been shown in recent years to be a key intermediate in the biosynthesis of nucleic acids (*34*).

The accumulated data reveal a thermal pathway as shown in Fig. 2. These reactions, with some minor modification, represent also some of the early steps in biosynthesis. Many inferences may be drawn from these relationships (*35*).

Considerations of the primordial origins of the biochemical world are necessarily subject to a long process of variation and retesting before they are fully acceptable. Justification of such activity lies in the possibility that it "limits the range of speculation," as expressed by Rubey (*3*) in a discussion of the origin of the biogeochemical world. It can be expected that the limits of speculation on the origin of the biochemical world may be set most accurately by the framework of dynamic biochemistry and a back-extrapolation of biological evolution, as is contemplated elsewhere (*35*).

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News of Science

Nuclear Weapons Tests

A recent statement emanating from the National Academy of Sciences that nuclear test explosions could be increased tenfold "without causing any serious genetic danger" has resulted in the publication (*Washington Post,* 26 Oct.) of the following letter from A. H. Sturtevant of California Institute of Technology.

"I have just seen the news item in your issue of Oct. 15, headed 'Tenfold Rise in A-Tests Seen as Safe.'

"This account implies that the National Academy of Sciences Committee on the Genetic Effects of Radiation concluded that a tenfold increase in fallout would not be serious.

"As a member of that committee I wish to state that the report of the committee reaches no such conclusion, and that I, for one, would have been unwilling to sign a report that could reasonably have been so interpreted.

"Further, since the committee reported, Commissioner Libby has indicated (Oct. 12) that the danger from radioactive strontium in fallout is greater than the information available to the committee led us to suppose. For this reason, our conclusions about the danger from fallout need revision upward."

In recent weeks other statements by scientists about the testing of hydrogen bombs have appeared in the press:

Bentley Glass of Johns Hopkins University, who like Sturtevant was a member of the NAS Committee on the Genetic Effects of Radiation, cautioned that uncontrolled testing of nuclear weapons could become a genetic threat through competitive snowballing. Glass proposed an international agreement on the number of nuclear explosions allowed to each nation, as a needed safeguard for the protection of the human race (*Washington Post,* 17 Oct.).

In supporting the proposal to discontinue large nuclear tests, ten scientists at California Institute of Technology said: "It appears to us that this might be a useful way to get the negotiations [on nuclear arms restrictions] out of the deadlock stage by taking a step that would not endanger our security" (Associated Press, 14 Oct.).

Later (Associated Press, 21 Oct.), 73 scientists at Argonne National Laboratory added their signatures to the C.I.T. declaration.

In contrast, five others said: "As citizens, we wish to express our approval of the test program as handled to date" (*New York Times,* 22 Oct.).

Lee A. DuBridge, president of C.I.T., followed his colleagues' endorsement of a test ban with the observation that from "my own official Government contacts, I have become convinced [that] large-scale tests are an important part of our weapons-research program. . . . Discontinuance [of such tests] should . . . not precede enforceable international agreements" (Associated Press, 15 Oct.).

Thirty-seven faculty members of the City College of New York, including 14 scientists, "warmly endorsed" the test ban proposal (*New York Times,* 19 Oct.).

Describing the outlook as "alarming," 24 scientists at Washington University in St. Louis commented that the nation's atomic policy has been made "in a vacuum of public information." They urged "intensive scientific study and public discussion" (Associated Press, 18 Oct.).

However, Arthur H. Compton, also of Washington University, and one of the principal figures in the development of the A-bomb, has stated that continued H-bomb tests are necessary "to maintain our freedom" (*Christian Science Monitor,* 25 Oct.).

Eleven members of the physics department at Columbia University, including Nobel laureate Polykarp Kusch, added their support to the proposed ban on tests and urged the President to join in the "clarification of public thinking on this crucial issue" (*New York Times,* 17 Oct.).

Nineteen members of the Atomic En-