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Synthesis of Uracil under Conditions of a Thermal Model of Prebiological Chemistry

Abstract. Uracil has been formed under thermal conditions which yield other materials of theoretical prebiochemical significance. The chemical evidence for the identity of uracil rests on chromatography, spectrography, color tests, and melting points and analyses of isolated material. The place of uracil in a scheme of biochemical origins, including carbon dioxide as an intermediate, is discussed.

A thermal model of biochemical origins has yielded a network of chemical pathways resembling a flowsheet of biosynthesis (*1*), amino acid copolymers having many of the properties of proteins (*2*), and microspherical units with some of the properties of bacterial boundaries (*1*). Part of the uniqueness of this model lies in the fact that these phenomena emerge in a continuum of physical conditions (*1*). One of the first products in the thermal experiments was ureidosuccinic acid (*3*), an intermediate in the biosynthesis of pyrimidines (*4*). This report describes the direct synthesis of uracil itself under conditions consistent with the other physical details of the model and of its recapitulationist emphasis on malic acid (*5*) which is in turn derivable from simpler compounds (Fig. 1).

In the light of the recent finding that polyphosphoric acid promotes the thermal condensation of amino acids (*1*) and the recognition of the many biological activations by polyphosphate, earlier studies of the reaction of malic acid and urea were extended by testing polyphosphoric acid as a reactive solvent. In the first experiments hypothetical hydrogen acceptors such as nicotinamide were included, but controls showed that the reaction proceeded in the presence of polyphosphoric acid alone. No chromatographic evidence of uracil was obtained in numerous experiments in which ureidosuccinic acid or oxaloacetic acid was included instead of malic acid.

Chromatography was carried out with ascension in one dimension on 3 MM Whatman chromatography paper with butanol-1 saturated with 10-percent urea or butanol-1 saturated with 7*N* NH_4OH. The R_F values of authentic uracil and of one spot was 0.32 in the former solvent and 0.16 in the latter solvent. The spots were identified by fluorescence in the rays from a Mineralight R-51 lamp. Uracil was isolated from a simultaneous series of bar chromatograms by elution with 0.1*N* HCl.

The conditions studied in the synthesis of uracil included temperatures in the range of 100° to 140°C, heating periods of from ¼ to 2 hr, and a range of ratios of polyphosphoric acid (Victor Chemical Co.) to equimolar proportions of malic acid and urea. Uracil was obtained over the entire range of temperature. Increase in proportion of polyphosphoric acid had the most pronounced effect of the factors tried. The variation in one experiment is shown in Table 1. The yield was determined by elution after chromatography and estimation against a standard curve at 260 mμ on a Beckman DU spectrophotometer.

From one of these samples was isolated by bar chromatography 86 mg of white recrystallized (H_2O) uracil of melting point and mixed melting point, 335°C, decrease uncorrected. Calculated for $C_4H_4N_2O_2$: C, 42.85; H, 3.59; N, 24.99. Found (by the Mikroanalytisches Laboratorium, Bonn, Germany): C, 42.84; H, 3.71; N, 24.84.

The uracil synthesized in the presence of polyphosphoric acid had a melting point and mixed melting point with authentic material of 335°C, decrease uncorrected. The Wheeler-Johnson color test with bromine-water and barium hydroxide (*6*) was positive

Table 1. Yields of uracil from varying amounts of polyphosphoric acid with 0.010 mole of malic acid and 0.015 mole of urea at 130°C for 60 min.

Amount of polyphosphoric acid (ml)	Yield of uracil (mmole)
0.5	Negligible
1.0	0.04
2.0	0.06
4.0	0.4
8.0	1.2
16.0	1.4

Ultraviolet absorption spectra of the newly synthesized material in acid, in neutral, and in alkaline solution were identical with the corresponding spectra of authentic material. The infrared absorption in Nujol mull of the new material was also identical with that of authentic uracil.

The thermal formation of urea from the inorganic substances ammonia and carbon dioxide is well documented as an industrial reaction and can thus explain the primordial origins of urea (*7*). Malic acid can be visualized as producible in the primitive context from acetic acid (*8*) which could arise in turn from methane, water, and hydrogen (*9*) or from glycine by radiolysis (*10*) or in other ways (*11*). Although the presence of carbon dioxide in the original atmosphere has been a controversial issue (*11*), it should be recognized that carbon dioxide would probably become available as the result of reactions of other compounds—for example, Miller has found substantial proportions of carbon dioxide in a sparked atmosphere which contained none at the outset (*9*).

The results reported lead to a revised thermal flowsheet, as shown in Fig. 1. The now enlarged picture of prebiochemical reactions, including synthesis

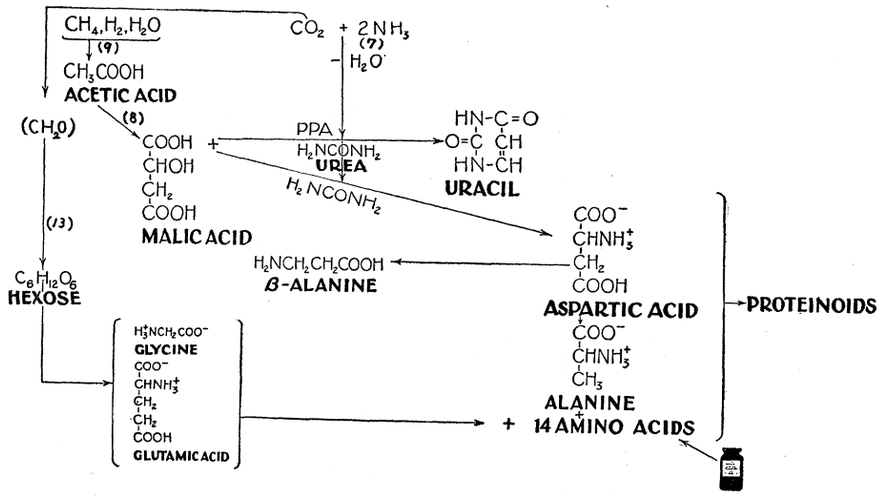

Fig. 1. Flowsheet, predominantly thermal, of suggested prebiochemical reactions. Unnumbered reactions are documented in (*1*).

of the nucleic acid component and of polymers with many properties of proteins (*2*), is consistent with a unified concept of the origin of biochemical pathways in a predominantly phosphoric medium (*14*).

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3 February 1961

Quantal and Graded Analysis of Dosage-Effect Relations

Abstract. Loewe's recommendations regarding treatment of stimulus-response relations are criticized. Conditions are described where quantal analysis is justified. Loewe's interpretation of his graded analysis must be modified in the light of the fact that response curves for individuals frequently cross. Superior lines of attack on the problem are suggested.

Loewe (*1*) has examined the logical consequences of an investigator's decision whether to treat responses to drugs as graded (ordinally scaled) or quantal (dichotomized). He concluded that the two analyses bring out different relationships, and that since the chief concern in most experiments is to determine the relation of response strength to stimulus strength, the quantal analysis is inappropriate. Especially because the conclusion, if valid, would apply widely in behavioral and biological research, it requires close scrutiny.

Loewe regards as unsatisfactory analyses which discard an appreciable amount of important information from the data, and this view is beyond dispute. Two situations, however, may be distinguished: either differences in response strength all along the scale are important to the investigator (as Loewe assumes), or there is some response level E_0 of special significance, such that attaining it or not attaining it is far more important than differences in response level elsewhere on the scale. The second case is not infrequently encountered. In personnel selection a dichotomous criterion of job performance appropriately represents utility to the firm, if differences between satisfactory men and those who must be discharged are far more critical than differences in output among the satisfactory men (*2*). In a tryout of advertising, the consumer's decision to buy or not to buy the product should sometimes be studied without taking into account degrees of interest in the product above or below this point. Surely there are drugs (for example, anesthetics, insecticides) where a certain level of response marks the transition from useless to useful effect. There may be theoretical as well as utilitarian reasons for preferring to quantize data; Estes (*3*) argues for analyzing learning in terms of the appearance or nonappearance of a response rather than in terms of a scaled measure such as latency.

If there is a critically important E_0, the information desired is the proportion of subjects giving a response equal to or greater than E_0, as a function of stimulus strength (dosage, D, in Loewe's case). There may be more than one critical E; if so, more than one probability function can be plotted.

Even if gradations of response are important, Loewe's recommendations require re-examination. His mathematical model consists of a surface representing effect E as a monotone increasing function of dosage D and individual tolerance T. In the model, T is not clearly defined; we know only that the individual's responsiveness is expressed as a percentile relative to others under study. To summarize the surface conveniently, Loewe would use D,E cross sections (T constant at 50, 16, and 84, or other such values). Loewe then describes an experiment whose data are coordinated with this model. The proposed single-dose experiment consists of drawing random samples and giving to each sample a different dosage. This produces a distribution of E for each D, which can be converted into a curve representing cumulative probability p_E as a function of E. The curves for various D form a surface. Loewe identifies p_E with T, though one is a group statistic and one is a constant associated with the individual; hence he identifies the D,E,p_E surface with the D,E,T model. He takes cross sections with p_E constant as the desired summary curves.

Loewe's model appears to be overly restrictive. His surface represents actual data only if all individuals having a certain tolerance T have the same D,E curve, within the limits of experimental error. This can occur only if the set of curves for all individuals is disjoint, that is, if no two curves cross each other within the range of D under study. When curves are disjoint, the only fault in his recommendation is that he preserves too little information to satisfy the person for whom some E_0 is of prime importance.

As a matter of fact, however, learning curves, drug-response curves, and so forth, for individuals often cross (*4*, *5*). Some measure of the individual (for example, strength of response at some arbitrarily chosen dose) may be used to represent T, but for every T there will be numerous D,E functions, and a distribution of E against D, not a curve, will be obtained. Only when curves are disjoint is it correct to identify p_E with T. A single-dose experiment does not permit a test of disjunction. If such an experiment is performed, and if utility considerations make gradations of response important, Loewe's analysis is an acceptable method of summarizing the distribution of E as a function of D even though it probably does not represent the relation of E to D for constant T.

Wherever the risk of unwanted order effects can be disregarded, it is much more informative to carry out an individuals-times-levels experiment in which several points on the curve for each individual are determined by successive dosings. If the data support the assumed disjunction of curves, one can give Loewe's analysis the strong interpretation he proposes. If they do not, a more powerful analysis should be sought. One possibility is to divide persons into groups such that the set of curves within any group is disjoint, after which Loewe's analysis can be applied. Even more powerful is the technique of establishing a limited number of prototype curves and describing each individual's record in terms of one curve or a combination of them (*5*). Such techniques for recognizing individual variation in shape of curve as well as differences in threshold level are still in an early stage of development.

Loewe's argument is consistent only if we accept his hidden assumptions: that no level of effect is especially important, that D,E curves for individuals are disjoint, and that a single-dose ex-