Table II
Excretion of Noradrenaline, Adrenaline, and Hydroxytyramine in Various Psychiatric Patients

Subject	Noradrenaline (µg/100 mg creatinine)	Adrenaline (µg/100 mg creatinine)	Hydroxytyramine (µg/100 mg creatinine)
1	2·80	0·60	39·00
2	4·90	1·10	28·90
3	2·20	0·30	24·50
4	1·90	0·50	26·00
5	2·45	0·38	24·80
6	7·90	1·32	39·30
7	6·85	0·95	31·00
8	8·00	1·67	46·00
9	6·25	1·50	52·50
10	4·10	0·02	36·00

Further specificity can be obtained by using a double-label technique. Urine extracts can be acetylated with tritium labeled acetic anhydride to convert adrenaline, noradrenaline, and hydroxytyramine quantitatively to the tritium labeled triacetate. A measured amount of authentic triacetate adrenaline-C^{14}, triacetate noradrenaline-C^{14}, and triacetate hydroxytyramine-C^{14} is added to each sample and the double labeled catecholamines are identified and purified by paper chromatography. The tritium and carbon^{14} content of the purified catecholamines is assayed by simultaneous counting in a liquid scintillation spectrometer. The amount of adrenaline, noradrenaline, and hydroxytyramine in the original extract can be calculated from the determination of the amount of carbon^{14} indicator lost during the purification, the yield of tritium radioactivity, and the specific activity of the tritium labeled acetic anhydride.

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New York University College of Medicine, Department of Psychiatry and Neurology, Psychopharmacology Research Unit, New York, November 3, 1958.

Zusammenfassung

Es wird eine neue Methode zur Bestimmung der Katecholamine im Harn beschrieben. Die Katecholamine werden im Harn acetyliert und die Acetylderivate papierchromatographisch im Bush-«C»-System getrennt. Nach erfolgter Trennung werden die einzelnen Katecholamine mit Äthylendiamin kondensiert und fluorimetrisch untersucht. Eine Spezifitätserhöhung der Methode durch Anwendung doppelt markierter Isotopen wird in Betracht gezogen.

Informations - Informationen - Informazioni - Notes

THEORIA

Thermal Polymerization of Amino Acids and a Theory of Biochemical Origins[1]

By S.W. Fox, Kaoru Harada, and A. Vegotsky[2]

The possibility of synthesizing peptides and perhaps protein (Fox and Middlebrook[3]) by simple heating of unsubstituted amino acids is one which has not been favored by chemical experience (Katchalski[4], Noguchi and Hayakawa[5], Curphey[6], Meggy[7], Bamford, Elliott, and Hanby[8]). When this goal was approached as a theoretical problem in prebiological molecular evolution, it became possible to visualize how the energetic barrier for the synthesis of protein could be overcome thermally (Fox, Johnson, and Vegotsky[9]), and how precise ordering of residues might result from selective influences of the reactant amino acids themselves (Fox[10]). The experiments which have resulted indicate that it is possible to prepare copolymeric peptides thermally if aspartic acid or glutamic acid is a reactant. It is furthermore possible, by using a considerable molar excess of both aspartic acid and glutamic acid, to copolymerize the eighteen common amino acids into a *proteinoid* which, after being hydrolyzed, reveals on paper chromatography the same qualitative composition as casein and other proteins.

In considering this knowledge in detail, attention should first be focussed on the chemistry of each of the dicarboxylic amino acids. It has long been known that heating glutamic acid alone yields the inner lactam, pyrrolidonecarboxylic acid (pyroglutamic acid). In this research it has been learned that heating glutamic acid with each of a number of other amino acids produces a copolymeric linear peptide when the temperature of reaction is 170°C for 3 h (Harada and Fox[11]). The polymer of glutamic acid and glycine has a mean chain weight of 10,000 to 20,000 after dialysis. During such reactions, the glutamic acid is converted to pyroglutamic acid which is molten in the presence of other materials at 170°C, and functions therefore as a solvent. The glutamic acid and pyroglutamic acid probably contribute also as acid catalysts. In addition, comparative yields suggest that the pyroglutamic acid is the more reactive species.

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[2] The Oceanographic Institute and Department of Chemistry of The Florida State University, Tallahassee.

[3] S. W. Fox and M. Middlebrook, Fed. Proc. *13*, 211 (1954).

[4] E. Katchalski, Adv. Protein Chem. *6*, 123 (1951).

[5] J. Noguchi and T. Hayakawa, J. Amer. chem. Soc. *76*, 2846 (1954).

[6] E. G. Curphey, Chem. & Ind. *1956*, 783.

[7] A. B. Meggy, J. chem. Soc. *1956*, 1444.

[8] C. H. Bamford, A. Elliott, and W. E. Hanby, *Synthetic Polypeptides* (Academic Press, New York 1956).

[9] S. W. Fox, J. E. Johnson, and A. Vegotsky, Science *124*, 923 (1956).

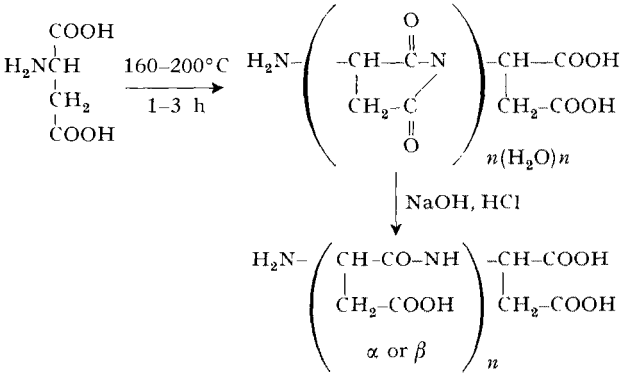
[10] S. W. Fox, Amer. Scientist *44*, 347 (1956).

[11] K. Harada and S. W. Fox, J. Amer. chem. Soc. *80*, 2694 (1958).

Relative Proportions of Glutamic Acid in Total Composition and N-Terminal Position in Thermal Polymers of Glutamic Acid and Aspartic Acid

Mols aspartic acid in reaction mixture	Mols glutamic acid in reaction mixture	Proportion of glutamic acid in whole polymer (A)	Proportion of glutamic acid in N-terminal position (B)	B/A
0·01	0·01	29%	59%	2·0
0·02	0·01	19%	54%	2·8
0·03	0·01	15%	54%	3·6

The heating of aspartic acid does not lead to a liquid product below 200°C, but it can also copolymerize with other amino acids. The copolymers with aspartic acid do not include as large proportions of neutral and basic amino acids as do the copolymers with glutamic acid.



An unique feature of the thermal polymerization of aspartic acid is that it yields a polyimide (KOVACS and KOENYVES[12], VEGOTSKY, HARADA, and FOX[13]). This polyimide can be gently split at each of the imide linkages by excess dilute cold alkali to yield a true peptide. The tendency of aspartic acid to copolymerize appears to be greater when heated with glutamic acid and this is probably explainable on the basis that all of the aspartic acid and glutamic acid is in a clear liquid at 170°C.

The copolymerization of glutamic acid and aspartic acid proceeds with exceptional facility. In this combination was first observed a salient relationship – the amino acids arrange themselves in a nonrandom style. This is illustrated in the experiment detailed in the Table.

The analyses were performed by hydrolysis of the whole polymer, DNPylation, and estimation of the DNP-amino acids. The N-terminal amino acids were determined on the hydrolyzed DNP-polymers. The results show that the proportion of glutamic acid in the N-terminal position is from 2 to $3^1/_2$ times as great as in the total composition. This substantiates the original working hypothesis mentioned at the outset and answers in principle the question of how precise order might have arisen in primordial protein before there were enzymes or nucleic acids on the earth to guide the order.

By the time this much knowledge had accumulated, the original goal of synthesis of protein was more precisely defined as the synthesis of polymeric peptide material which contains all of the eighteen amino acids common to protein (FOX[10]), without regard to configuration of the amino acid residues (FOX, JOHNSON, and VEGOTSKY[9]). Although other working premises can be adopted (AKABORI, OKAWA, and SATO[14]), material of this description would be qualitatively indistinguishable from current protein and perhaps more like original protein than current protein. Part of the reason for believing that the first protein was so like current protein is the concept of the 'unity of biochemistry' (KLUYVER and VAN NIEL[15]) which has been found to apply to proteins as well as to other substances (FOX[10], FOX[16]).

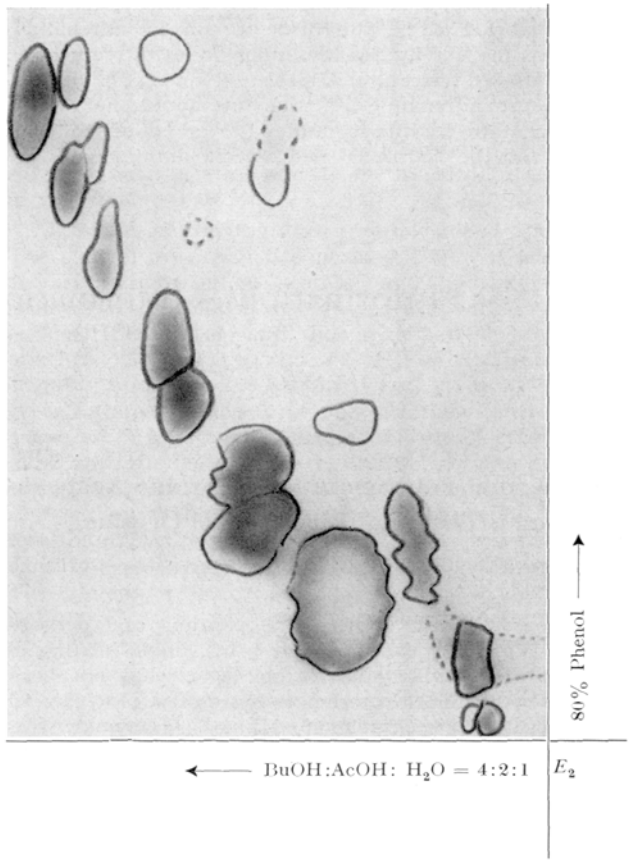


Fig. 1. Two-dimensional chromatogram of hydrolyzate of proteinoid

Attempts to pyropolymerize all the common amino acids have been made from time to time in this program. These attempts were based usually on unimolar ratios of amino acids. After the effect of a dominant glutamic acid-aspartic acid ratio, as well as the unique copolymerization effects of each were recognized, such attempts were again repeated with these two amino acids in large excess. The

[12] J. KOVACS and I. KOENYVES, Naturwissenschaften *41*, 333 (1954).

[13] A. VEGOTSKY, K. HARADA, and S. W. FOX, J. Amer. chem. Soc. *80*, 3361 (1958).

[14] S. AKABORI, K. OKAWA, and M. SATO, Bull. chem. Soc. Japan *29*, 608 (1956).

[15] A. J. KLUYVER and C. B. VAN NIEL, *The Microbe's Contribution to Biology* (Harvard University Press, Cambridge, Mass. 1956).

[16] S. W. FOX, J. chem. Education *34*, 472 (1957).

results were immediately successful (FOX and HARADA[17]) and each of a series of more than twenty subsequent trials has likewise been successful. Renewed attempts to obtain such polymers with unimolar ratios of the dicarboxylic amino acids reveal that even if peptide is formed, the simultaneous formation of unwanted products such as diketopiperazines is so dominant as to make isolation of the peptides difficult at best. Critical need for high proportions of glutamic acid and aspartic acid is consistent with the principle of unity in biochemistry and the facts of (1) disproportionately high ratios of the dicarboxylic amino acids in virtually all proteins and (2) the large amounts of glutamine and asparagine in metabolic pools.

The proteinoids are typically produced simply by heating two parts of glutamic acid by weight, two parts of aspartic acid, and one part of a mixture of amino acids in equimolar proportions at 170°C for 3 h. The unreacted material and smaller molecules are removed by dialysis through a period of five days. In some cases the initial product was sterilized prior to dialysis by immersion of the dialysis sac in methanol or ethanol for 24–48 h. The product yielded a major insoluble fraction and a soluble fraction. The insoluble fraction was dissolved as the sodium salt and dialyzed. The products from this, from the originally soluble fraction, and from the runs which were sterilized were each dried, hydrolyzed, and chromatographed. The chromatograms appeared as in Figure 1 in all cases (FOX and HARADA[17]). The reaction is not delicate, and many variations are possible. Glutamic acid can be replaced by glutamine. Orthophosphoric acid improves the yield and lowers the necessary temperature by typically 20°C, but the reaction proceeds successfully in the absence of phosphoric acid, in yields by weight which exceed 15%. The proteinoid replaces peptone in the broth culture of *Lactobacillus arabinosus* at a somewhat reduced rate of growth, a fact which indicates that the linkages are susceptible to the attack of bacterial proteases. The product is undoubtedly a mixture and it will be some time before it is possible to state how the amino acids are distributed among individual molecules. The product has in peptide linkage all of the common amino acids, except of course that these are optically inactive, and the hydrolyzate behaves in qualitative chromatography like that from protein. This relationship is one which resembles closely that for any two current heterologous proteins. It is significant that this proteinoid was ob-

[17] S. W. FOX and K. HARADA, Science *128*, 1214 (1958).

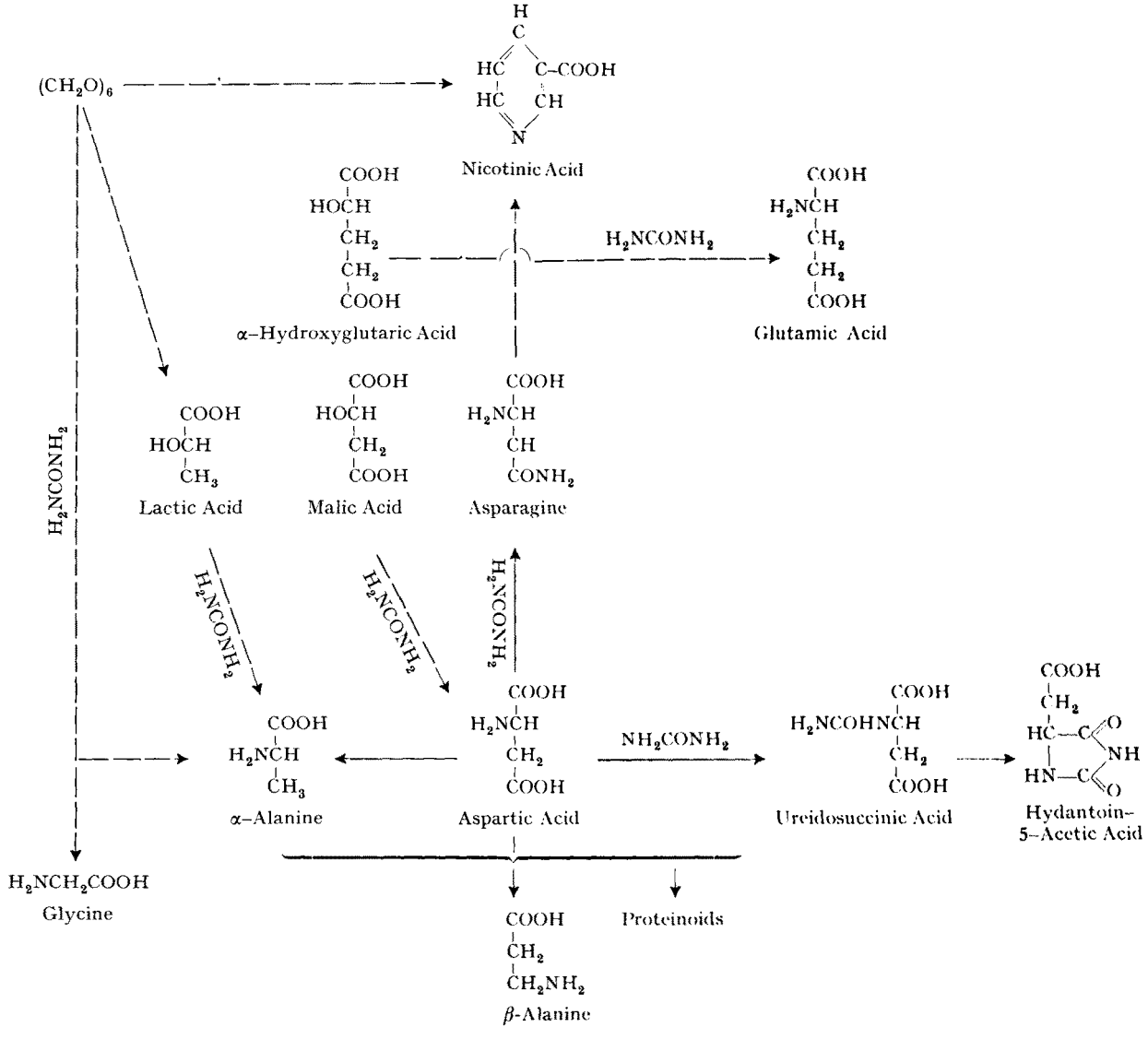


Fig. 2. Thermal pathways which resemble generalized biosynthesis

tained in a hypothetical framework of prebiological conditions. Added support for a protein-like structure of the polymer is found in positive biuret tests, infrared absorption spectra, and a mean chain weight of 4900.

Shortly after this project on thermal syntheses of peptides was begun, unexpected results under these same conditions led to other investigations (FOX, JOHNSON, and MIDDLEBROOK[18]). There was observed the appearance of amino acids, α- and β-alanine, not present in the original. This result suggested that our known family of amino acids may have originated by thermal conversion and rearrangement. One biochemical thing led to another and a thermal reaction flowsheet as in Figure 2 was assembled. These are some of the reactions which have been studied; the solid lines represent those for which the details have been published. It was found that aspartic acid can arise thermally from the Krebs Cycle acid, malic acid, by reaction with ammonia or urea and that aspartic acid is converted to α- and β-alanine. An unexpected product of especial interest was ureidosuccinic acid (FOX, JOHNSON, and VEGOTSKY[9]).

This partial chart includes many thermal products identical to those found in organisms, except for lack of optical activity in some. The reactions are also similar and, most strikingly, the sequences of thermal reactions resemble those of a generalized biosynthesis.

These unified results indicate in principle how the anabolic reactions and protein, somehow functioning as enzyme, could arise jointly. The appearance of ureidosuccinic acid suggests the biosynthesis of genic nucleic acid inasmuch as ureidosuccinic acid is now a recognized biological intermediate for pyrimidines (LIEBERMAN and KORNBERG[19]). From this it is possible to visualize the generation of anabolic reactions, enzymes, and genes in a reflexive chemical memory mechanism (FOX, VEGOTSKY, HARADA, and HOAGLAND[20]). This picture is essentially the reverse of the gene-enzyme-reaction system of BEADLE[21] *et al.* The entire integrated picture is of course incomplete, but it is brought into sharper focus by the finding that all of the common amino acids can be copolymerized under selected thermal conditions into a single product suggestive of protein.

Purely biological evidence for the thermal origin of organisms, although not conclusive, can be found in the literature. The proposal that life began in thermal waters has been offered by several biologists, notably COPELAND[22]. One criticism of chemical or biological theories emphasizing thermal origins is that biological systems cannot withstand such elevated temperatures. One answer to this problem is the fact that some bacteria and algae normally inhabit nearly boiling hot springs. These, according to Copeland, are the most primitive organisms. Secondly, GREENSTEIN and HOYER[23], and also HAMER[24], showed that many substances inhibit the thermal coagulation of protein; notably nucleic acids do this. In attempting to understand the origin of physiological systems, it may be necessary therefore to understand the special influences of molecular interactions quite as fully as the generation of substances and reactions.

Despite increasing numbers of experimental demonstrations, more than usual uncertainty attends any interpretations in the subject matter of biochemical origins. A true understanding of origins however offers much promise of systematizing the tremendously ramified mass of biochemical knowledge and is to be sought for this reason alone.

At the most the picture which has been inferred represents essentially the way in which biochemical systems originated. At the least, it presents in relatively complete outline an internally and externally consistent picture of how biochemical systems, and by self-directed extrapolation, life (FOX[16]) could have originated, when one invokes a modulation from a hypohydrous magma to an aqueous system.

At the non-interpretative level, the studies have revealed ways and means of producing with relative ease a large variety of peptides which command interest in a number of potential applications.

Zusammenfassung

Bei Überschuss von Glutaminsäure und Asparaginsäure werden zahlreiche Aminosäuren thermisch kopolymerisiert. Das Studium der Reaktionen und Nebenreaktionen führt zu einer Theorie über den thermischen Ursprung biochemischer Systeme.

[18] S. W. FOX, J. E. JOHNSON, and M. MIDDLEBROOK, J. Amer. chem. Soc. *77*, 1048 (1955).

[19] I. LIEBERMAN and A. KORNBERG, Biochim. biophys. Acta *12*, 223 (1953).

[20] S. W. FOX, A. VEGOTSKY, K. HARADA, and P. D. HOAGLAND, Ann. N. Y. Acad. Sci. *69*, 328 (1957).

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[22] J. J. COPELAND, Ann. N. Y. Acad. Sci. *36*, 1 (1936).

[23] J. P. GREENSTEIN and M. L. HOYER, J. biol. Chem. *182*, 457 (1950).

[24] D. HAMER, Biochem. J. *56*, 610 (1954).

Corrigenda

ROSHAN J. IRANI and K. GANAPATHI: *The Effect of Glycerol on the Biosynthesis of Benzylpenicillin by the Washed Cells of Penicillium chrysogenum*, Exper. *14*, fasc. 9, 329 (1958).

The third entry in Table III should read: PA (0·05%) + glycerol (1%) + KCN (0·005 *M*).

H. GROSSFELD: *Fat Formation and Glycolysis in Tissue Culture. Action of Hydrocortisone.* Exper. *14*, fasc. 10, 371 (1958).

There has been an error in the first column of page 372 in line 9 from below. This line should read: ... treated with hydrocortisone in concentration of 125 micrograms/ml.