The origin of intermediary metabolism

Harold J. Morowitz*†, Jennifer D. Kostelnik‡, Jeremy Yang§, and George D. Cody¶

*Krasnow Institute of Advanced Study, George Mason University, Fairfax, VA 22030; ‡Kline Science Library, Yale University, New Haven, CT 06520; §Daylight Inc., Santa Fe, NM 87501; and ¶Geophysical Laboratory, Carnegie Institution of Washington, Washington, DC 20015

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The core of intermediary metabolism in autotrophs is the citric acid cycle. In a certain group of chemoautotrophs, the reductive citric acid cycle is an engine of synthesis, taking in CO_2 and synthesizing the molecules of the cycle. We have examined the chemistry of a model system of C, H, and O that starts with carbon dioxide and reductants and uses redox couples as the energy source. To inquire into the reaction networks that might emerge, we start with the largest available database of organic molecules, Beilstein on-line, and prune by a set of physical and chemical constraints applicable to the model system. From the 3.5 million entries in Beilstein we emerge with 153 molecules that contain all 11 members of the reductive citric acid cycle. A small number of selection rules generates a very constrained subset, suggesting that this is the type of reaction model that will prove useful in the study of biogenesis. The model indicates that the metabolism shown in the universal chart of pathways may be central to the origin of life, is emergent from organic chemistry, and may be unique.

The chart of metabolic pathways (1) is an expression of the universality of intermediary metabolism. The reaction networks of all extant species of organisms map onto a single chart, the great unity within diversity of the living world. There are a number of possible explanations.

(*i*) The chart is the reaction network of the universal ancestor, which has survived in all branches of the evolutionary radiation. It is thus a virtual fossil that has persisted because changes deep within the system tend to be lethal, owing to the high degree of connectivity.

(*ii*) The chart has emerged from a facile interspecific sharing of genes by horizontal transfer across the taxa.

(*iii*) The chart represents an optimally successful solution to designing biochemical networks.

(*iv*) Some combination of the above explanations.

All of the possibilities suggest that the metabolic chart or parts thereof can be traced to the earliest organisms and contain information about the chemistry of biogenesis and the prebiotic planet some 4 billion years ago. This period is the preenzymatic domain. A paradox to be faced is that, at present, enzymes are required to define or generate the reaction network, and the network is required to synthesize the enzymes and their component monomers.

In trying to model the beginnings of biochemistry, we assume a vat with the appropriate chemicals, catalytic surfaces, a source of energy, and an energy sink. The source must provide energetic enough quanta to drive reactions involving covalent bond change. In carrying out the modeling, we use generalizations from biochemistry and ecology such as the metabolic chart and the carbon cycle, the notion of fitness, and insights from thermal physics such as the cycling theorem (2) and the notion that the flow of energy from a source to a sink organizes the intermediate system (3).

There has been an ongoing argument as to whether the earliest organisms were autotrophs or heterotrophs. Autotrophy requires metabolic pathways from environmental, one-carbon, minimum free-energy compounds to all intermediates. Heterotrophy in earliest metabolism requires the synthesis of high concentrations of nutrients in an environment free of specific biocatalysts. There is an intermediate case in which a small number of high-probability intermediates arise in the environment and are used by otherwise autotrophic systems. This paper generally assumes autotrophy with the possibility that there may be a preferred reaction network that bridges the gap between environments and cells.

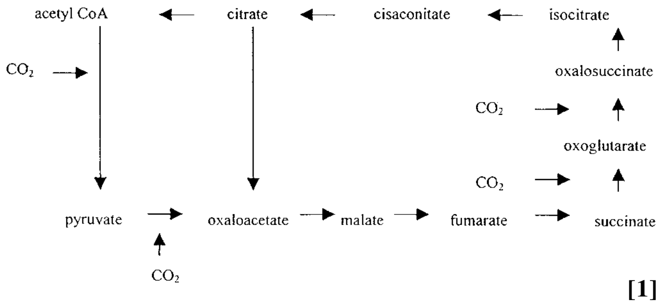
For autotrophs, the metabolic chart has a shell structure (4). The core is the citric acid cycle and related reactions. The first shell is the synthesis of amino acids, which comes from amination of core keto acids. The second shell involves sulfur incorporation into amino acids. The third shell requires the synthesis of dinitrogen heterocycles. We assume that metabolism recapitulates biogenesis; the number of steps from CO_2 incorporation to a given biochemical indexes the appearance of that molecule in biogenesis.

At the core of the metabolic chart is the citric acid cycle, which is the pathway to efficient oxidation in aerobic heterotrophs. In autotrophs, the citric acid cycle is the central pathway to all biosynthesis. Lipids come from acetyl CoA, sugars from phosphoenol pyruvate, and amino acids from keto acids and other compounds in the cycle. Nucleic acid components are synthesized from amino acids and sugars. In autotrophs, the citric acid cycle is an engine of synthesis.

Over the past 15 years, a number of chemoautotrophs have been isolated that operate by using the reductive citric acid cycle (5–8). Such organisms gain their energy from environmental redox couples and incorporate CO_2 in those steps where CO_2 is given off in the oxidative citric acid cycle. These organisms may provide clues as to the origin of metabolism in biogenesis.

The reductive citric acid cycle is found in both eubacteria and archea and in both aerobes and anaerobes. It is found in both mesophiles and thermophiles (9).

The cycle may be represented as follows.



[1]

Two features of this cycle should be noted.

(*i*) It is network autocatalytic (as distinguished from template autocatalytic), and the overall reaction may be represented by,

$$\text{citrate} + 6CO_2 + 9H_2 \rightarrow 2\text{citrate} + 5H_2O. \qquad [2]$$

Abbreviation: TCA, tricarboxylic acid.

See commentary on page 7678.

†To whom reprint requests should be addressed at: Krasnow Institute for Advanced Study, Mailstop 2A1, George Mason University, Fairfax, VA 22030. E-mail: morowitz@gmu.edu.

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Any of the substrates is autocatalytic for its own synthesis. This type of autocatalysis may be a crucial step on the route to metabolism.

(*ii*) If the network occurs in a chemical reaction system, then it is a sink for carbon going from CO_2 to more complex molecules. It is the simplest extant route for CO_2 going to biochemicals.

In chemoautotrophs, the citric acid cycle is the central starting point on the route to all biochemicals. Energy must be supplied from outside the citric acid cycle by reactions going from environmental redox couples to ATP, reduced NAD^+, reduced NADP, and reduced FAD. Given this energy, the cycle is the central feature of the metabolic chart.

One approach to the origin of metabolism is therefore a prebiotic nonenzymatic reductive citric acid cycle. In the prebiotic domain, CH_3CO-SR can play the role now carried out by acetyl CoA and pyrophosphate can replace ATP. The model we are looking at is a vat of water, CO_2, nitrogen, phosphorus, and sulfur and an energy source that will pump the ground state (equilibrium state) to excited states where they will react. We are interested in the occurrence of the reductive tricarboxylic acid (TCA) cycle under possible prebiotic conditions. The vat may contain catalytic surfaces such as pyrite and other metal sulfides (10, 11). The energy source can be photons or environmental redox couples. Carbon is supplied as CO_2 and reductants are available.

The task is to find a set of physically motivated selection rules that will lead to a vat with a high concentration of reductive citric acid cycle intermediates and to analyze what the conditions must be for these rules to govern the system in the absence of enzymes. Because at a substrate level the molecules of interest in the citric acid cycle are $C_xH_yO_z$, this is the universe we deal with first.

The guidance for restricting the domain to CHO comes from certain universal features of present-day metabolism, biochemistry, and chemical ecology. For example, almost all flow of nitrogen into the biosphere involves a series of oxidations and reductions to NH_3, followed by the reaction of ammonia with keto acids to form amino acids. This finding strongly suggests the necessity of a network to produce keto acids before nitrogen incorporation and the synthesis of amino acids. Biological phosphorus almost universally occurs in the oxidation states as orthophosphates and pyrophosphates and attaches to intermediates by phosphate ester bonds. The phosphorus is not part of the carbon backbone structure or the small molecules at the core of metabolism. Sulfur also is restricted to the cofactor level in CoA, acetyl CoA, and succinyl CoA.

To study the chemistry within the reaction vessel, the list of all possible compounds can, in theory, be obtained in two ways.

(*i*) It can be algorithmically generated from the rules of organic chemistry or ultimately from the rules of quantum mechanics.

(*ii*) It can be extracted from databases of organic chemistry such as the Beilstein handbook (12) or the *Dictionary of Organic Compounds* (13). Both of these references are now available electronically.

The object of the selection rules is to generate the emergence (14) of the reductive citric acid cycle from the master list of compounds. These rules may be physical, chemical, biological, informational, or a combination of the above.

Because of the difficulty of deriving the network from the fundamental theory of organic chemistry, we have opted to search CROSSFIRE (12), an online version of the Beilstein handbook rather than the algorithmic approach. We start by looking at $C_xH_yO_z$ for which:

$$\begin{gathered}1 \leq x \leq 6,\\ 1 \leq y < 99, \text{ and}\\ 1 \leq z < 99.\end{gathered} \qquad [3]$$

The value 99 is chosen to show that there is no upper limit at this stage. The selection for low-molecular weight compounds embodies an assumption that the beginning of biochemistry starts with C_1 compounds and develops into compounds of higher molecular weights. The first cut yielded 2,790 compounds and included all of the intermediates of the reductive TCA cycle.

The next cut came from examining the oil-water partition coefficient and selecting for water solubility. This preference for aqueous solubility deals with phase separation in the original reactions and assumes at some time the capture of the reactions in vesicles of bilayer membranes made of amphiphilic molecules. Partition coefficients are contained in a database maintained by Biobyte Software (Claremont, CA). The quantity p represents the ratio of the concentration in water-saturated octanol divided by the concentration in octanol-saturated water, where those two phases are in equilibrium with each other. Values are available for log p obtained either experimentally or by computation *c*log p. Negative values of the logarithm are designated as hydrophilic and positive values as lipophilic. Biobyte can be accessed by using the SMILES representation of molecules maintained by Daylight Inc.

The next selection rule is for low heats of combustion, to look first for compounds energetically close to CO_2 because this represents the initial domain accessed in the energetic pumping of CO_2, water, and reductants. Thermodynamic data can be obtained from experimental databases (15, 16) and by calculation from group contributions (17).

After an examination of a number of $C_xH_yO_z$ compounds, we discovered two informatic selection rules that include the oil-water partitions and thermodynamic selection without the necessity of using the other databases.

The two rules are:

$$\begin{gathered}x/z \leq 1 \quad y/z \leq 2 \quad \text{for } 1 \leq x \leq 3 \text{ and}\\ x/z \leq 1 \quad y/z \leq 1.5 \quad \text{for } 4 \leq x \leq 6.\end{gathered} \qquad [4]$$

In general for these compounds, the more reduced the molecules, the more hydrophobic and the greater the heat of combustion. Thus, the informatics rules embody the thermodynamic selections and are much easier to apply.

The next selection is to exclude compounds that have no carbonyl groups. The essence of biochemistry of CHO molecules is the domain of carbonyl reactivity, and the set of molecules is restricted to those that can participate in such reactions.

The next selection is to exclude cyclic compounds and compounds with C—O—C on the basis of being difficult to synthesize nonenzymatically in this C, H, O domain.

The next step excludes C≡C and O—O on the grounds of stability. Radicals and ions are present in the Beilstein list (12) and are not included here. Chiral pairs are treated as single molecules.

The application of the primary rules results in a set of only 153 compounds containing the 11 intermediates of the reductive TCA cycle (see Table 1). Starting with the 3.5 million compounds of Beilstein and applying a small number of pruning rules motivated by physical and chemical considerations, we arrive at a small subset of organic compounds that includes all of the reductive TCA intermediates.

One feature of the reductive TCA cycle that immediately attracts attention is that it is network autocatalytic. Any molecule in the cycle is catalytic for its own synthesis. Another feature is that all reactions either are monomolecular or involve substrates interacting with environmental molecules. Because the substrates are at low concentrations, these reactions are kinetically favored over substrate–substrate reactions by an order of magnitude. For these reactions to proceed, therefore, does not require a vesicle to trap the reaction products. The core chemistry can proceed without an envelope. It is a consequence of the

Table 1. Compounds selected from Beilstein

No.	Molecular formula	Chemical name	Chemical Abstracts Service registry number
1	CH_2O	Formaldehyde	50-00-0
2	CH_2O_2	Formic acid	64-18-6
3	$C_2H_2O_2$	Ethanedial	107-22-2
4	$C_2H_2O_3$	Oxo-acetic acid	298-12-4
5	$C_2H_2O_4$	Oxalic acid	144-62-7
6	$C_2H_4O_2$	Acetic acid	64-19-7[†]
7	$C_2H_4O_2$	Hydroxy-acetaldehyde	141-46-8
8	$C_2H_4O_3$	Dihydroxy-acetaldehyde	631-59-4
9	$C_2H_4O_3$	Hydroxy-acetic acid	79-14-1
10	$C_2H_4O_4$	Dihydroxy-acetic acid	563-96-2
11	$C_3H_2O_3$	2-Oxo-malonaldehyde	497-16-5
12	$C_3H_2O_4$	2,3-Dioxo-propionic acid	815-53-2
13	$C_3H_2O_5$	2-Oxo-malonic acid	473-90-5
14	$C_3H_4O_3$	2,3-Dihydroxy-propenal	636-38-4
15	$C_3H_4O_3$	2-Hydroxy-acrylic acid	19071-34-2
16	$C_3H_4O_3$	2-Hydroxy-malonaldehyde	497-15-4
17	$C_3H_4O_3$	2-Oxo-propionic acid	127-17-3[†]
18	$C_3H_4O_3$	3-Hydroxy-2-oxo-propionaldehyde	997-10-4
19	$C_3H_4O_3$	3-Hydroxy-acrylic acid	65034-30-2
20	$C_3H_4O_3$	3-Oxo-propionic acid	926-61-4
21	$C_3H_4O_4$	2,2-Dihydroxy-malonaldehyde	4464-20-4
22	$C_3H_4O_4$	2,3-Dihydroxy-acrylic acid	2702-94-5
23	$C_3H_4O_4$	2-Hydroxy-3-oxo-propionic acid	2480-77-5
24	$C_3H_4O_4$	3,3-Dihydroxy-acrylic acid	177594-62-6
25	$C_3H_4O_4$	3-Hydroxy-2-oxo-propionic acid	1113-60-6
26	$C_3H_4O_4$	Malonic acid	141-82-2
27	$C_3H_4O_5$	2-Hydroxy-malonic acid	80-69-3
28	$C_3H_4O_6$	2,2-Dihydroxy-malonic acid	560-27-0
29	$C_3H_6O_3$	1,1-Dihydroxy-propan-2-one	1186-47-6
30	$C_3H_6O_3$	1,3-Dihydroxy-propan-2-one	96-26-4
31	$C_3H_6O_3$	2,3-Dihydroxy-propionaldehyde	453-17-8
32	$C_3H_6O_3$	2-Hydroxy-propionic acid	50-21-5
33	$C_3H_6O_3$	3-Hydroxy-propionic acid	503-66-2
34	$C_3H_6O_4$	2,2-Dihydroxy-propionic acid	1825-45-2
35	$C_3H_6O_4$	2,3-Dihydroxy-propionic acid	473-81-4
36	$C_4H_2O_4$	2,3-Dihydroxy-buta-1,3-diene-1,4-dione	7472724*
37	$C_4H_2O_4$	2,3-Dioxo-succinaldehyde	97245-29-9
38	$C_4H_2O_6$	2,3-Dioxo-succinic acid	7580-59-8
39	$C_4H_4O_4$	2,4-Dioxo-butyric acid	1069-50-7
40	$C_4H_4O_4$	2-Hydroxy-4-oxo-but-2-enoic acid	114847-32-4
41	$C_4H_4O_4$	2-Methylene-malonic acid	4442-03-9
42	$C_4H_4O_4$	3,4-Dioxo-butyric acid	20602-39-5
43	$C_4H_4O_4$	4-Hydroxy-2-oxo-but-3-enoic acid	1748936*
44	$C_4H_4O_4$	But-2-enedioic acid	6915-18-0[†]
45	$C_4H_4O_5$	2-Hydroxy-but-2-enedioic acid	7619-04-7
46	$C_4H_4O_5$	2-Oxo-succinic acid	328-42-7[†]
47	$C_4H_4O_6$	2,3-Dihydroxy-but-2-enedioic acid	13096-38-3
48	$C_4H_4O_6$	2-Hydroxy-3-oxo-succinic acid	5651-05-8
49	$C_4H_4O_7$	2-Carboxy-2-hydroxy-malonic acid	44968-58-3
50	$C_4H_6O_4$	1,4-Dihydroxy-butane-2,3-dione	162369-87-1
51	$C_4H_6O_4$	2,3-Dihydroxy-succinaldehyde	34361-91-6
52	$C_4H_6O_4$	2-Hydroxy-3-oxo-butyric acid	37520-05-1
53	$C_4H_6O_4$	2-Hydroxy-4-oxo-butyric acid	62386-30-5
54	$C_4H_6O_4$	2-Methyl-malonic acid	516-05-2
55	$C_4H_6O_4$	3,3-Dihydroxy-2-methyl-acrylic acid	69858-40-8
56	$C_4H_6O_4$	3,4-Dihydroxy-2-oxo-butyraldehyde	496-56-0
57	$C_4H_6O_4$	3-Hydroxy-2-oxo-butyric acid	1944-42-9
58	$C_4H_6O_4$	3-Hydroxy-4-oxo-butyric acid	10495-18-8
59	$C_4H_6O_4$	4-Hydroxy-2-oxo-butyric acid	22136-38-5
60	$C_4H_6O_4$	Succinic acid	110-15-6[†]
61	$C_4H_6O_5$	2,3,4-Trihydroxy-but-2-enoic acid	1928462*
62	$C_4H_6O_5$	2,3-Dihydroxy-4-oxo-butyric acid	10385-76-9
63	$C_4H_6O_5$	2-Hydroxy-2-methyl-malonic acid	595-48-2
64	$C_4H_6O_5$	2-Hydroxymethyl-malonic acid	4360-96-7
65	$C_4H_6O_5$	2-Hydroxy-succinic acid	6915-15-7[†]
66	$C_4H_6O_5$	3,4-Dihydroxy-2-oxo-butyric acid	114579-56-5
67	$C_4H_6O_6$	2,2-Dihydroxy-succinic acid	60047-52-1
68	$C_4H_6O_6$	2,3-Dihydroxy-succinic acid	526-83-0
69	$C_4H_6O_6$	2-Hydroxy-2-hydroxymethyl-malonic acid	54472-64-9
70	$C_4H_6O_8$	2,2,3,3-Tetrahydroxy-succinic acid	76-30-2
71	$C_5H_2O_5$	2,3,4-Trioxo-pentanedial	97245-30-2
72	$C_5H_4O_5$	2-Formyl-but-2-enedioic acid	111598-98-2
73	$C_5H_4O_5$	4-Oxo-pent-2-enedioic acid	6004-32-6
74	$C_5H_4O_6$	2-Carboxy-but-2-enedioic acid	4364-81-2
75	$C_5H_4O_7$	2,3-Dihydroxy-4-oxo-pent-2-enedioic acid	89712-64-1
76	$C_5H_4O_7$	2-Carboxy-3-hydroxy-but-2-enedioic acid	1785338*
77	$C_5H_4O_7$	2-Carboxy-3-oxo-succinic acid	4378-81-8
78	$C_5H_4O_7$	2-Hydroxy-3,4-dioxo-pentanedioic acid	89282-33-7
79	$C_5H_4O_8$	2,2-Dicarboxy-malonic acid	193197-67-0
80	$C_5H_6O_5$	2-Formyl-succinic acid	5856-44-0
81	$C_5H_6O_5$	2-Hydroxy-3-methyl-but-2-enedioic acid	148716-85-2
82	$C_5H_6O_5$	2-Methyl-3-oxo-succinic acid	642-93-3
83	$C_5H_6O_5$	2-Oxo-pentanedioic acid	328-50-7[†]
84	$C_5H_6O_5$	3-Oxo-pentanedioic acid	542-05-2
85	$C_5H_6O_6$	2,3,5-Trihydroxy-4-oxo-pent-2-enoic acid	5425275*
86	$C_5H_6O_6$	2-Carboxy-succinic acid	922-84-9
87	$C_5H_6O_6$	2-Hydroxy-2-methyl-3-oxo-succinic acid	1777463*
88	$C_5H_6O_6$	2-Hydroxy-4-oxo-pentanedioic acid	1187-99-1
89	$C_5H_6O_6$	2-Hydroxymethyl-3-oxo-succinic acid	89323-48-8
90	$C_5H_6O_7$	2,3,4-Trihydroxy-pent-2-enedioic acid	91113-90-5
91	$C_5H_6O_7$	2,3-Dihydroxy-4-oxo-pentanedioic acid	1787046*
92	$C_5H_6O_7$	2-Carboxy-2-hydroxy-succinic acid	110863-50-8
93	$C_5H_6O_7$	2-Carboxy-3-hydroxy-succinic acid	80754-80-9
94	$C_5H_6O_8$	2-Carboxy-2,3-dihydroxy-succinic acid	639-51-0
95	$C_5H_8O_6$	2,2-Bis-hydroxymethyl-malonic acid	173783-71-6
96	$C_5H_8O_6$	2,2-Dihydroxy-3-methyl-succinic acid	4980495*
97	$C_5H_8O_6$	2,2-Dihydroxy-pentanedioic acid	23788-98-9
98	$C_5H_8O_6$	2,3,4-Trihydroxy-5-oxo-pentanoic acid	114375-57-4
99	$C_5H_8O_6$	2,3,5-Trihydroxy-4-oxo-pentanoic acid	134616-21-0
100	$C_5H_8O_6$	2,3-Dihydroxy-2-methyl-succinic acid	15853-34-6
101	$C_5H_8O_6$	2,3-Dihydroxy-pentanedioic acid	82864-78-6
102	$C_5H_8O_6$	2,4-Dihydroxy-pentanedioic acid	82864-77-5
103	$C_5H_8O_6$	2-Hydroxy-2-hydroxymethyl-succinic acid	2957-09-7
104	$C_5H_8O_6$	3,4,5-Trihydroxy-2-oxo-pentanoic acid	110902-88-0

Table 1. (continued)

No.	Molecular formula	Chemical name	Chemical Abstracts Service registry number
105	$C_5H_8O_7$	2,3,4-Trihydroxy-pentanedioic acid	608-55-9
106	$C_5H_8O_7$	2,3-Dihydroxy-2-hydroxymethyl-succinic acid	6115630*
107	$C_6H_4O_6$	4,5-Dioxo-hex-2-enedioic acid	6123412*
108	$C_6H_4O_8$	2,3-Dicarboxy-but-2-enedioic acid	4363-44-4
109	$C_6H_6O_6$	2,5-Dihydroxy-hexa-2,4-dienedioic acid	1725831*
110	$C_6H_6O_6$	2,5-Dioxo-hexanedioic acid	25466-26-6
111	$C_6H_6O_6$	2-Carboxy-3-methyl-but-2-enedioic acid	1781603*
112	$C_6H_6O_6$	2-Carboxy-3-methylene-succinic acid	1779647*
113	$C_6H_6O_6$	3,4-Dioxo-hexanedioic acid	533-76-6
114	$C_6H_6O_6$	3,6-Dihydroxy-2,5-dioxo-hex-3-enoic acid	2443471*
115	$C_6H_6O_6$	3-Carboxy-pent-2-enedioic acid	499-12-7[†]
116	$C_6H_6O_7$	3-Carboxy-2-hydroxy-pent-2-enedioic acid	1792255*
117	$C_6H_6O_7$	3-Carboxy-2-oxo-pentanedioic acid	1948-82-9[†]
118	$C_6H_6O_8$	2,3-Dicarboxy-succinic acid	4378-76-1
119	$C_6H_6O_8$	3,4-Dihydroxy-2,5-dioxo-hexanedioic acid	1794752*
120	$C_6H_6O_8$	3-Carboxy-2-hydroxy-4-oxo-pentanedioic acid	3687-15-8
121	$C_6H_8O_6$	2-Carboxy-2-methyl-succinic acid	39994-39-3
122	$C_6H_8O_6$	2-Carboxy-3-methyl-succinic acid	61713-72-2
123	$C_6H_8O_6$	2-Carboxy-pentanedioic acid	4756-09-6
124	$C_6H_8O_6$	2-Hydroxy-2-methyl-4-oxo-pentanedioic acid	19071-44-4
125	$C_6H_8O_6$	2-Hydroxy-5-oxo-hexanedioic acid	13095-45-9
126	$C_6H_8O_6$	3-Carboxy-pentanedioic acid	99-14-9
127	$C_6H_8O_7$	2,3,5,6-Tetrahydroxy-4-oxo-hex-2-enoic acid	5478036*
128	$C_6H_8O_7$	2,3,5-Trihydroxy-4,6-dioxo-hexanoic acid	4746-27-4
129	$C_6H_8O_7$	2,3-Dihydroxy-5-oxo-hexanedioic acid	26566-33-6
130	$C_6H_8O_7$	3,4,6-Trihydroxy-2,5-dioxo-hexanoic acid	2595-33-7
131	$C_6H_8O_7$	3-Carboxy-2-hydroxy-pentanedioic acid	320-77-4[†]
132	$C_6H_8O_7$	3-Carboxy-3-hydroxy-pentanedioic acid	77-92-9[†]
133	$C_6H_8O_7$	4,5,6-Trihydroxy-2,3-dioxo-hexanoic acid	7683-53-6
134	$C_6H_8O_8$	2,3,4-Trihydroxy-5-oxo-hexanedioic acid	149250-15-7
135	$C_6H_8O_8$	2-Carboxy-2,4-dihydroxy-pentanedioic acid	82848-19-9
136	$C_6H_8O_8$	3-Carboxy-2,3-dihydroxy-pentanedioic acid	6205-14-7
137	$C_6H_8O_9$	2-Carboxy-2,3,4-trihydroxy-pentanedioic acid	1801017*
138	$C_6H_{10}O_7$	2-(1,2-Dihydroxy-ethyl)-2-hydroxy-succinic acid	1790363*
139	$C_6H_{10}O_7$	2,3,4,5,6-Pentahydroxy-hex-2-enoic acid	113892-19-6
140	$C_6H_{10}O_7$	2,3,4,5-Tetrahydroxy-6-oxo-hexanoic acid	6814-36-4
141	$C_6H_{10}O_7$	2,3,4,6-Tetrahydroxy-5-oxo-hexanoic acid	13425-57-5
142	$C_6H_{10}O_7$	2,3,4-Trihydroxy-2-hydroxymethyl-5-oxo-pentanoic acid	1711202*
143	$C_6H_{10}O_7$	2,3,4-Trihydroxy-2-methyl-pentanedioic acid	469-44-3
144	$C_6H_{10}O_7$	2,3,4-Trihydroxy-hexanedioic acid	4382-48-3
145	$C_6H_{10}O_7$	2,3,5,6-Tetrahydroxy-4-oxo-hexanoic acid	54911-28-3
146	$C_6H_{10}O_7$	2,3,5-Trihydroxy-hexanedioic acid	13427-52-6
147	$C_6H_{10}O_7$	2,3-Dihydroxy-2-(2-hydroxy-ethyl)-succinic acid	1790420*
148	$C_6H_{10}O_7$	2,4-Dihydroxy-2-hydroxymethyl-pentanedioic acid	98574-40-4
149	$C_6H_{10}O_7$	3,4,5,6-Tetrahydroxy-2-oxo-hexanoic acid	73803-83-5
150	$C_6H_{10}O_8$	2,3,4,5-Tetrahydroxy-hexanedioic acid	7558-19-2
151	$C_6H_{10}O_8$	2,3,4-Trihydroxy-2-hydroxymethyl-pentanedioic acid	1712927*
152	$C_6H_{10}O_8$	3,4,5,5,6-Pentahydroxy-2-oxo-hexanoic acid	7808083*
153	$C_6H_{10}O_{10}$	2,2,3,4,5,5-Hexahydroxy-hexanedioic acid	1801900*

*Beilstein registry numbers.

[†] Member of the TCA.

type of reactions at the center of the metabolic chart and will no longer apply as soon as a reaction is required that is between two substrates. The dominant reactions are oxidation–reduction, hydration–dehydration, carboxylation–decarboxylation, and splitting; they operate independently of an enclosure.

From the domain of all possible reactions in a reduced world containing H_2O and CO_2, there emerges through certain physically motivated pruning rules a small set of 153 compounds that includes all of the citric acid cycle intermediates. Another few molecules, such as hydroxypyruvate, occur as intermediates along neighboring metabolic pathways. Thus, the subset of emergent molecules is highly favored as metabolites. In any case, the reductive citric acid cycle is embedded in the emergent subset.

From a point of view of general complexity theory, the Beilstein compendium is a highly structured database of end-points of reaction networks. The fact that it can be used to generate heuristic approaches to biogenesis indicates a possible approach to the theory of directed database mining. It is facilitated by the rich knowledge of chemistry that accompanies the database.

Efforts have been made to analyze the TCA cycle from the point of view of efficiency (18). They are oriented to acetate oxidation rather than to operating in the reductive direction. We suggest alternative cycles that contain several compounds from our group of 153 and some others that we excluded because they had too high a content of hydrogen. Note that the approach in our study is oriented toward reductive autotrophic metabolism and concentrates on anabolism.

If one wishes to study biogenesis from the bottom up, the first step is to reason from atoms of the periodic table to those molecules that form the core of biochemistry, those molecules central to the chart of intermediary metabolism in chemoautotrophs. We have started with the assumption that the core molecules are made of CHO, possibly supplemented by −SR and polyphosphates. We have assumed that biogenesis moves from simplicity to complexity, from low free energy to high free energy, and from autotrophy to heterotrophy. We convert these assumptions to primary rules as to the kinds of molecules to be selected for and apply this selection to the primary database of organic molecules, Beilstein (12). What emerges is a set of 153 molecules that include the 11 members of the reductive citric acid cycle, as well as some other molecules from the metabolic chart. We argue that there is an enormous simplification as well as indication that the chemistry at the core of the metabolic chart is necessary and deterministic and would likely characterize any aqueous carbon-based life anywhere it is found in this universe. Experiments to find corollaries of these results are in progress.

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1. Nicholson, D. E. (1997*) Metabolic Pathways* (Sigma, St. Louis).
2. Morowitz, H. J. (1966) *J. Therm. Biol.* **13,** 60–62.
3. Morowitz, H. J. (1968) *Energy Flow in Biology* (Academic, New York).
4. Morowitz, H. J. (1999) *Complexity* **4,** 39–53.
5. Evans, M. C. W., Buchanan, B. B. & Arron, D. I. (1966) *Proc. Natl. Acad. Sci. USA* **55,** 928–934.
6. Ivanovsky, R. N., Sintsov, N. V. & Kondratieva, E. N. (1980) *Arch. Microbiol.* **128,** 239–241.
7. Shiba, H., Kawasumi, T., Igarashi, Y., Kodoma, T. & Minoda, Y. (1985) *Arch. Microbiol.* **142,** 198–203.
8. Shima, S. & Suzuki, K. I. (1993) *Int. J. Syst. Bacteriol.* **43,** 703–708.
9. Danson, M. J., Hough, D. W. & Lunt, G. G., eds. (1992) *The Archaebacteria: Biochemistry and Biotechnology* (Portland, London), pp. 14–20.
10. Wächterhäuser, G. (1988) *Microbiol. Rev.* **52,** 452–484.
11. Wächterhäuser, G. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 200–204.
12. Beilstein Informationssysteme (1998) Beilstein CROSSFIRE (Springer, Berlin), update no. BS 9902PR.
13. *Dictionary of Organic Compounds* (1999) (Chapman & Hall, London), 6th Ed, CD-ROM.
14. Holland, J. H. (1998) *Emergence* (Helix, Reading, MA).
15. Marsh, K., ed. (1999) *TRC Thermodynamic Tables* (Thermodynamic Research Center, Texas A & M Univ., College Station, TX).
16. Stull, D. R., Westrum, E. F. & Sinke, G. C. (1969) *The Chemical Thermodynamics of Organic Compounds* (Wiley, New York).
17. Mavrovouniotis, M. L., Prickett, S. & Constantinov, L. (1992) *Comput. Chem. Eng.* **16**, 5353–5360.
18. Melendez-Hevia, E., Waddell, T. G. & Cascanta, M. (1996) *J. Mol. Evol.* **43,** 393–303.