

## CHAPTER 10

### FUTURE RESEARCH

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#### 1.A. Overall Definition of Natural Systems

What field studies need to be carried out in order to test the hydrothermal model for the origin of life? This question was already raised in 1989 by the workshop in Prague on 'Chemical Evolution and Neo-Abiogenesis in Marine Hydrothermal Systems' (Holm, 1990). The full range and diversity of hydrothermal environments can only be revealed if more exploration work is carried out on a world-wide basis. Much progress has been made in understanding the controls on the hydrothermal fluids of individual areas, but extension to a global context remains difficult (cf. Von Damm, 1990). In Chapter 2 off-axis hydrothermal systems are discussed, pointing out that only minor surveys have been directed towards this type of hydrothermal activity, even though the amount of water which circulates through off-axis systems is estimated to exceed that of on-axis systems by a factor of between 20 and 25.

A list of possible studies was compiled at the Prague meeting in order to identify future research opportunities (field studies in italics):

1. *Chemical monitoring of deep-sea hydrothermal systems.*
2. *Comparative studies of terrestrial systems.*
3. *Comparative studies of shallow marine systems.*
4. *On-station experiments.*
5. *Injection-capture experiments.*
6. *On-line pumping of deep-sea hydrothermal water to the sea surface for analysis and experiments.*
7. *Drilling activities on the seafloor.*
8. *Survey of areas other than ridge axes - primarily ridge flanks* (see above).
9. Modelling of hydrothermal systems with O<sub>2</sub> excluded.
10. Modelling of hydrothermal conditions in laboratory experiments.
11. Adsorption/desorption and catalysis experiments at high pressures and temperatures.
12. Construction and operation of quenching systems.

Edmond (1991), in his recent review article about research on hydrothermal chemistry, has remarked that on a general level information is lacking from the super-fast spreading centers which occur mainly in the southern oceans. It is possible that hydrothermal systems present at fast-spreading centers are more representative of systems on the early Earth than other, better studied settings on the present Earth. Information is also scarce from most of the numerous types of environments encountered in back-arc basins. Diversity in substrate composition and in the rate and extent of reactions may broaden the constraints on chemical and thermodynamic interpretations. For example, the report by Fouquet and coworkers (1991) on high temperatures (400°C) and high proton activities (pH 2) in hydrothermal fluids of the Lau back-arc spreading center indicates that these systems may differ considerably in chemical composition and hydrologic variability from many of the best-studied ridge-crest environments.

The report of the first meeting in 1990 of InterRIDGE, the international framework of research related to the U.S. Ridge Inter-Disciplinary Global Experiments (RIDGE) science program, concluded that 'biological investigations of hydrothermal systems thus directly concern an understanding of the origin and evolution of life on Earth'. Some of the central program components for future research of InterRIDGE concern the development of an inventory of hydrothermal activity on a global scale and the characterization of environments in which life forms can be sustained. The second InterRIDGE meeting in 1992 identified a set of current scientific problems that are essential components of a comprehensive understanding of temporal patterns of ridge processes. One such problem is whether or not the chemical environments of hydrothermal systems yield viable environments for the origin of life. This is, of course, in line with the spirit of the work of SCOR Working Group 91. Within SCOR there is a continuous discussion of future participation in large-scale science programs, and one such program that has been mentioned

lately is InterRIDGE. It is the opinion of Working Group 91 that such an engagement should be strongly encouraged.

One of the first steps of the spatial characterization of mid-ocean ridges on a global scale would be to carry out off-axis bathymetric and across-axis geophysical surveys. The bathymetry and geophysics should eventually be followed by investigations of individual hydrothermal (off-axis) sites for the evaluation of the ranges of, for instance, temperature, pH,  $f\text{H}_2$ , and flow rates. On the basis of mineral alteration, maximum values for the longevity of hydrothermal systems at off-axis positions have been estimated to reach up to and beyond  $10^7$  years (Fehn and Cathles, 1986). Persistent upwelling flow in the temperature range 14-40°C has been demonstrated in  $11 \times 10^7$  years old oceanic crust of the Bermuda Rise in the Atlantic (Muehlenbachs, 1979; Lawrence, 1979; Alt and Honnorez, 1984). According to some hydrologic models, the temperatures reached by convecting fluids in off-axis systems are not expected to exceed 200°C (Fehn and Cathles, 1986). A vast distribution system in that temperature region has, however, yet to be confirmed. The East Pacific Rise Detailed Planning Group (ERDPG, 1991) of the Ocean Drilling Program (ODP) has recommended a strategy of cross-axis array drill sites at approximately 9°30'N on the East Pacific Rise. The strategy includes a submersible fluid sampling program and hydrological sealing of the drilled holes for re-entry and multiple sample recovery. The drill-ship JOIDES Resolution will temporarily leave the Pacific by January 1993 and work in the Atlantic Ocean for perhaps about three years. Plans for participation in further East Pacific Rise drilling legs should thus be developed within the next few years.

### 1.B. Physical and Chemical Characterization of Natural Systems

To study the possibility that complex organic syntheses and perhaps life itself could have originated within the Earth's crust, it is necessary to acquire data from laboratory and in situ experiments to advance present knowledge. What follows is a non-comprehensive set of suggestions for further experimental research activities which are considered to be 'reasonable, feasible and timely' enterprises to better characterize the physics and chemistry of natural systems within the Earth's crust.

The detailed study of hydrologic regimes (temperature, pressure, permeability, porosity, preferential pathways, fracture systems, fluid velocities, etc.) operating within small segments (m to 100 m) of the Earth's crust is warranted. This is necessary because an understanding of fluid dynamics at small scales is required for the study of abiotic organic synthesis and the origin of life. It is important to understand whether and where fluids can mix, where they can be trapped, and where boiling or degassing can occur. In particular, the migration velocity of fluids in the crust is of importance because it determines, among other things, how far a fluid can travel before approaching chemical and/or physical equilibrium. It is also important to realize that hydrology, based on a fluid reference frame, in the Earth's crust is strongly scale dependent. For example, a large (km

scale) hydrothermal field may be quite stable for long periods of time and one could assume that within such a system most physical and chemical equilibria could easily be approached. However, at a smaller scale (m) within some portions of the same hydrothermal system, it becomes difficult to defend the idea that any physical or chemical equilibrium could be approached, due to the ever changing dynamic conditions. In addition, little is known of the longevity of hydrologic regimes, particularly in proximity to active hydrothermal systems. Hydrologic information should be acquired preferentially from in situ measurements. The experience acquired by hydrologists and petroleum reservoir scientists should be extensively applied. Drilling log tests (gamma-ray, X-ray, resistivity, etc.), tracer tests and long term pumping tests should be conducted at selected sites. Long term observation (years) of small hydrothermal features such as hydrothermal seeps or vents should also be conducted.

Characterization of the types of fluids circulating in the Earth's crust would be of great interest. Aqueous and non-aqueous phases are circulating in the crust. For example, the magnitude of circulation of supercritical CO<sub>2</sub> or other supercritical non-aqueous fluids is virtually unknown. Sampling technology designed for collecting non-aqueous fluids should be developed. Laboratory studies should characterize non-aqueous fluid migration in material representative of the Earth's crust. The detailed chemical composition of non-aqueous phases should be determined, and chemical reaction mechanisms within such phases should be investigated.

Laboratory studies should be conducted to investigate abiotic organic synthesis in all the phases known to exist in the Earth's crust, as well as at interfaces of immiscible phases. The effects of phase transition (appearance or disappearance of a phase) on organic syntheses deserves to be studied. In addition, analytical techniques should be developed to identify and quantify organic compounds within naturally occurring fluid inclusions, since fluid inclusions may represent encapsulated environments for the study of natural abiotic organic chemistry.

### 1.C. Supercritical Fluids

Supercritical fluid research is a fascinating field. Observed and/or postulated supercritical fluids in hydrothermal systems are CO<sub>2</sub>, H<sub>2</sub>O, CH<sub>4</sub> (with C<sub>2</sub>H<sub>6</sub>, C<sub>3</sub>H<sub>8</sub>, etc.), H<sub>2</sub>S, and perhaps NH<sub>3</sub>, as well as mixtures of these components. The divergence between the characteristics of supercritical fluids and liquids at room temperature and atmospheric pressure has been discussed in several of the previous chapters (see Chapters 2, 4, 5 and 6). Supercritical fluids can be excellent solvents and extractors of organic compounds (Hawthorne, 1990); they have much better mass transfer characteristics than liquid solvents (Hawthorne, 1990); the heat capacity of any substance approaches infinity at its critical point; both oxidative and reductive reactions can easily be carried out in modified supercritical fluids (see Chapter 4); etc. Much fundamental research needs to be done, however, in order to achieve an improved understanding of the thermodynamic and

molecular properties in the supercritical region. Shaw and coworkers (1991) remarked that we must study in supercritical water reactions that are well understood in other media. We also have to elucidate the effects of pressure on the kinetics of well-characterized reactions of simple molecules, and separate such effects from the interaction with solvents and solvent properties. In addition, it is necessary to understand the extent to which diffusion controls the rate of reactions and the associated problem of cages of solvent molecules that surround solute molecules. Such solvation cages may hinder molecular breakup by promoting the recombination of solute fragments (Shaw *et al.*, 1991).

### 1.D. Mineral Catalysis of Organic Transformations in Hydrothermal Systems

It is most likely that organic compounds percolating through hydrothermal systems will be adsorbed and desorbed many times on and off mineral surfaces. Some organic compounds may adhere to the mineral until they undergo a chemical transformation to another compound which does not bind. The mineral surfaces may catalyze the transformation of some of these organic compounds to higher molecular weight compounds, and surfaces may affect decomposition to simpler derivatives. An example of the formation of higher molecular weight compounds is the conversion of activated mononucleotides to oligonucleotides in aqueous solution in the presence of montmorillonite clay (Ferris *et al.*, 1990). This reaction has, however, not been investigated under hydrothermal conditions.

Experimental studies of the mineral catalyzed transformations of organic compounds at the higher temperatures and pressures present in hydrothermal systems should be explored in the laboratory. Minerals which are present in hydrothermal systems such as zeolites, smectite clays and metal sulfides (cf. Chapter 9) should be investigated as possible catalysts. Care should be taken to use a chemically inert reaction vessel to avoid catalysis by the container material at high temperatures and pressures (Shaw *et al.*, 1991). Examples of chemical transformations to be studied include:

1. Ester formation between an alcohol and carboxylic acid, since this process has been reported to occur in supercritical water (Chapter 6, Section 3.2). If ester formation is successful then one should proceed to investigate amide formation between simple acids and amines as the first step towards a more complicated and detailed study of polypeptide formation from amino acids.
2. An approach similar to that outlined for polypeptide formation could also be devised for the investigation of oligonucleotide synthesis.
3. The potential for Fischer-Tropsch type (FTT) syntheses on montmorillonite, metal sulfides and other minerals should be high on the list for investigation, especially if such reactions are studied at realistic hydrothermal conditions. FTT synthesis may provide a route to linear fatty acids, compounds which have not been detected in the potential prebiotic reactions that have been studied to date. The FTT synthesis of linear hydrocarbons and acids has been reported to take place using montmorillonite

and metal sulfides as catalysts (Chapter 6, Sections 4 and 5.4.2). The formation of linear fatty acids is necessary for the construction of lipid membranes that may have been required to encapsulate the first life. FTT reactions using a mixture of CO and NH<sub>3</sub> as reactants may provide the heterocyclic bases and amino acids necessary for the formation of polypeptides and polynucleotides (Chapter 6, Section 5.3.2).

## 2.A. Organic Synthesis-Simulations

Abiotic synthesis experiments are notorious for their lack of geochemical or geologic relevance. Synthesis experiments at properly controlled hydrothermal conditions would represent a significant break with this historical trend. However, it must be kept in mind that there is considerably more to hydrothermal systems than hot water. Compositional constraints, including oxidation states, are imposed by fluid/rock reactions. Therefore a constructive effort to test the possibility of hydrothermal synthesis of organic compounds should emerge from the long history of hydrothermal experimentation developed by geochemists and petrologists who have studied these systems for many decades.

Design of hydrothermal synthesis experiments can be guided by theoretical calculations which identify the ranges of the many descriptive variables of hydrothermal systems (temperature, pressure, salinity, metal-ion composition, oxidation state, pH, etc.) which are conducive to organic synthesis. Calculations of this type are available and numerous further calculations are possible with data already available (see Chapter 5). Coupling theoretical predictions with experimental tests will facilitate enormously the general understanding of the geochemical processes which control whether or not organic synthesis is possible in hydrothermal systems.

The primary experiments which should be done would be designed to answer the fundamental yes-or-no questions concerning hydrothermal organic synthesis. Promising indications from work by French (1964) and others (see Chapters 7 and 8) show that hydrothermal organic synthesis can occur. Maybe confirmation of some of these early results should be sought in similarly designed experiments, where the analytical emphasis would be shifted more strongly to the organic compounds.

The key first step to test the hypothesis of hydrothermal organic synthesis is to demonstrate the conversion of CO<sub>2</sub> to organic compounds. This should be followed with experiments on the fixation of N<sub>2</sub> and CO<sub>2</sub> to organic products. If these are successful then all the other reactions described above should also be possible.

## 2.B. Experimental/Analytical Issues

Initial experimentation will probably be carried out in batch reactors such as various types of autoclaves/bombs. However, flow reactors need to be adapted and developed in order to better simulate the natural systems. This permits single pass interaction of reagents and immediate removal of the products from the reaction zone. Such systems could also

be cycled in terms of thermal regime. Reaction and contact times need to be maximized for the highest product yield by matrix experimentation. The temperature range under typical marine hydrothermal system pressures (100-400 bars) for optimal organic synthesis must also be established by the same experimental design.

As mentioned above, container effects such as catalysis or redox reactions should be minimized by choosing inert materials or appropriately buffering the reaction medium. Artifact formation should be prevented and rigorously proven that it does not occur. The minimization of organic contamination in the experimental and analytical procedures is absolutely critical. For example, the report by French (1964) of organic matter formation in the hydrothermal experimentation with ferrous oxalate could also be interpreted as alteration of traces of organic contaminants such as lubricating hydrocarbons if they were in the system. Hydrocarbons are ubiquitous contaminants and are converted in part to oxygenated derivatives such as ketones, aldehydes, and acids by water in the 250-350°C range (Leif *et al.*, unpublished data). Such compounds were detected in the organic products reported by French (1964). Thus control of potential organic contamination is a key aspect in the future experimental design to test this hypothesis. Organic contamination in the analytical procedures can be minimized by applying the established microanalytical techniques of organic geochemistry.

Once organic products have been demonstrated to be synthesized in the laboratory simulations, their confirmation must be extended to the natural systems. This would involve field checks and field simulations with isotope labeled tracers.

### 3. Macromolecules and Membranes

#### 3.1. SYNTHESIS

It is axiomatic that in order to generate enzymes non-enzymatic synthesis must have occurred. At the moment there is some evidence demonstrating the synthesis of polypeptide chains from amino acids linked by peptide bonds. What is needed is a demonstration not only that such a process can occur but also careful definition of the conditions that are favorable to such polymerization. An indication of the temperature, and of which amino acids are most likely to form peptide bonds would be significant factors for the design of experiments focussed on examining the stability and catalytic ability of minimal enzymes (see below).

Similarly, the generation of nucleotides from simpler chemicals, and the aggregation of phospholipids and their synthesis will also be significant. Another area which is relatively unexplored at elevated temperature is the way in which phospholipids, or indeed any amphipathic compounds, are capable of forming membranes or vesicles at temperatures around 100°C. Given the ubiquity of hydrocarbon-containing compounds (lipids) in modern membranes this seems to be a sensible starting compound class in this area. The difficulties of carrying out such experiments should not be underestimated. It may be

necessary to explore an appreciable temperature and pressure range (probably from 40°C to 300°C and from 1 to 300 atmospheres), as well as trying to control the redox and pH of the system throughout this range.

### 3.2. THE MINIMAL REQUIREMENT OF A FUNCTIONAL SYSTEM

The minimal requirement for the origin of life can be said to be the appearance of systems able to evolve under natural selection, suggesting that genetic, catalytic and membrane materials of some sort were provided by the hydrothermal environment at the outset. The usual assumption that any materials must have been similar to nucleic acids, proteins and lipids is by no means clear. As discussed in Chapter 9 one of the exciting prospects for hydrothermal research is in looking carefully at the components of mineral assemblages for possible materials from which components of the first evolvers might have been made. One could then see, through laboratory simulations, if such materials have genetic, catalytic or membrane forming potential. For example, it is known that thin membranes of colloidal iron sulfide vesicles are semipermeable. The significance of conditions under which present day biochemical materials can survive and function remains: the idea is that such primitive mineral components would subsequently have been updated when the means for making polynucleotides or peptides had evolved.

In this context it is reasonable to expect 'protoenzymes' to be much simpler in composition and structure than modern day enzymes, the ubiquity of enzymes suggests that the development of effective protein catalysts is likely to have been an early stage in the development of life. While the discovery of ribozymes is exciting, and has apparently offered the possibility of an evolutionary track that started with nucleic acids rather than with enzymes, the relative inefficiency of ribozymes and their very limited functions still point to the early development of catalytic proteins. Although a considerable amount of work is now being carried out in this field we still do not have a good understanding of, for example, how a few different amino acids can be used to make an effective catalytic protein, and how small a polypeptide chain is likely to be functional. Research on catalytic antibodies will certainly help to define the minimal requirements for catalytic function. It may be that tracing back the evolutionary tree of enzymes may give us some clues about which enzymes or enzyme types were most dominant or important in very early life forms. Certainly within hydrothermal systems we may expect hydrolysis/condensation reactions to be extremely important. It is difficult to see what other constraints hydrothermal systems may have placed on the functions of protoenzymes. However, the ready availability of metals and the general involvement of these in modern enzymes participating in redox reactions may also be suggestive and of importance.



### 3.3. STABILITY

Much more information is needed on the hydrothermal stability of the small biomolecules (amino acids, sugars, nucleotides) as well as the larger biomolecules (polypeptide chains). For example, it is currently an open question whether, among all the protein structures available, there exist proteins of a nature which will resist the temperatures to be found in some hydrothermal systems (e.g., 150°C upwards) and which will also retain a structure capable of functioning catalytically. The evidence we have on protein stability is mostly based on proteins which have evolved to function well below 105°C. These are presumably highly adapted to these conditions and to their particular functions, so the results may not be applicable to much more stable proteins. In general even high temperature stability experiments have not been carried out with much pH or redox buffering. A variety of artificial peptides need to be examined for resistance to degradative reactions under conditions approximating those in thermal systems. The next stage would be to determine the upper temperature limit for the maintenance of some degree of tertiary structure.

Experiments involving the stability of membranes, both natural and artificial, and of polymeric information storage systems such as DNA and RNA are also needed to fill gaps in our knowledge. Again the conditions under which the stabilities are examined will be crucial. It is likely to be necessary to consider the effects of high pressure as well as temperature although to date the evidence concerning the stabilizing effect of pressure is unconvincing. pH and redox buffering, however, will be crucial and to date it is not possible to measure pH directly much above 120°C. We know, however, that pH is likely to have a large influence on stability to hydrolytic degradation.

## 4. The Ultimate Limits for Life

The first pure culture of an organism from a hot spring was grown in a laboratory in the late 1960s. Since then we have seen a steady rise in the upper temperature at which we can culture micro-organisms, from 75°C or so in those early days to 110°C at present. We have only recently had access to high temperature (over 100°C) aqueous environments stable over substantial periods, and our techniques for handling organisms at these high temperatures are relatively undeveloped. There is every prospect that the upper growth temperature for life can be raised from its present 110°C to a figure significantly higher. Efforts in this field will influence our views regarding the original temperature at which life evolved, not least because current results indicate that it is these high temperature organisms that are the most primitive found so far.

Not many laboratories are equipped to grow organisms above 80°C, and even fewer to do so at significant pressures. Nevertheless, since many of these high temperature systems are at substantial pressures, it may be that growth experiments will have to be carried out at significant pressures if it is hoped to culture successfully the most extreme thermophiles. To date it has been possible to culture all of the known extreme ther-

mophiles at atmospheric pressures, but it is difficult to be sure whether this is a convenient feature of these organisms or whether we have simply failed to grow those organisms that demand high pressures.

It is imperative that more field and laboratory experiments are carried out not only to seek organisms from high temperature hydrothermal systems but also to find a wider variety of such systems including those 'off-axis' and in sedimentary basins. Non-marine hydrothermal systems will continue to be an extremely important area for exploration, because of the relatively low cost, ready accessibility, and wide variety of such systems.

Many key aspects of thermotolerance and/or thermophily remain to be identified. So far, no significant biochemical differences between extreme thermophiles and mesophiles have been found that can convincingly be attributed to temperature rather than taxonomy. Nevertheless, differences between modes of life at 20°C and 110°C, if they can be found, will certainly shed an important light on the origin of life.

Although the evidence to date seems relatively convincing, further confirmation that thermophily is a primitive characteristic should be sought. Not all of the RNA sequences available have been flawless, many others are not generally available, and taxonomic trees for the archaebacteria are not only variable year to year but are also the subject of some contention. Given the recent development of these trees and the relatively small number of organisms which have been worked on from what is certainly a large population, this is hardly surprising.