



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

BETHESDA 14, MD.

NATIONAL INSTITUTES OF HEALTH
O Liver 6-4000

April 5, 1960

Professor Joshua Lederberg
Dept. of Genetics
Stanford University
Stanford, California

Dear Josh:

You probably remember that I have had an interest in the origin of life for some years, and published a paper on the subject in the *American Naturalist* in 1957. My interest has grown steadily. Since 1954 it has seemed to me that even feeble autocatalytic systems or "reflexive catalysts" should in some instances be detectable in the laboratory under suitable conditions. Apparently most biologists doubt that such systems exist, but admit their importance if they did exist. Chemists admit their probable existence, but either deny that they are interesting or, as in the case of Melvin Calvin, see no way to detect them except by conventional chemical approaches.

I am ready to undertake some long-term experiments designed to take advantage of the organism-like properties of these hypothetical reflexive catalysts, approaching the problem as a biological one rather than as a chemical one. But the only chemist in our institute who has any idea what I want to do says the results will be meaningless unless I work out the kinetics in detail; that I won't even know what mixtures (see experimental description accompanying) are promising without analyzing them.

I maintain that an autocatalytic synthesis will propagate itself in such an unmistakable way as to obviate chemical analysis, although of course that would be an important subsequent line of investigation. I also suspect that there are numerous possible reflexive catalysts among rather simple compounds, and that given sufficient time, probably millenia in some instances, almost any chemical mixture, constantly renewed, would eventually be dominated by them. My experiments are based on the optimistic assumption that some mixtures, under conditions described, will develop reflexive catalytic systems within two to five years. All I need to find is one to open the way for further studies.

I think I will be permitted to do the research here if I insist, but since nobody will back me up, I am in a tight spot. This is not meant to bias you, but to emphasize how much help your considered opinion, favorable or not, may be to me personally.

Sincerely yours,

Gordon Allen

G. Allen
3/22/60

PROJECT DESCRIPTION

I. Project Title.

A search for reflexive catalytic phenomena in aqueous mixtures of organic compounds.

II. Purpose.

Enzymes and enzyme-catalyzed reactions are among the most important characteristics of modern organisms. The activity of enzymes depends largely on steric specificity and on ordered sequences of amino acids, and these in turn depend on other enzymes and on specific nucleic acids. While the deoxyribonucleic acids appear to impress their own specificity on new molecules of DNA as they are formed, the synthesis and assembly of the components is effected by other protoplasmic constituents. Even in viruses, life as it exists today therefore requires the coordinated action of nucleic acids and multiple specific enzymes. It is difficult to see how nucleic acids could ever have reproduced themselves without a highly organized protoplasm.

If nucleic acids characterized the first living organism, its origin would seemingly have required the aggregation by chance or mass action of a large array of specific nucleic acids and proteins, each of which was itself highly improbable. A more attractive hypothesis, if tenable, is that catalysts and hereditary determiners occurred first as much simpler molecules which nevertheless permitted the early operation of natural selection and the gradual evolution of more intricate mechanisms. Instances are known of catalysis by very simple compounds such as ethanol and methylamine, and it has been shown that, theoretically, any catalyst that facilitates a step in

its own synthesis, referred to here as a reflexive catalyst, can fill the roles simultaneously of enzyme and gene, albeit very inefficiently.

The number of simple catalysts known at present would be altogether inadequate to maintain a primitive protoplasm, and no cases of reflexive catalysis have been demonstrated. The purpose of the experiments described here is to extend present knowledge in these areas. The experiments will attempt to study mixtures of simple chemical reagents under conditions that permit the spontaneous formation of rare and relatively complex compounds. The required conditions are thought to be (1) moderately high concentrations and temperatures, (2) a nearly steady state, (3) a duration measured in years. Perhaps most important is the application of sensitive tests at the end of that time for the presence of catalytic activity.

III. Hypothesis

Although rare or nonexistent in present biological systems, a large variety of simple organic catalysts await discovery, and some of these are capable of catalyzing steps in their own synthesis from more abundant compounds.

IV. Assumptions underlying the experimental design.

1. Among products in a favorable mixture of reactive compounds, some may be expected to have catalytic action on the reactions occurring.

2. Among such catalysts, a small proportion might be expected to act upon steps in their own synthesis; each such catalyst would start an autocatalytic process.

3. As long as starting materials are continually renewed, most reactions in the mixture will be sustained, and among these sustained reactions, any that were autocatalytic would tend to accelerate with time and to become more and more prominent. Hence, the desired compounds would tend to select themselves. Although reflexive catalysis may be rare among catalytic phenomena, it would be less rare among detectable catalyses.

4. After an appropriate interval, perhaps two years, when an aliquot of a mixture containing such an autolytic cycle was added to a fresh solution of the starting materials, all the compounds within that system would increase more rapidly than in a control solution to which none of the old mixture was added.

5. If one of the compounds in the reflexive catalytic cycle produced a measurable effect on optical or other properties of the mixture, its relation to an autocatalytic process could be recognized either by its exponential rate of increase or by its relatively great capacity to "seed" itself in a third or fourth flask of the starting materials.

6. Anything that increases the rate and diversity of reactions in a mixture will, in the average case, increase the probability of origin of catalytic systems. However, diversity of reactions will at the same time tend to diminish the prominence and detectability of individual catalysts.

While favorable mixtures and conditions may be exceptional, properties of modern living systems are not a dependable guide to the favorable experiment. Conditions that existed at the time of the origin of life on the earth (temperature, dilution, etc.) may have been relatively unfavorable even for that event, and might be quite incompatible with the

emergence of reflexive catalysts in short-term laboratory experiments. Hence, high concentrations, high temperatures, and even an abundance of compounds or elements poisonous to modern organisms need not be avoided if they have any merits.

7. The probability of discovering reflexive catalysts, if they exist, depends largely on the number of different mixtures and conditions studied and on the time during which their reactions are allowed to proceed.

V. Tentative experimental design

1. Time requirements. The project will have three phases, the third to be carried out only if some positive findings are obtained in the first two.

The first phase of perhaps two years will be used for a pilot experiment, requiring only about one day a week for maintenance of six reaction mixtures. The second phase of about three years will continue the original reactions if they have shown any tendency to yield diverse products, and will explore methods of describing the constituents without necessarily identifying them (e.g. with chromatography and spectroscopy). Also, with the experience gained in the first two years and with nearly full time available, a large number of additional mixtures will be made up and studied, possibly a hundred. In the third phase, if any of the mixtures studied to that time have shown evidence of catalyzed syntheses, attempts will be made to isolate and identify the catalysts and their products. All mixtures which show continuing changes will be studied further and new mixtures and new reaction conditions will be tried.

2. Reagents. In addition to certain electrolytes, each mixture will contain four to eight compounds of carbon, nitrogen and/or sulfur. All of these compounds will have at least one moderately reactive site, some two, and some will be highly reactive. Combinations will be carefully selected to insure a large number of primary reactions, none of which will be a quantitative polymerization or precipitation. To the extent that they can be predicted, secondary combinations of primary products and other side reactions will be maximized. Mixtures in which known biological catalysts might occur will be favored but not expressly sought, at least in early experiments, since the likelihood of such a compound being detectably catalytic under the given conditions may be smaller than that for the aggregate of unpredicted compounds.

3. Experimental conditions. Mixtures will be held at a temperature of 90°C or 95°C to favor high reaction rates and to maintain bacteriological sterility. Each flask will carry a reflux condenser to trap volatile reagents and products, and a constant flow of inert gas will be provided over the upper end of each condenser to maintain constant pressure and to eliminate oxygen in systems where reducing conditions are desired.

Each flask will be started with 100 milliliters of solution containing reagents at a concentration of .02 molal or 0.1 saturation, whichever is less. At weekly intervals one ml. of water will be added containing fresh reagents at a concentration of .18 M. or 0.9 saturation, and additional water to compensate evaporation. After 100 such additions, the volume will be 200 ml. and the concentration of any unused reagents will be 0.1 M. or half-saturated; this is referred to below as standard concentration. At that time 100 ml. will be removed from each flask and

frozen for future study. Mixtures that are to be maintained longer will be replenished thereafter by weekly addition of 2 ml. of water containing all reagents in standard concentration.

4. Detection of catalysts. This will be attempted only after a mixture has been maintained for two years or longer, and the methods will be essentially biological rather than chemical.

The first requirement is a detailed description of the mixture as a whole in terms of spectral absorption, chromatographic fractionation, and possibly electrophoresis. In this way it is anticipated that almost all compounds present in a concentration of more than .001 M. will be registered in sufficiently distinctive fashion to permit presumptive recognition in subsequent experiments. Chemical identification will not be attempted at this stage, and the descriptive data for any mixture will be treated as a set of properties rather than as a list of constituents.

When a mixture (a in the accompanying diagram) is ready for testing, two new flasks, b and c, will be made up containing 100 ml. of the reagents at standard concentration. One of these flasks, b, will be inoculated with a milliliter of the original mixture and both will be incubated under reflux. Full descriptive data will be obtained as a base line, and development of new properties will be followed in both flasks at weekly intervals.

With due allowance made for the amount of the original mixture added, any great relative precocity or delay in appearance of a property in the inoculated flask will be presumptive evidence of catalysis or inhibition. An inhibition would be of some interest as a possible reflection of a

catalyzed competing process, the product of which required different methods of detection. In some instances it might be possible to observe the rate of increase in a property. If the rate of increase diminished with time, the responsible reaction would generally (but not always) involve a simple catalysis or a reaction of something in the inoculum with something in the fresh solution. If the rate of increase increased with time, the responsible reaction might be either one step in a reflexive catalytic cycle or a reaction whose catalyst was being synthesized by another catalyst.

A primary test for an autocatalytic process would be applied, as shown in the diagram, whenever the first inoculated flask acquired properties at a different rate or in a different order than the control flask. The first inoculated flask would be subinoculated ^{as soon as a difference was apparent} into a new flask (d) while a control (e) was inoculated with one ml. from the first control and .01 ml. from the original mixture. If a reflexive catalyst was present, flask (d) would acquire the distinguishing property or properties appreciably faster than the control, and the differences would be equal or greater in a second serial inoculation, comparing flasks (g) and (h).

As further proof, and as a first step toward identification of an autocatalytic system, the compound responsible for the distinguishing property would, if possible, be obtained in fairly pure state by chromatography or electrophoresis. Addition of this fraction to a fresh standard solution should have an effect qualitatively similar to that of inoculation with the original mixture, unless the isolated compound was a noncontributing by-product of the autocatalytic cycle.

VI. Significance of possible findings.

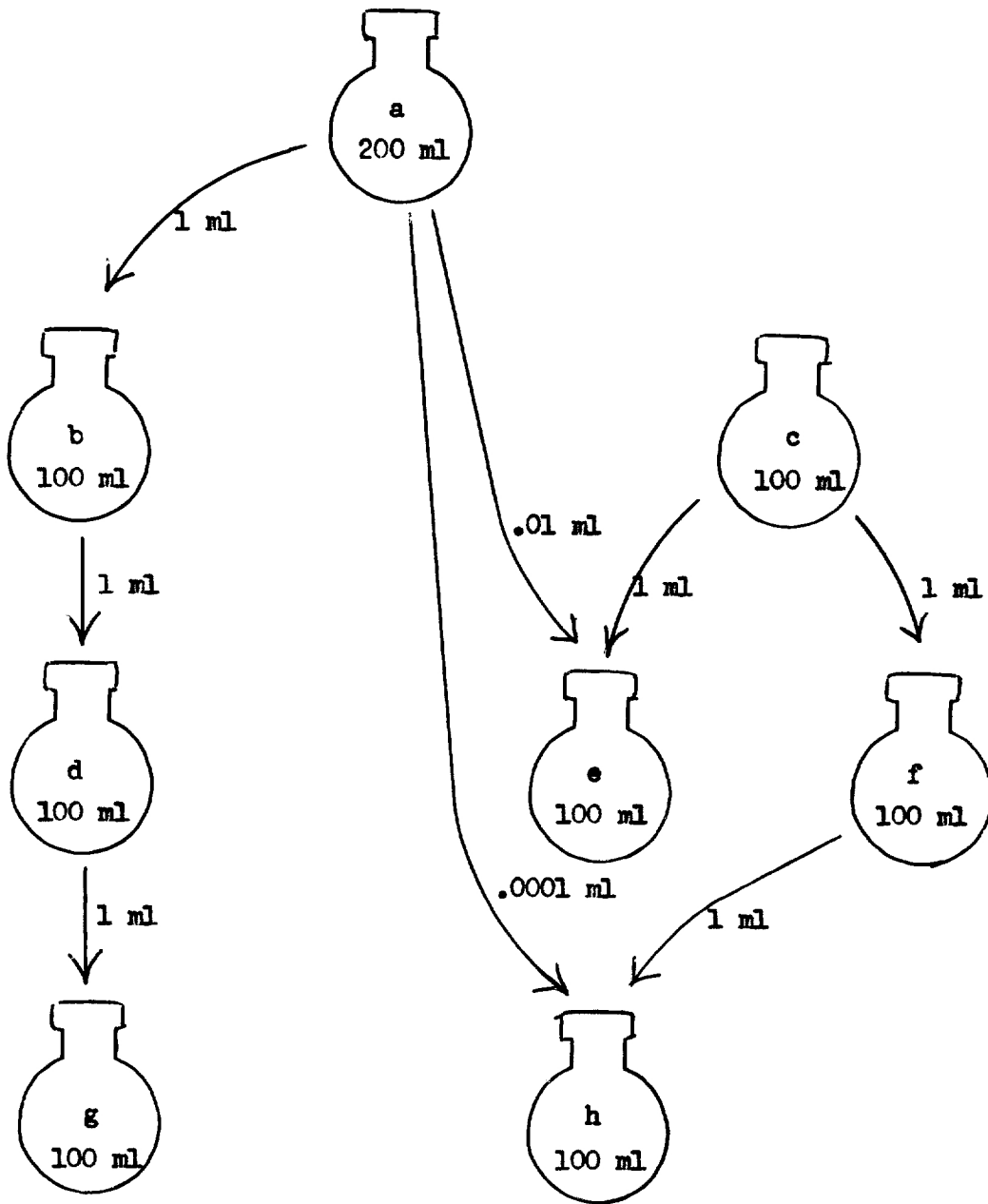
The discovery of any catalytic phenomena at all in mixtures of simple organic compounds might be of interest and potentially useful. The discovery of a reflexive catalyst would support one theory of the origin of life and would make possible the study of natural selection among chemical reactions in a solution as among genes in a population.

VII. Personnel and budget.

In the first two years I shall devote only about a fifth of my time to this project and shall not need technical assistance. Dr. Seymour Friess of the National Naval Medical Center is interested and is helping me to plan the experiments. If progress in the third or fourth year seems to warrant it, I may at that time ask for a part or full-time laboratory assistant.

Modest laboratory facilities will have to be installed, including possibly a hood. Little equipment will be needed in the first two years except a constant temperature bath, a moderately precise laboratory balance, and glassware. A larger item on the budget will be reagents, some of which will be fairly expensive but required only in small amounts. The total budgetary requirement for the first two years should not exceed \$1000. In the third year more space and equipment will be needed for maintenance of test solutions, and I shall request access to recording spectrophotometers and similar apparatus.

Test for Catalysis and Reflexive Catalysis
by serial inoculations



Note: Repetition of (c) (an uninoculated control flask) at each level would, in some ways, be better control than (e) or (h). There are several ways of controlling, all ~~at the same time~~ in one test.