PROTOBIOCHEMISTRY - THEORETICAL AND EXPERIMENTAL CONSIDERATIONS CONCERNING PRIMORDIAL BIOCHEMICAL DEVELOPMENT

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ABSTRACT

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An extensive theory was formulated to define the relationship of information accumulation and entropy. From this, the evolution of mass and energy was proposed and the connection of the electron to this development was also suggested. Likewise, the relationship of the electron to protobiochemical evolution was indicated.

On the basis of these fundamental theoretical considerations, experiments were proposed and carried out to test the final concept discussed in the theory pertaining to the role of the electron in evolution. First of all, it was observed that aqueous solutions of alcohols, when sparked, displayed the characteristics of carbohydrates. Glycerol was selected as the reactant for the greatest portion of the study and upon sparking, it responded positively to tests usually employed to identify amino sugars such as ninhydrin, Elson-Morgan, and Fehling procedures.

Secondly, organic acids were sparked. The sparked product of acetic acid indicated a glycine-like substance.

Finally, a mixture of glycerol, acetic acid, ethanol, and ammonium sulfamate, when sparked for a long period of time, yielded a high molecular weight product. This polymer-like substance had many of the characteristics of mucopolysaccharides.

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- PRELIMINARY REPORT -
FORMATION AND MECHANISM OF CONDENSATION PRODUCTS FROM
FORMALDEHYDE AND ACETALDEHYDE

by
Gary Stevens

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Gary Steinman

A THESIS

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PREDICTING PROTEIN-DNA INTERACTIONS

THEORETICAL AND EXPERIMENTAL CONSIDERATIONS CONCERNING DNA

- ABSTRACT -

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"Physical and chemical approaches to problems in biology have become increasingly productive in recent years. Major advances in the understanding of life processes have been made through research in such specialties as biophysical chemistry, molecular biology, biophysics, and electrophysiology. Continuing progress will require an even more perceptive study of the interactions of matter, energy, and information in biological systems."

- J.L.Oncley, editor, Biophysical Science - A Study Program, John Wiley and Sons, Inc., New York, 1959, p.1.

I. GENERAL INTRODUCTION

This thesis represents a systematic line of scientific reasoning and experimentation. It is designed to present plausible explanations for certain problems which science has tried to resolve for many years.

The objectives of this work are threefold. First of all, from the standpoint of organic chemistry, the synthesis and analysis of basic reactions will be investigated. A study of the effect of high voltage electrical sparking on compounds of organic interest will augment present knowledge of the characteristics of these molecules.

Secondly, laboratory experimentation will be used to demonstrate certain desired syntheses as a possible indication to the origin of biochemically significant structures and compounds. This would, of course, supply essential links in the explanation of the origin of biological life itself. These first two goals will be discussed more thoroughly in the section on experimental objectives.

Finally, this thesis will be used to introduce a theory which is intended to provide a rational definition of some of the dynamics of the physical world. The significance of the photon as an energy supplier for promoting atomic and molecular evolution with the ultimate appearance of the electron and its assumption of the role of the photon as a reaction stimulator will be proposed. The importance of the electron in relation to physically significant functions such as entropy, information, and organization, as well as its possible connection to biophysical evolution will also be discussed.

II. FUNDAMENTAL THEORETICAL CONSIDERATIONS

The real world, as is evident to any astute observer, is a conglomerate of various physical and thermodynamic interrelationships. These would include material transfer, interconversion of the various forms of energy, constructive and destructive co-activities, and the like.

A reflection upon these functions entices one to analyze the causes behind the observed effects. For example, the conception of the forces in the physical world rests entirely upon sense perception. Therefore, no matter what may be the actual conditions of a given event, absolutes, even into the realm of abstractions, must be proposed within the framework of sensual limitations, whether this be by first hand observation or with the assistance of analytical instruments and aids.

Mental codification is based on interpretations of external impressions by which perception determines resultant values. Thus, for example, the degree to which an observer realizes the organization or disorganization of the units of a given system is subject to the manner in which the stimuli are received, the relative magnitudes of each class of data reaching the observer, and the value system he employs in drawing conclusions, whether this be arbitrary or prejudiced. Since man must draw conclusions from perceptions received, the expanse of these conceptions is subject to the limitations and the inaccuracies of the receptor or the means of transmission so that what is concluded to occur in a certain physical event may either not represent the actual phenomenon or may not give a large enough overview of all the factors significant to defining the meaning and relevance of the given event.

Based upon such functions, many aspects of the physical environment have been perceived and concluded to be in continuous flux. It is

possible, however, that the primary absolute which sense perceptions have failed to register because of a limited scope of observation is the tendency of progressive development to replicate primordial constancy, for if such change were unlimited and unidirectional, then primordial constancy would be dissipated forever. (This term will be defined later in this section).

From this, it is possible to make the distinction between that which is absolute and that which is relative. Let it be assumed that the tendency toward primoridal constancy, the destined realm of potential development, is a reality which is so, with or without an observer to perceive it. This makes it an absolute for no matter how the observer goes about defining such a reality, the intrinsic properties of it remain unchanged. Should the observer, however, misinterpret the data which he receives concerning the characteristics of primordial constancy, the conclusions he draws will define it in a manner which does not represent a real picture of this phenomenon so that the observer's definition is relative to him and those he affects by his conclusions. Even in such a circumstance, the real character of the phenomenon remains an absolute and its definition is still a relative concept, dependent on the observer.

Information, Entropy, and Organization

A consideration of the essential features of change would include evaluation of the relationship between organization and disorganization. For example, metabolism is the result of the opposing forces of anabolism, or biological construction, and catabolism, the destruction of molecular complexes and food stores for the purpose of conversion to required energy supplies (1). Thus, in a developing bacterial cell, growth is readily apparent so that anabolism predominates whereas in a performing muscle, catabolism overrides its counterpart.

The relative complexity of the physical world can be viewed as a reflection of a process similar to metabolism but entailing a much broader scope. First of all, the buildup process may be interpreted as an accumulation of information. Information may be defined as that which sets or reflects the pattern of organization of a system. DNA (2) carries the information which will ultimately set the pattern for the sequence of amino acids in cellular proteins as well as a template for its own duplication. Likewise, as a hereditary determinant, DNA establishes the manner in which the given cell can react to a variety of environmental possibilities. It is observed that the greater the complexity and abundance of proteins in a given cell, the more extensive and sophisticated must be the inherent information of the nuclear DNA. Similarly the more functional the DNA molecule itself is, the greater must be the systemization of the units of its somposition.

To clarify this point, one can consider a series of interlocking pieces of a jigsaw puzzle whose units form the number sequence 1-2-3-4-5. This series is composed of five members, each of which when standing alone bears little significance relative to the final pattern and when sacttered randomly, are found in almost complete disorder and disorganization. The potential for creating many different sequences, each with its own particular, restricted significance, is initially large. From the disorganized conglomerate of the numbers of which the series 1-2-3-4-5 is composed could results such patterns as 5-1-2-3-4, 4-5-1-2-3, 3-4-5-1-2 and so forth. Each possibility is more than just a regular series of units since such a system can also express a concept of something very real beyond the immediate association of parts. Then, in only one sequence of interactions by which the 2 follows the 1 and the 3 follows 2, is the series 1-2-3-4-5 formed. The first member bears

the information that if the desired pattern is to be formed, a 2 must follow it. This, if all possible units were available to be in juxtaposition to the 1, the inherent quality of this informational entity by which the desired sequence would be formed sets the pattern whereby only a 2 can and will follow it. Likewise, the 2 possesses such information by which only a 1 can come before it, and it, in turn, must follow the 1.

Therefore, the information of the 1-2 system is threefold:

1) The 1, in order to lead to the formation of the series 1-2-3-4-5, must be the first of the sequence and by so doing, carry with it all its geometrical significance.

2) The 2, by this scheme, must appear in the system because of the architectural importance it entails as well as the restrictions it inherently possesses with regard to the types of combinations that may precede or follow it.

3) The sequence 1-2 is not 2-1 nor 1-3 nor 1-4 so that the creation of this sequence (1-2) develops the information both as an extrinsic property of its immediate appearance and significance, and as an intrinsic property by which only a 3 can follow it so that ultimately the desired series will be formed, similar to the theory of epigenesis (3).

Now, let it be assumed that for every two members of the series which are placed in proper sequence, one falls out of line. As more units become organized into the sequence, there are more places in which a disarrangement could result and thus a greater number of permutations of possible resultant distributions and errors in the system. Ultimately the desired series will be formed but initially it would appear to the neutral observer that all is chaos and confusion. It would seem to him that the process was more inefficient than the value any possible result could merit since he could not, at the point of one-third or even one-half

completion, comprehend the enhanced information resulting from the process or the effect which such accumulation of information could have on overcoming the disorder noted at first.

One case of this is found in proteins. This example is given merely as an illustration of the concept of information and its relationship to organization, not as a proposal to explain protein evolution. It is noted that nearly all natural proteins are composed of L-amino acids exclusively (4). It can be speculated that primordially there was an equal possibility of amino acid polymers being composed of D or L stereo-isomeric members. As it may have turned out, the first polypeptides were formed from L-amino acids. To maintain the stability of the a-helix, all other members of tha t polymer would also have to be L substances. From there, these initial proteins catalytically determined the L character of all other proteins to be synthesized. Thus, biological evolution utilized the proteins composed of L-amino acids because of their abundance. Initially the high randomness and low organization of the racemic modification indicated a small degree of information in the system. As polypeptides appeared with specific characteristics, the information increased. Each L-amino acid had the inherent information that it could polymerize only with another L-molecule, but this system did not formerly have the information that nearly all such polymers would be of the L variety. As L polymers increased in number and size, this information became an inherent aspect of the system. Each polymer not only had the information that it was itself composed of L members but also that it could serve as a template for the systemization of the units of another polymer from unorganized masses of potential members. The information of the first members set restrictions and specifications on the future systems to be evolved.

This analogy can be used to explain certain properties in

the dynamics of original thinking. The nervous system is primarily a network of interconnected patterns by which appropriate stimuli are channeled to bring about the corresponding response (5). Except for the autonomic system which is genetically determined, the patterning of responses is formed by trial and error. A baby flops its arms about until appropriate nerve patterns are established to distinguish the action of arms, fingers, and so forth. Thus, human experience patterns the nerve channels by which thinking itself originates. Concepts without suitable past experience and conditioning are almost untenable. For example, when the sound of the word "tuberculosis" reaches the brain by way of the auditory system, a prearranged pattern stimulates the response,"Oh, that's a disease." If additional patterning has set up the system of thought that diseases are evil, the stimulus of the original word will continue to meander through the brain's complex network and eventually come upon this pattern as well, yielding the corresponding verbal response. To an unenlightened aborigine, this word will most likely elicit no response since no patterns for reaction have been developed from his past experience.

Deep original "thinking," the synthesis of new information, is not quite this spontaneous since more prearranged patterns must be traversed before the "idea" emerges. An idea is original with a given person only by the fact that his unique experience and ability to establish corresponding patterns has set up the appropriate circuit to organize elements of information into a new system bringing about the emergence of the new concept to the corresponding stimulus. Therefore, inessence, thinking does not originate inside the brain but is merely a response to appropriate stimuli traveling through prearranged individualized nerve patterns.

This concept can be extended to analyze certain properties in

physico-chemical processes. The first point of consideration is entropy (6). This is defined as the function by which the overall effect of physical interactions is to lead to greater disorganization, or increased randomness, so that entropy indicates a system's randomness or tendency toward disorganization. Thus, the eternal job of living cells, where energy flow is a homeostatic process, is to resist entropy which would, if unchecked, lead to the total disorganization of the cellular system.

The second law of thermodynamics (6) may be viewed as that whenever energy is transferred from a state of higher temperature to one of lower temperature, it is impossible to regain all of the energy of the first state in the second since some of it will be lost in the process.

$$\Delta S = q/T$$

Entropy may also be observed in the case of a freely expanding gas. Here the gas molecules are going from a state of less randomness to one of greater disorganization which implies increased entropy.

$$\Delta S = R \ln V_2/V_1$$

In this irreversible case, the probability of finding a given gas molecule within a certain unit volume is decreased by the process.

Evolution of Entropy and Information

Entropy, however, when considered in a much wider scope, is merely a relative concept. In other words, it is the observation of the results of a given number of phenomena during a certain time span that is relative to the observer. This becomes more readily apparent within the consideration of the concept of information. As noted, this value may be defined as that which sets or reflects the pattern of organization. For example, all of the units of information necessary to construct an atomic bomb were available to a prehistoric man. He did not, however, have the organizational information with which to pattern the units of information into a utilizable assembly. The units were already in existence, such as the physical

phenomenon of critical mass, but his level of experience restricted his capabilities to even recognize this information, an organizational process itself, so that it might be used effectively. Thus, because of a lack of organization of information, the units with which to assemble a greater system of information remained unorganized for a time.

If information is that which sets the pattern for all organization or is a function of the organization itself, inherent information is necessary before energy acquired by agiven material system can be used to the advantage of the scheme. By this, information represents a degree of organiztion of units of information into a coordinated system. By a decrease of randomness of a finite number of units through the evolution of coordinating information, there is a corresponding decrease in the choice of alternatives. This information serves to promote further quantitative and qualitative changes by systematizing a response, inducing the formation of additional unique informational assemblies, duplicating information, coordinating and maintaining a system, and so forth. Therefore, information is seen to be an intrinsic characteristic of a system. It is that which not only indicates its own significance when standing alone but also its relationship to the total systemization of the parts, which is a unit of information itself, as well as determining and restricting possibilities of organization with other available units.

As evolutionary time advanced, more units of information were recognized and organized but at the same time the possibility for this organization to be disrupted likewise increased as aresult of the expanded complexity of the system. Therefore, the permutations of potential disorganization increased as the number of organized units in the system increased.

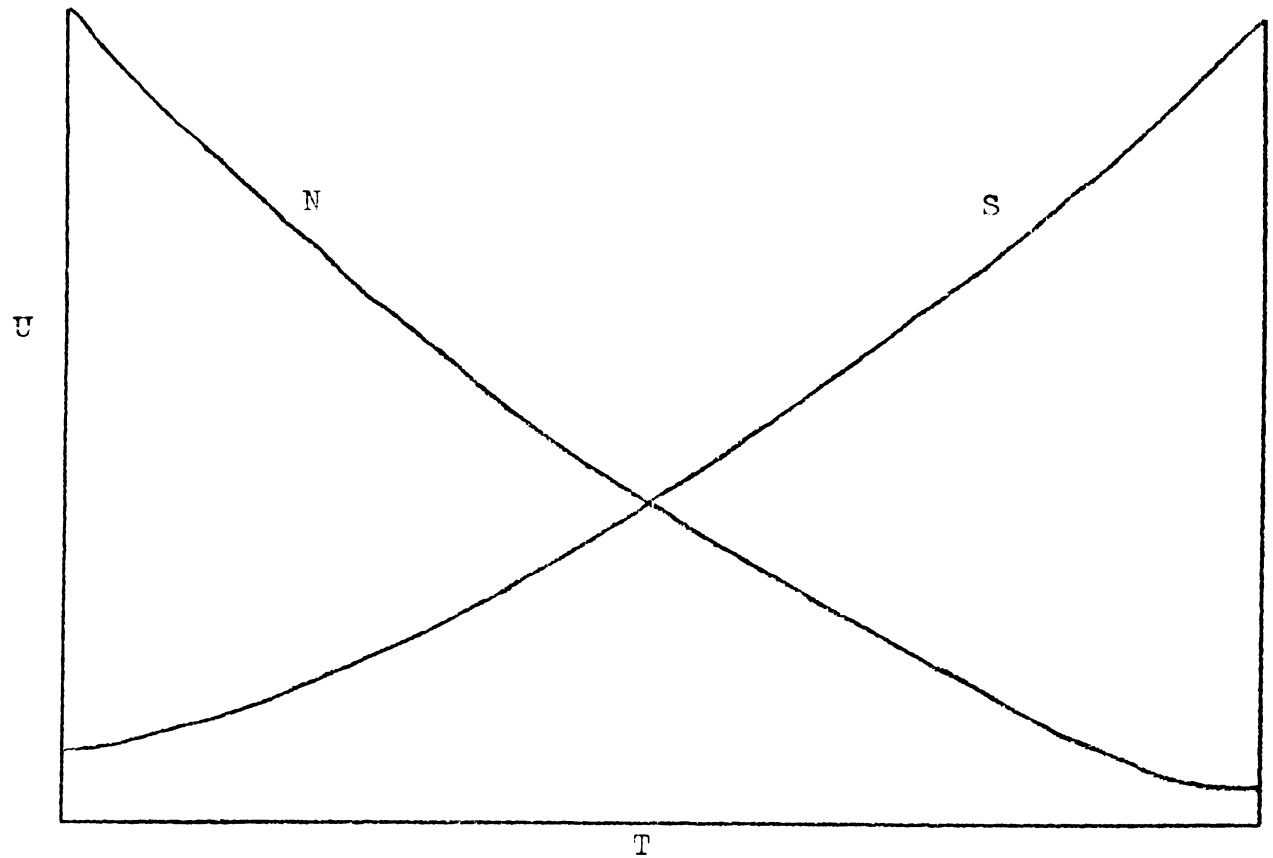
At this point, the much larger view of the total universe must be

considered. With time, it may be noted that the increased organization of information comes into conflict with the increase of disorganization, or entropy. This is indicated in figure 1. Here the value, ΔN, is a function of organization whereas ΔS is a function of negative organization ($\Delta N = f(0)$; $\Delta S = -f(0')$). Since the necessary condition for any equilibrium is $f(0) = f(0')$ and by the graphic representation such a balance is observed to approach absolute existence as time becomes infinitely large, then it may be concluded that at such points in time the real world approaches homeostatic constancy because organizational variation is no longer an overall variable, thus replicating and recreating primoridal constancy.

The progression of such sophistication is readily observed in biological nature and evolution. The amoeba carries on the same basic general functions as man in order to survive as a living organism such as respiration, excretion, reproduction and the like. However, the essential difference between man and the amoeba is that the former, through evolution, has become more complex overall and more specialized in each specific function. These functions individually operate at less relative efficiency and independence than the corresponding simplified functions in the amoeba but give man greater versatility as a result. The entropy, as a function of specific inefficiency, is greater in man than the amoeba but the organizational information wrapped up in man is evidently greater. Thus, maximized simplicity approaches the state of complete non-entropy.

Absolute Disposition

The entropy indicated here is actually relative entropy since it is that which appears to the neutral observer and is relative only to his point of reference and realm of observation. However, from the consideration of the counterforce of information, it may be concluded that



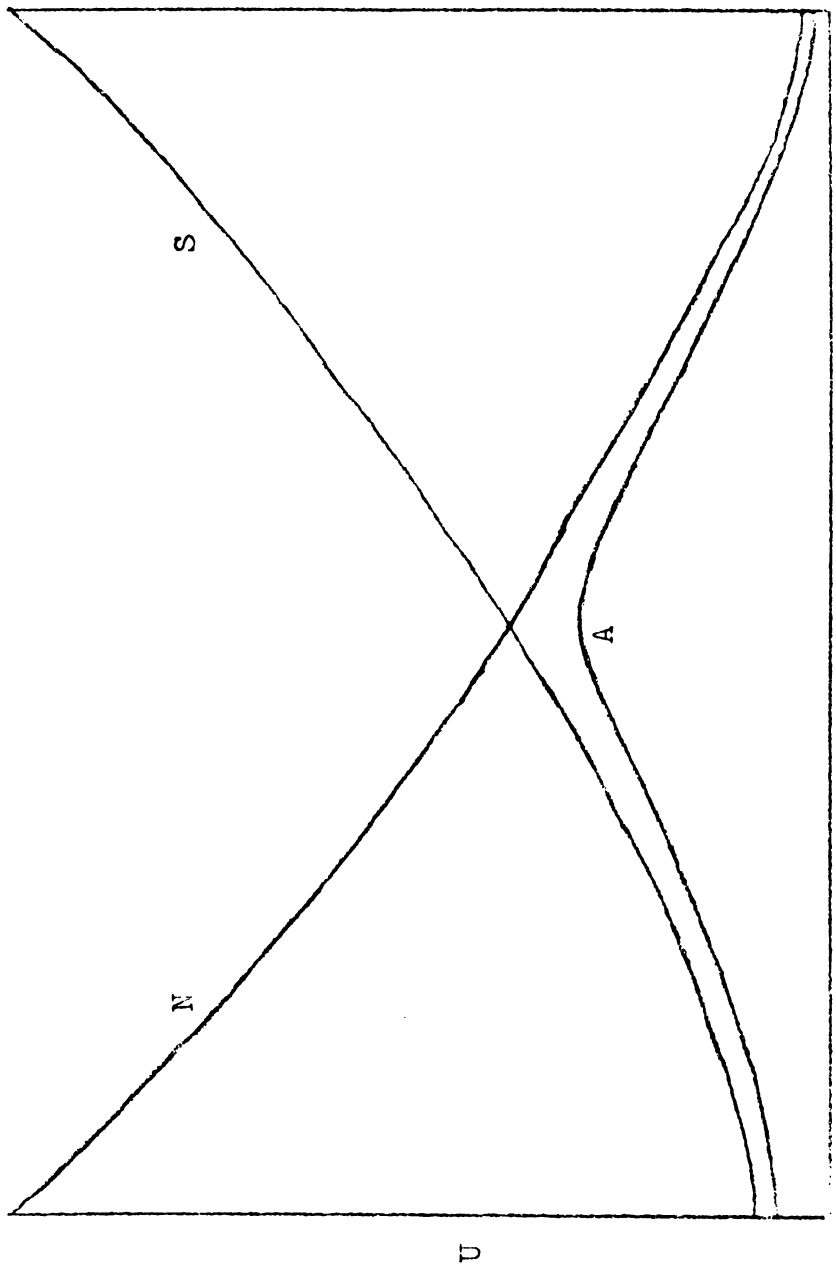
S = disorganizational entropy
T = time coordinate parameter
U = units of non-organization
N = non-organization of information

Fig. 1 - Relationship between Entropy and Information

the actual resultant of these two functions is a third value, absolute disposition (A) which expresses the change in the relationship of information and relative entropy to the total state of the physical world. Thus, it gives a means by which the actual order-disorder dichotomy can be elucidated in its true perspective. For example, as the imbalance between entropy and information increases, absolute disposition increases. This relationship is indicated in figure 2.

Whereas information or entropy may be thought of as a measure of the randomness of an isolated event or system of events, absolute disposition is taken as an overall indication of the relationship between information and entropy of all isolated physical phenomena combined at any given instant. The absolute disposition, with respect to the X and Y axis coordinates, approximately follows a normal Gaussian probability distribution (7). In a continuum, the area under such a curve is taken as one square unit.

Another way of viewing this concept would be through distribution functions. For example, if O is the coordinate of the organization parameter and N(O) is the number of units in each organization segment, where it is assumed that the total number of units in the entire system is constant at all times, then figure 3 indicates such an arrangement. This would be taken as a representation of the units at a given time classified according to the degree of organization (or disorganization) relative to primordial (t=0) conditions. The point A on the x-axis represents the locus of all units of the system on the organization scale at time t_o. As time advances and thermodynamic processes take place, some units become relatively more organized because of enhanced information while the remaining units either do not vary or else decrease in organization taking on greater entropy, such as at t=3. Because of the initial

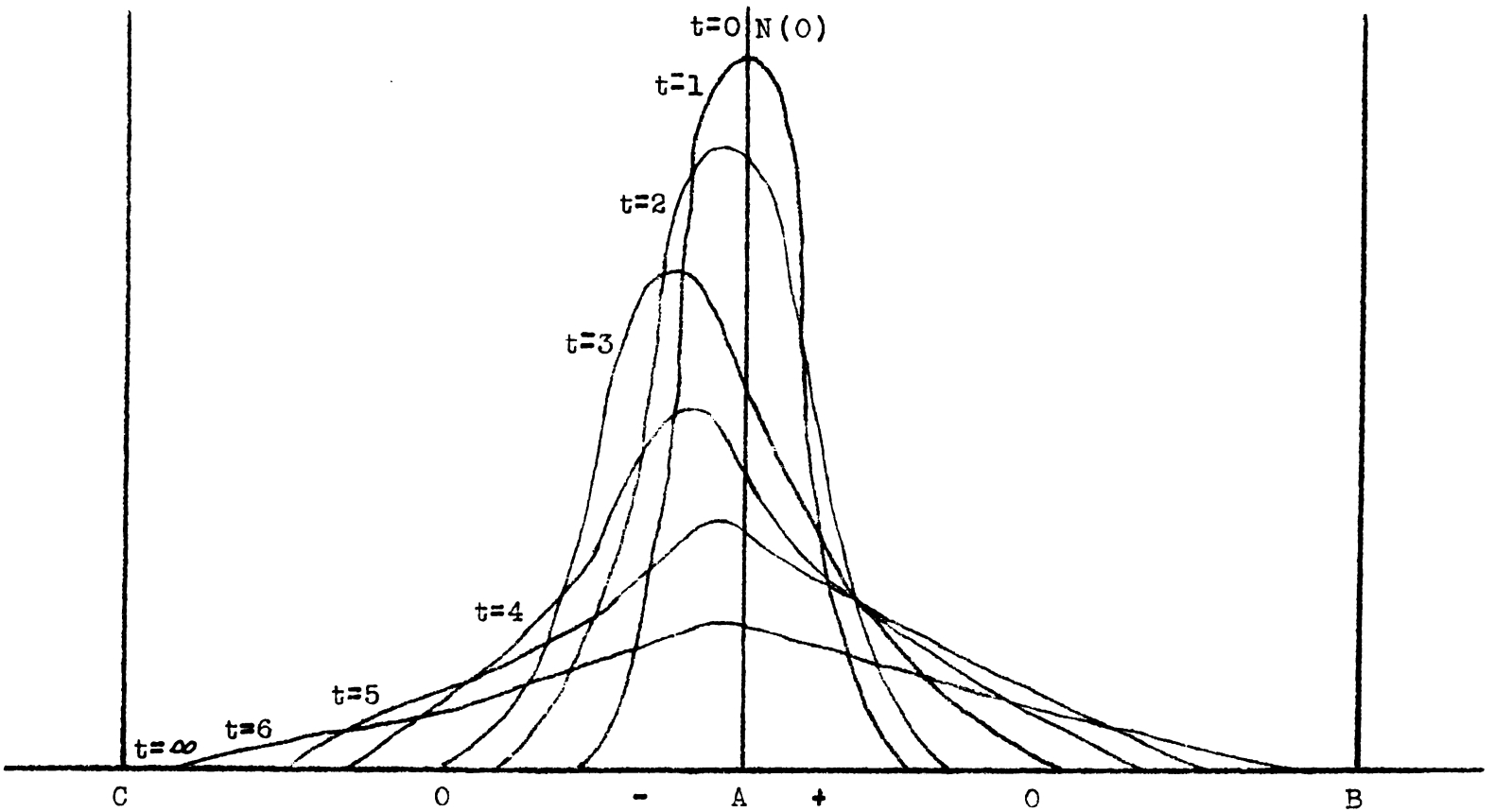


A = absolute disposition
T = time coordinate parameter

Fig. 2 - Formation of Absolute Disposition

overpowering effect of entropy, the center of gravity shifts toward C. However, as higher positive organizational segments become more heavily populated with the complexation of information, the trend of the center of gravity of the units reverses back toward A. If this ever reached A, primordial constancy, the exact balance between information and entropy, would be recreated, although it is here postulated that this could happen only when time would reach infinity. This indicates that enhanced complexation compensates fof earlier trends toward the overall increase of entropy and shifts the previous tendency of maximum disorganization into the opposite direction. Since it is assumed that the primordial constancy is never completely recreated within finite time, the reversed trend is the tendency toward a homeostatic steady state by which greater numbers of units become more complex and more highly organized while other units possess increased entropy. Therefore, units are always available for greater or less organization. The expanded information of the system allows it to compensate for earlier accumulations of disorganization and thus reverse the overall entropy trend.

Of course, if such a shift did not take place, a new constancy function would appear at C when all units would reach that level of organization at one time implying a breakdown of information complexes in the process. Then C could be taken as the new arbitrary zero and the process would start again. On the other hand, if the shift did take place but did not stop at A, a new arbitrary zero would be created at B as complexation reached an upper limit for all the units of the system and likewise the process could start again in either direction from B. If such shifts alternated, the center of gravity would vary about A as waves where A would serve as the mean center of gravity of these waves. The maximum of each successive wave would be slightly farther from A than



t = time
N(O)= units per organization increment
O = organization increment

Fig. 3 - Proposed Organization Distribution of a Finite System

the previous corresponding counterpart so that greater degrees of complexation would be attained with each shift into the positive direction.

If it is assumed that only one overall shift takes place and it is a composite of several oscillating waves, then figure 3 is upheld and the original hypothesis corroborated. It is important to note that during such a shift, the individual changes of entropy and information are, by themselves, unaltered but their overall effect, as reflected by the absolute disposition curve, is affected. Since at the present time it is found that entropy appears to overshadow information accumulation in the physical world, it may concluded from this hypothesis that the world, within the realm of empirical observation, is either before or almost in the midst of this shift. Absolute disposition is a measure of the degree to which the center of gravity of distribution deviates from **A** whereby entropy and information are not in homeostatic balance. The return to **A** indicates a decrease in absolute disposition and the re-establishment of such a balance. If time is to be defined in terms of the trend of entropy, then after such a shift, time would be reversed due to the increasing prominence of information accumulation. Finally, since finite time, by definition, never reaches infinity, the return to the homeostatic steady state of primordial constancy between entropy and information is never quite achieved. The curve note in figure 3 would approach the x-axis and continue to grow broader at its extremities but would never be exactly superimposed upon the axis.

Origin of Energy and Mass

The law of conservation of matter and energy is a law only as long as no observation is made of some phenomenon which disobeys it. Thus, even if such phenomena do occur but have not been observed either because of limitations of observation or lack of sufficient sampling experience, the law would still be quoted even though it was not actually true. Also

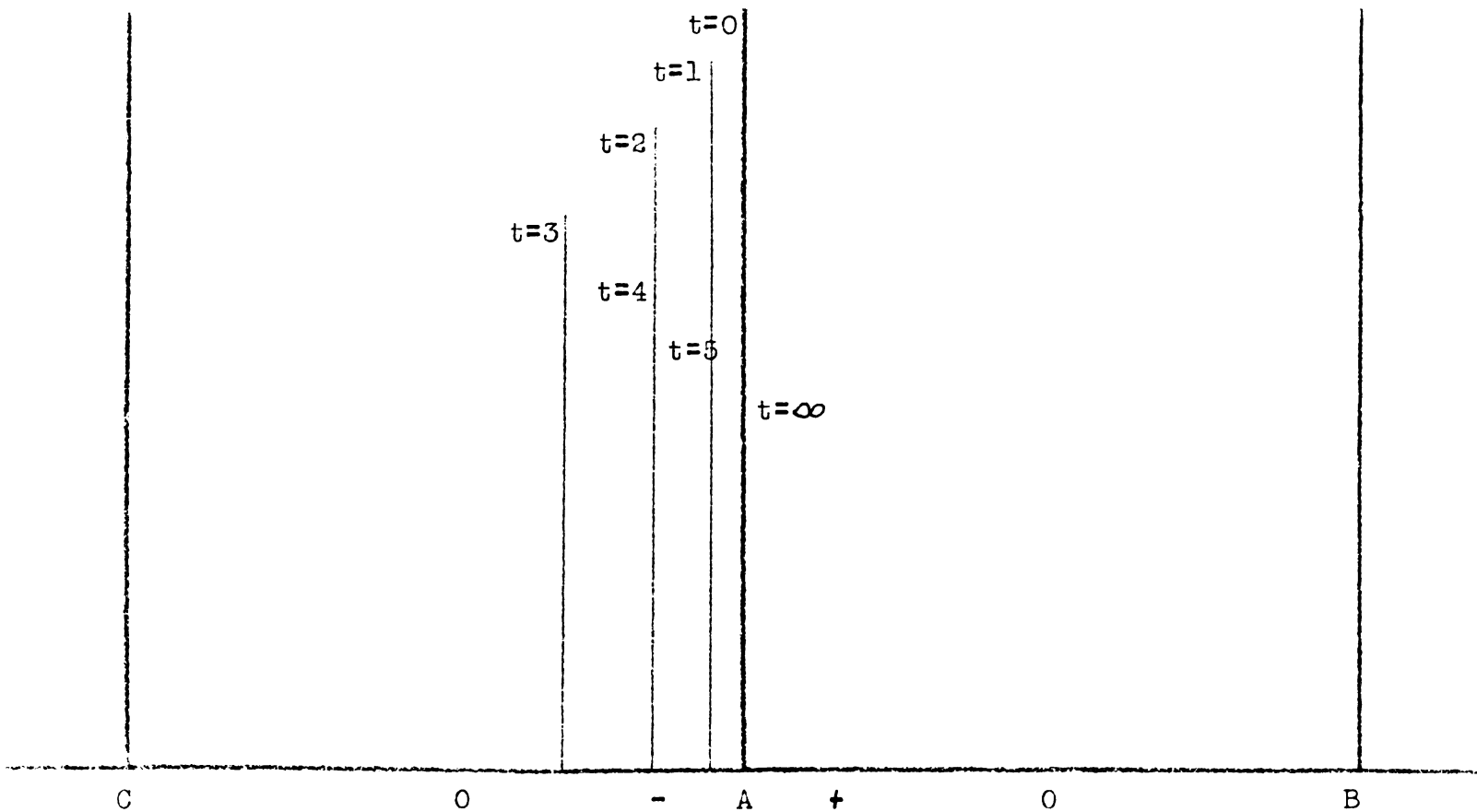


Fig. 4 - Proposed Shift of the Distributional Center of Gravity in a Finite System with Time (from Fig.3)

it is possible that the law is true today but at some previous time it was not, yielding the plausibility of spontaneous generation of mass and/or energy. In this case, a previously undefined concept of absolute nothingness would have to be postulated. This principle is more easily understood if instead of customarily accepting time as being infinitely divisible, it should rather be considered quantized. In other words, a point of division of time increments could be reached by which no further segmentation would be possible. The unit at that point would be the fundamental quantum of time. Combinations of these quanta constitute various segments of time passage such as the second. Undoubtedly, the fundamental quantum of time is much smaller than the second. It is here assumed that primordially the fundamental quantum was not the same as it is now and was in fact infinitely large.

Different velocities may be compared in two ways:

1) constant distance with variable time;

2) variable distance with constant time.

The first case can be applied to the point at hand. Since $c = \Delta x/\Delta t$ and Δx, the distance traversed by a light photon during a quantum of time, is held constant, the only way c can vary would be if and when the quantum of time (Δt) is not constant because c is a function of Δt. Since it has been postualated already that primordially this Δt was infinite, by the Einstein expression, energy was then nonexistent:

$$E = mc^2 = m(\Delta x/\Delta t)^2 = mx^2/\infty = 0.$$

For time to pass, this quantum would have had to become finite which would likewise cause energy, and from this, mass, to appear.

The primordial state of absolute nothingness, where the time quantum was infinitely large, was not a realm of an absence of something since time patterns were lacking and the realm itself did not even exist in

which something could function in the first place. By this deficiency, physical laws were also nonexistent. This motionless primordial state gradually took on the dimension of moving time ($\Delta t < \infty$) which led to the appearance of energy. From the concept of absolute nothingness, it may be noted that energy is as interrelated with time as it is with mass. The apparent stationary character of the physical world today with regard to the constancy of total mass and energy would indicate that the quantum of time is, for all practical purposes, now constant on the Earth.

The concept of quantized time could also be used to explain other physical phenomena. For example, it has been observed that light emitted from distant galaxies has a tendency to appear shifted toward the red end of the spectrum when suitably analyzed (8). One explanation of this may be found in the following derivation where a hypothetical finite system is considered:

h = Planck's constant
V = light frequency
L = light wavelength
Δx = distance traversed by a light photon during a quantum of time
Δt = fundamental quantum of time

1) $E = mc^2$

2) $E = hV$

3) $V = c/L$

4) $mc^2 = hV = hc/L$

5) $L = h/mc = h/m\Delta x/\Delta t$

h, m, Δx held constant

6) $h/m\Delta x = k$

7) $L = k\Delta t$

Thus, as Δt goes from large to small,

L goes from I.R. to visible.

It has been observed that the red shift increases the greater the distance of the light source is from our Earth (8). Therefore,

it may be hypothesized that somewhere in the vicinity of our solar system was the location of the first point in evolution where the quantum of time went from infinity to some finite value. By drawing concentric spheres about that point, the spread of this phenomenon may be visualized as outflowing waves. This would account for the observation of the increasing magnitude of the red shift with distance away from our Earth. Consequently, the universe is exapnding in relation to the rate at which the realm of absolute nothingness is oversome by the spherically spreading deviation of the quanta of time from infinity.

If it is tentatively accepted that initially the real physical world was a giant blob of energy lacking mass, an additional factor must be included at this juncture. Such a possibility has empirical and theoretical foundations. The materialization of energy (**9**) has been observed in the case of high energy photons bombarded upon a heavy nucleus from which results a positron-negatron pair. In 1900, Planck proposed the concept of quantized energy which Einstein took up in 1905 to explain the photo-electric effect (**6**). This is quantitatively expressed by $E = hV$ where V is the frequency, h is Planck's constant, and E is the energy of the photon. Thus, the photon is considered a quantum of energy which, when bombarded upon a suitable target, induces an expulsion of electrons (**9**). If the energy of the photon is above 1.02 Mev, the photon disappears within the field of the nucleus and a positron-negatron pair results, conforming to the equation $hV = 2mc^2 + E_1 + E_2$ where E is the kinetic energy of the particle at the time of production, mc^2 equals 0.51 Mev or the rest-mass energy of each of the resultant particles, and hV is the energy of the photon.

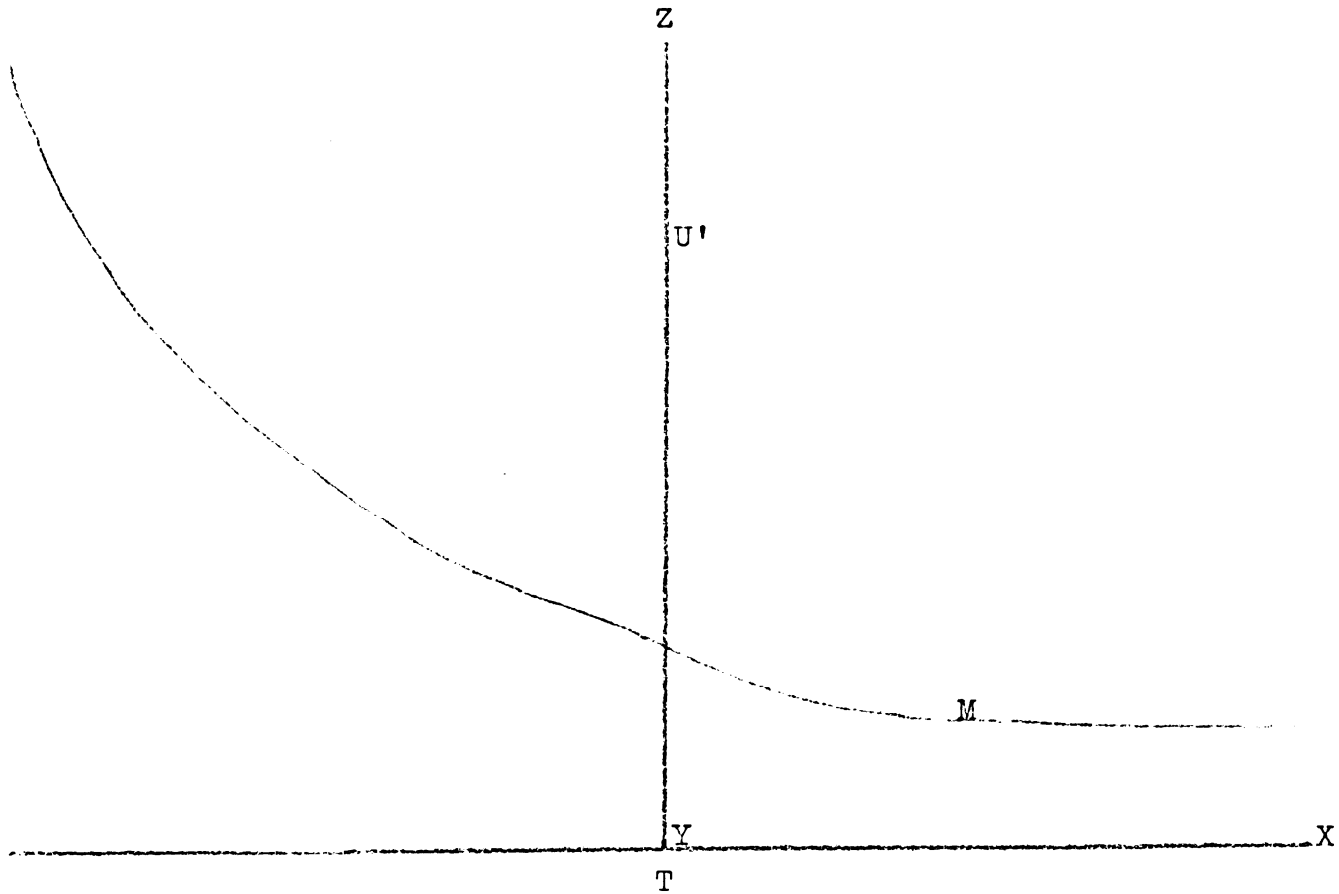
Annihilation of matter is observed when a positron collides with a negatron, yielding two gamma rays each of energy 0.51 Mev. Similarly, it is possible for a positron-negatron collision to result in the production of a neutrino-antineutrino pair which also has no mass (**10**).

The positron may be viewed as an electron of higher positive energy, greater than $2mc^2$, while bearing positive charge and the same rest mass as the electron (11). On the other hand, a photon has no charge, no rest mass, and possesses energy by virtue of its motion at the speed of light. The photon is a quantum of energy at any one of a number of frequencies so that to define a gamma ray or an X-ray is merely to designate the frequency of the given group of photons.

The production of a positron-negatron pair by photon-photon collision has been proposed (12). Such a process requires sufficient energy. It is also of significance that when the energy of the photons is of the order of billions of elecron volts (Bev), the products of their collision can be a proton and an antiproton, each possessing identifiable mass and charge. As a result of the collision process, both photons disappear.

Pair production by photon-photon collision is very difficult to observe and perform experimentally because of the high energy ($h\nu_1+h\nu_2>2mc^2$) and the very sensitive equipment required (13). However, positron-negatron pair production becomes appreciable in the interior of stars where radiation density and temperature are both very high. The value kT is approximately equal to mc^2 at $T = 5 \times 10^9 C^o$ and above this the density of beta particle pairs becomes equal to the density of the light quanta themselves.

In photon-photon collision, the production of identifiable mass from energy without the need for an original mass has fundamental theoretical significance especially in the hypothesis now under consideration. Not only does such a process give a plausible explanation for the materialization of non-matter from primordial energy sources but it also suggests strongly the real significance of the electrical charge dichotomy observable in nature. One of the most outstanding examples is the electron



U' = units of non-organization
M = non-organization of the primordial energy system

Fig. 5 - Organization of the Primordial Energy System

and proton.

The graphic representation of the change of organization of the primordial energy system by photon conversion (figure 5) is given in organizational units at any given time. This indicates that the change of absolute disposition is affected by the change in the number of material units available to be ordered or disordered. As already noted, the number of material units is affected both by the appearance of energy with the change in the time quantum and the conversion of that energy to mass by photon-photon collision.

If it is postulated that in the materialization of energy by photon-photon collision an instantaneous unstable intermediate particle N is formed, the relationship of absolute disposition to information and relative entropy is clarified.

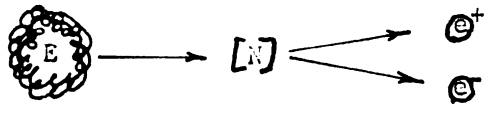


Figure 6 - Materialization of Energy

By this scheme, primordial energy E, representing two interacting photon quanta, is formed into a single unit, the intermediate N. This is the ultimate of organization for this particular event. Disorganization ensues when the single particle divides into the beta pair. Information is greatest in the intermediate, but since mass remains at the end of the event, absolute disposition is altered by it even though some relative entropy is noted. The more often such transformations take place in a given unit of time, the greater is the alteration of absolute disposition when considering only this type of event. Thus materialization of energy is an example of the increase of organization or information, whereas the annihilation increases disorganization, or entropy.

Of course, on a much broader, the systemization of the resultant

beta particles also affects the total absolute disposition, whether that be annihilation or further material construction. Materialization of energy puts a greater number of units into the system to be affected by the interactions taking place. As long as materialization overrides annihilation, the flux of this factor is significant to the alteration of absolute disposition.

It could be concluded from this theory that primordial energy lacked any semblance of order and organization. However, even in the greatest possible degree of disorder there is some measure of organization. This is partly based on the inherent information of each of the components of the system as well as the relationship of one seemingly unordered unit to another with regard to such interacting factors as energy, position, relative velocity, apparent momentum, and so forth. From this consideration, it may be noted that the primordial energy system possessed a measure of organization with regard to the state of its constituents and that as materialization progressed, with its resultant charge dichotomy and attraction-repulsion phenomena from particle interactions, this degree of organization changed and, in turn, information fluxuated. When this aspect is included on the original order-disorder graph (figure 1), a third dimension on the z-axis is introduced which directly affects the total change of absolute disposition with time by defining it over a surface of possible variations of organization at any instant. Ultimately, once the balance between materialization-dichotomization, annihilation, entropy and information complexation is achieved, an overall steady state is established (figure 7). It is noted that as time approaches infinity, the absolute disposition, or total interplay of order-disorder, reaches a homeostatic balance and all subsequent finite changes would alter the entire system in only infinitesimally small degrees. Thus,

absolute disposition is the true measure of the flux of the order-disorder dichotomy when considered on an overall universal scale and not relative to an observer's limited realm of perception. Absolute disposition, within the limits of finite time, never reaches 0 since the function approaches zero asymptotically. The instant at which absolute disposition reaches a maximum is likewise theoretically determinable and is the point in the time of the entropy shift noted earlier.

The Electron in Protobiochemical Evolution

Electricity is recognized as the flow of electrons in which negative and positive regions are defined by an abundance of or deficiency in electrons respectively (14). Here the electron serves not only as a unit of energy-mass but also as a transport medium for energy from one site to another. As noted earlier, the electron may be postulated to be the primary and most primitive mass particle in the physical world resulting from positron-negatron pair production by photon-photon collision. Thus, any subsequent transformations and fabrications which occurred depended on the electron both as a site of available mass and as an energy source.

As the abundance of negative and positive particles increased in physical evolution, the importance of photons as reaction stimulators decreased and the subsequent importance of the electron as a reaction inducer became more significant and apparent. One of the most outstanding remnants by which the photon acts as a reaction inducer is in photosynthesis.

The basis for chemical reactions is the activity of planetary electrons and spectroscopy depends on the phenomenon of electrons absorbing and emitting quanta of energy (6). Thus, the electron appears to serve as an energetic stimulator to induce and promote reactions whose course is predetermined by inherent patterns for organization, much

the same as in the number example given earlier where the individual jigsaw pieces carried the inherent information by which the ultimate sequence of their interaction would be ordered. Assuming that the course of biochemical evolution reflects the inherent patterns within the constituents, it may be hypothesized that if basic structural units could be placed under the influence of electrical stimulus, the course of biochemical development could be, at least in part, reproduced. Thus, primitive atoms and molecules seem to have had a wide range of possible forms to assumeupon complexing and evolving, but, much the same as in Darwinian-type survival of the fittest, only those which found use in the further development of biochemically significant structures continued to appear repeatedly in the course of chemical sophistication and evolution. The degree to which a given atom or molecule served as a useful site for further evolution was determined by its natural tendencies as controlled by developed intrinsic informational units as well. Thus, the biologically significant compounds and structures which appear today are those which evolution has found by experience to be the most efficient and most useful from the entire repertoire of possible combinations.

The abundance of energy in an electrical environment permits reactions which would otherwise be prohibited without such conditions, and thus allows the reacting units to exhibit their inherent informational tendencies. Likewise, such an experiment would give some indication as to the significance of fundamental molecular units and structures to larger systems such as macromolecules, as well as shedding light on the plausibility of certain energy sources having been primordial reaction inducers.

In any consideration of the origin of biological life, the means for the synthesis of biochemically significant structures must first be established. Therefore, if the postulation is tentatively accepted that electrons are among the principal stimulators for the fabrication of

fundamental structures from basic atomic and molecular units, then the projection of this concept into the realm of molecular biology provides a substantial foundation upon which to explain biochemical evolution. Thus, it is the essential purpose of this series of experiments to provide support to this specific hypothesis as well as to the general theory already proposed.

III. HISTORICAL REVIEW

Even though the intended purpose of the experimental portion of this study is to deal with specific reactions within a highly specialized realm of the physical sciences, the more important goal here proposed is to introduce some coordinating concepts from a broad point of view and thence provide specific laboratory procedures which tend to substantiate the theory advanced.

Paul Wiss, of the Rockefeller Institute, in his article, "Cellular Dynamics," has pointed out the need to attack big problems (15):

> "... we frequently try to fit our questions to the very limited answers which our fragmentary knowledge has been able to provide, instead of boldly facing the much broader questions posed by living systems and phrasing them in such a way that still more penetrating answers may be found in the future."

Therefore, since the theory put forth in this thesis concerns itself with a comprehensive topic of this nature, it is necessary to seek support and impetus not only from current efforts in the laboratory but also from the work of previous researchers whose findings have significant bearing upon the subject now under consideration. In this sense, the job of the researcher is to observe the trends in the present and from these, extrapolate backward and forward in order to logically theorize on various aspects of the physical world, its origin, dynamics, and destiny.

The enigma of the origin of life and the materials necessary to sustain life has been pondered for many years. Some rational suggestions were advanced as early as the days of the ancient Greeks. For example, Democritus thought that life began in the water as the result of an inherent movement of the atoms (16). The question of spontaneous generation engendered especially high fervor during the debates between

Spallanzani and Needham in the 1760's (17).

In 1931, Holiday and Nuttingham demonstrated that methane (believed to be one of the components in the primordial atmosphere), when heated to 1000°C, is converted to acetylene (17).

$$2CH_4 = CH{\equiv}CH + 3H_2$$

Tchitchibabin described how acetlene, in the presence of water, produces oxidized hydroxy derivatives, specifically acetaldehyde (18).

$$CH{\equiv}CH + H_2O = CH_3CHO$$

Oparin suggested that such reactions could also lead to other oxidation products of hydrocarbons, such as alcohols, aldehydes, ketones and organic acids (17). He also noted that such oxidized derivatives can enter into a variety of chemical reactions with reduced forms of nitrogen and give rise to amines and other nitrogen-containing compounds.

The Cannizaro type of reaction gives a specific means by which aldehydes can react to form alcohols and organic acids (19). An oxidation-reduction reaction between two acetaldehyde molecules plus water can yield ethyl alcohol and acetic acid, according to Oparin (17). Acetaldehyde and formaldehyde can also react under the Cannizaro type of reaction to produce the polyhydroxy compound, pentaerythritol (20). Sabatier reported that aldehydes have a great tendency towards polymerization (17). Thus, it is reasonable to postulate that primordial conditions could have provided the necessary conditions and materials for the production of various types of alcohols and organic acids specifically.

A number of chemically induced techniques have been found for the artificial in vitro synthesis of sugars. Kiliani discovered that it is possible to synthesize a sugar-life material by his cynamohydrin method, in which cyanide intermediates provide additional carbons to build up the size of the molecule (21). Similarly, under the influence

of a weak base, formaldehyde produces formose, a mixture of hexoses (22).

Fischer found that a several step oxidation-reduction manipulation of glycerol resulted in the synthesis of hexose sugars which involves aldol condensations between carbonyl groups and the hydrogen atoms adjacent to the carbonyl groups(23). Acrolein, resulting from the oxidation of glycerol, also produces hexoses in alkaline solution due to the formation of glycerose, a mixture of glyceraldehyde and dihydroxyacetone, with subsequent condensation of these products to acrose. In 1902, Fischer and Leuche achieved the synthesis of glucosamine from arabinose (24). Peat found that by rupturing the vic-oxide group of an anhydro sugar in the presence of ammonia, both a 2-amino sugar derivative and a 3-amino sugar derivative result (20).

It has been determined that as part of the in vivo photosynthetic carbon cycle, glyceraldehyde and dihydroxyacetone serve as significant intermediates within an enzyme regulated system. The production of hexoses is here induced and controlled by the input of energy, in this case, light(25).

The original form of nitrogen needed to produce amino groups, such as in amino acids, as well as the means by which such substances were synthesized has been a topic of investigation. Urey noted that molecular nitrogen could very well have been present in the primordial atmosphere (26). He also suggested that absorption of ultraviolet light in the upper atmosphere provided the free energy by which critical syntheses were induced through free radical reactions. S.L. Miller, a student of Urey, published the results of an experiment in which he synthesized amino acids by passing a gaseous mixture of hydrogen, methane, ammonia, and water vapor through an electric spark (27). At the end of a week, glycine and alanine were identified in his solution

by chromatography. From these data he proposed that ammonia was the fundamental primordial source of nitrogen. Similar results have been observed when such a gaseous mixture was exposed to ultraviolet light (28). Ctozai and co-workers were able to polymerize glycine under the influence of a Tesla coil discharge (29).

Hughes and Ingold synthesized alanine from lactic acid by means of bromination and azide formation (20). The product was unnatural since it turned out to be of the D-form. Natural L-alanine was prepared chemically and resolved by Wolfrom using suitable cleavages of glucosamine with lead tetraacetate(20).

Fieser and Fieser have reviewed other chemical methods for the in vitro synthesis of sugars and amino acids (20). The Gabriel synthesis, utilizing potassium phthalimide and suitable halo esters, has been employed in the production of amino acids. Amination of aldehydes by means of cyanide addition to yield amino acids has been shown in the Strecker synthesis. For example, acetaldehyde would give alanine by this process. Suitable treatment of malonic ester, such as with phthalimide and nitrous acid, was shown to result in amino acids. Aldehyde condensations of appropriate aromatic reagents was used to synthesize amino acids with aromatic groups by making use of the activated methylene groups.

The possible primordial sources of energy to bring about essential reactions has also been investigated quite extensively. While a number of expetiments have been carried out on the effect of both ultraviolet light and electrical dishcarge, few have had significant bearing on the problem of the origin of life and the substances necessary for the generation of life. However, some workers such as Miller have shown that in the gaseous phase, cyanides and aldehydes play an important role as intermediate

products of these reactions.

There appears to be little difference in the products of chemical transformation by various forms of high-energy bombardment (30). For example, it was observed that high-energy alpha particles, gamma rays, and ultraviolet light can all produce a variety of forms, such as formaldehyde and formic acid, from the same initial materials.

In 1962, Calvin reported that he had submitted a gaseous mixture of methane, ammonia, hydrogen and water to alinear beta accelerator where C^{14} methane was employed. He found that the products of this reaction,when analyzed as an autoradiographic paper chromatogram, matched up favorably in certain cases with the product from the reaction of formaldehyde in base (31). This latter material is known to be a source of formose. In 1958, Hasselstron and co-workers irradiated aqueous solutions of ammonium acetate with the beta accelerator whereby glycine and some other amino acids were produced (32).

Under the influence of ultraviolet rays of less than 2000 Å, methanol and ethanol were found to yield formaldehyde and acetaldehyde respectively with a quantum yield between one and two(33), while isopropyl alcohol gave acetone (34). In the presence of ultraviolet light and oxygen, Cartieni found that ethylene glycol was oxidized to a peroxide(35). Henti and Ranc, in 1912, observed that irradiated glycerol gave a positive phloroglucinol reaction for glycerol aldehyde (36).

Reactions induced by X-ray bombardment bear much resemblance to those produced by a stream of high velocity electrons (37). Fricke noted that a peculiarity of X-ray action is that it frequently involves the production of molecules with unusually high energy states, such as water molecules with at least 91 kilocalories per gram molecule (38).

Ellis and Wells noted that electromagnetic radiation of appropriate wavelength can be used to bring about chemical reactions involving free radicals (37). Such mechanisms are often initiated by the homolytic splitting of a bond to yield free radicals which then proceed to various other reactions utilizing these reactive units (39). An example of this would be the photolysis of acetic acid where hydrogen free radicals play an important part in the essential production of ethane, water and carbon dioxide finally. Chain propagation is also possible in this general type of reaction (37).

Glen and Hansen, in 1962, found that ionizing gamma radiation causes b-lactoglobulin to unfold. Since a free radical reaction ensued, the unfolded species tended to dimerize. Similar effects were observed under the influence of electrons from a linear accelerator (40).

Dhar and Mukherjee, in 1934, claimed that exposure to sunlight or a mercury-vapor lamp of 0.5N solutions of ammonium, potassium or sodium nitrate containing glucose, tartaric acid, glycerol, arabinose, fructose, mannose or galactose gave positive colorimetric tests for amino acids where the best yields were with ammonium nitrate(41). This is similar in nature to the work carried out by Hasselstrom and co-workers already noted. Dhar and Mukherjee also found that the use of ammonium hydroxide as the nitrogen source proved unsuccessful.

In 1866, Berthelot examined the behavior of alcohols, aldehydes, and organic acids in the presence of nitrogen under the influence of silent electrical discharge. He found that small amounts of nitrogen became fixed to these substances in the processs(42). Also, nitrogen-containing molecules were found to fix more nitrogen in the effluve.

In 1875, Cavendish discovered that an electric spark passed through a mixture of oxygen and nitrogen induced the formation of

nitric acid(43). Birkeland and Eyde developed a high-tension discharge arc furnace which formed nitrogen oxide from air containing nitrogen and oxygen at 3000°C with yields of about 1%. When cooled, the nitric oxide combined with oxygen giving nitrogen dioxide and tetroxide. The dioxide, when dissolved in water, formed nitric acid and, when brought into contact with slaked lime, yielded calcium nitrate.

Lewis and Randall noted that thermodynamics has been responsible for the introduction of many significant concepts among which entropy is of noteworthy importance. The primciple resulted from the pioneering work of Carnot, Kelvin, and Clasius and ultimately found its place in physical science as the second law of thermodynamics (44). As already noted, this concept relates to the randomness of a system, although a precise interpretation of entropy cannot be given easily. Gibbs, as well as Helmholtz and Maxwell freely employed this concept in their contributory works. Lewis and Randall commented on the development and significance of the first law of thermodynamics:

> "The first law of thermodynamics, or the law of conservation of energy, was universally accepted almost as soon as it was stated, not because the experimental evidence in its favor was at that time overwhelming, but rather because it appeared reasonable and in accord with human intuition. The concept of the permanence of things is one which is possessed by all."

(The underlining in the previous quotation is that of the author of this thesis. The reader is here referred back to the section on theoretical considerations dealing with human observation in relation to absolute and relative concepts.)

From these considerations, a very decisive point can be drawn: The origin of life and the primordial synthesis of biochemically significant compounds have been topics of considerable scientific concern.

However, no set of experiments has thusfar decisively established the answer to this enigma, or has strictly limited the realm of speculation in this problem. Therefore, as a greater number of possible solutions are presented, the true explanation will eventually become clearer. The function of critical speculation, based on sound scientific reasoning and empirical justification, is to present plausible perspectives by which progress may be stimulated and the knowledge of man will ultimately elucidate the solutions to his deepest problems.

IV. EXPERIMENTAL

EXPERIMENTAL OBJECTIVES

This series of experiments has been undertaken to investigate many aspects of the topics under consideration:

1) To determine the feasibility of using high voltage electrical sparking to bring about the synthesis of biochemically significant compounds and functional groups in the aqueous phase such as:

a) Primary amino groups from atmospheric nitrogen (and not ammonia)
b) Amino acid-like molecules from organic acids
c) Reducing aldehydic groups from hydroxyl groups
d) Sugar-like molecules from mono- and poly-hydroxyl alcohols
e) Amino sugar-like molecules
f) High molecular weight polymers.

2) To investigate the effect of electrical sparking on known molecules and polymers.

3) To present a possible means by which the compounds essential to the initiation of biological life under primordial conditions may have originated.

4) To lend initial support to an encompassing theory concerning basic concepts of the dynamics of the physical world as a plausible explanation for its origin, course, and destiny (see Theoretical Considerations).

5) To suggest a course of attack in solving associated problems in biophysical and molecular research.

6) To gain experience and insight with analytical procedures which are important in organic, biochemical, and biophysical research.

EXPERIMENTAL METHODS

For expediency and clarity in explanation, the experimental procedures and data are presented together in this section. When analytical tests were run with a specific purpose (e.g., to identify carbohydrates) and the test was positive, it was intended to show only that carbohydrate-like substances were present in the tested material. However, no single test can be taken as conclusive or absolute. References to "T-number" (e.g., T-1) indicate the analytical techniques and reagents described in detail in the Appendix.

A. Apparatus

The primary investigation problem, as noted in the objectives, was to examine the various aspects of the use of electrical sparking to initiate the synthesis of biochemically significant compounds. It is important here to distinguish between the linear beta accelerator, silent electrical discharge, which is found in vacuum tubes, and electrical sparking, where a visible spark jumps a gap between two electrodes as employed in this series of experiments. For this, closed setups were designed to approach this problem. The schematic drawing of the electrical system is shown in Fig. 8 .

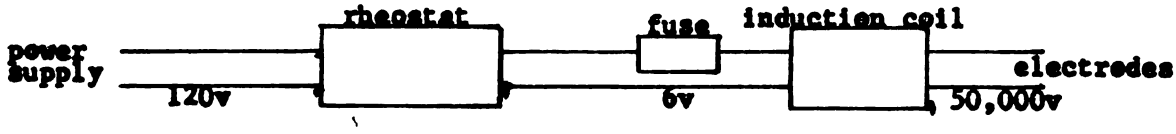


Fig. 8 - Schematic drawing of electrical sparking system.

In this way, a common electrical source of 110 volts was utilized and suitably converted to supply a voltage high enough to arc a gap of about 1 centimeter. Since all syntheses were carried out in the aqueous

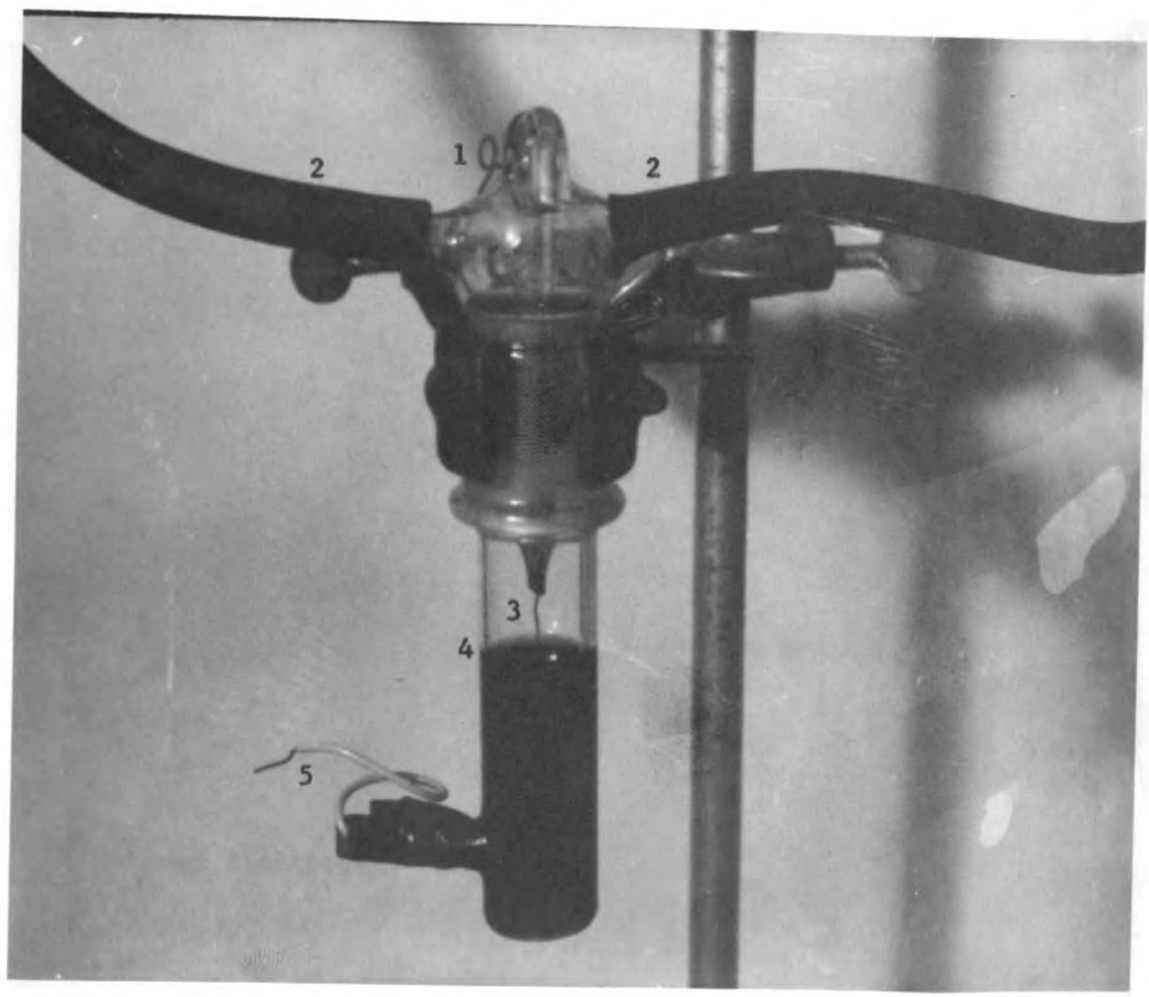


Figure # 9 - Reaction Chamber
1: Connection for upper electrode
2: Ventilation connections
3: Upper electrode
4: Experimental solution level
5: Connection for lower electrode

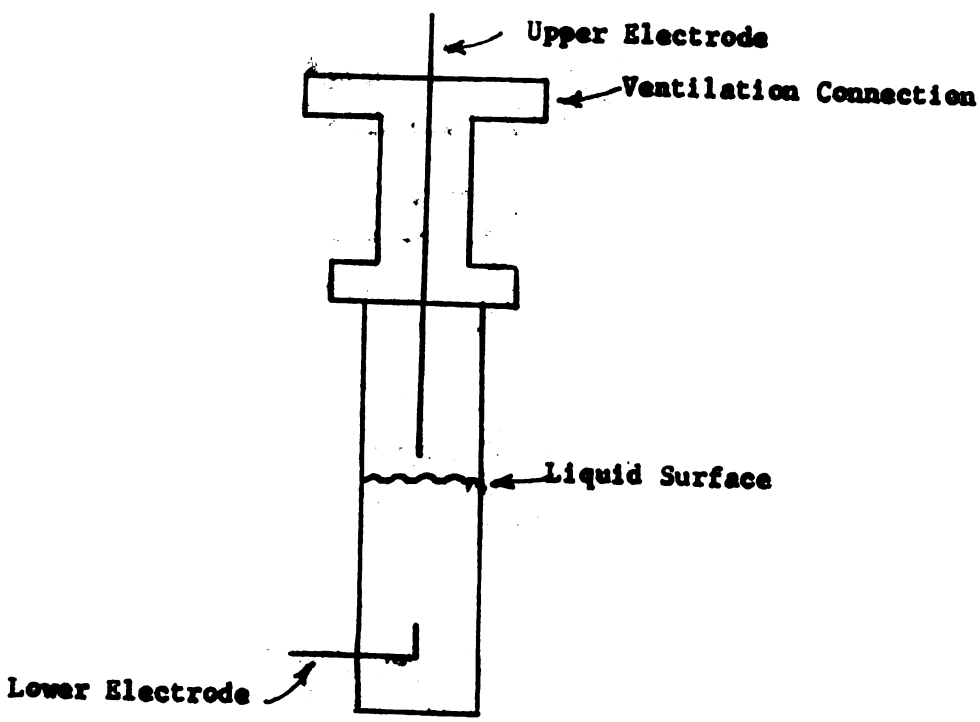


Fig.10 - Schematic drawing of reaction chamber

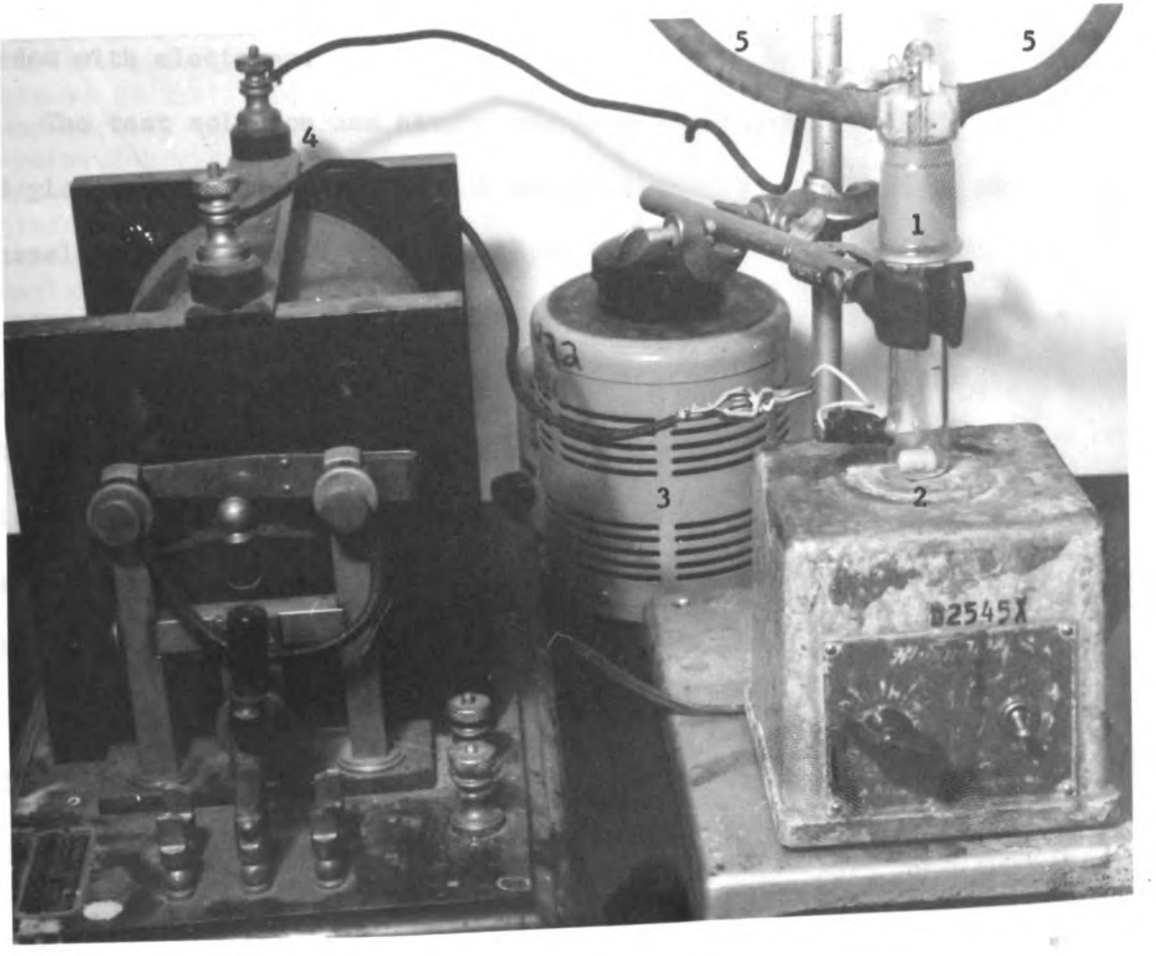


Figure # 11- Experimental Setup
1: Reaction chamber
2: Magnetic stirrer
3: Power supply
4: Induction coil
5: Ventilation

phase, one of the electrodes was submerged within the solution and the other was suspended above. Thus, the entire solution served as one of the poles and a continuous electrical sparking current passed from the upper electrode directly to the surface of the test solution. Consequently, the molecules in the path of the current were directly bombarded with electrons.

The test solution and electrodes were contained within the closed glass vessel and open only to ventilation ports at the top of the vessel bringing in and releasing acid- and thymol-filtered air under pressure. This arrangement removed undesired gases resulting from the sparking process. Filtration of the air before entry insured its microbiological purity. To accommodate large amounts of test reagents, vessels of 1-liter and 2-liter capacity were used, whereas for smaller samples, a vessel of 10- to 12-ml. capacity was employed (see Fig. **9,10** and Fig. **11**).

B. Chemicals and Reagents

The following list includes the experimental reagents of at least C.P. grade employed in this work. The chemicals starred were tested for purity before use in experiment no. 5, to be described. For a listing of analytical reagents and equipment, refer to the Appendix. Experimental equipment not mentioned in the Appendix will be cited in the appropriate experiments.

Methanol, absolute * (Central Scientific Company)
Ethanol, 95%* (Commercial Solvents Corporation; C.P.)
Ethylene glycol* (Fisher Scientific Company; Fisher Certified Reagent)
n-Propanol* (Baker and Addison; C.P.)
iso-Propanol* (Baker and Addison)
1,2-Propanediol* (Eastman Organic Chemicals)
1,3-Propanediol* (Eastman Organic Chemicals)
Glycerol* (Central Scientific Company; USP)

Acetic acid, glacial (Mallinchrodt Chemical Works; Analytical Reagent)
Bovine blood serum, freshly drawn and citrated
n-Butanol (Fisher Scientific Company; Fisher Certified Reagent)
n-Amyl alcohol (S.T. Baker Chemical Company; Baker Analyzed Reagent)
Ribose (Nutritional Biochemicals Corporation)
Glucosamine-HCl (General Biochemicals)
Ammonium sulfamate (LaMotte Chemical Products Company)
Glycine (Nutritional Biochemicals Corporation)
Valine (Nutritional Biochemicals Corporation)
Alanine (Nutritional Biochemicals Corporation)
Histidine (Nutritional Biochemicals Corporation)
Cysteine (Nutritional Biochemicals Corporation)
Phenylalanine (Nutritional Biochemicals Corporation)
Tyrosine (Nutritional Biochemicals Corporation)
Lysine (Nutritional Biochemicals Corporation)
Arginine (Nutritional Biochemicals Corporation)
Nitric acid (Allied Chemical Company; C.P.)
Phenylacetic acid (Eastman Organic Chemicals)

C. Procedures and Data

The experiments and data to be presented after experiment no.1 are arranged into three major groups of compounds produced by sparking techniques.

i) Sugars and amino sugars (experiments 2 to 10)

ii) Amino acids (experiments 11 to 12)

iii) Polymers (experiments 13a to 13c)

Each experiment is introduced with a statement of its purpose, followed by the procedure, and concluded with the resulting data usually in tabulated form. The analysis and significance of these data is presented under the Discussion section.

Wherever appropriate, certain experiments (such as 5 and 14) were included to rule out nonspecific effects as well as to provide evidence for specific points put forth in the theory and the discussion (such as in experiment no.15).

Several measures were employed to minimize microbial contamination including use of high purity reagents as well as chemical and/or autoclave

sterilization of glassware. Standard bacteriological assays (see experiment no. 14) verified the effectiveness of these precautionary methods.

Experiment 1

To carry out studies on the effect of electrical sparking on solutions of known biological substances or compounds, the following procedures were performed:

a) Several known amino acids including glycine, alanine, histidine, cysteine, phenylalanine, tyrosine, lysine, and arginine were mixed together into aqueous solution so that the final concentration of each was 1% (w/v) and with no sparking were tested by ultraviolet spectrophtometry in the Beckman Model DK-2 instrument by scanning between 220 mu and 340 mu (T-32).

b) Amino acids such as in a) were mixed together into a solvent composed of glycerol:95% ethanol (50:50) instead of water and tested without sparking by ultraviolet spectrophotometry.

c) A mixture like that in b) was prepared and heated at 110°C for 24 hours, not sparked, and then submitted to ultraviolet spectrophotometry.

d) A mixture of amino acids as in b) was sparked for 18 hours and subsequently tested by ultraviolet spectrophotometry.

e) To 2 ml. of ethanol (95%) and 1 ml. of glycerol was added 7 ml. of a 1% (w/v) aqueous solution of alanine which in turn was examined as follows:

(1) About 1/2 of this solution as a control was measured directly by ultraviolet spectrophotometry between 220 mu and 340 mu.

(2) The remaining portion of the solution was sparked for 2 hours and scanned similarily by ultraviolet spectrophotometry.

f) To 2 ml. of ethanol (95%) and 1 ml. of glycerol was added 7 ml. of a 1% (w/v) aqueous solution of phenylalanine and divided in the following manner:

(1) About 1/3 of this solution as a control was directly scanned by ultraviolet spectrophotometry between 220 mu and 340 mu.

(2) About 1/3 of this solution was sparked for 2 hours and subjected to ultraviolet spectrophotometry.

(3) The remaining portion of the solution was added to 2 ml. of hydrogen peroxide (5%) and tested by ultraviolet spectrophotometry.

g) A sample of freshly drawn citrated bovine blood serum was divided into two portions:

(1) The first portion served as a control and was analyzed for its protein fractions in a Perkin-Elmer Model 38 Tiselius electrophoresis apparatus (T-26) at pH 8.6 and ionic strength of 0.01 in veronal buffer.

(2) The second portion was sparked for 24 hours and submitted to Tiselius electrophoresis under the same conditions.

A summary of the results of the various parts of experiment 1 is presented in table 1 .

TABLE 1

A COMPARISON OF THE EFFECTS OF SPARKING AND OTHER PHYSICAL TREATMENTS ON SELECTED BIO-ORGANIC COMPOUNDS AND SUBSTANCES

(table on next page)

TABLE 1

A COMPARISON OF THE EFFECTS OF SPARKING AND OTHER PHYSICAL TREATMENTS ON SELECTED BIO-ORGANIC COMPOUNDS AND SUBSTANCES

Test	Substance	Treatment	Analysis	Change from Control
a	a.a.*	none	UV scan	control
b	a.a.	g.-e.**	UV scan	none
c	a.a.	110°C - 24 hr.	UV scan	none
d	a.a.	spark - 18 hr.	UV scan	yes
e-1	alanine	none	UV scan	control
e-2	alanine	spark - 2 hr.	UV scan	none
f-1	phenylalanine	none	UV scan	control
f-2	phenylalanine	spark - 2 hr.	UV scan	yes
f-3	phenylalanine	H_2O_2 - 5%	UV scan	yes***
g-1	serum	none	Tiselius	several peaks
g-2	serum	spark - 24 hr.	Tiselius	one peak

* The solution was a mixture of a number of selected amino acids including glycine, alanine, histidine, cysteine, phenylalanine, tyrosine, lysine, and arginine each in 1% (w/v) concentration.

** The solvent was a 1:1 solution of glycerol and 95% ethanol instead of water.

***This differed from f-2 as well as from the control.

SUGARS AND AMINO SUGARS

Experiment 2

This experiment was undertaken to test for the production of reducing groups from various monhydroxy alcohols as well as glycerol by sparking as well as changes in solubility as a result of this process. Aqueous solutions of each selected alcohol were arced for 30 minutes and then spectrophotometrically analyzed for reducing groups by the ferricyanide test (T-1). The results to this test are found in table 2.

TABLE 2

THE APPEARANCE OF REDUCING GROUPS AS OBSERVED BY THE FERRICYANIDE TEST AND CHANGES IN MISCIBILITY CAUSED BY SPARKING VARIOUS ALCOHOLS

Sample	Concentration (v/v)	Ferricyanide Absorbancy	Miscibility before	Miscibility after
blank	-	0.03		
methanol	10%	0.17	total	total
ethanol	5%	0.24	total	total
n-propanol	10%	0.32	total	total
n-butanol	10%	0.20	partial	total
n-amyl alcohol	10%	0.30	partial	total
glycerol*	10%	inf.	total	total

* Glycerol was sparked for 60 minutes.

Experiment 3

The purpose of this experiment was to observe the rate of production of reducing groups from glycerol by sparking and to note the effects of ammonia on this synthesis.

A 10% (v/v) aqueous solution of glycerol was sparked for 2 hours. Also, an aqueous solution of glycerol plus 2.5% ammonia was sparked for 2 hours. Samples were removed from both solutions after 0, 1, 5, 15, 30, 60, 90, and 120 minutes of sparking. All samples were analyzed by paper chromatography using ethyl acetate:pyridine:water (60:25:20) as solvent and benzidine to develop the reducing sugars (T-10). The chromatogram from this experiment where only the 10% aqueous glycerol was sparked is seen in Fig. **12**. A sample of the product from the sparking of glycerol with ammonia gave the same kind of chromatogram as with glycerol alone.

The samples from the sparking of 10% aqueous glycerol were further checked with Fehling's solution (T-3). These were made

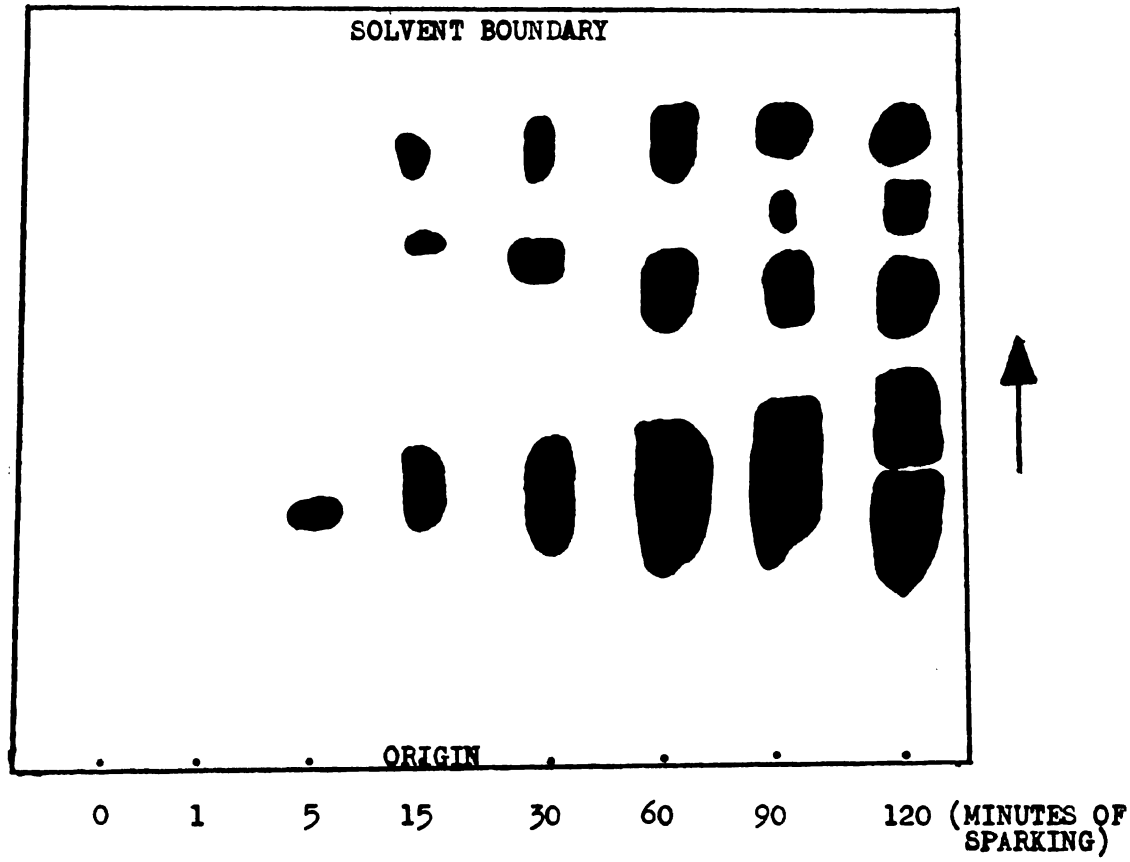


Fig.12 - Sketch of a chromatogram on Whatman #1 paper using ethyl acetate:pyridine:water (60:25:20) solvent showing the increase of benzidine-positive material produced from glycerol with time of sparking.

semi-quantitative by reading their absorbancy at 570 mu in the spectrophotometer before the iron oxide precipitated completely out of the solution. The results are summarized in table 3 .

TABLE 3

PRODUCTION OF REDUCING SUBSTANCES BY THE SPARKING OF GLYCEROL AS NOTED BY SPECTROPHOTOMETRIC READINGS OF THE FEHLINGS TEST PRODUCT

Sample	Sparking time (min.)	Absorbancy	Approximate percent*
1	water blank	0.000	0.000
2	0	0.036	0.054
3	1	0.063	0.095
4	5	0.129	0.194
5	15	0.208	0.312
6	30	0.347	0.522
7	60	0.553	0.833
8	90	0.600	0.902
9	120	0.985	1.480
10	0.2% glucose	0.133	0.200

* The percentages are approximate since the readings are only semi-quantitative. The percentages are calculated in comparison to 0.2% (w/v) glucose. Sample calculation:

$$0.200/x = 0.133/0.600 \qquad x = 0.902$$

Experiment 4

The purpose of this experiment was to indicate the production of carbohydrate-like compounds from the sparking of glycerol. A 10% (v/v) aqueous solution of glycerol was arced for 2 hours and checked for sugars by the o-aminodiphenyl test (T-4), change in optical rotation from the initial solution (T-6), and solubility of the product in ether (T-12). Also, solutions of known glucose concentration were included for comparison with the products from sparked glycerol.

The possibility that the product might be ascorbic acid was

ruled out by a negative response to the dichlorophenol-indophenol test (T-8).

TABLE 4

TESTS FOR THE PRODUCTION OF SUGARS FROM THE SPARKING OF GLYCEROL AS COMPARED WITH GLUCOSE

Sample	Concentration	Sparking time	Dilution (after)	Absorbancy (o-Aminodiphenyl)	Ether sol.	Opt. rot.
glucose	25 mg%	0	0	0.655*	-	+
glucose	50 mg%	0	0	1.360	-	+
glucose	100 mg%	0	0	inf.	-	+
glycerol	10% (v/v)	0	1:100	0.000	-	none
glycerol	10% (v/v)	120 min.	1:100	0.623*	-	none

* Calculation (dilution factor = 100): 655/623 = 25/x
x = 23.78 mg%
23.78 X 100 = 2.38 gm%

Experiment 5

From the preliminary results on the alcohols in experiment 2, it became desirable to carry out a more systematic study of the effect of the spark on various mono-, di-, and tri-hydroxy alcohols. Selected 10% (v/v) aqueous alcohol solutions were each sparked for 2 hours and checked by various tests for organic functional groups. Also, the purity of the reagents before use was ascertained.

The results are found summarized from these tests in table 5.

Experiment 6

To test for the production of amino sugars from glycerol by sparking with selected co-reagents and the effects of the arcing process on hydrogen ion concentration (pH), the following aqueous solutions in 10 ml. total volume were each sparked for 2 hours:

a) glycerol (1 ml.) plus ammonium sulfamate (500 mgm.)
b) glycerol (1 ml.) plus 95% ethanol (1 ml.)

TABLE 5

Tests for the Appearance of Functional Groups in Sparked Alcohol Solutions from Experiment 5

Before Spark

Tests	Alcohols								
	1-Methanol	1-Ethanol	1,2-Ethanediol	1-Propanol	2-Propanol	1,2-Propanediol	1,3-Propanediol	1,2,3-Propanetriol	water blank
Molisch (T-5a)	-	-	-	+	-	+	-	-GR	-
Fehling (T-3)	-	-	-	-	-	-	-	-	-
Elson-Morgan (T-15)	-	-	-	-	+	-	-	-	-
2,4-DNPH*(T-9b)	-	-	-	-	-	-	-	-	-
Ninhydrin (T-13a)	-	-	-	-	-	-	-	-	-

After Spark

Molisch (T-5a)	+	+	+	+	+	+	+	+
Ninhydrin (T-13a)	+	+	+	+	+	+	+	+
Benzidine (T-10a)	+	+	+	+	+	+	+	+
Fehling (T-3)	+	+	+++.	+f	+f	+++.	+++.	+++.
Osazone (T-9a)	+G	+G	+++O.	+G	+G	+++Y.	+++O.	+++D.
Elson-Morgan (T-15)	-	-	+++I	-	-	+++I	+	+++I
2,4-DNPH*(T-9b)								
reaction	+.Y	+.Y	+.R	+.R	+.O	+.R	+.Y	+.D
melting point	160	151	T	141	110	>245	>245	>245
Benzidine-IO_4(T-10d)	-	-	+d	-	-	+d	+d	+d

*KEY

f = faint reaction
. = precipitation without heating
O = yellow-orange precipitate
R = orange precipitate
D = deep orange precipitate
G = greenish yellow precipitate
2,4-DNPH = 2,4-dinitrophenylhydrazone
T = 155->245
Y = yellow precipitate
d = double spot
I = immediate reaction
+ = positive reaction
- = negative reaction
> = greater than
GR = green

Before and after sparking, pH readings were taken and the solutions were analyzed by the Elson-Morgan test for amino sugars (T-15). The results were compared with data obtained from known samples, as noted in table 8.

Experiment 7

To test for the production of primary amines, a 10% (v/v) aqueous solution of glycerol was sparked for 2 hours and tested by the nitrous acid procedure (T-14).

TABLE 6

RESULTS OF THE NITROUS ACID TEST FOR PRIMARY AMINES

Sample	Observation
glycine	+
sparked glycerol	+
unsparked glycerol	-
water	-

Experiment 8

In order to support the assumption that a portion of the sparked glycerol products was a pentose, a 10% (v/v) aqueous solution arced for 2 hours was analyzed by the Molisch (T-5a), Bial (T-5b), and Tauber (T-5c) methods. The findings are reported in table 7.

TABLE 7

QUALITATIVE INDICATIONS FOR THE PRESENCE OF A PENTOSE IN SPARKED GLYCEROL

Test	Sample	Result
1) Molisch	sparked glycerol	+
	ribose (1% solution)	+
2) Bial	sparked glycerol	+ (for pentose)
	ribose (1% solution)	+ (for pentose)
3) Tauber	sparked glycerol	+ (for pentose)
	ribose (1% solution)	+ (for pentose)

TABLE 8

Results of pH and Elson-Morgan Determinations on Sparked Samples of Glycerol and Co-reagents in Experiment 6

Sample Name	Sample Conc.	Co-reagent Name	Co-reagent Conc.	Conditions	pH	Dilution (after)	Elson-Morgan Test Color	Elson-Morgan Test Absorbancy	Elson-Morgan Test Conclusion
water blank				no spark			clear	0.023	-
Glucosamine	25 ug/ml			no spark			red	0.158	+
	50 ug/ml			no spark			red	0.267	+
	100 ug/ml			no spark			red	0.475	+
	150 ug/ml			no spark			red	0.640	+
Glycerol	10% (v/v)	$NH_4SO_2NH_2$	5%(w/v)	no spark	5.2		yellow		-
	10% (v/v)		5%(w/v)	2 hr.spark	2.5		deep red	inf.	++
	10% (v/v)		5%(w/v)	2 hr.spark		1:10	red	0.418	+
	10% (v/v)		5%(w/v)	2 hr.spark*		1:10	red	0.395**	+
Glycerol	10% (v/v)	Ethanol	9.5%(v/v)	no spark	5.2		yellow		-
	10% (v/v)		9/5%(v/v)	2 hr.spark	2.5		deep red	inf.	++
	10% (v/v)		9.5%(v/v)	2 hr.spark		1:10	red	0.313	+
	10% (v/v)		9/5%(v/v)	2 hr. spark*		1:10	red	0.290	+

* Correction = 1:10 dilution reading - reading for water blank

** The following is the calculation for sparked glycerol with ammonium sulfamate based on 50 ug/ml and 100 ug/ml glucosamine readings:

$0.395/0.267 = x/50$; $x = 74$ ug/ml

$0.395/0.475 = x/100$; $x = 83$ ug/ml

with dilution factors: conc. = 74 mg% cpmc. = 83 mg%

Experiment 9

This experiment was performed for the purpose of testing the effect of sparking on a known pentose sugar solution without glycerol in relation to the sugars synthesized from glycerol. A 10% (w/v) aqueous solution of ribose was sparked for 2 hours after which paper chromatography of the ribose product and that also synthesized from glycerol by sparking was carried out using ethyl acetate:pyridine:water (60:25:20) as solvent and ninhydrin as the spot(s) indicator (T-10c). Several ninhydrin-positive spots appeared from the sparked ribose, two of which matched the sugars synthesized from sparked glycerol. The results are depicted in Fig. **13**.

Experiment 10

The purpose of this experiment was to effect separation of the sugar products of sparked alcohols by means of column chromatography. Separate 10 ml. 20% (v/v) aqueous solutions of glycerol and ethylene glycol were each sparked for 4 hours and then applied to columns containing Dowex 50-X4 resin (T-17). The acid displacement eluates were tested by the ninhydrin (T-13a), ferricyanide (T-1), and Elson-Morgan (T-15) procedures. Also, a sample of sparked glycerol was adsorbed on Amberlite IRA-400 and separated by gradient elution of the column. The results of the various tests on the eluates are presented in Figs. **14, 15, 16.**

A more specific identification of the main sugar fraction eluted from the Dowex columns was carried out by paper chromatography using ethyl acetate:pyridine:water (60:25:20) as solvent and ninhydrin indicator (T-10**e**). The fraction from sparked glycerol showed an Rf value of 0.500 and that from ethylene glycol exhibited an Rf of 0.529.

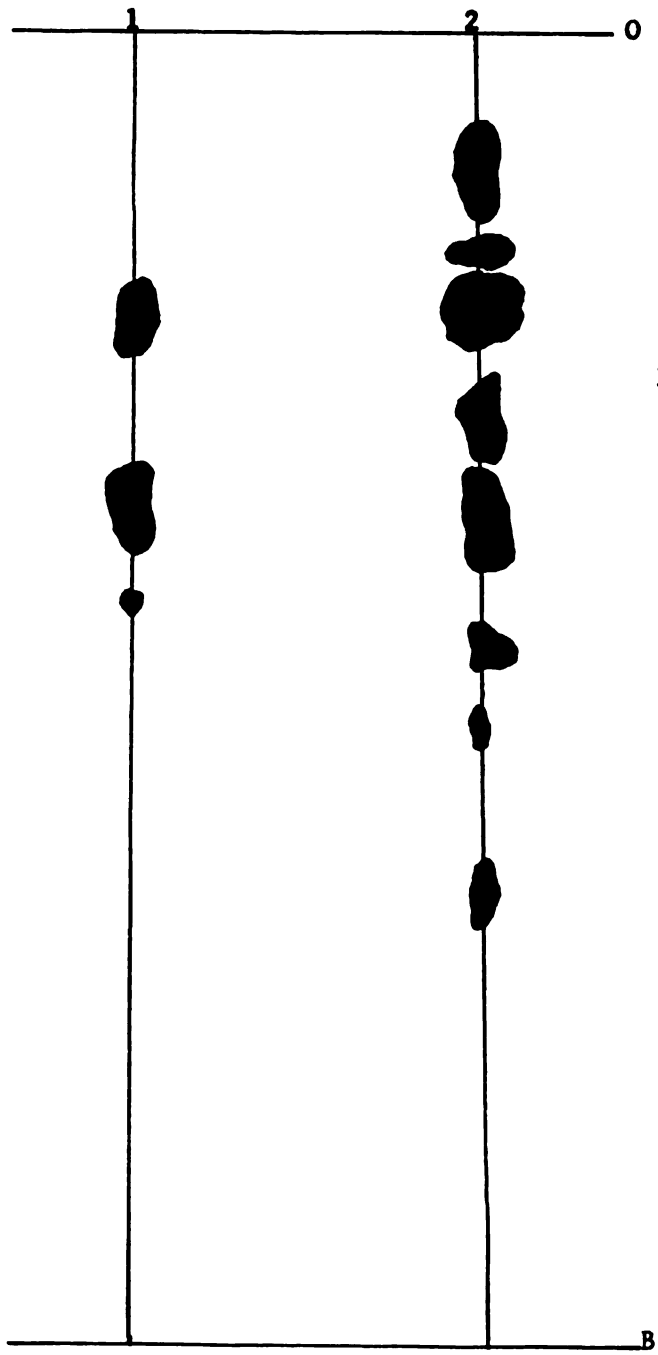


Fig.13 - Approximate sketch of a paper chromatogram on which sparked ribose and sparked glycerol have been resolved

KEY

O = origin
B = solvent boundary
1 = sparked glycerol
2 = sparked ribose

Solvent:
ethyl acetate:pyridine:water (60:25:20)

Developer: Ninhydrin

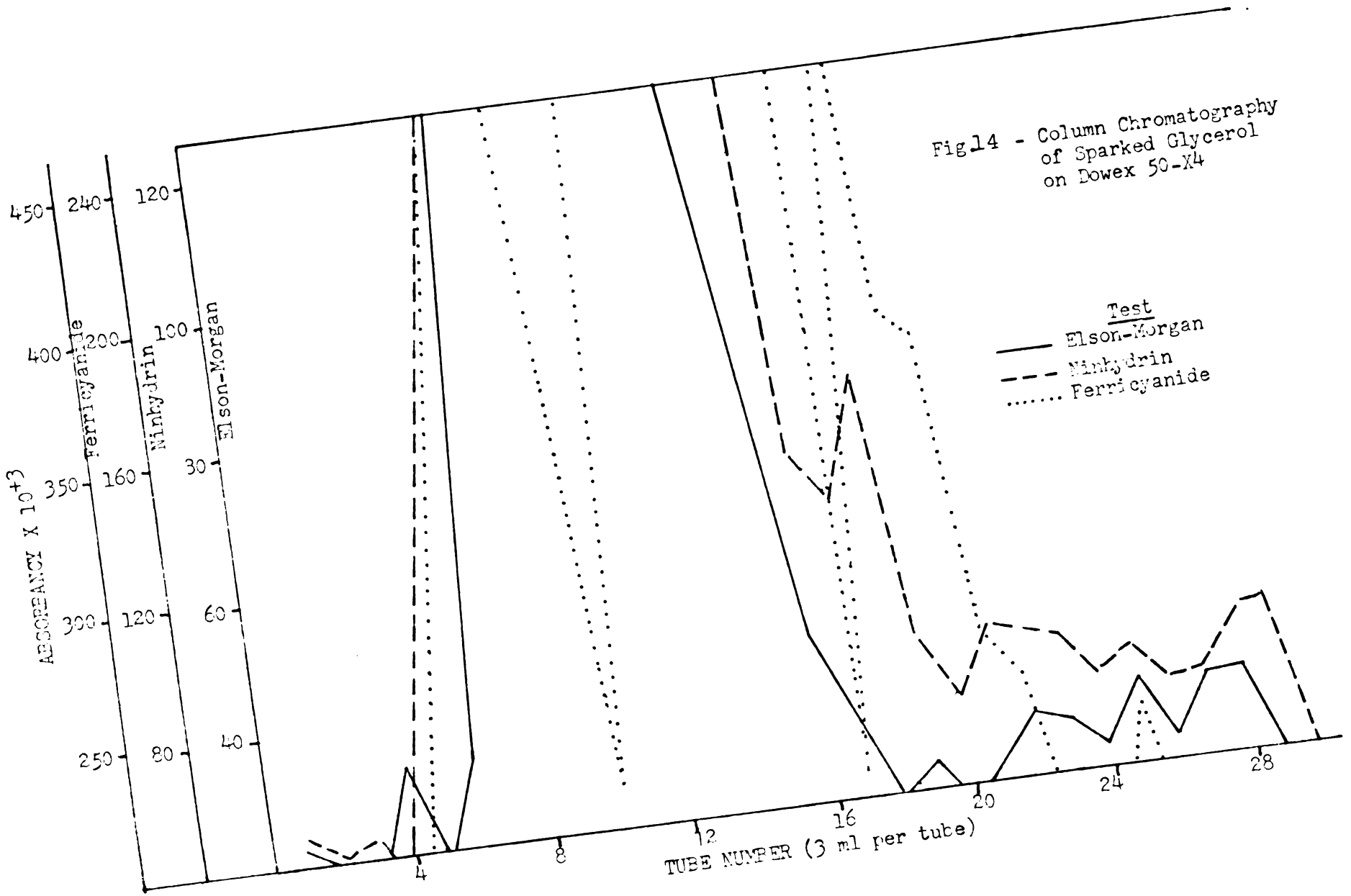


Fig 14 - Column Chromatography of Sparked Glycerol on Dowex 50-X4
Test
Elson-Morgan
Ninhydrin
Ferricyanide
ABSORBANCY X 10+3
450
400
350
300
250
240
200
160
120
80
120
100
60
40
Ferricyanide
Ninhydrin
Elson-Morgan
4
8
12
16
20
24
28
TUBE NUMBER (3 ml per tube)

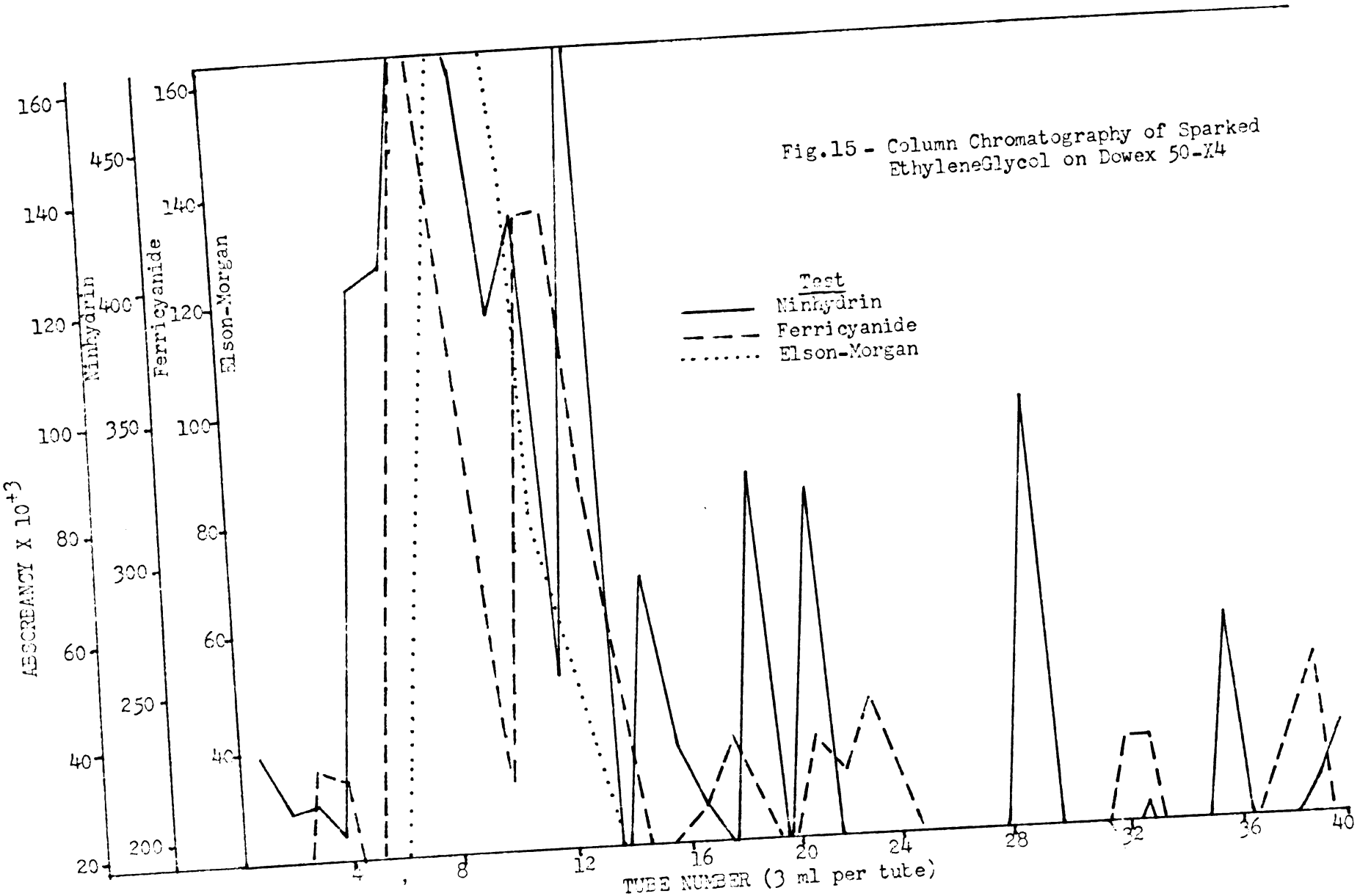


Fig.15 - Column Chromatography of Sparked EthyleneGlycol on Dowex 50-X4

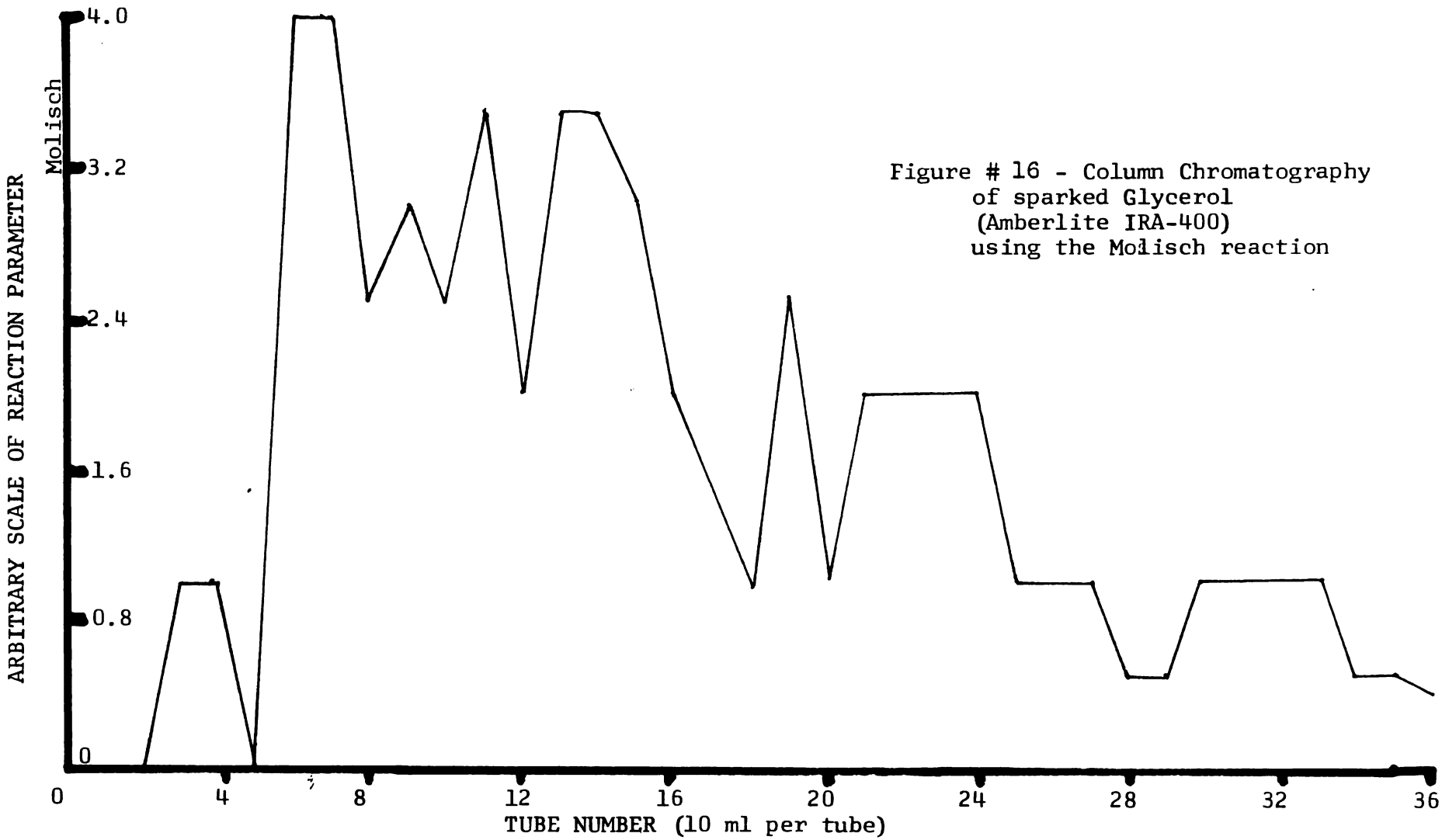


Figure # 16 - Column Chromatography of sparked Glycerol (Amberlite IRA-400) using the Molisch reaction

AMINO ACIDS

Experiment 11

This experiment was carried out to test the possibility of producing amino acids by sparking organic acids. Separate 10 ml. aqueous solutions of 10% (v/v) acetic acid and 10%(v/v) isovaleric acid were each sparked for 2 hours and then checked by unidimensional descending paper chromatography (T-10) using n-butanol:acetic acid:water (4:1:5) as solvent and ninhydrin as the spot indicator. The acetic acid product had a spot which matched the one for glycine. The isovaleric acid product yielded a spot which matched the one for glycine and another weaker spot suggesting valine. Also, when using phenol:water (88:12) or n-butanol: pyridine:water (6:6:6) solvents, the acetic acid product gave a spot which coincided with the one for glycine (see Figs.**17, 18, 19).**

When phenylacetic acid was used as a reactant, a histidine-like product was observed with paper chromatography together with two other unidentified ninhydrin-positive substances.

Experiment 12

The amino acids produced from arcing the acetic acid solution were separated by column chromatography and and characterized with ninhydrin (T-13a) and Tosyl chloride (paratoluenesulfonyl chloride; T-22). A 10 ml. portion of a 20% (v/v) aqueous acetic acid solution sparked for 4 hours was applied to a Dowex 50-X4 column (T-17). They were eluted with citric acid buffer and followed with ninhydrin reagent. The results were plotted as in Fig. **20.**

Tosyl derivatives were made with the amino acid products from the combined column elution fractions of tubes 5-9, 10-14, and 15-20 of sparked acetic acid.

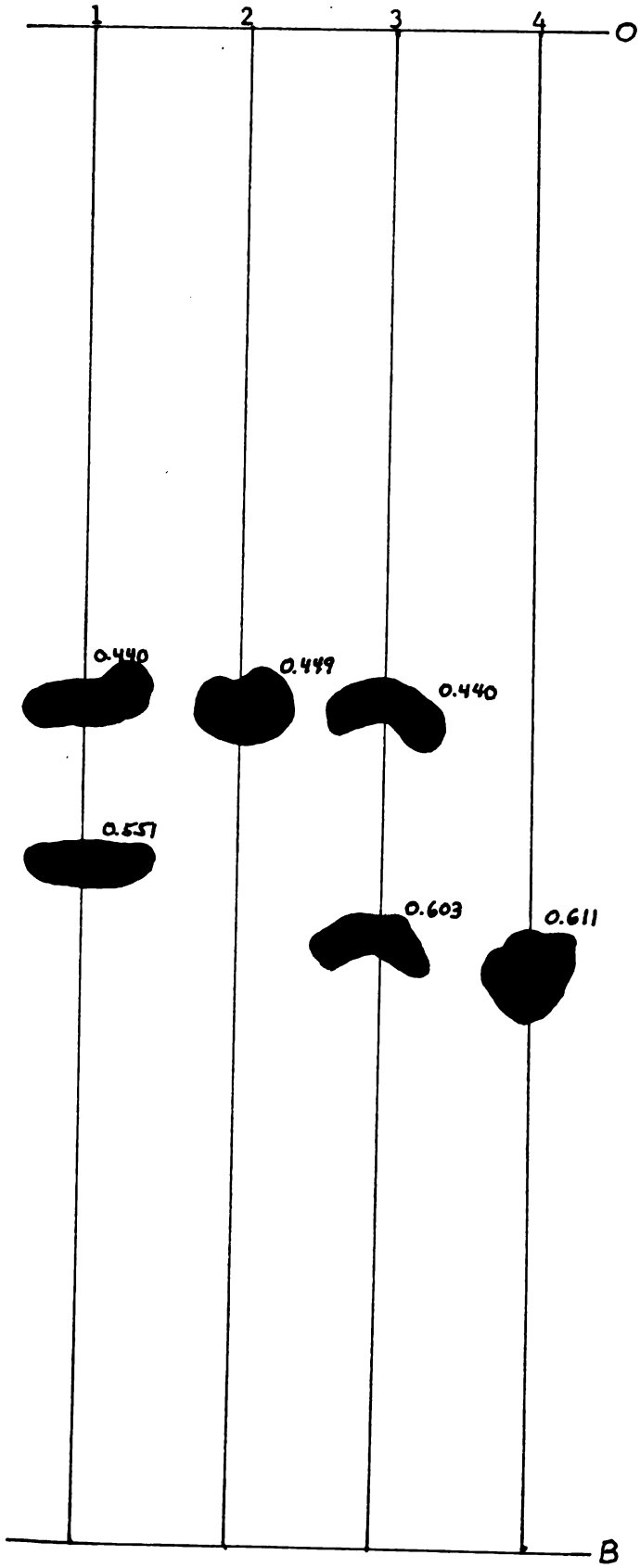


Figure #17- Chromatogram Facsimile

Code: O = Origin
B = Solvent boundary
1 = Sparked acetic acid
2 = Glycine
3 = Sparked isovaleric acid
4 = Valine

Solvent system:
n-butanol:acetic acid:water
4 : 1 : 5

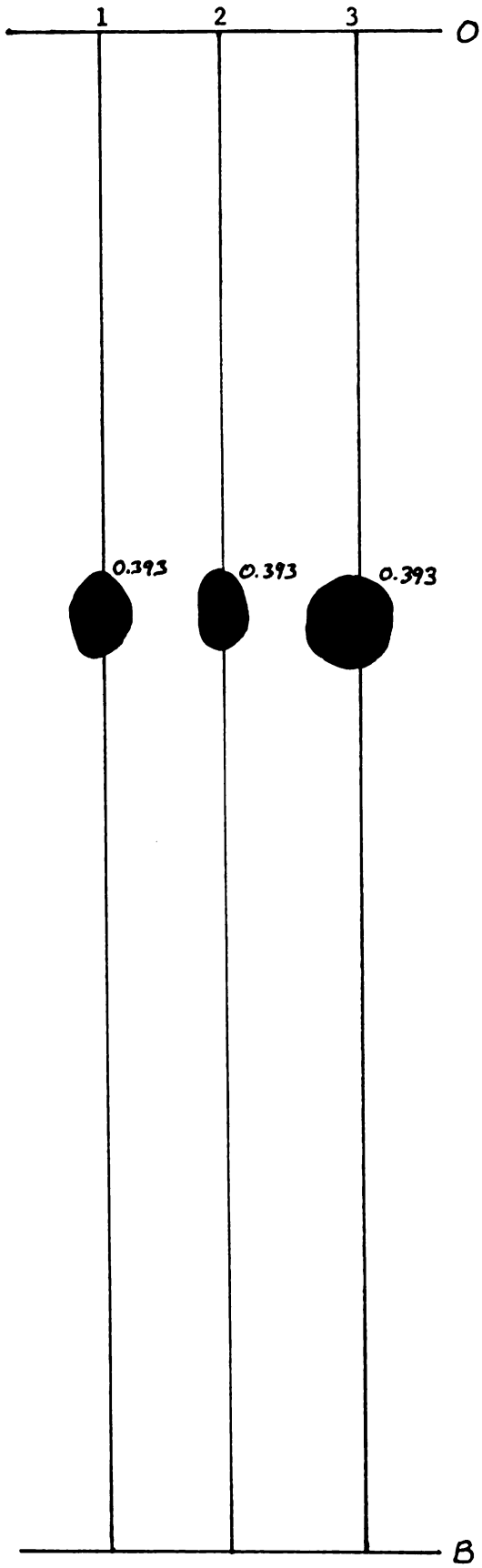


Figure #18 - Chromatogram Facsimile

Code: O = Origin
B = Solvent Boundary
1 = glycine
2 = Sparked acetic acid
3 = Mixture of 1 and 2
Numbers are Rf values.

Solvent system:
phenol:water
88 : 20

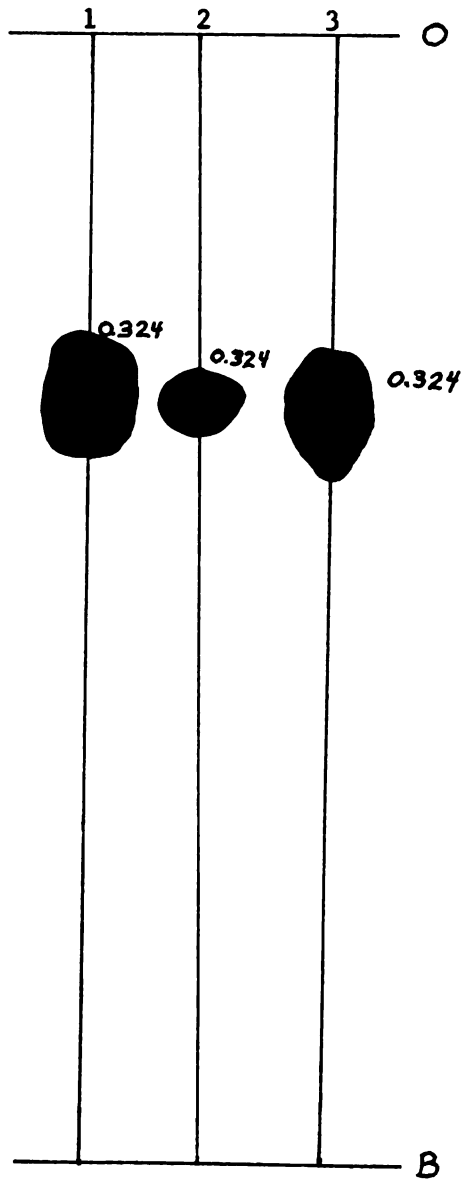


Figure #19- Chromatogram Facsimile

Code: O = origin
B = Solvent boundary
1 = Glycine
2 = Sparked acetic acid
3 = Mixture of 1 and 2
Numbers are Rf values.

Solvent system;
n-butanol:pyridine:water
6 : 6 : 6

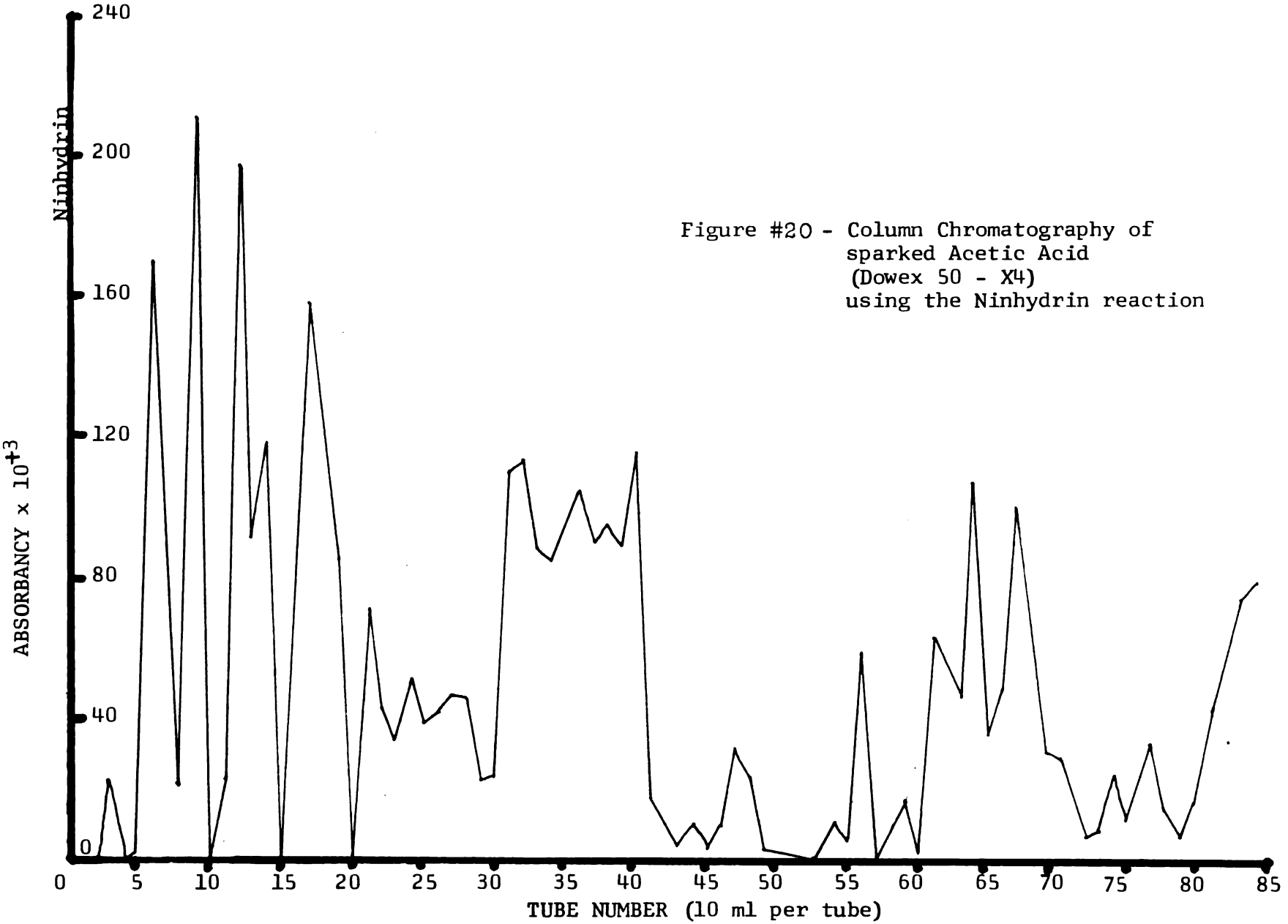


Figure #20 - Column Chromatography of sparked Acetic Acid (Dowex 50 - X4) using the Ninhydrin reaction

TABLE 9

PRODUCTION OF PARATOLUENESULFONYL CHLORIDE DERIVATIVES FROM THE ELUTION FRACTIONS OF SPARKED ACETIC ACID

Sample	State of derivative	Melting Point °C
glycine (control)	needles	145
tubes 5-9	oil	(not determined)
tubes 10-14	needles	(not determined)
tubes 15-20	needles	148

POLYMERS

Experiment 13

The purpose of this experiment was to search for a biopolymer being formed by sparking a solution of 5% (w/v) ammonium sulfamate, 5% (v/v) acetic acid, 2.5% (v/v) glycerol, and 5% (v/v) ethanol for 250 hours using inert platinum electrodes. With dialysis followed by lyophilization, a large amount of white material assumed to be polymeric was isolated and analyzed by several tests to be described.

When the same experiment was repeated but with copper electrodes, the white material was no longer found in the dried isolated product. Instead, a small quantity of a reddish-brown substance was observed and no further tests were performed on this product.

a) General tests and characterizations

A soluble concentrate of the pervaporated product solution gave a positive biuret reaction (T-28). When the white polymer was checked by the tryptophan-anthrone test (T-34), an absorbancy of 0.330 for the test product was observed at 520 mu against a water blank.

A 20 ml. portion of the isolated polymer dissolved in a 0.67% (w/v) aqueous solution was titrated with 0.1 N HCl. The titrant was added in 0.01 ml. aliquots with a microsyringe and the pH change was followed with a Beckman Meter equipped with a calomel electrode. Another 20 ml. portion of the polymer solution was titrated with 0.1 N KOH. To a third 20 ml. portion was added 2 ml. of 30% formaldehyde and then titrated with 0.1 N KOH. The data were plotted as in Fig. **21**. The results indicated the typical graph for a dipolar ion as with amino acids or proteins.

A standard Kjeldahl (T-31) determination indicated the polymer to contain 0.5% nitrogen. This value was confirmed by a Conway micro-diffusion adaptation of the same method.

b) Polysaccharide and Aminopolysaccharide Characteristics

A sample of the white polymer was partially hydrolyzed by refluxing in 6 N HCl for 30 minutes. Two aliquots of neutral hydrolysate were paper chromatographed side by side using n-butanol:acetone:water (4:1:5) as solvent after which one side was developed with silver nitrate and the other with ninhydrin (T-10). Each showed a common spot at an Rf value of 0.10. Using n-butanol:acetic acid:water (4:1:5) as solvent for two similar parallel chromatograms of the same neutralized polymer hydrolysate, three spots were observed on one chromatogram after development with the Elson-Morgan spray with the strongest spot at Rf 0.64. When the other chromatogram was stained for acidic groups with toluidine blue, a single spot was noted at Rf 0.62.

In order to further test for possible carbohydrate-like properties of the white polymer produced by sparking, a 0.1% (w/v) aqueous solution was divided into three equal portions. One portion was oxidized by sodium periodate solution (T-30). The second was hydrolyzed in 6 N HCl for

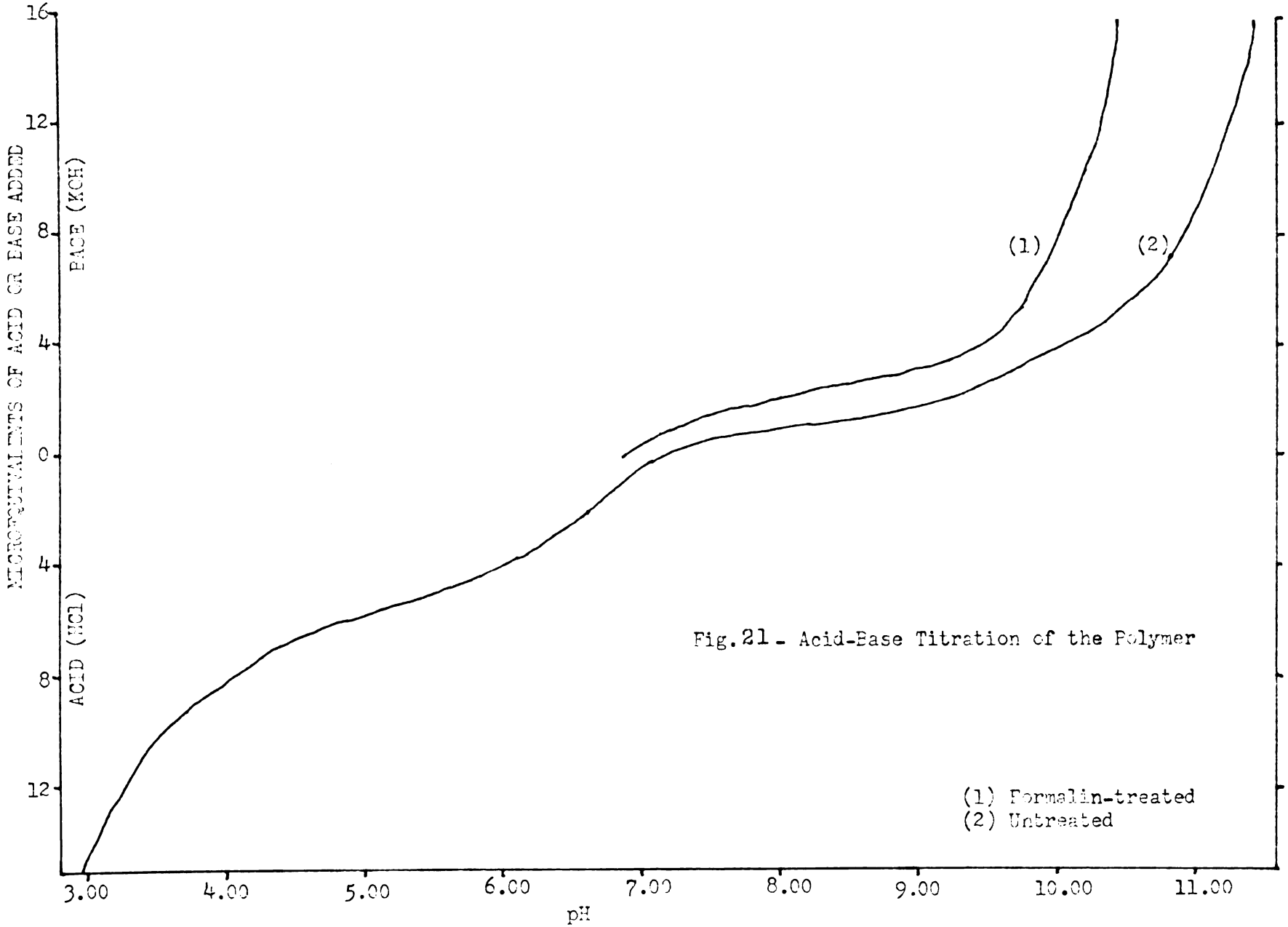


Fig. 21 - Acid-Base Titration of the Polymer

5.5 hours at 100°C. The third portion remained untreated and was used as a control. Both the periodate-treated and control samples gave a positive Molisch reaction (T-5a) whereas the hydrolyzed sample did not. Also, the hydrolysate did not produce a positive benzidine reaction (T-10a).

To test for the possibility of linear polysaccharide-like structure within the polymer, a 50 mgm. portion of the product was submitted to periodate oxidation (T-30). The oxidized product became neutralized with 0.34 ml. of 0.01 N sodium hydroxide solution. This result would suggest a molecular weight of 50/0.0034 = 14,706 if only one formic acid molecule were released per polymer chain.

It was thought desirable to compare the hydrolysate of the white polymer to the amino sugar-like products synthesized earlier as in experiment 3. A 10 ml. aqueous solution of 10% (v/v) glycerol was sparked for 60 minutes and analyzed for amino sugars by paper chromatography using ethyl acetate:pyridine:water (60:25:20) as solvent (T-10). After development with ninhydrin on one strip and periodate benzidine on another strip, spots with identical Rf values were obtained. A 1% (w/v) aqueous solution of the white polymer was partially hydrolyzed by refluxing for 30 minutes in 1 N hydrochloric acid and then submitted to paper chromatography identical with that of the sparked glycerol sample. The outcome was the formation of a spot with the same Rf as from sparked glycerol.

c) Physico-Chemical Analysis

Tiselius electrophoresis (T-26) of the polymer product in 1% (w/v) aqueous solution after dialysis against phosphate buffer (pH = 6.75; $\Gamma/2 = 0.01$) gave a single peak with a mobility equal to 1.48×10^{-5} $cm^2 volt^{-1} sec^{-1}$.

The original product solution was made 6 molar with respect to urea for 72 hours and then dialyzed against water. Finally, in the same phosphate

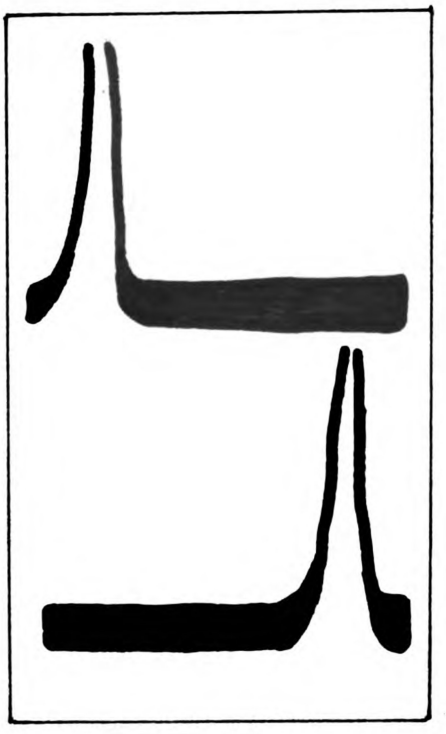


Figure #22 - Tiselius electrophoresis pattern of the polymer in phosphate buffer at pH 6.75.

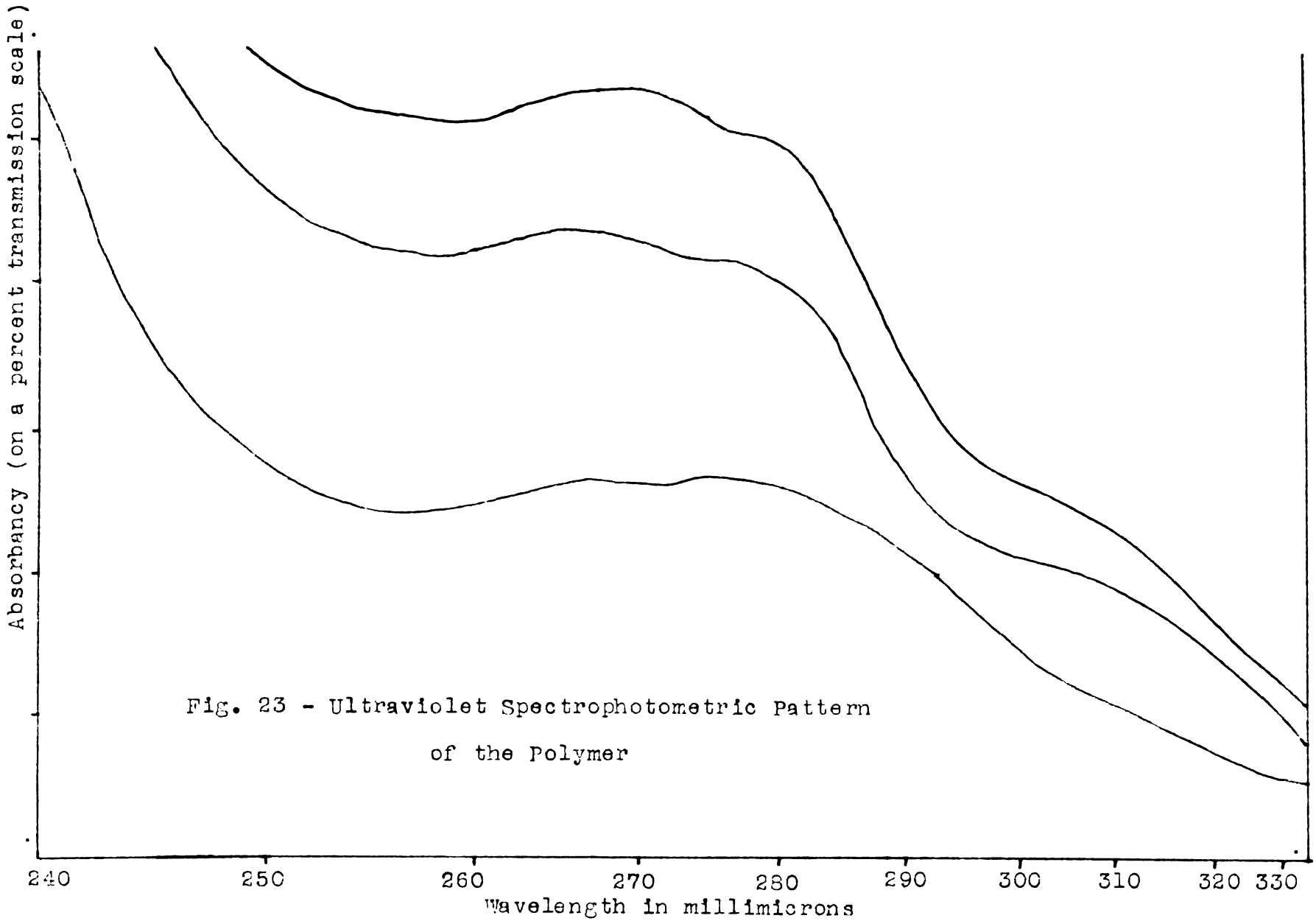


Fig. 23 - Ultraviolet Spectrophotometric Pattern of the Polymer

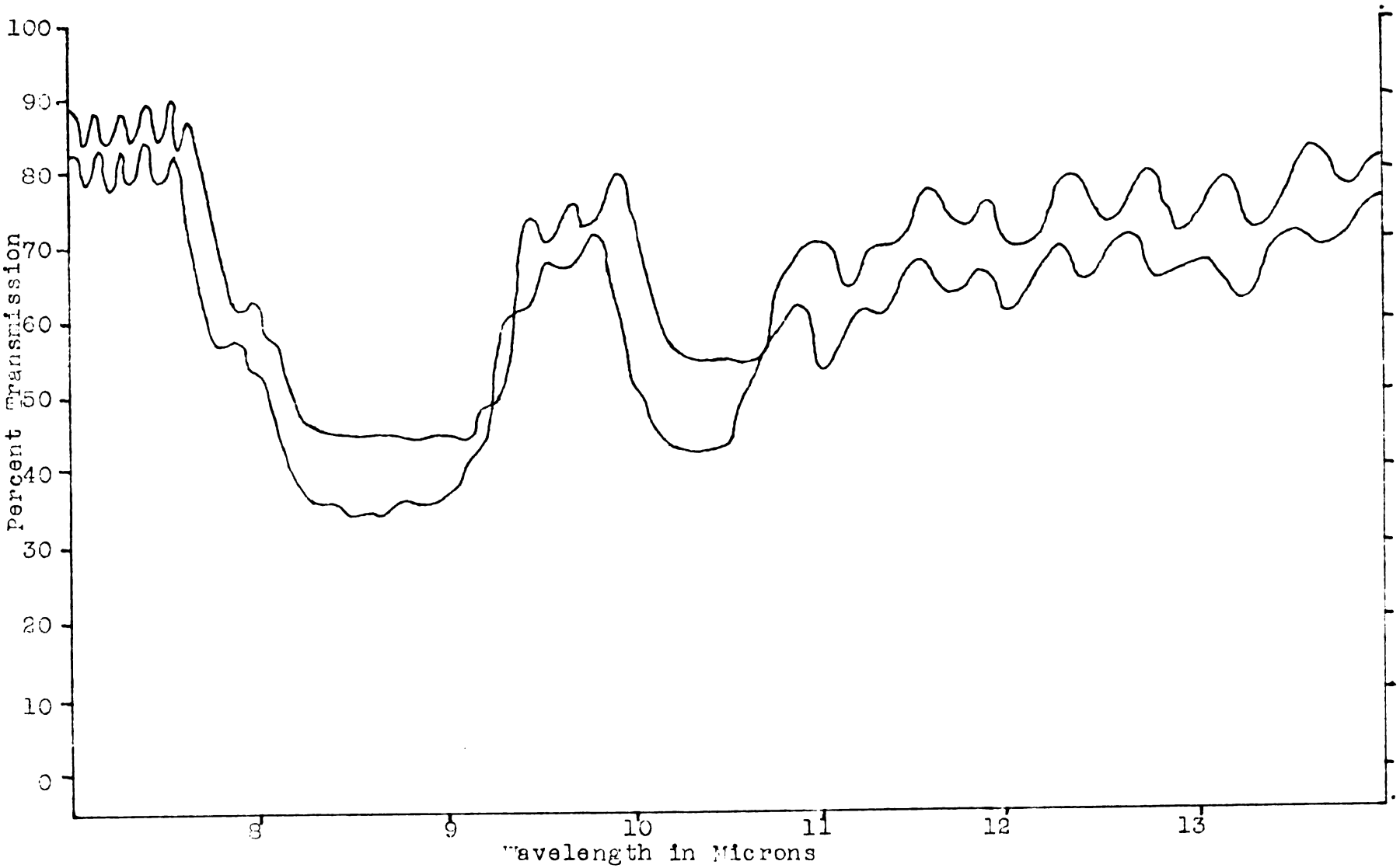


Fig.24 - Infrared Spectrophotometric Pattern of the Polymer

buffer, the same unaltered Tiselius peak was observed as before urea treatment. Age stability was tested after storing the polymer solution at 4°C for one full year and then subjecting it to Tiselius electrophoresis again. Once more the same unaltered single peak was observed as with the fresh sample.

In an effort to determine the essential reactants for the production of the polymer, a 1 liter aqueous solution of 150 ml. of glacial acetic acid and 150 ml. of glycerol was sparked for 300 hours, dialyzed against water, concentrated by pervaporation, and tested by Tiselius electrophoresis in phosphate buffer buffer (pH = 6.0; $\Gamma/2 = 0.01$). No peak was observed as it had been in earlier syntheses.

Ultraviolet and infrared double beam spectrophotometric scan patterns were determined for the dried polymer (T-32; see Figs.

To further characterize the mass properties of the polymer, ultracentrifugation (T-24) was carried out on a 1% (w/v) solution of the isolated product in 0.1 M sodium chloride. Partial specific volume was measured with a pycnometer and classical calculations were then made for sedimentation coefficient with data collected by sedimentation centrifugation. Molecular weight was determined by Archibald equilibrium centrifugation. The results obtained were as follows:

1) Sedimentation Coefficient (59,780 RPM; see Fig. **25**)

$$d \log x/dt = 1.319 \times 10^{-4} \text{ at } 25^{\circ}C$$

$$s_{25} = d \ln x/dt\, w^2 = (1.319 \times 10^{-4})(9.794 \times 10^{10})$$

$$s_{25} = 1.29 \times 10^{-13} \text{sec} = 1.29$$

$$s_{20} = s_{25}\eta_{25}/\eta_{20} = (1.29)(0.8937)/1.005 = 1.15$$

2) Initial concentration (low speed; see Fig. **26**)

$$x_{inner} = 119.87 \text{ mm} \qquad x_m = 131.24 \text{ mm}$$

$$c_o = \Delta x/\lambda_o \varepsilon \Delta Y = (0.1)(73.105)/2.103 = 3.476$$

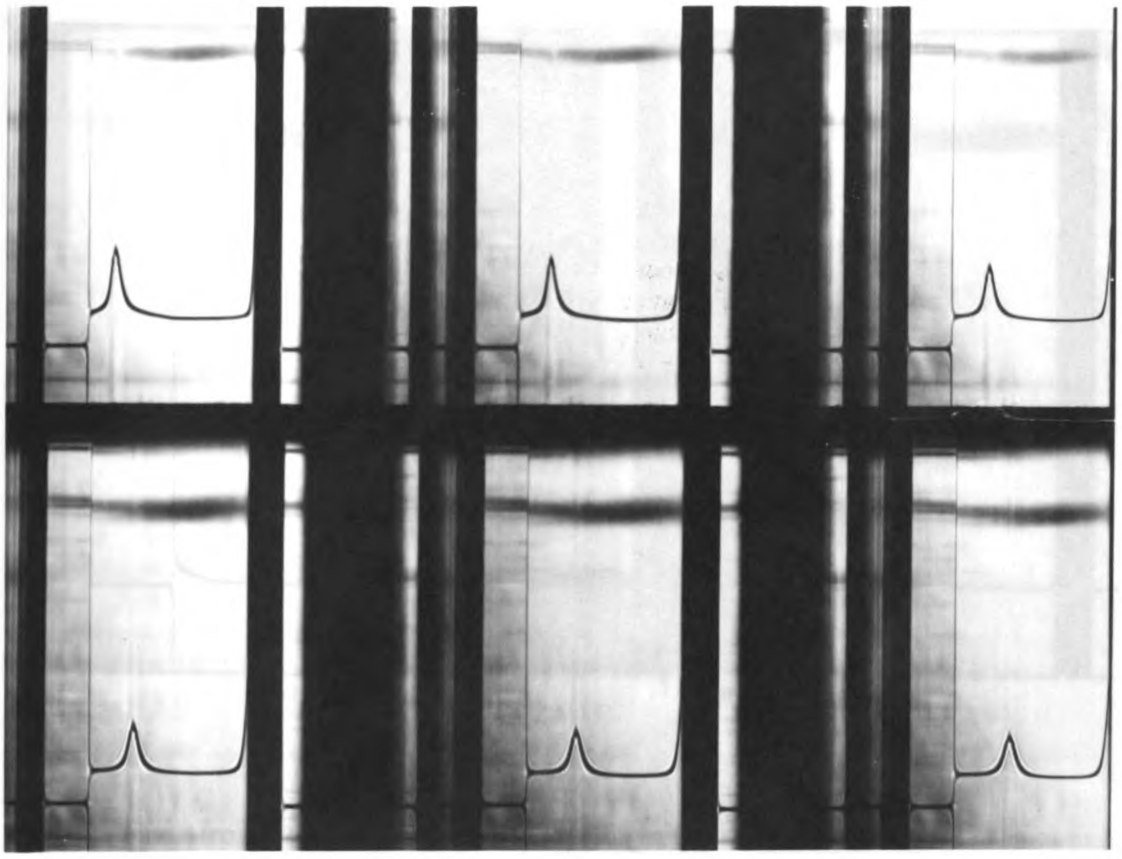


Fig. 25 - Ultracentrifugal patterns used in determining the sedimentation coefficient of the polymer.

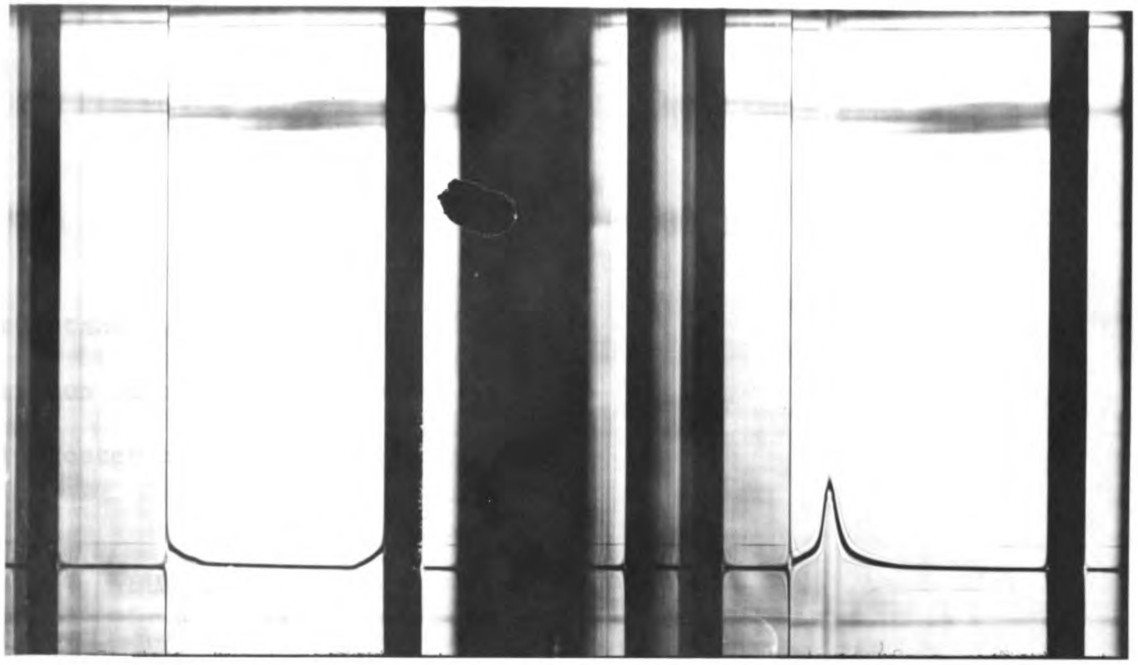


Fig. 26 - Ultracentrifugal patterns used in determining the molecular weight of the polymer.

3) Concentration at the Meniscus

$$c_m = c_o - (1/x_m^2)(dx/H_o)\Sigma x^2\Delta Y$$

$$c_m = 0.03476 - 0.01(55.3295)/41.0506(2.103) = 0.02835$$

4) Molecular Weight

$$M = RT(dc/dx)_m/(1-v_{sp}\rho)\omega^2 x_m c_m)$$

$$M = 8.314 \times 10^7(298)(100)(1.200)/(1 - (0.346)(2.014))0.03476(131.24)$$

$$M = 31,027$$

To test the polymer for immunogenicity, antibodies for the substance were prepared by the standard procedure (T-27) with rabbits and run on Ouchterlony plates against the antigen. Initial experiments indicated no immunological specificity.

TABLE 10

SUMMARY OF PHYSICO-CHEMICAL ANALYSES OF THE SYNTHESIZED POLYMER

Analysis	Concentration	Solvent & Treatment	Peaks	Derived Values
Electrophoresis	1%(w/v)	Phosphate buffer (pH 6.75; u = 0.01)	1	u = 1.48
	1%(w/v)	Phosphate buffer; 6 M urea-treated	1	u = 1.48
	1%(w/v)	Phosphate buffer; 1 year storage	1	u = 1.48
Ultracentrifugation	1% (w/v)	0.1 M sodium chloride	1	S_{20} = 1.14
	1% (w/v)	0.1 M sodium chloride	1	M_{20} = 31,027

Experiment 14

The purpose of this experiment was to test for microbial contamination in the reactants and the biological significance of the synthesized products in supportin life. Separate 10% (v/v) aqueous solutions of glycerol and acetic acid were each sparked. During the sparking period, samples were taken from the glycerol to check for the presence of yeasts, molds, and bacteria (T-35). Following the sparking period, both test solutions were exposed to the open air for 48 hours and

then observed for colony growth. No contamination was found in the glycerol sample by standard bacteriological techniques during the sparking period. However, the exposed solutions were both observed to support microbial growth. In the case of the glycerol, the colonies were transferred aseptically to a fresh solution of 60% (v/v) unsparked glycerol and 1% (v/v) nitric acid but growth was observed to cease entirely.

Experiment 15

This experiment was carried out to compare the effects of electrons and photons on the specific reagents used in this study. Samples of an aqueous 20% (v/v) solution of glycerol were each submitted to a separate type of treatment as described below. Also, portions of a 20% (v/v) aqueous solution of acetic acid were treated in a similar fashion.

Sample	Treatment
1) glycerol	3.72×10^6 Reps. (General Electric Linear Beta Accelerator)
2) glycerol	4 hours of spark (electrical sparking apparatus)
3) glycerol	21,000 roentgens (General Electric X-ray Treatment Apparatus)
4) glycerol	44 hours of UV light (Ultra-Violet Products Inc. Mineralight UV lamp - model R5 - 0.6 amps)
5) acetic acid	3.72×10^6 Reps. (Linear Beta Accelerator)
6) acetic acid	2 hours of spark (electrical sparking apparatus)
7) acetic acid	45 hours of UV light (UV lamp)

The products were tested by the Molisch (T-5a), Tauber (T-5c), Fehling (T-5), Elson-Morgan (T-15), and ninhydrin (T-13a) procedures. The purity of the reagents had already been ascertained in previous experiments. The results are summarized in table 11.

TABLE 11

COMPARISON OF ALL ASSAYS OF PAPER AND CHROMATOGRAPHIC ON

CERTAIN SUBSTANCES

Sample**	Ninhydrin	Molisch	Bauer	Fehling	Elson-Morgan
1) glycerol		++	++p*	++	++
2) glycerol		++	++p	++	++
3) glycerol		f*	++p	+	++
4) glycerol		+	+p	++	++
5) acetic acid	+				
6) acetic acid	++				
7) acetic acid	++				

* The symbol "p" indicates that the test was positive for pentose-like substances and the symbol "f" represents a faint response to the test.

**For treatments, refer to previous page.

Sample	Treatment
1	beta accelerator
2	spark
3	X-ray
4	UV
5	beta accelerator
6	spark
7	UV

V. DISCUSSION

A review of the experiments performed together with the results indicates, as the general introduction proposed, that this investigation represents a systematic line of scientific reasoning and experimentation. Each experiment was carried out with an explicit purpose intended and thus led to specific as well as general conclusions based upon the results.

The first experiment gave initial evidence as to the effect, if any, that electrical sparking had on selected bio-organic compounds in aqueous solutions. Whereas heating appeared to do little to change the ultraviolet patterns of known amino acids, sparking significantly altered them. Likewise, the electrophoretic components of bovine serum proteins were diminished by the spark. It was observed that the spark had a greater effect on the ultraviolet pattern of aromatic rather than aliphatic amino acids. It may be concluded that the spark did have appreciable effect on the structural and charge properties of the samples employed.

Sugar- and Amino Sugar-Like Compounds

From this point on, carbohydrate synthesis was considered. Experiment 2 indicated that the spark, by converting a hydroxy group to a carbonyl, could have an oxidizing effect. This was confirmed qualitatively with the ferricyanide test. Together with experiment 3, it was shown that there was a direct relationship between the time of sparking and the magnitude of the production of reducing groups since the Fehling test gave increasingly positive responses with longer arcing. Ammonia did little to alter this effect. The observation that electrical sparking enhanced the solubility in water of higher alcohols was seen in this experiment. Also, the oxidizing effects of the spark on a number of one to five carbon monohydroxy alcohols was demonstrated. Glycerol was included in order

to see any effect which the arcing process might have on a polyhydroxy alcohol. All of the sparked alcohols, by the ferricyanide test, exhibited the appearance of reducing groups.

Sugars are relatively insoluble in ether and experiment 4 indicated that such was also the case for the glycerol product. This carbohydrate-like compound resulting from the sparking of glycerol, however, showed no change in optical rotation from the initial reagent. This might mean that a racemic modification was produced, assuming unsymmetrical carbons were present. The orthoaminodiphenyl test gave further evidence for the carbohydrate-like properties of the sparked glycerol product.

It was tentatively speculated that ascorbic acid might be one of the products of the sparking of glycerol but this possibility was ruled out by the negative response to the dichlorophenol-indophenol test.

Characterization of Sparked Alcohols

Experiment 5 yielded a variety of conclusions as to the effect that the spark had on different types of alcohols. All of the mono-, di-, and tri-hydroxy alcohols tested gave the usual responses for carbohydrates and reducing sugars with the Molisch, benzidine, Fehling, and osazone reactions. But an additional, very significant observation brought about by the ninhydrin test was that sparking caused the synthesis of amino groups. Since no nitrogen compounds were in the experimental reagents, it was concluded that atmospheric nitrogen had been extracted from the air during the sparking and suitably added to the carbohydrate-like products. This observation was further substantiated by the Elson-Morgan test which is specific for compounds with hydroxyl and amino groups in close proximity. It is of significance that osazones were derived from all the products. Only four products and those were from 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, and glycerol, indicated vicinal hydroxyl

groups. This observation was based upon paper chromatography of the products and development with periodate-benzidine. Where a reagent containing no vicinal hydroxyl groups was sparked, one spot had to appear by chromatography if a product with adjacent hydroxyls were present. In the case of a reagent with vicinal hydroxyl groups, at least two spots were necessary for the product to be considered a glycol, since one of the spots would represent the residual unchanged reagent, as employed. Except for 1,3-propanediol, it was found that only those reagents which did have cis hydroxyl groups gave glycol products as seen by two spot chromatograms.

Qualitative Aspects of Amino Sugar-like Production

The production of sugars by sparking glycerol was observed in experiment 4 and that of amino sugars in experiment 6. The addition of ammonium sulfamate as a reduced form of nitrogen in the latter experiment appeared to bear little, if any, effect on the production of amino groups since Elson-Morgan-positive material was produced in its absence. In this experiment it was determined that a greater quantity was present as reducing sugar in general than specifically as an amino sugar (3.4% of the total reducing sugars). This indicated that not all of the reducing carbohydrates produced were aminated. Experiment 7 established that at least some of the amino groups were primary as noted by the nitrous acid test.

Carbohydrate Classification

Based on positive Molisch, Bial, and Tauber tests in experiment 6, it was observed tha the sugars produced by sparked glycerol were pentose-like. However, it is important to remember that these tests give the same response for hexuronic acids.

A lowering of pH occurred during the sparking process, as noted

in experiment 6 and may indicate the displacement of hydrogen that would be necessary for two glycerol molecules to condense into a five or six carbon sugar-like molecule. It could have also indicated the formation of a sugar derivative bearing a carboxylic acid group such as a hexuronic acid. A sample of known ribose was sparked in experiment 9 in an attempt to aminate it. Paper chromatography with ninhydrin yielded similar spots for both the sparked ribose and sparked glycerol samples.

Column chromatography in experiment 10 of both sparked glycerol and sparked ethylene glycol using the ninhydrin, ferricyanide, and Elson-Morgan tests to follow the eluates confirmed the observation that the same specific molecules within the product possessed both the properties of reducing sugars and amino compounds. The largest fraction after column chromatography of each of these substances indicated very slight differences between one another when analyzed by paper chromatography.

Amino Acid-like Products

The second section of this experimentation was carried out in order to investigate the possibility of synthesizing amino acids in the aqueous phase. By sparking as in experiment 11, acetic acid yielded glycine or a glycine-like product and isovaleric acid indicated valine- and glycine-like substances when analyzed by paper chromatography and ninhydrin.

A closer study of the acetic acid products separated by column chromatography as in experiment 12 showed the formation of several ninhydrin-positive fractions. One of these (tubes 15-20), when tested for the formation of a tosyl chloride derivative, supported the suggestion that a glycine-like substance had been synthesized. In 1951, Moore and Stein (63) reported this to be the general range in which glycine would be

expected to elute from a Dowex 50-X4 column. It is important to note that no nitrogenous reagent was initially used with the sparked acetic acid; hence, the material for the synthesis of amino groups most likely came from atmospheric nitrogen.

Polymers

The third phase of this work involved the production of polymeric substances by electrical sparking with about a 5% yield for the amount of sparking and reagents employed. The polymer formed in experiment 13 had many significant properties. First of all, copper ion seemed to inhibit the production of the polymer. By titration with acid and base, the polymer appeared to act like a dipolar ion. The decrease in basic titration of the product following formalin treatment further supported this point.

Polysaccharide and Aminopolysaccharide Characteristics of the Polymer

By paper chromatography after acid hydrolysis, the polymer exhibited amino-sugar-like composition. Nitrogen determination by the Kjeldahl method showed too low a value for the polymer to be a chain of amino sugars units only. If it were assumed that all the units were aminopentoses, the polymer should be 10.7% nitrogen, but the Kjeldahl determination indicated only 0.5% which is 4.7% of the predicted amount. If the assumption is correct that only some of the units were aminated, then only one unit in thirteen, on the average, had an amino group. It is interesting to recall that the analysis of the sparked glycerol products made in earlier experiments showed that 3.4% of the total reducing sugars synthesized were amino sugars.

Titration of the polymer indicated that the substance had acidic groups in addition to the amino groups. Therefore, it is possible that many of the remaining units of the chain could be hexuronic acids, for example.

Periodate oxidation of the polymer suggested a molecular weight of about 15,000 which is approximately one-half of the value found by ultracentrifugation (to be discussed later). This would mean that two formic acid molecules, in the average, were released per polymer chain. One explanation for this might be to assume that the polymer is some combination of any or all of the following monomeric units: aminopentopyranose, penturonic acid, aminohexopyranose, and hexuronic acid. Branching would have to be minimized and the linkage exist as 1-3 or 1-4. In the case of 1-4 linkage, the unit on the reducing end would need to have an amino group at carbon 2; however, this requirement is unnecessary in a 1-3 linkage. It is quite possible that other rationalizations could be proposed to explain the data.

The carbohydrate-like properties of the polymer were further observed by the Molisch test. The hydrolysate of the polymer gave a ninhydrin-positive and a benzidine-periodate-positive spot in the same location by paper chromatography. A sample of sparked glycerol without polymer producing coreagents from experiment 3, since it gave the same spots, suggested that at least some units of the polymer were amino sugar-like.

Physical-Chemical Analysis of the Polymer

The product resisted dialysis indicating its high molecular weight. It gave a single distinct peak by Tiselius moving-boundary electrophoresis suggesting molecular homogeneity. Urea treatment had no noticeable effect on the polymer's electrophoretic properties as it might have had on something like a protein.

Sedimentation velocity ultracentrifugation of the polymer indicated an S_{20} of 1.15 and Archibald equilibrium centrifugation

suggested a molecular weight of 31,027 further exhibiting its macromolecular proportions. Initial investigations indicated that the polymer had no immunogenic specificity.

Microbiological Considerations

By the aseptic precautions noted at the beginning of the experimental section and the bacteriological procedures carried out in experiment 14, the chance of contamination causing non-specific effects was regarded as minimal. Also of significance was the observation that the sugar- and amino acid-like products had apparent biological significance in that under suitable conditions, they could be used to support the existence of living micro-organisms, as noted in experiment 14. Either the products were of a form by which they were readily employed or they could be suitably converted to utilizable substances by the micro-organism consuming them. This nutritional observation was also made by Miller in his gaseous amino acid synthesis (**27**).

Photon-Electron Relationships

One of the most important investigations carried out in support of the theory presented earlier in this dissertation was found in the utilization of various energy sources as in experiment 15. Here reagents were submitted to bombardment by both electrons and photons. The production of amino sugar- and amino acid-like substances was noted under both conditions in that the products synthesized by the two energy sources exhibited the same characteristics by the analytical procedures used. For photon bombardment, X-ray and ultra-violet light were used; for electron source, a linear beta accelerator and the electrical sparking system employed throughout this study were utilized. Similarities in the effect of all four energy sources were noted in the results of

this experiment. It gave a favorable comparison of the effects of the two forms of energy supply and therefore indicated close similarities between the electron and the photon as reaction inducers, as predicted by the theory. Of course, further evidence will be needed before any final, definite conclusions can be drawn, but these data offer strong support for the theoretical proposals.

Prospectus

From the experimental results, several possibilities for future investigation become evident. First of all, it is proposed that if the polymer formed bears some of the characteristics of aminopolysaccharides, these structures could possibly serve as a suitable template by which nucleotides would be lined up for synthesis into DNA- and RNA-type polymers. This might result from an electrostatic bond being formed between the hydrogen of the phosphate and the amino group under suitable conditions.

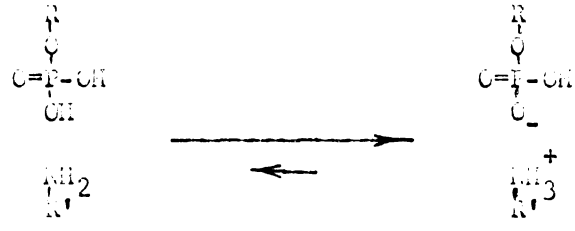


Fig.27 - Proposed bonding between a nucleotide and an aminopolysaccharide

Steric and thermodynamic factors would determine which nucleotide would be bonded to which amino groups. Once such an arrangement would occur with several nucleotides, inter-nucleotide phosphoesterification would take place between adjacent nucleotides. Then the electrostatic bonds would be broken with the mucopolysaccharide skeleton and an independent, information-rich polynucleotide (DNA-type) molecule would result. Such speculation, however, requires further experimentation.

The evolutionary antiquity of mucopolysaccharides seems evident from their occurrence in crustacean and insect chitin, as well as cartilage, which often constitutes the more ancient sections of skeletons. The combination of hexuronic acids and aminosugars is also found in chondroitin sulfate and hyaluronic acids. Essentially, nitrogen-containing polysaccharides are not very wide spread in nature today. In the same way that less efficient prototypes of biological species disappeared through the course of evolution, so too protobiochemicals used to establish higher forms of essential substances disappeared with time and are now infrequently observed in nature.

Also, it may be speculated that some type of polysaccharide could have been the primordial template for the production of polypeptides. The possibility of this is suggested by the complementary stereochemical characteristics of naturally occurring species of these types of molecules most of which are now found as D-sugars and L-amino acids, respectively.

Another possible subject for further experimentation would be the use of high energy electrical sparking discharge for the synthesis of ATP from ATP and ADP. Initial experiments by this investigator indicate the good possibility of such being possible although more extensive work will have to be done before any definite conclusions can be drawn.

Gaffron, in a recent review (**45**), noted that the synthesis of porphyrins could have started with a condensation between glycine and acetate. Since this class of compounds is very significant in living systems, it is possible that the products of the sparking of acetic acid, as formed in this series of experiments, could have provided the foundation upon which porphyrins were synthesized primordially.

Since it is postulated that irradiation of the proper reagents would reproduce essential primordial reactions, it might be desirable

to orbit such samples as glycerol and acetic acid outside of the Earth's atmosphere and then analyze their products upon recovery. Such a method could also be used to determine what the chances are that some other planet bears the proper conditions by which life systems similar to the types found on the Earth could inhabit that planet or might have evolved there at all.

SUMMARY AND CONCLUSIONS

The results of these experiments which were reported in this dissertation seem to indicate certain conclusions at the present time. It is important to emphasize that the purpose of these experiments was to synthesize certain types of reactive groups and classes of substances rather than specific compounds.

1) Electrical sparking served as an effective means for oxidizing an alcohol into a reducing substance.

2) Molecular nitrogen could have been the primordial source of nitrogen for the synthesis of amino groups, rather than ammonia as Miller (27) has suggested.

3) An alcohol, under sparking, can be made to take on many of the characteristics of carbohydrates, amino sugars in particular. The primary example used in this series of experiments was the formation of an aminosugar from glycerol.

4) Amino acid-like substances can be produced from organic acids in the aqueous phase by electrical stimulation. This evidently shows that primordial amino acid synthesis did not exclusively have to be the result of a gaseous reaction.

5) High molecular weight mucopolysaccharide-like polymers were formed by extensive sparking. When synthesized from a mixture of ethanol, glycerol, ammonium sulfamate, and acetic acid, the polymeric substance formed appeared to act like like a dipolar ion.

6) There appears to be a very close relationship between the photon and the electron as reaction inducers. Not only did both serve as general stimulators but also the results exhibited by substances

bombarded with each seemed to indicate that the modes of behavior and effect of these energy suppliers are very similar with respect to the materials employed as reagents as well as the groups tested for in the products. This tends to support the proposition set forth in the theory that there might be an integral relationship between the electron, the photon, and protobiochemical evolution.

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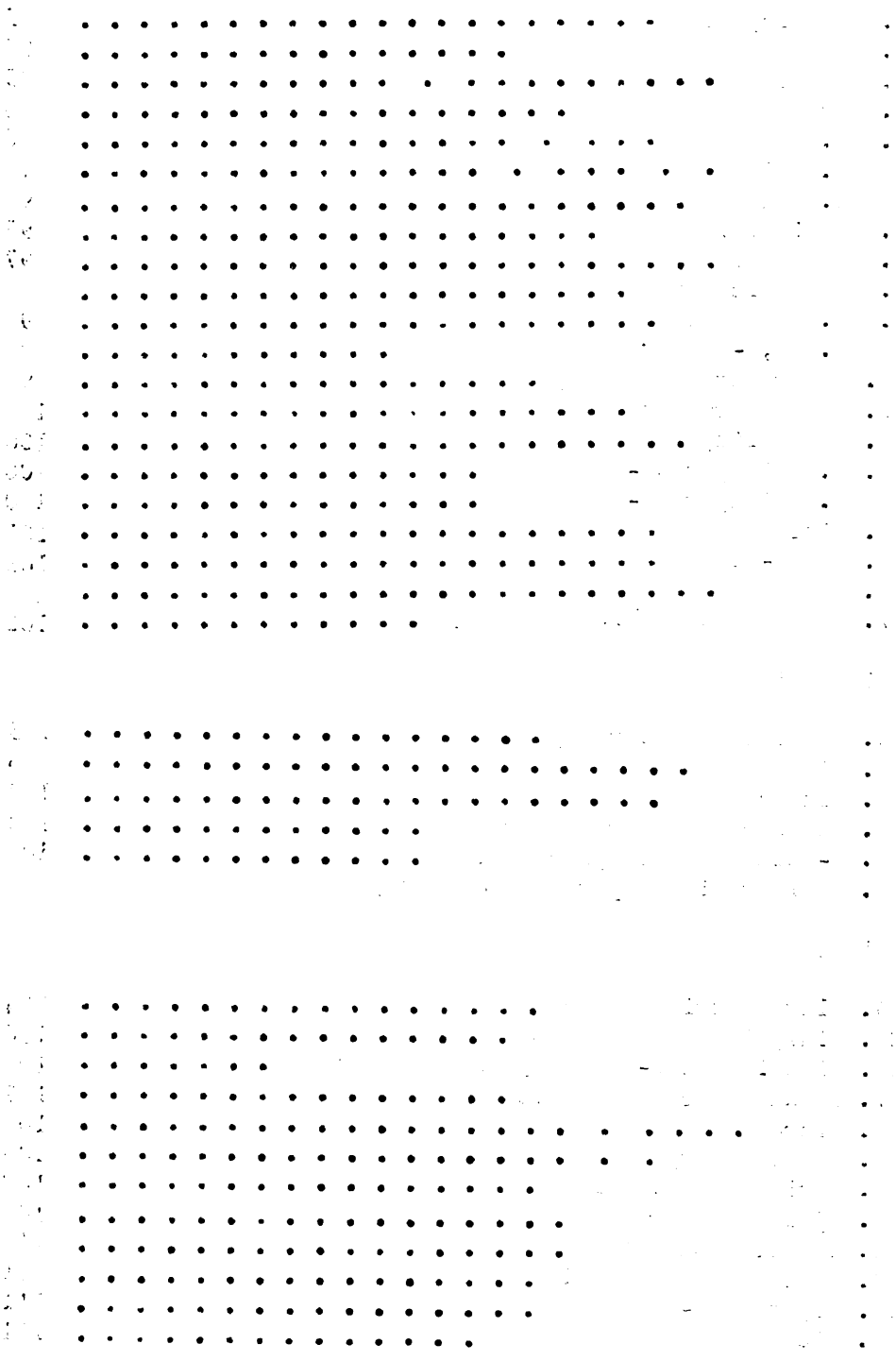
APPENDIX I

Analytical Techniques

Index to Analytical Techniques



A. Sugar and Amino Sugars

1. Ferricyanide test, based on the reduction of the ferricyanide ion (46)

a. Reagents

1) A solution of 0.5 grams of potassium ferricynaide in 1 liter of water.
2) A solution of 5.3 grams of sodium carbonate plus 0.65 grams of potassium cyanide in 1 liter of water.
3) A solution of ferric ammonium sulfate in 1 liter of 0.05 normal sulfuric acid.

b. Procedure

1) Add 1 milliliter of reagent 2 to 1 milliliter of sample and 1 milliliter of reagent 1.
2) Heat in boiling water bath for 15 minutes.
3) Cool and add 5 ml. of reagent 3.
4) Read in spectrophotometer at 690 mu after 15 minutes of development.

2. Alkline decomposition, for total amino grps.(47)

a. Reagents

1) Saturated solution of sodium phosphate and sodium borate.
2) Nessler's reagent: 4.55 gm. of mercuric iodide plus 3.49 gm. of potassium iodide dissolved in as little water as possible, followed by 8 gm. of sodium hydroxide and made up to 100 ml. of final solution with water.
3) A 2% boric acid solution.

b. Prodedure

1) Add 2 ml. of sample to 5 ml. of reagent 1 and 2 ml. of water and 1 ml. of reagent 3.
2) Heat in boiling water bath for 2 minutes.
3) Cool in ice water bath and add 1 ml. of reagent 2.
4) Stand for 30 minutes and then read in spectrophotometer at 490 mu.

3. Fehling test, for aldehyde reducing groups (48)

a. Reagents

1) A solution of 34.6 gm. of copper sulfate in 500 ml. of water.
2) A solution of 173.0 gm. of sodium potassium tartrate plus 60 gm. of sodium hydroxide in 500 ml. of water.

b. Procedure

1) Add 0.5 ml. of reagent 1 to 0.5 ml. of sample, 0.5 ml. of reagent 2, and 4 ml. of water.
2) Heat in boiling water bath for 15 minutes.
3) Observe for red precipitate of cuprous oxide.

4. Orthoaminodiphenyl test, for sugars (49)

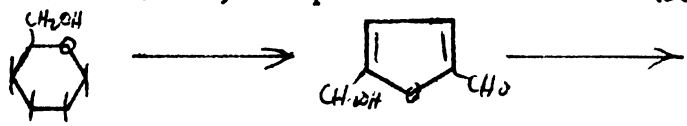
a. Reagent

1) A 100 ml. solution of 0.4% o-aminodiphenyl in glacial acetic acid.

b. Procedure

1) Add 1 ml. of sample to 5 ml. of reagnt 1.
2) Heat in boiling water bath for 45 minutes.
3) Read in spectrophotometer at 380 mu and observe the color of the solution.
   a) Arabinose, ribose, and xylose give a brownish yellow solution.
   b) Galactose and glucose give a green solution.
   c) Rhamnose gives a brown solution.

5a. Molisch reaction, for pentoses and hexoses (50)



hexose hydroxymethyl furfural

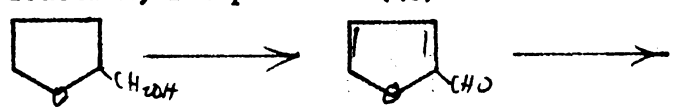
a. Reagent

1) A 5% solution of a-naphthol or thymol in 95% ethanol.

b. Procedure

1) Add four drops of reagent to 2 ml. of sample.
2) Stratify solution over concentrated sulfuric acid.
3) Observe for violet ring at the plane of contact, an indication of the production and liberation of furfural.

5b. Bial reaction, for pentoses (48)



pentose furfural

a. Specificity - for pentoses, 2-deoxypentoses, 6-deoxypentoses, hexuronic acids, trioses, and certain heptoses.

b. Reagent

1) A solution of 1.5 gm. of orcinol and 30 drops of a 0.1% aqueous solution of ferric chloride in 500 ml. of concentrated hydrochloric acid.

c. Procedure

1) Add 1 ml. of sample solution to 3 ml. of reagent.
2) Heat in a boiling water bath until color change occurs.
3) Observe color produced.
   a) Yellow or brown - hexose, aldehyde, hydroxy aldehyde.
   b) Emerald green, blue-green, or blue - pentose.

5c. Taber test, for pentoses and hexoses **(51)**

a. Mechanism - similar to the Molisch and Bial reactions.

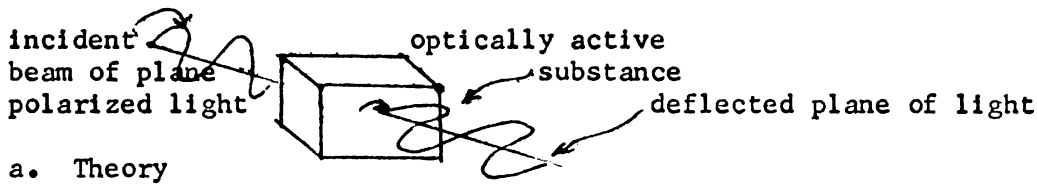
b. Reagent

1) A solution of 1.5 gm. of benzidine in 500 ml. of concentrated sulfuric acid.

c. Procedure

1) Add 1 ml. of sample solution to 3ml. of reagent 1.
2) Heat in a boiling water bath until color change occurs.
3) Observe color produced.
   a) Cherry red - pentoses and uronic acids.
   b) Yellow or brown - hexoses.

6. Optical rotation **(48)**



a. Theory

Optical rotation is a measure of a given molecule's asymmetry as defined by its ability to bend a plane of polarized light. For example, a carbon atom is asymmetrical if the four substituents bonded to it are all different in polarizability, as is the case with carbon 2 of glucose. A substance is known as dextrorotatory if it bends the polarized light to the right and levorotatory if to the left. The degree of rotation is a function of temperature, concentration, and solution density. An equal mixture of a dextrorotatory and a levorotatory species of a given molecule will result in no optical rotation in the polarimeter.

b. Procedure

1) Fill cell and place in polarimeter.
2) Take reading and compare unknown to knowns.

7. Anthrone reaction **(52)**

7. a. Reagent

   1) A 0.2% solution anthrone in 95% sulfuric acid.

   b. Mechanism - similar to Molisch and Bial reactions.

   c. Procedure

   1) Add 2.5 ml. of sample to 5 ml. of reagent.
   2) Heat in boiling water bath for 10 minutes.
   3) Cool in cold water bath.
   4) Read in spectrophotometer at 620 mu and observe for color reaction

8. Test for ascorbic acid (46)

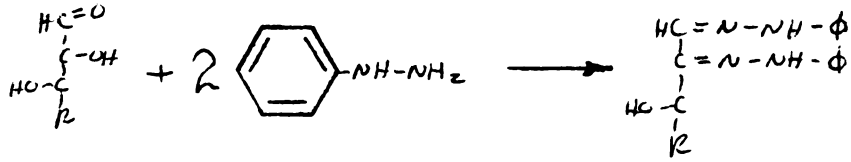
   a. Reagent

   1) A 5% metaphosphoric acid solution.
   2) A solution of 2.5 mg. of 2,6-dichlorphenol-indophenol in 100 ml. of water.
   3) A solution of 4.53 gm. of sodium acetate in 100 ml. of water plus 0.26 ml. of 0.5 normal acetic acid.

   b. Procedure

   1) Mix equal volumes of all three reagents and sample.
   2) Read in spectrophotometer at 520 mu.

9a. Osazones, for a-hydroxyketone and b-hydroxyaldehydes (53)



   a. Reagents

   1) A solution of 0.4 gm. of phenylhydrazine plus 0.6 gm. of sodium acetate in 4 ml. of concentrated sulfuric acid.

   b. Procedure

   1) Mix equal volumes of reagent 1 and sample.
   2) Heat in boiling water bath and note time for yellow precipitate formation.

9b. 2,4-dinitrophenylhydrazones (53)

   a. Mechnaism - same as with osazones except that 2,4-dinitrophenylhydrazine is used and substitution occurs only once per molecule, at the carbon 1 position.

b. Procedure

1) Mix equal volumes of reagent 1 and sample.
2) Heat in boiling water bath and note time for precipitate formation.
3) Collect precipitate, recrystallize from 95% ethanol, and take melting point.

10. Paper chromatography (54)

a. Solvent systems

1)	Ethyl acetate:pyridine:water	(60:25:20)
2)	Isopropanol:water	(80:20)
3)	Butanol:pyridine:water	(40:40:20)
4)	Butanol:acetone:water	(40:10:50)
5)	Butanol:acetic acid:water	(40:10:50)
6)	Phenol:water	(80:20)
7)	Phenol:water	(88:12)
8)	Butanol:pyridine:water	(60:60:60)

b. Procedure

1) Spot sample on Whatman #1 paper and set up for run.
2) Pass solvent either ascending or descending.
3) Dry and develop with appropriate indicator.
   a) Benzidine, for reducing groups
      (1) A solution of 0.1 gm. of benzidine in 4 ml. of glacial acetic acid plus 3 gm. of trichloro-acetic acid in 4 ml. of water.
      (2) Use as spray and heat to develop.
      (3) Observe for brown spots.
   b) Elson-Morgan, for amino sugars
      (1) Spray #1 - 0.5 ml. of a solution of 5ml. of 50% aqueous potassium hydroxide and 20 ml. of ethanol added to 10 ml. of a solution of 0.5 ml. of acetylacetone and 50 ml. of butanol.
      (2) Spray #2 - 1 gm. of dimethylaminobenzaldehyde in 30 ml. of ethanol added to 30 ml. of hydrochloric acid and then to 180 ml. of n-butanol.
      (3) Spray with #1 and heat for 5 minutes.
      (4) Spray with #2 and heat for 5 Minutes.
      (5) Observe for red spots
   c) Ninhydrin, for amino groups.
      (1) A 0.2% solution of 1,2,3-triketohydrindene in n-butanol
      (2) Use as a spray and heat to develop as purple spots.
   d) Periodate-benzidine, for viscinal glycols ( )
      (1) Dip #1 - 0.5 gm.of sodium periodate in 100 ml. of water.
      (2) Spray #1 - 0.5 gm. of benzidine in in 100 ml. of ethanol-acetic acid (80:20).
      (3) Submerge in dip #1 and dry.
      (4) Coat with spray #1 and observe for white spots on a blue background.

e) Silver nitrate, for sugars
   (1) Spray #1 - 1 ml. of a saturated silver nitrate solution is added dropwise to 20 ml. of acetone with stirring.
   (2) Spray with #1 and keep in a light-free ammonia atmosphere for one hour.
   (3) Heat to develop.
   (4) Wash in 10% sodium thiosulfate solution
   (5) Wash in running water and dry.

c. Calculation of Rf - Divide distance of migration of fraction spot by distance of migration of solvent front.

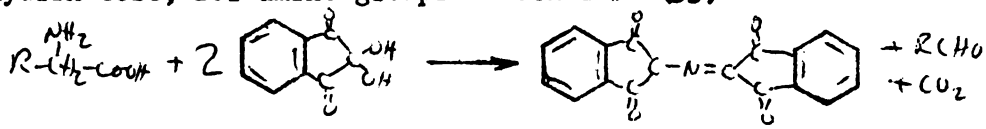
11. pH measurement **(48)**

a. Adjust meter with standard buffer.
b. Submerge electrodes in test solution and take reading
c. pH = $-\log (H^+)$

12. Solubility of a sugar

a. Dissolve sample in water.
b. Bring solution into contact with another immiscible solvent such as ether.
c. Test second solvent for presence of sugars by Molisch test (technique 5).

13a. Ninhydrin test, for amino groups - method #1 **(55)**



Ninhydrin

a. Reagents
   1) A solution of 0.8 gm. of stannous chloride in 500 ml. of 4 molar sodium acetate buffer added to 20 gm. of 1,2,3-triketohydrindene in 500 ml. of methyl cellosolve.
   2) A 50% solution of ethanol in water.

b. Procedure

   1) Add 1 ml. of reagent 1 to 1 ml. of sample solution.
   2) Heat in boiling water bath for 20 minutes.
   3) Add a 5 ml. portion of reagent 2 and stand for 15 minutes.
   4) Read in spectrophotometer at 570 mu.

13b. Ninhydrin test - method #2 **(52)**

a. Reagents

   1) Add 2 ml. of 0.01 molar potassium cyanide solution to 98 ml. of pyridine.
   2) An 80% solution of phenol.
   3) A 5% solution of 1,2,3,-triketohydrindene in absolute ethanol.
   4) A solution of 60% ethanol.

b. Procedure

1) Add 0.5 ml. of sample solution to 0.8 ml. of reagent 1 and 0.8 ml. of reagent 2.
2) Heat in boiling water bath for 3 minutes.
3) Add 0.4 ml. of reagent 3.
4) Heat in boiling water bath for 5 minutes.
5) Add 7.5 ml. of reagent 4.
6) Read in spectrophotometer at 570 mu.

14. Nitrous acid test, for primary amines (56)

$$NaNO_2 + HCl = NaCl + HNO_2$$

1^o: $R\text{-}NH_2 + HONO = ROH + N_2$

2^o: $R_2\text{-}NH + HONO = R_2\text{-}N\text{-}NO$ (yellow)

3^o: $R_3\text{-}N + HONO =$ (no reaction)

a. Reagents

1) A 2 normal hydrochloric acid solution
2) Solid sodium nitrite.

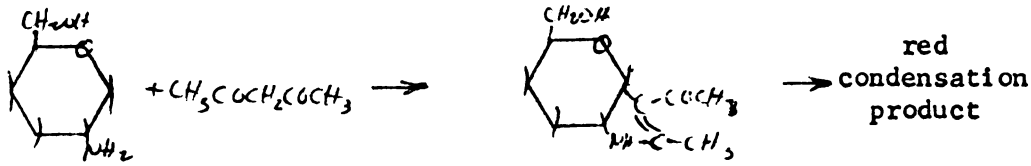
b. Procedure

1) Add 1 ml. of sample solution to 1 ml. of reagent 1.
2) Add 250 mg. of reagent 2.
3) Observe for release of nitrogen gas bubbles.

c. Alternative application

1) Treat sample with nitrous acid to remove amino group and replace it with a hydroxyl group.
2) Use product for paper chromatography of non-aminated species.

15. Elson-Morgan reaction, for amino sugars (48)



a. Reagents

1) A solution of 0.75 ml. of acetylacetone in 25 ml. of 1.25 normal sodium carbonate.
2) A solution of 1.6 gm. of p-dimethylaminobenzaldehyde in 30 ml. of hydrochloric acid added to 30 ml. of 96% ethanol.

b. Procedure

1) Add 0.5 ml. of sample to 0.5 ml. of reagent 1, heat in boiling water bath for 20 minutes, and add 4 ml. of 96% ethanol and 0.5 ml. of reagent 2.
2) Stand for 1 hour.
3) Read in spectrophotometer at 525 mu and observe for red color.

16. Sulfate test (47)

a. Reagent

1) A 10% aqueous solution of barium chloride

b. Procedure

1) Add 1 ml. of sample and 1 ml. of water to 1 ml. of reagent 1.
2) Observe for turbidity and read in the spectrophotometer at 550 mu.

17. Ion exchange chromatography for amino sugars and amino acids (48,57,) (63)

A. Theory

This procedure depends heavily upon the principle that oppositely charged ions attract each other. The supporting material for the ion exchange is usually a macromolecular polyelectrolyte whose free groups are ionizable. Synthetic polyelectrolytes are generally employed with smaller molecules, such as amino acids and amino sugars. Examples of supporting resins are Dowex-50, a nuclear sulfonic acid cation exchanger, and Amberlite IRA-400, a strong base anion exchanger.

Dowex-50 acts as a sulfonated cross-linked polystyrene resin, making it insoluble but permeable to other ions and capable of changing size by swelling. An ion is bound to the resin until a suitable solvent breaks the electrostatic bond by replacing the held ion with an exchanging ion of the solvent.

The rate of elution is governed by temperature, swelling, pH, and ionic strength of the solvent, as well as the flow rate of the system.

b. Preparation of Dowex 50-X4 (200-400 mesh) for amino sugar analysis.

1) Wash with a 2 normal sodium hydroxide solution.
2) Wash with a 2 normal hydrochloric acid solution.
3) Wash with water.
4) Repeat steps 1, 2, and 3 several times.
5) Wash with a 0.3 normal hydrochloric acid solution after finishing the last wash cycle with stepl, followed by step 3.
6) Apply 28 cubic centimeters of resin as a 1:1 (v/v) suspension with the 0.3 normal hydrochloric acid solution to the column.

c. Preparation of Dowex 50-X4 (200-400 mesh) for amino acid analysis.

1) Prepare buffer #1 - 21.008 gm. of citric acid in 200 ml. of one normal sodium hydroxide solution is diluted to 500 ml. total volume and added to 110 ml. of one normal hydrochloric acid solution and 390 ml. of water (pH = 3.52).

c. 2) Wash resin as for amino sugar analysis except that buffer 1 replaces the 0.3 normal hydrochloric acid solution.
3) Apply 90 cubic centimeters of resin to the column.

d. Preparation of Amberlite IRA-400 (HSO_3^-form) for sugar analysis.

1) Wash resin as for Dowex 50-X4.
2) Apply 13.5 cubic centimeters of resin to column.

e. Elution and collecition of fractions

1) Apply sample solution to column.
2) Apply suitable solvent.

a) A 0.3 normal solution of hydrochloric acid for amino sugars on Dowex 50-X4.
b) Buffer 1 (see 17.c.1) for amino acids on Dowex 50-X4.
c) Gradient elution between 99.5% ethanol and 95.0% ethanol for sugars on Amberlite IRA-400.

3) Collect samples with an automatic fraction collector table based on siphon volume control (3 ml. or 10 ml. fractions).
4) Analyze fractions.

B. Amino Acids

18. Paper chromatography (see technique 10).

19. Ninhydrin (see technique 13).

20. Nitrous acid (see technique 14).

21. Ion exchange chromatography (see technique 17).

22. p-Toluene sulfonyl chloride derivatives (53)

1^o: $p\text{-}CH_3C_6H_4SO_2Cl + R\text{-}NH_2 = p\text{-}CH_3C_6H_4SO_2NH\text{-}R$

2^o: $p\text{-}CH_3C_6H_4SO_2Cl + R_2\text{-}NH = p\text{-}CH_3C_6H_4SO_2NR_2$

3^o: $p\text{-}CH_3C_6H_4SO_2Cl + R_3\text{-}N =$ (no reaction)

a. Reagents

1) A 1 normal sodium hydroxide solution.
2) An 8% solution of p-toluenesulfonyl chloride in ether.

b. Procedure

1) Add smaple to 20 ml. of reagent 1 and 25 ml. of reagent 2.
2) Stir for 4 hours and then acidify with dilute hydrochloric acid.
3) Filter, recrystallize from 60% ethanol, and take melting point.

C. Polymer

23. (All appropriate tests for amino acids, sugars, and amino sugars.)

24. Ultracentrifugation (52)

a. Theory

Ultracentrifugation can generally be divided into two sections:

1. Equilibrium centrifugation
2. Sedimentation centrifugation

In the first type of study, a sufficient speed is attained so that the diffusion of the substance is exactly counterbalanced by centrifugal force. In the latter type of study, the speed is increased from that in the former study so that the molecules being investigated actually sediment out of solution.

The cell of the ultracentrifuge is in the shape of a sector so that migration of the molecules away from the center of the rotor is not impeded by collision with the walls of the cell which would set up disturbing countercurrents within the solution.

In sedimentation studies, the migration of particles under the influence of the high centrifugal field created by the speed of the moving rotor may be followed as the movement of the boundary between the plateau, the region of uniform distribution of molecules, and the solvent, in particular that portion which has been cleansed of the test molecules by the sedimentation toward the bottom of the cell.

The Schlieren lens system of the ultracentrifuge is such that an image of the differential of concentration with distance is transmitted. Theoretically, this division would be an infinitely sharp line at a given distance from the center of the rotor. However, diffusion causes the peak to broaden out, giving significance to the area under the curve in various analytical considerations.

The primary formula in ultracentrifugation is the Svedburg equation. With the contributions of Einstein and Sutherland, the Svedburg equation indicates the relationship between species molecular weight and the sedimentation coefficient, which in turn is defined by the radial acceleration of the centrifugal field in a sedimentaition study.

$$S = \frac{d \log x(2.303)}{w^2 dt}$$

$$S = \frac{v}{w^2x} = \frac{(x_2-x_1)2}{(x_2+x_1)w^2(t_2-t_1)}$$

$$M = \frac{RTS}{D(1-\bar{v}_{sp}p)}$$

Archibald has taken the Svedburg equation and applied it to equilibrium ultracentrifugation

$$M = \frac{RT(dc/dx)_m}{(1-v_{sp}p)w^2x_mc_m}$$

Several factors are necessary in the Archibald equation for which Shachmann and others have determined the means of calculation.

1. Partial specific volume

$$\bar{v}_2^* = v_p(1/m_o - 100(1/m_o - 1/m)/p)$$

$\bar{v}_2^*$ = apparent partial specific volume
v_p = volume of the pycnometer
m_o = weight of contents with solvent
m = weight of contents with solution
p = percent of macromolecules in solution
Values are graphically extrapolated to o concentration to get partial specific volume.

2. Initial concentration (synthetic boundary cell, see figure **26**)

Readings of ΔY taken at regular intervals of X

$c_o = \Delta X/M_o\Sigma\Delta Y$
$M_o = 2.103$ (magnification factor)

3. Concentration at the meniscus (low speed equilibrium study, see figure **26**)

$c_m = c_o - (1/x_m^2)(dx/M_o)\Sigma x^2\Delta Y$
x_m = distance of meniscus from center of rotor.

4. The value of $(dc/dx)_m$ (equilibrium study)

$(dc/dx)_m = \Delta Y_m$ = change of concentration at the meniscus

5. Additional necessary values

R = gas constant = 8.314×10^7 ergs/C°/mole
T = absolute temperature
p = density of solution
w = radial velocity

25. Dialysis and polymer isolation (52)

a. Purpose - to separate small ions from solutions of macromolecules and to determine the minimum value for molecular weight of the polymer.

b. Procedure

The solution containing the polymer and the undersired smaller ions and the initial reagents is placed in a dialyzing membrane which is suspended in water. The pores of the membrane chosen are of such size that molecules of less than 6,000 molecular weight diffuse out into the water and the macromolecules remain within the bag. The surrounding water is changed often. The resultant polymer solution is condensed by air pervaporation and the product is isolated as a dry solid by freeze-drying lyophilization.

26. Tiselius free-boundary electrophoresis (58)

a. Theory

Electrophoresis may be defined as the migration of charged particles within the influence of an electrical field gradient. Tidelius, in 1937, developed a practical means for using electrophoresis in the study of high molecular weight substances, which is now available as the Perkin-Elmer Model 38 Apparatus, among others. The cell is in the shape of a "U". When suitably prepared with samples and buffers, the migration of particles within the electrical field can be viewed through the Schlieren lens system in the apparatus. Since, at a given buffered pH, different proteins of a mixture, for example, exhibit correspondingly different degrees of net charge, the rate of migration of each protein species varies. Since the lens system depends on areas of more or less dense concentration, the movement of these proteins, seen as boundaries, can be quantitatively analyzed. The Schlieren lens system projects the change of concentration from point to point as a series of differential curves. Thus, as with the sedimentation coefficient in ultracentrifugation, the rate of migration of each species is measurable and calculable.

b. Procedure

1) Dialyze the sample solution in buffer.
2) Prepare and fill the cell.
3) Place the cell in the ice bath chamber filled with water within the apparatus and connect the electrodes.
4) Align the channels of the cell.
5) Compensate the initial boundary and photograph it.
6) Apply measured voltage and amperage, noting time as well.
7) Stop theelectric current after suitable migration and photograph the peaks.
8) Measure the resistance of buffer in conductivity cell.
9) Measure distance of migration from the photographs.

c. Calculations

$u = dAK/tIRm$

u = mobility (cm^2/volt/sec)
d = distance of migration (cm)
A = cross-sectional area of cell (0.30 cm^2)
K = conductivity cell constant (0.850)
t = time (sec)
I = current (amp)
R = resistance of buffer (ohms)
m = magnification factor of optical system (1)

27. Immunological analysis (**52**)

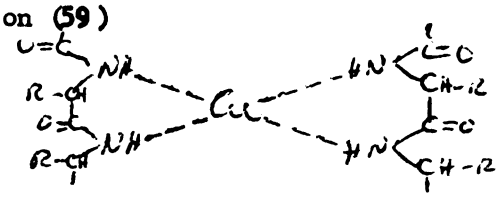
a. Theory

A macromolecule often has the property of inducing the production of specific antibodies against itself which may be analysed by the Ouchterlony plate technique. By this method, antigen diffuses from a source well through the agar of the plate toward the antibody diffusing from the other direction. At the points where specific antibodies meet corresponding antigens, precipitation occurs. Since different types of antigens diffuse at different rates, precipitation lines arise at different locations so that a count of these lines indicates the number of specific components in the antigen mixture for which antibodies have been produced.

b. Procedure

1) Inject a 5% solution of polymer in complete adjuvant:water (50:50) suspension into the rabbit.
2) Two weeks later inject a 5% solution of polymer in an incomplete adjuvant:water (50:50) suspension into the same rabbit.
3) One week later, bleed the rabbit, allow the blood to clot and settle overnight.
4) Collect serum and use against a 1% solution of polymer antigen in the Ouchterlony agar plates.
5) Observe the plates for the appearance of precipitation lines.

28. Biuret reaction (**59**)



colored complex

a. Theory

Compounds containing two or more peptide bonds give a characteristic purple color when treated with dilute copper sulfate in an alkaline solution. The name of the reaction comes

from the compound biuret which gives a typically positive test. The color is apparently due to the coordination comples of the copper and four nitrogen atoms, two from each of the two peptide chains. Steric factors are also significant.

b. Reagent

1) A 500 ml. solution is made up of 0.75 gm. of copper sulfate, 3.00 gm. of sodium potassium tartrate, 150 ml. of a 10% sodium hydroxide solution, and enough water tomake up the remaining volume of solution.

c. Procedure

1) Add 1 ml. of sample solution to 5 ml. of reagent 1.
2) Stand for 30 minutes.
3) Read in photometer at 570 mu.

29. Turbidimetry **(59)**

a. Reagent

1) Low concentration solution of acetic acid and iron potassium cyanide.

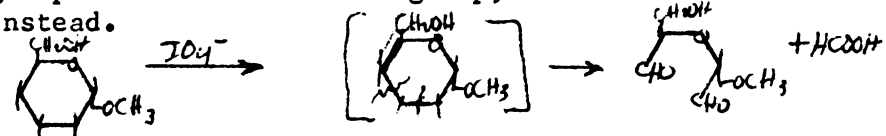
b. Procedure

1) Add 1 ml. of sample to 5 ml. of reagent 1.
2) Measure photometrically at 600 mu.

30. Periodate oxidation **(48,52)**

a. Theory

Periodate ion oxidizes the carbon to carbon bond between combinations involving hydroxyls, aldehydes, ketones or primary amines. For example, the oxidation of a mole of ethylene glycol would yield two moles of formic acid. An a-acetal of a pyranose aldohexose is not affected by such action nor is the bond between C1 and C2 altered. However, cleaving the C2-C3 bond and C3-C4 bond produces a molecule of formic acid. If the hydroxyl group of C3 were an amino group, ammonia would be produced instead.



In a polysaccharide such as amylose with its 1,4-glycosidic bonds, formic acid is liberated from both ends of the chain but from no internal members. Thus, the number of moles of formic acid produced can be used to determine the average chain length of a given sugar polymer.

b. Reagents

1) A 0.37 molar aqueous solution of sodium metaperiodate
2) Ethylene glycol
3) A solution of carbon dioxide-free 0.01 normal sodium hydroxide.

c. Procedure

1) Dissolve 50 milligrams of sample in 5 milliliters of water.
2) Cool to 3°C and add 5 milliliters of reagent 1.
3) Keep in the dark for 30 hours under refrigeration.
4) Add 1 ml. of reagent 2.
5) Titrate potentiometrically to pH 6.0 with reagent 3.

d. Calculations

$U = w/vNP \qquad UP = M = w/vN$

U = average number of units per chain
w = weight of sample used
v = volume of base used to titrate
N = normality of base
P = weight of each unit
M = average molecular weight of chain

31. Kjeldahl nitrogen determination (52)

a. Reagents

1) Powdered sodium sulfate with some available mercuric ion contributor such as mercuric oxide.
2) Concentrated sulfuric acid.
3) A solution of 12.5 normal sodium hydroxide.
4) Boric acid, water saturated.
5) A mixture of 5 parts 0.2% bromocresol green and 1 part 0.02% methyl red.

b. Procedure

1) Add sample to 1 teaspoon of reagent 1 and 12 ml. of reagent 2.
2) Digest for 1.5 hours.
3) Dilute with 200 ml. of water.
4) Add 30 ml. of reagent 3.
5) Distill into a flask containing 25 ml. of reagent 4 and 5 drops of reagent 5.
6) Titrate against standard acid.

c. Calculations

$$\% \text{ Nitrogen} = ANM/W$$

A = volume of standard acid used to titrate
N = normality of standard acid
M = milliequivalent weight of nitrogen (0.014)
W = dry weight of sample (gm)

32. Spectrophotometry (60,61)

a. Theory

The spectrophotometer, as the name implies, is an instrument for making quantitative measurements of the transmission of light at various wavelengths by a given medium. The principal parts are the source of electromagnetic radiation, the monochromator (usually a prism or a grating), the cell compartment, the photoelectric detector, a variable slit to control light intensity and limit the spread of wavelengths, and a device for indicating the signal from the detector such as an electrical meter, potentiometer, or recording potentiometer.

Various chemical structures are known to absorb light at certain wavelengths. Therefore, when a solution containing a structure which absorbs light at a given wavelength, is placed in a beam of that wavelength, the dector notes that less light is transmitted than when the solution is not there. A double beam spectrophotometer is so constructed that absorbance due to the solvent is ruled out and only that due to the substance being tested is recorded.

The Beer-Lambert law gives quantitative significance to spectrophotometry:

$$\log (I_o/I) = A = abc$$

where A is absorbancy, I_o is the intensity of light of a particular wavelength with no sample in its path, I is the light intensity with the sample, a is the absorbancy index (a solute proportionality constant which varies with light wavelength, temperature, and solvent), b is the thickness of the solution, and c is the concentration. Automatic spectrophotometers are so constructed that they directly measure absorbance or percent transmission.

It is possible to test a given substance in the ultraviolet, infrared, or visible range with a suitable spectrophotometer. In the ultraviolet and visible range, quartz cells are used; in the infrared range, sodium chloride cells are used so that all test materials must be emultions or solutions in nonpolar solvents. Rotation and vibration-rotation spectra are observed in the infrared range whereas the ultraviolet and visible ranges exhibit the electronic spectrum of a given substance.

In the Beckman DK-a (ultraviolet and visible) and Beckman IR-5 (infrared) spectrophotometers, it is possible to either take a reading at one wavelength or set the apparatus to automatically take readings within a range of wavelengths.

b. Procedure

1) Adjust 0% and 100% transmission and fill cells.
2) Take necessary readings as absorbancy or percent transmission.

33. Trichloracetic acid precipitation, for proteins (62)

a. Reagent

1) A 5% aqueous solution of trichloroacetic acid.

b. Procedure

1) Add 5 ml. of sample solution to 10 ml. of reagent 1.
2) Observe for precipitate.

34. Tryptophan-anthrone test, for mucopolysaccharides (52)

a. Reagents

1) Asolution of 95% ethanol.
2) A 0.1% aqueous solution of tryptophan.
3) A 0.15% solution of anthrone in 95% sulfuric acid.

b. Procedure

1) Add 2 ml. of reagent 1 and 1 ml. of reagent 2 to sample.
2) Chill for 15 minutes.
3) Add 6 ml. of reagent 3.
4) Heat in boiling water bath for 20 minutes.
5) Cool for 10 minutes and then read in spectrophotometer at 520 mu.

35. Tests for solution contamination (64)

a. Test for bacterial contamination

1) Add 23.5 gm. of Plate Count Agar (tryptone glucose yeast agar - Difco Laboratories) to 1 liter of water.
2) Heat to dissolve and sterilize for 15 minutes at 15 pounds pressure and 121°C.
3) Aseptically mix the warm fluid agar in a Petri dish with 1 ml. of sample solution and allow to harden; store at constant 32°C.
4) After 3 days, inspect the dish for colonies.

b. Test for yeast and mold contamination

1) Add 39 gm. of Potato Dextrose Agar (Difco Laboratories) and 1 ml. of a 10% solution of tartaric acid (to adjust to pH 3.5) to 1 liter of water.
2) Heat to dissolve and sterilize for 15 minutes at 15 pounds pressure and 121°C.
3) Aseptically mix warm fluid agar in Petri dish with 1 ml. of sample solution and allow to harden.
4) Store at constant 23°C.
5) After 7 days, inspect the dish for colonies.

APPENDIX II

VITA

The author was born in Detroit, Michigan on June 1,1941. He graduated as valedicoerian from Flint Northern High School in June, 1959 and entered Michigan State University the following fall on the Distinguished Alumni Scholarship.

In 1960, the author entered the Honors College program and later became a University Scholar. His undergraduate research was carried out under the auspices of the National Science Foundation program at Michigan State. He also was employed as a research assistant in the Department of Food Science.

During the spring and summer of 1961, he continued his research at Tel Hashomer Hospital in Tel Aviv, Israel. In March of 1962, he entered the graduate program of the Department of Biochemistry at Michigan State University.

The author plans to marry on May 12, 1963 and expects to complete both his bachelors and masters degree in June, 1963. From there, he will begin a doctoral program in biophysics.

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