**Title**

Chemical Evolution of Life and Sensibility



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**Publication Date**

1966-09-01

UCRL-17132
Preprint C. 2

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Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48



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September 1966

## DISCLAIMER

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## INTRODUCTION

It is something of the nature of asking a "silly question" that I seek to discuss the problem of chemical evolution, but it is not going to be asking it of you in the audience, but rather of a broad area of science. Only someone as ignorant as I am of many of the fields in which I am asking these particular questions would dare to ask the questions and do the kind of speculating that I am going to do today. There will be among you, I am sure, those who know a good deal more about certain bits of the areas with which I have contact, and, therefore, will recognize some of the questions with which I am concerned and the formulations I propose as being naive. But I don't really offer a preliminary apology. I am merely trying to say to you that I feel a certain kind of concern and determination to ask these naive questions, because I have seen what happened to me when my students come fresh each year (they are just as fresh every year as they were the year before -- sometimes they are fresher!) and the fact that the students are that way and have the temerity to ask the kind of questions they do has helped me to what I hope are fundamental understandings which I would never have arrived at otherwise. The reason for this is that in the course of the evolution of the computer system of the mind, over the years

of an individual life, certain kinds of programs have been built-in and you tend not to monkey with those programs in any way. And these programs may or may not be right -- but they are there! These "silly questions" that the students ask every year push me to go back and change the settings on the dials, or even the dials themselves, and this gives rise very often to entirely different points of view which I normally would not arrive at. And it is this type of thing which encourages me to do what I am going to do today.

In the past ten years or so I have been concerned with the problem of the evolution of living things from the nonliving world, with which we presumably began roughly five billion years ago. Until this particular talk, my normal procedure was to begin with a solid earth roughly five billion years ago, having a primitive reducing atmosphere which was subjected to a variety of energy inputs (generally in the form of cosmic radiation, ultraviolet radiation from the sun and heat giving rise to electrostatic discharges in the earth's atmosphere and possibly the radioactive ionizing energies in the earth's rocks). These various sources of energy, all taken together, would produce a series of changes in the primitive atmosphere of the earth, and I have tried to trace what those changes are, doing experiments whenever it is possible to do a laboratory experiment suggested by the proposed series of events. If an experiment can't yet be done, I assume the proposition and go on to the next step until an experiment can be done. What this actually means is devising a chain of events from the primitive atmosphere to the first living cell, and testing the hypothesis of that chain of events whenever it impinges upon a circumstance which is susceptible to laboratory test, or observational test, as the case may be.

Up until today that discussion usually terminated when we arrive at a living cell, a system of molecules surrounded by a boundary (barrier, membrane) which is capable of reproducing itself and transforming energy in a directed way. Generally that is the end of the discussion. Today, however, I hope that that point will come roughly in the middle of the discussion, because while one can describe the sequence of transitions from the primitive simple molecular atmosphere of the earth to the functioning living cell in a series of chemical and physical changes, and one can talk about critical points along the time sequence, it seems to me that looking at the problem more broadly this appearance of the defined cell (defined by an active membrane separating the internal contents of the cell from the rest of the world) is simply one stage in a much longer chain. Thus, the next important transformation which ultimately gave rise to man is the evolution of a system for transmitting information from the environment into the cell, so that the cell can react to that environment. I would like to carry the discussion, then, another stage further to the evolution of that information-gathering, information-transmitting, information-processing, information-storing function which we now know to be evident/in the nervous system of the primitive animals and on even ultimately to the higher animals, including man. In a sense, the appearance of a cell, together with its membrane boundary, should be only one of the critical stages in the transformation of inanimate, initial simple molecules to the kind of information processing apparatus which is represented by the human neural system.

You can see that this is a much bigger job and must necessarily be more speculative for two reasons. One is that the real mechanism by which the

neuronal system works is not yet really known to us in its intimate details in the same way as what little we do know about the energy transforming system which is part of the earlier evolutionary pattern. However, be that as it may, I am going to undertake this speculation and see where we come out.

## PRIMITIVE (PREBIOTIC) CHEMISTRY

We begin, therefore, with the idea of the primitive earth in its initial form, insofar as we understand it today. The geological history of the earth, beginning roughly about five billion years ago, is depicted in FIGURE 1. The primitive atmosphere formed shortly thereafter was believed to contain the simplest molecules -- atoms of the first row of the periodic table, generally in their most reduced form; carbon attached to hydrogen (methane), oxygen attached to hydrogen (water), nitrogen attached to hydrogen (ammonia), and hydrogen itself, as well as some partly oxidized carbon, such as CO (see FIGURE 2). The period of chemical evolution leading to the single-celled organism presumably occurred somewhere in the early region, between five and three billion years ago. The complexity of organic chemical organization is increasing during the period of chemical evolution while the complexity of the inanimate organic environment decreases after the appearance of living things, i.e., after organic evolution has begun. This cross-over point between chemical evolution and organic evolution (after the single cell had appeared) is now believed to be at about three billion years, or perhaps even earlier.

An answer to the question of when the nervous system -- these specialized instruments for gathering and transmitting information -- appears is totally

unknown, and as yet there is no chemical record (fingerprint) that can identify the nervous system. The nervous system presumably began somewhere around two billion years ago, at least the information-transmitting cells appeared then, but that is pure guesswork at this time. By the time we have reached 500 million years ago, the well-known fossils have appeared. It now appears fairly certain that there may be earlier fossils, one billion years ago, and we now believe that single-celled organisms have been seen as early as two billion years ago. In any case, no multicellular organism is unequivocally known earlier than 500 million years ago, and by the time such evolved there must be organisms which clearly have neuronal systems in them; at least they have them today, and their early counterparts probably had them as well. A unique paleobiochemical identification of the nervous system, as I said a moment ago, is still undone.

We have been able to examine this problem by going backwards in time and looking at the fossils which were present, and have been able to find "molecular" fossils as early as three billion years ago. These molecular fossils are in the nature of hydrocarbons of very particular structures; not random hydrocarbons but hydrocarbon chains and rings (very highly specialized structures) related to the steroids of today's living organisms. The fact that these molecular fossils were present as early as three billion years ago tells us that complex metabolic machinery was in existence at that time, so the single cell must have come into being before then.

The other way of examining chemical evolution by formulating a series of chemical events which led up to the point of appearance of "molecular" fossils is to begin with the simple molecules of the primitive atmosphere (FIGURE 2) and try to reproduce some of the chemical transformations. This,

of course, is where our experimental science can play a role. Roughly fifteen years ago we did some of the first experiments of this type, starting with carbon dioxide, water and hydrogen, and we were able to show that with ionizing radiation we could make reduced carbon. In fact, carbon-to-carbon links could be created in this way, following the ionization and breaking of the water/molecules and hydrogen and construction of reduced carbon. Shortly after that, Miller put ammonia into the primitive reducing system and was able to show even more complex compounds. There has been a whole series of experiments in which examinations have been made of the products that are formed when high energy (or ionizing) radiation in the form of UV energy, electrical discharge, etc. is introduced into the primitive atmosphere of the earth. FIGURE 2, in the top row, shows the simple primordial molecules, and in the second row are depicted the compounds which are obtained when the ionizing radiations are introduced into the gaseous system shown in the top row. You can see that the molecules in the second and third rows of FIGURE 2 are the very molecules of which the modern day living cell is constructed and upon which it operates. The amino acids in the bottom row are the typical ones of which proteins are ultimately constructed. There should be a fourth row in this figure which would show even more complex small molecules, such as the heterocyclic purine and pyrimidine rings which are the basic units for the construction of the nucleic acids, another type of biopolymer of major importance in the reproduction of living things, just as the polypeptide and protein molecules, which are the biopolymers made of the amino acids, are also macromolecules essential to the construction and function of living organisms.

This type of experiment has been done repeatedly, by many people, with a wide variety of energy inputs into the simple molecules. However, this in itself does not give rise to the biopolymer(s) or, in the next stage, to the selective energy converting apparatus that we now know in the living cell, nor does it give rise to the limiting membrane of the living cell.

The next stage in chemical evolution is that leading to the biopolymer(s), and a quick examination of the kinds of chemical reactions that are required to lead to the essential biopolymers (polypeptides, nucleic acids, polysaccharides and lipids) are shown in FIGURES 3 and 4. These show something common to all of these chemical reactions. First of all, in the polymerization reactions leading to proteins, there is an elimination of a water molecule between the carboxylic acid end of an amino acid and the amino end of an amino acid, giving rise to a peptide linkage. This produces a dimeric molecule with the corresponding original functions at each end -- an acid group at one end and an amino group at the other. This reaction can continue by adding units at either end. For the construction of the polysaccharides, which are the biopolymers which we recognize as the structural elements of the cell wall in plants as well as one of the energy storage units of plants and animals, a dehydration condensation (the characteristic single reaction of all these biopolymers which are formed from the simple molecules) takes place between a semiacetal hydroxyl group and some other hydroxyl from a carbohydrate molecule to give a linkage involving the elimination of water. Thus, a disaccharide which then has the semiacetal hydroxyl and alcoholic hydroxyl at either end, and which can thus grow on to the polymer, is made. Lipids, the fat-type

molecules, again show the same type of reaction, i.e., the elimination of water between an acid hydroxyl group and an alcoholic hydroxyl, to create the ester bond. This, however, does not give rise to a potentially indefinitely long polymer but can give rise to rather large molecules. With the polysaccharides and proteins, polymers can be created which can grow indefinitely, and because of the variety of R groups can make an enormous variety of molecular structures.

The last great class of biopolymers shown in FIGURE 4 involves three different kinds of dehydration reactions. There is a purine ring (adenine) which can be formed in the first stage of chemical evolution, on which it is now possible to have a dehydration condensation between one of the NH groups in the molecule and the semiacetal hydroxyl of a five-carbon sugar, thus giving rise to one kind of linkage. Another kind of dehydration condensation can take place between a phosphoric acid molecule and the primary alcohol group at the endof the sugar molecule, thus giving rise to a phosphate ester. And, finally, a second phosphate ester group can be formed between another one of the hydroxyl groups of the phosphoric acid and the secondary alcohol on another sugar molecule.

You will notice that the three dehydration condensations have now given rise to a unit which is capable of still further growth, indefinitely, much in the same way as the polypeptide. There is still another hydroxyl which can undergo a condensation, i.e., there is an additional hydroxyl on the sugar molecule (a secondary hydroxyl) which can undergo the same kind of condensation as in the first case. We therefore have a unit which has two functional groups (hydroxyl and phosphoric acid) which can condense with each other, at either end, and thus grow indefinitely to make very long linear arrays.

The question now is: How can we induce that kind of a dehydration polymerization in water? We know that this kind of dehydration condensation takes place today in water; the living organism performs this type of reaction all the time. How could this kind of dehydration condensation have happened in the absence of a living organism? Is there any kind of system we can devise in the laboratory which will do this with the materials available from the initial energy input on the methane-ammonia-hydrogen-water system? Today's organisms use phosphoric anhydride linkages in order to induce dehydration condensation in an aqueous milieu. But the formation of such phosphoric anhydride linkages in a nonliving system, while it can be achieved,can be achieved only in special circumstances. It may very well be that these special circumstances pertained. However, I find it more satisfying to use the kinds of molecules made generally by the introduction of high energy radiation into the initial chemical milieu of the primitive reducing atmosphere to achieve these phosphoric anhydride linkages.

A series of those very interesting molecules is represented by the cyanamides. HCN itself is formed in the primitive atmosphere, and two molecules of HCN reacting with ammonia can form dicyanamide. These dicyanamides, in turn, are now energy rich materials since they have a carbon-nitrogen linkage having more than one bond between the two atoms (two or three bonds between the carbon and the nitrogen). This multiple bonding between the carbon and the nitrogen can be used to accomplish the dehydration condensation. The bond between the carbon and the nitrogen is unsaturated, that is, can take up water among other things, and do this in a specific manner. We have used dicyanamide (an ammonia molecule with two cyano

groups replacing two of the hydrogen atoms) as a condensing agent to induce all of the dehydration condensations (polymerizations) in one form or another in dilute aqueous solution, using the dicyanamide as the only energy source. It is useful because the dicyanamide groups do not react very rapidly with water itself but will react with phosphoric acid, with carboxylic acids, and with the alcohols more rapidly than they will react with water itself. We can therefore use (di)cyanamide in water solution.

The series of reactions by which a peptide is formed by dehydration condensation reactions is shown in FIGURE 5. You can see dicyanamide in its various tautomeric forms. Notice that one of the tautomeric forms can be written as a carbodiimide and thus corresponds to the well known reagent which has been used most frequently in a nonaqueous medium for the same reaction series. Here an amino acid will add to the carbodiimide linkage, and once the oxygen atom is attached to the carbon it stays there, and the carboxyl carbon is then subject to nucleophilic attack by another amino group, the nitrogen attacking that carbon and forming the peptide link. Notice that what was originally the cyano group of the carbodiimide has become urea. A water molecule has been added to the carbon-nitrogen triple bond (carbodiimide), giving the dehydration condensation to the dipeptide. The other reactions shown in FIGURE 5 are side reactions which should be minimized in order to make this a useful reaction. However, the overall reaction may be of the order of ten percent, and this is efficient enough for our purposes.

The same kind of dehydration condensation reaction can be used to form phosphate esters, glycosidic linkages, fatty acid esters, and the like, and all these things have been performed in the laboratory. We are

thus able to achieve a certain kind of dehydration polymerization, even in water solution. We have now reached the stage of the biopolymers, dissolved/water. The question now is: Can some higher degree of order be obtained from this?

## GENERATION OF HIGHER DEGREES OF MOLECULAR ORDER

Let us examine what happens when a polypeptide becomes eight or ten units long, i.e., when it begins to have built into it factors which may give rise to a secondary order. The primary order is simply the sequence of amino acids which is hooked together, and the secondary order grows out of that. FIGURE 6 shows the nature of some of the amino acids, giving rise to the primary polypeptide (or protein) structure -- the $R_1$, $R_2$, $R_3$, etc., sequence. The figure shows some of the varieties of R group which are available. The secondary structure of the polypeptide, however, is shown in FIGURE 7, which depicts a helical coiled structure whose nature is built right into the peptide structure by virtue of the carbonyl groups of the amide linkages which form hydrogen bonds with the hydrogen of the amide link three or four amides removed from it down the chain. The helix is what we call the secondary structure of the linear array and it is thermodynamically stable under suitable conditions. This characteristic is demonstrated in FIGURE 8, showing the result of an experiment in which conditions were arranged for melting out the helix, producing a random coil, and then reversing the conditions, causing the secondary structure to return. By adjustment of the pH, the structure can be changed from a random coil at alkaline pH to an alpha helix at pH 4.9, and this is perfectly reversible. Thus, some of the evidence appears that the secondary struc-

ture is built right into the linear array and does not have to be wound up by hand, so to speak. The secondary structure is an element of "information", if you like, an element of structure built into the linear array.

We can go one step further. Following the secondary helical structure, the folding of that helical component into some secondary folding is also built in and can be reversibly removed, as shown in FIGURE 9. This tertiary structure is itself built into the secondary structure which, in turn, is a function of the primary structure. This can be shown by melting out, or destroying, the tertiary structure by a rise in temperature and havint the tertiary structure reformed upon cooling (FIGURE 9). In FIGURE 9 is shown the folded helix melting out of chymotrypsin as a function of temperature. At pH 3.5, the folded helix is not melted out until a temperature of about 50°C is reached, and it goes right back upon cooling down again, to get back the folded structure such as the one shown for myoglobin in FIGURE 10. We are not melting out the alpha helix, now; we are melting out the tertiary structure. We can go one step further and show that the aggregation and de-aggregation of these tertiary structures into quaternary structures is also a reversible thing. FIGURE 10 actually shows a quaternary structure in which four chains of a protein (hemoglobin) all folded up into a tertiary structure are packed together in what might be called a quaternary structure.

For polypeptides we have thus traced a degree of order from the amino acids, which are randomly formed, to the polypeptides, to the helix, to the folded helix, to the packed, aggregated folded helices; in other words, from primary to secondary to tertiary to quaternary structure.

The ordering of the polynucleotides, for the components of which the mechanism of formation by random procedures was shown earlier, is seen in FIGURE 11. This indicates how they organize into a secondary helical structure in much the same way as a polypeptide organizes itself in a secondary structure. Two nucleic acid chains are here involved in the formation of the secondary helical structure of the nucleic acids, the reversibility of which is shown in FIGURE 12 which demonstrates a temperature melting out process. By raising the temperature from 22° to 99° the double helix of the nucleic acid units melts into a random coil, and when it is cooled down again to 22° it reconvenes into the helical structure, which is the second degree of structure corresponding to the helix-coil transition in the proteins. The linear array of the nucleic acids has built into it the second order structural feature.

## AUTOCATALYSIS

The next step we must take in our evolutionary development is to introduce the notion that once certain kinds of structures appear they, in turn, can in some way control, or induce, their self-formation, or formation from their component units. This is what the chemists call autocatalysis, and a very nice example is shown in FIGURE 13. Here in diagrammatic form are shown the results of a number of experimental observations. If an attempt is made to induce a hexamer of thymine (diagramatically indicated along the top) to undergo a dehydration condensation between a hydroxyl group on one end of the hexamer and the phosphate on another one, with a dehydrating condensing agent such as the carbodiimide, you find that they will not condense. Instead, a variety of other events occur, and the yield of condensation products is extremely small. However,

if a polyadenylic acid, which contains the complementary base of thymine, is added to the reaction mixture, the condensation reaction is much more efficient, with about a ten percent yield of the condensation product, that is, the one obtained by hooking together the two hexamer unit. Here we have the case in which the polymer is catalytic for its own complementary polymer formation. In the same way, the polythymidylic acid would catalyze the condensation of adenylic acid to polyadenylic acid. Here is the cross autocatalysis which is the essential feature of the self-reproducing genetic system and which is here demonstrated in a simple nonliving laboratory system. This autocatalytic quality is an essential feature of the chemical evolutionary scheme, and the reactions shown in FIGURE 13 are one of the first cases I have been able to extract from the literature which is really pertinent. Formerly in discussing autocatalysis I had used other examples (Fe catalysis of oxidation leading to the efficient catalysis by Fe-Pt-Me) with which you are all familiar, but even though some of them were experimentally observable they illustrated the basic idea, but on systems which were not of such direct interest in the evolution of the genetic system of living organism. So much, then, for the introduction of autocatalysis in the control of the generation of the kinds of molecules which are necessary for organic evolution.

## APPEARANCE OF VISIBLE STRUCTURE ------ LINEAR ARRAY

We now need to go to a higher degree of organization and order, and we can get, so to speak, a fifth degree of order which brings us into the region of visible structure. FIGURE 14 shows the example of taking apart the protein material of collagen and reassembling it into visible collagen

fibrils. By readjusting the salt concentration of the solution, the collagen fibrils, after being dismantled, reaggregate into visible units which can be seen as identical with the naturally-occurring collagen fibrils. We have now reached the level of visible structure which is contained implicitly within the units of the molecules themselves. We have thus traveled all the way from methane-ammonia-water-hydrogen to visible structure without the introduction of Maxwell's demon.

The next step is the reconstitution of linear structures from molecules, linear biologically interesting structures from relatively simple small molecules. One such example is the reconstitution of TMV virus particle, shown in FIGURES 15, 16 and 17. FIGURE 15 shows the native TMV particle, and the size is quite definite both in diameter and in length. In FIGURE 16 is shown the reconstituted TMV protein without RNA. The TMV particle has been taken apart and dissolved, the nucleic acid has been removed, and the protein molecules have been reaggregated. Here we can see that the protein molecules of the TMV virus particle reaggregate, but at random lengths. If, however, the separated protein molecules and the separated nucleic acid TMV/molecules are combined and reaggregated in the presence of each other, the reconstitution is more precise (FIGURE 17). Here we have reconstitution in what is a linear array. The thing that determines the length is the nucleic acid; the protein tends to aggregate but its length is indeterminate.

Another type of reconstitution of a still higher order is that of the flagellum of a bacterium. In FIGURE 18 four different types of bacterial flagella (I, II, III, IV) are shown. You can see that three of the bacteria have flagella with quite long, wavy shapes, and the fourth one has a shorter, curly shape. It is possible to separate the flagella,

take them out of the bacteria and dissolve them into molecular solution, with a molecular weight of about 40,000. These can then be reconstituted -- get back the flagella, so to speak -- which process is shown in FIGURE 19. The monomer from III and seed from III (that is, the monomer is seeded with fragments of III) and a III type flagellum results from the monomer. In the lower part of FIGURE 19 is the monomer from IV (the "curly" one) seeded with III, and the curly type predominates. This shows that the protein structure of IV is different from III, and because even when you use the same seed nuclei for both solutions, the flagellar growth pattern is determined, in this case, by the protein molecular structure itself.

Much more complex spontaneous reassembly processes have been demonstrated, i.e., the enzyme complex of alpha-keto acid oxidases. Here, a complex made of three different proteins (the decarboxylase, the acyltransferase and the flavoprotein) is reassembled into a very specific, catalytically active complex, using the component molecules in the ratio 12:1:6; this is the elegant work of Reed in Texas.

The flagella (and virus) which we have been describing above are still essentially linear arrays. A two-dimensional array would be the next stage in the development. We would thus be on the way to a film, or a membrane structure, which would surround the energy conversion (enzyme complex) and structure determining apparatus (genetic material). This is the beginning of our analysis of the membrane structure.

## THE NEXT HIGHER ORDER ---- STRUCTURE OF A MEMBRANE

FIGURE 20 shows an electron micrograph of one of the individual membranes, lamellar structures, within the chloroplast (a model for a membrane, if you like) and you can see that the membrane is not a smooth structure but a granular one. It is composed of granular units of protein, probably hooked together in the first approximation by virtue of properties of their own structure and then covered by some kind of lipid layer. In FIGURE 21 this same type of structure is shown in even greater detail, and the cracking of the lamellar structures can be seen at various levels. You can see that the lamellar structures are granular and that the granules are of different sizes at different levels, with alternating lipid and protein layers. The two-dimensional array is a result of the molecular structures from which it is built.

We have not yet reconstructed an energy converting membrane such as the chloroplast lamellae. We have taken it apart, but have not yet learned how to reconstruct it. A few efforts have been made, and I fully expect that in the not too distant future some reconstructions of the two-dimensional energy converting arrays will occur.

There has, however, been an effort to reconstruct another biologically interesting membrane by using as yet rather crude methods, but it is the first effort I have seen in the literature to reconstruct a biologically active membrane. This is shown in FIGURE 22, which gives the reconstruction of a membrane of Mycoplasma, a slime mold. The upper right shows the intact mold, the upper right the isolated intact membrane before it is taken apart, and in the lower part of FIGURE 22 is shown the attempted reconstruc-

tion of the membrane fragments. After the lipid and protein is redissolved it is reconstituted to give the type of structure shown. We can begin to see here the reconstruction of membranes in section which appear very similar to the intact initial membrane. This is a step toward the reconstruction of two-dimensional asymmetric membranes which are essential for the containment of the material functioning structure inside the living cell.

This is hardly a bare beginning. Not only must we probe much more deeply into the constitution, construction and reconstruction of the various types of biological membranes, but the basic physics and chemistry of synthetic, and partially synthetic, model systems requires the development of both synthetic and theoretical principles which are as yet extremely rudimentary.

## DEVELOPMENT OF THE NERVOUS SYSTEM

A next step in our evolutionary travels is the development of the nervous system. We have repeatedly heard that the factors which select for the survival of a system, whether it be a simple chemical system or a single living organism which is really a complex physical-chemical system of a certain sort, is the environment in which it resides. If one particular array of physical-chemical systems has survival advantage over another in a particular environment, it is clear that one will have more probability of reproducing itself and thus fully occupying the environment, than the other one. This is merely a statement to the effect that the physical-chemical system which has some reproductive survival advantage in a particular environment will eventually use up all the raw material of

that environment to the exclusion of other systems requiring the same materials. This is the basic process which has been taken for granted in all of the evolutionary steps which we have discussed up until this point. This discussion has brought us to the evolution of a single entity surrounded by a membrane which keeps it separated from its outside environment, i.e., a cell. Most of the responses of that cell, that single entity, to the environment are responses to chemical stimuli. A chemical stimulus can be some change in the chemistry of the environment and the organism senses that change by virtue of a physico-chemical reaction of its own, which is dependent upon the concentration of some chemical in its environment. Thus, a gradient of the concentration of that chemical in its surroundings can produce a reaction to that gradient. Initially, there is no "directionality" to that kind of sensing other than the gradient itself, but it does work so long as the organism is small -- single cells.

Even when cells aggregate linearly, every cell can sense the environment directly and does not need any help; it responds more or less chemically to the environment. An example of such a linear array is Nostoc, a unicellular algae which aggregates linearly, as does Nitella. Every single cell is exposed at least in two dimensions to the outside environment, so that every cell can sense the outside environment directly. So the linear aggregation of cells does not require any kind of specialized information gathering or transferring equipment. However, if the cells aggregate in two dimensions and then the sheet is allowed to close and form a hollow sphere, or tube, there will be cells inside which do not have direct access to the environment on the outside. Therefore, it is necessary to

have some information transferring apparatus to help the cells on the inside. All cells already have a membrane which is asymmetric because the inside of the single cell is different from the outside. Some of the cells on the outside of the tube, or sphere, could extend that membrane through the double or triple layer of cells into the inside and find a way of transmitting the information about the environment which they (the outside cells) can sense directly by some sensing device, into the cells on the inside of the multiple layers which cannot so sense directly the environment. The organism (system of cells) which devises a mechanism for doing this, with a minimum loss of information in the transfer, is the organism which can react to changes in the environment with the least loss to itself and its offspring. This is, I think, the focussing requirement for the evolution of the nervous system.

The evolution of a tansmitting system is the first step in the evolution of the nervous system. Presumably the sensing device on the outside is still chemical (it could be physical if it were responding to light, for example). The sensing device, the initial transducer for example, can still be the same as it was originally, but the transmitting system must, in some way, be improved beyond that of the single cell.

We already have an asymmetric membrane which was evolved in response to the requirement for the initial encasement of the cell and which is capable of keeping salts in or out, as the case may be, depending on where the organism is, as well as keeping certain organic molecules in or out. Thus will arise an electrically asymmetric membrane. For example, in order to move into the fresh water from salt water it will have to devise ways of keeping the salt that it needs for its functioning inside in the presence

of the osmotic gradient. Thus, almost simultaneously with the initial cellular enclosure, evolved semi-permeable membranes and, secondly, salt pumps. These are functions whose molecular mechanism we do not yet know. We do not know the mechanisms of semi-permeability or selectivity with respect to salt, nor do we know the mechanisms of the pumping apparatus, that is, the mechanism of pumping salt, or metabolites, from the low concentration back up to the high concentration which is needed inside the organism. A typical salt concentration inside the organism is of the order of 500 millimolar of NaCl. If you take the cell into fresh water there has to be a mechanism, first of all, for preventing immediately leakage of the NaCl out and, secondly, a mechanism for pumping back that material which does leak out, since no permeability barrier is perfect. These mechanisms were evolved even for the single-celled organisms.

What is needed now is the evolution of a device for transmitting the chemically or physically sensed environmental change along some direction without decrement. You might speculate that all that is necessary would be a confining tubule with the concentration gradient going down that tubule. However, you can see that a concentration gradient down a tubule, even though it is contained in a narrow passageway, transmits information content with a logarithmic decrement as it goes down the tubule. This is not a very satisfactory way of transmitting information. This is the crux of the next step in the evolution of the ability to transmit information.

Obviously, the next stage is to put repeating stations into the mechanism, with an energy input every so often, and thus rebuilding the signal back up over the noise as you go down the tubule. The electrically active asymmetric membrane was used by the living cell in just this fashion.

The essence of the idea is as follows: The nerve cell does not generally transmit an impulse by a gradient transmission but by a frequency code.

Before examining the mechanism of transmission, let us have a look at the physical apparatus which the specialized information transferring cell has become, i.e., the neuron. FIGURE 23 is a drawing (by Valverde) of a highly developed neuron, showing the apparatus whose function we are going to examine, and I think the molecular mechanism of the evolution of the apparatus will become apparent only from any molecular knowledge we have, or may gather, as to how it works. You can see the cell body, originally a little "blob" surrounded by a membrane; but now the membrane has extended processes in many directions, and most of these processes (dendrites) are those which are taking messages from other cells. There is one that is sending its own message (the axon). The small numbers on the processes count the endings of other cells which end on this particular one. This shows how complex the transmission system is for even this single cell; the single cell is receiving something from at least 500 other cells, and the message it sends out is synthesized from all the messages it receives. So, this single cell, for example, shows some of the complexity of a cortical cell and the system of which it is a part. FIGURE 24 shows what one of the interactions is like in detail, the transmitting end of one cell and the receiving end of another cell, a synapse. The message comes down the presynaptic tube and there is a gap between the two cells. The little circles are presumed to contain a chemical, and as the electrical message comes down the cell wall something happens at the gap (and I mean just something; we don't know exactly what), which lets a few of these vesicles loose into the gap between the two

cells. The chemical contained in the vesicles is released and it travels across the gap and does something to the post-synaptic surface which starts the message down the next cell. These are the two important components of nerve transmission: The axonic transmission, or, I should say, the nerve impulse itself, and the synaptic transmission from one nerve to the other. We must find ways and means of understanding these two essential features in molecular terms in order to devise evolutionary schemes for them. FIGURE 25 shows an electon micrograph of part of a nerve cell, and the vesicles in the presynaptic button are shown quite clearly; you can see the gap between the two cells, and the arrow points to the place in which it was believed a discharge took place The energy producing apparatus, the mitochondria, for remaking all the material which is discharged, is clear, and the post-synaptic cell which picks up the message and starts it toward the cell body is easily visible.

With that as the apparatus with which we must deal we can see in FIGURE 26 the frequency code which I mentioned a few minutes ago. The numbers represent relative intensities of light on Limulus eyes and the pulses are the signals which come out of the nerve attached to the crab's eye. At very low intensity there are very few signals, and as the intensity is increased the frequency increases but the signal which passes down the nerve is always the same signal in size -- all you get is a frequency change and not a size change. This is the result of the mechanism by which the information is transferred which is a repeating station, so there is always the same signal strength. But there are more signals for higher intensity on the transducer. FIGURE 27 shows at a higher magnification one of the signals shown on FIGURE 26, just to ob-

serve it in terms of the voltage changes involved; showing the voltage pulse as it passes a particular point on the nerve.

The question is: What causes that voltage pulse in the nerve and how is it controlled? It seems to be due to a salt concentration change of some sort, and this notion has already been introduced by virtue of the general nature of membranes. I am going to try to briefly outline the Hodgkin-Huxley theory of the nerve impulse conduction, one pulse of which is shown in FIGURE 27. FIGURE 28 depicts what occurs at a given point to the current flowing across the membrane as a pulse passes that point. At a particular point the current flows inward and then outward (curve A, FIGURE 28). By controlling the polarization of the membrane and the ions both on the inside and outside of the membrane it was possible to show that the current was made up of two components, sodium ions flowing inwards and potassium ions flowing outwards. Notice that for a particular potential difference imposed the sodium ion current going inwards grows rapidly and then decays, and the potassium ion current going outwards starts more slowly and then remains constant for that particular polarization (+ 56 mv). These currents are interpreted in terms of changes in the selective conductivity of the membranes for Na and K ions which can be measured separately, as shown in FIGURE 29. When a voltage is impressed from the outside, you can see what happens to the sodium ion conductivity. It rises very rapidly as the sodium leaks into the membrane, thus reducing the negative potential on the inside. The potassium then gradually begins to leak out. The sodium conductivity increases rapidly and then decreases again; the potassium conductivity increases more slowly and stays more constant, until the impressed voltage is reduced. You can see that there is a selective change in the

conductivity, the sodium ion conductivity increasing at first and then decreasing. This sodium ion decrease sets in more rapidly than the potassium ion increase and is thus not solely the result of a repolarization due to potassium leakage. The dotted lines in FIGURE 29 show what happens to the conductivity if the voltage clamp is released; the sodium conductivity drops very rapidly and the potassium ion conductivity drops more slowly.

If you take these two conductivity changes and put them into a suitable equation for corresponding voltage changes, the voltage being determined first electrochemically by the potassium and then by the sodium, you can get the passage of the nerve impulse pretty much as it was seen in FIGURE 28; this is shown in more detail in FIGURE 30. What is involved here is the requirement that something happen to the membrane when the potential across it is changed to make it quickly permeable to sodium with a quick recovery of sodium resistance and slowly permeable to potassium. As the potassium ion leaks out, that voltage is then returned to its original value, and as the polarization of the membrane is returned to its original value the potassium current is gradually shut off. Now, this voltage signal travels down the nerve by the mechanism shown in FIGURE 31. You start with the voltage negative on the inside of the nerve cell and positive on the outside. Then a reverse voltage is impressed at some point. Sodium ions flow from the outside in, thus pulling sodium ions from the neighboring part of the membrane; potassium ions flow from the inside out, but more slowly. The sodium ion flows over from B to A to close up the gap, depolarizing at B, which then becomes permeable to sodium ion. Some macromolecular change occurs in the polymer membrane

itself so that it becomes conducting to sodium ion immediately which is the cause of the migration of the depolarization wave down the nerve.

Where is the energy for this depolarization coming from? It is coming from the salt concentrations on the two sides of the cell. Inside the membrane there is a concentration of sodium of 50 millimolar and a concentration of potassium of about 400 millimolar; outside the membrane the situation is reversed, with 20 millimolar for potassium and 400 millimolar for sodium. It is that electrochemical concentration cell which stores the energy for driving and regenerating the deoplarization wave as it travels down the nerve. The amount of sodium and potassium which exchanges per square centimeter of membrane is very small (about 4 picomoles per pulse). Remembering that this is per square centimeter of nerve membrane and remembering that the concentration is of the order of millimolar (20 - 400 on both sides of the membrane) it can be seen that many pulses can be passed before the amount of sodium and potassium on either side of the membrane is appreciably changed. But a pump <u>is</u> required which gradually pumps the potassium back in from the outside, and pushes the sodium from the inside out, and that pump is a metabolic pump coupled to metabolic energy which, in some way, burning sugar eventually pumps ions against their gradient.

There is at least one place where electrochemistry and polymer chemistry can come together and that is to seek an answer to the prime questions: What conceivably could be the mechanism of the permeability change induced by changing the membrane polarization from -50 mv to +50 mv? How in this way an something be suddenly done to the permeability of sodium and potassium, which is gradually reversed by the leaking of the po-

tassium out of the cell and the sodium into the cell, thus returning the permeability and polarization to its original values? The polarization change across the membrane required to trigger the event is about 50 mv. Keep in mind that kT is about 25 mv. How big a dipole would be necessary to permit it to turn in this field gradient against the 25 mv of thermal energy? A dipole of 50 Debyes will turn completely in a 50 mv field over 100 Å, the approximate thickness of the cell membrane. By making that simple kind of energy calculation you can see that the protein molecules, for example, which have unsymmetrical charge distributions could very easily be involved in a switch opening or closing the membrane to sodium or potassium ion.

We saw earlier how we could generate the protein molecules, with their various side groups with various kinds of charges (both plus and minus), and how the protein molecules would pack generally under thermodynamic control. What appears to be happening here is this: The polarization is maintained by the salts (the K ion concentration), the resting potential of the nerve membrane being maintained primarily by a potassium ion electrochemical concentration cell. This, in turn, keeps that protein-lipid dipde, whatever it is, oriented in such a way that sodium (and to a lesser extent, potassium) cannot go through. The moment that the potential acros the membrane is reduced, there is a relaxation of the protein-lipid-carbohydrate complex, and the sodium-potassium can go through. As the potassiu m builds back up again to the ten-fold concentration difference, the potential is gradually restored, protein configuration is turned back, and the permeability of sodium and potassium is cut off.

It is quite astonishing that one should come up with the right size of dipolar separation corresponding roughly to the dimensions of the membrane, of the order of 100 Å, for the critical voltage which switches it

open and closed. This is a rather significant fact, which suggests that this is the kind of mechanism that is occurring; that it is probably not something more erudite than that. Be that as it may, it provides a model which we can perhaps seek to reconstruct by synthetic systems.

The other essential component of the information transferring system, synaptic transmission, can now be conceived as a further development of membrane sensitivity. It is no far cry from the turning dipole to its possible susceptibility to ions [acetylcholine, catechol amines, $Ca^{++}$, and other neurologically active ions (curare)] or potential ions (LSD) as well as the dipolar ions, gamma-aminobutyric acid, phosphocholine, etc. The interaction energy of what appears to be a necessarily highly polar permeability determining center in the membrane with such highly charged materials may be expected to be large. The evolution of a degree of specificity in these transmitter substances may be expected. Finally, a variability (plasticity) of membrane structure can be visualized in response to the electrical input to a cell or groups of cells mediated through changes in secondary, and even primary, protein structure, in turn under RNA and DNA control.

The organization of such a total system for higher animals is shown in FIGURE 32. There is a receptor cell, something which detects the environmental change and starts the signal. Various neurological levels of a higher organism are shown, and you can also see the signal traveling from cell to cell, with various kinds of circuits at different levels. At the bottom is a direct input-output system, on the muscle fibers, with

no processing. You can also see, however, that there are all kinds of levels of processing -- in the spinal cord (with feedback), in the cerebellum, the red nucleus, and, finally, up into the cerebral cortex, with all stages of filtration and processing in between. At this point I can go no further except to suppose that it is in some way the facilitation, or inhibition, of these various transmissions that is the information processing and storage device, keeping in mind that these "facilitations" and "inhibitions" are ultimately recorded in molecular structure.

## CONCLUSION

We thus see cellular aggregation and specialization arising from the selection pressure of efficiency in the use of available raw materials in the environment. Such aggregation, in turn, demands the development of devices for transmitting and processing information about the environment from sites where it can be detected to sites that can appropriately respond to it, lest the aggregate system (sensibility) disappear. Such devices are seen, insofar as we understand them, as extensions and ramifications of the molecular organizations evolved in the initial response of the primeval atoms of the earth's atmosphere to the succession of energy fluxes which are still continuing.

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The preparation of this paper was sponsored by the U.S. Atomic Energy Commission.

Presented at Boulder, Colorado, July 22, 1966 as part of the intensive study program of the Neurosciences Research Program.

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# FIGURE CAPTIONS

Figure 1 Time scale for total evolution.

Figure 2 Primeval and primitive organic molecules.

Figure 3 Dehydration condensation of amino acids.

Figure 4 Dehydration condensation of nucleic acids.

Figure 5 Series of reactions by which a peptide is formed by dehydration condensation reactions.

Figure 6 Primary protein structure.

Figure 7 Secondary protein structure.

Figure 8 Absorption spectrum of polyglutamic acid in both (a) helical and (b) random coil forms. (Tinoco, Halpern & Simpson, in "Polyamino Acids, Polypeptides and Proteins,"University of Wisconsin Press, Madison, 1962, pp. 147-157)

Figure 9 Change in extinction coefficient as a function of temperature for alpha-chymotrypsin at various pH. (Biltonen, private communication, 1965).

Figure 10 Structures of myoglobin and hemoglobin. (Kendrew, Science, 139, 1259 (1963); Perutz, Science, 140, 863 (1963).

Figure 11 Molecular drawing of components of DNA.

Figure 12 Hyperchroism on nucleic acid (Tinoco, Halpern & Simpson, 1962).

Figure 13 Autocatalytic reactions in chemical evolution (Naylor and Gilham, Information Exchange Group No. 7, Scientific Memo No. 277, March 1966)

Figure 14 Structure of collagen

Figure 15 Native tobacco mosaic virus (TMV) (Williams, Virus Laboratory, University of California, 1961)

Figure 16 Repolymerized TMV protein (Williams, 1961).

Figure 17 Reconstituted TMV protein and nucleic acid (Williams, 1961).

Figure 18 Reconstitution of bacterial flagellum (Asakura, Eguchi and Iino, J. Mol. Biol. 6, 302 (1966))

Figure 19 Reconstituted flagella (Asakura, Eguchi & Iino)

Figure 20 Electron micrograph of chloroplast lamella

Figure 21 Detail of lamellar structure of chloroplast, showing granular construction

Figure 22 Electron micrographs of reconstruction of membranes of slime mold (Razin, et al., Proc. Nat. Acad. Sci. 54, 219 (1965))

Figure 23 Diagram of the neuron (Valverde, J. Neurol., in press)

Figure 24 Diagram of synapse (deRobertis, 1962))

Figure 25 Electron micrograph of nerve cell (Palay, Exptl. Cell Research, Suppl. 5, 275 (1958))

Figure 26 Impulses in optic nerve fibre of Limulus by flash of light (Hodgkin, "Conduction of the Nervous Impulse", p. 12)

Figure 27 Higher magnification of one of signals shown in Figure 17 (Hodgkin, p. 39)

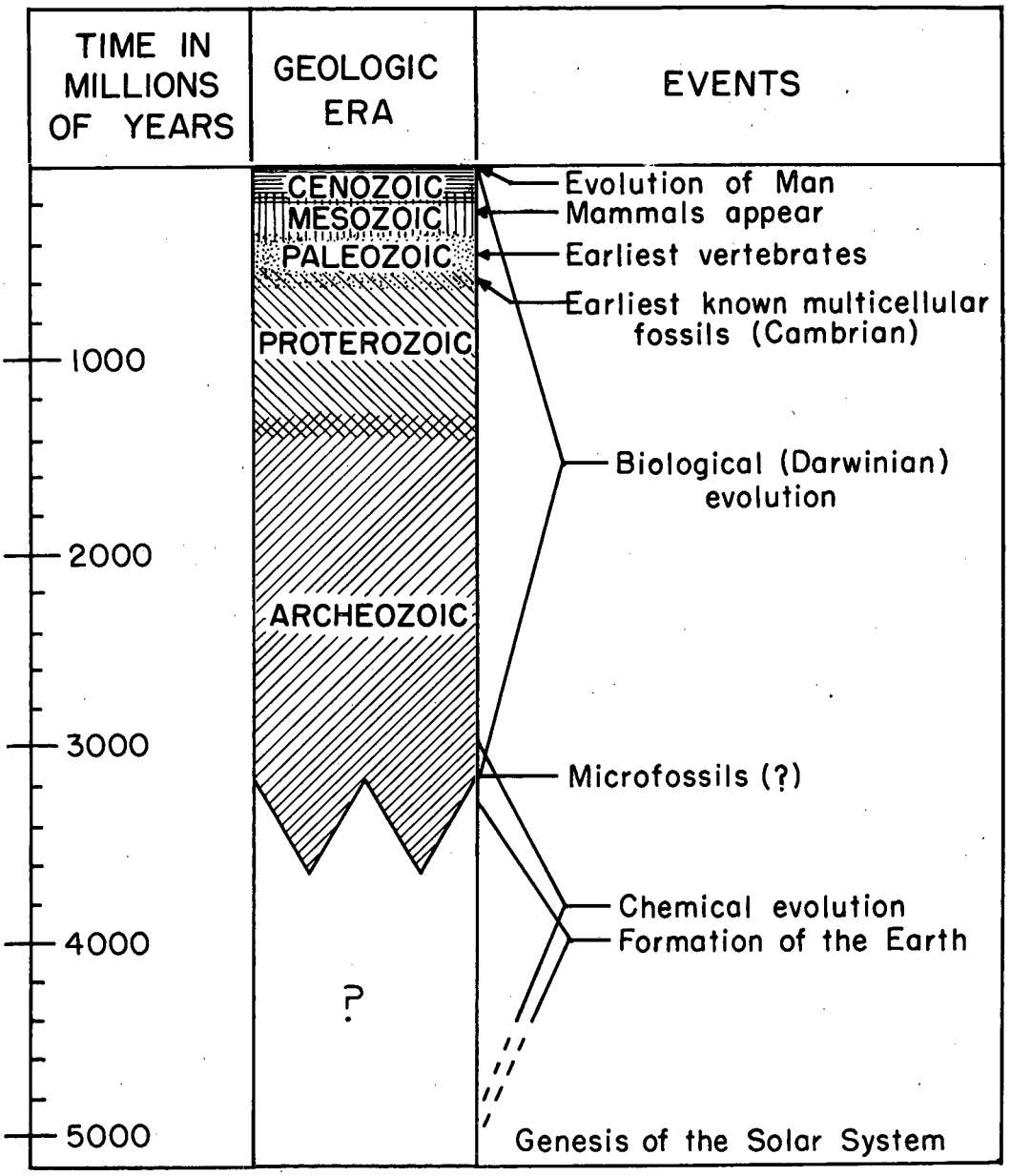
Figure 28 Separation of membrane current into components carried by Na and K (Hodgkin, p. 58)

Figure 29 Time course of sodium and potassium conductance associated with depolarization (Hodgkin, p. 59)

Figure 30 Theoretical solution for propagated action potential and conductances (Hodgkin, p. 63)

Figure 31 Diagrams illustrating the local circuit theory of nerve conduction (Hodgkin, p. 32)

Figure 32 Simplified flow diagram of the nervous system (Katz, Scientific American, Sept. 1961))




MU-9665-B

Fig. 1

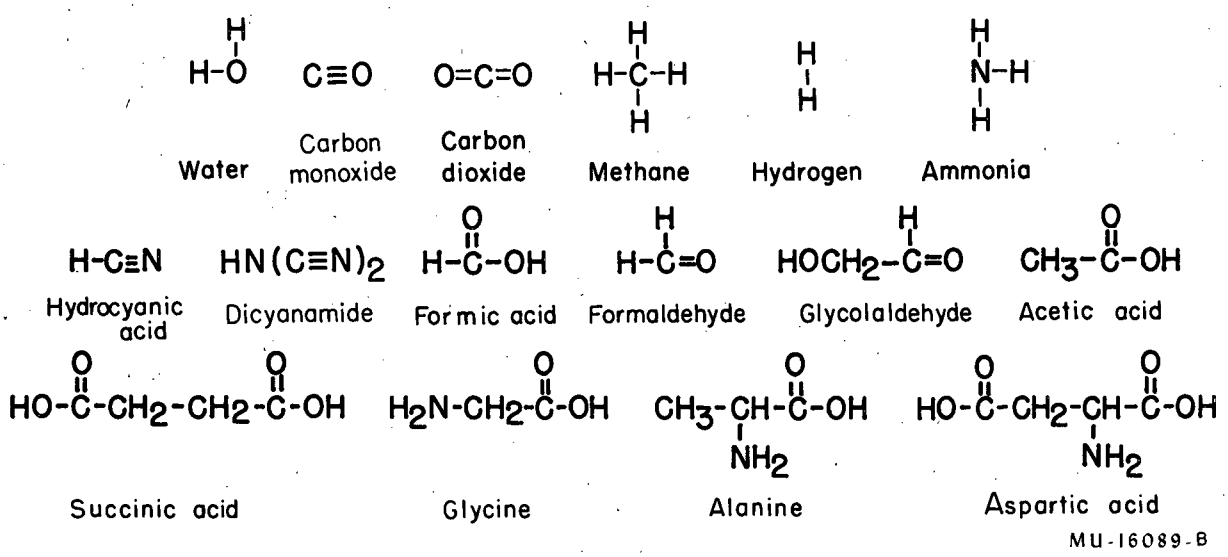

Water
Carbon monoxide
Carbon dioxide
Methane
Hydrogen
Ammonia
Hydrocyanic acid
Dicyanamide
Formic acid
Formaldehyde
Glycolaldehyde
Acetic acid
Succinic acid
Glycine
Alanine
Aspartic acid
MU-16089-B

Fig. 2

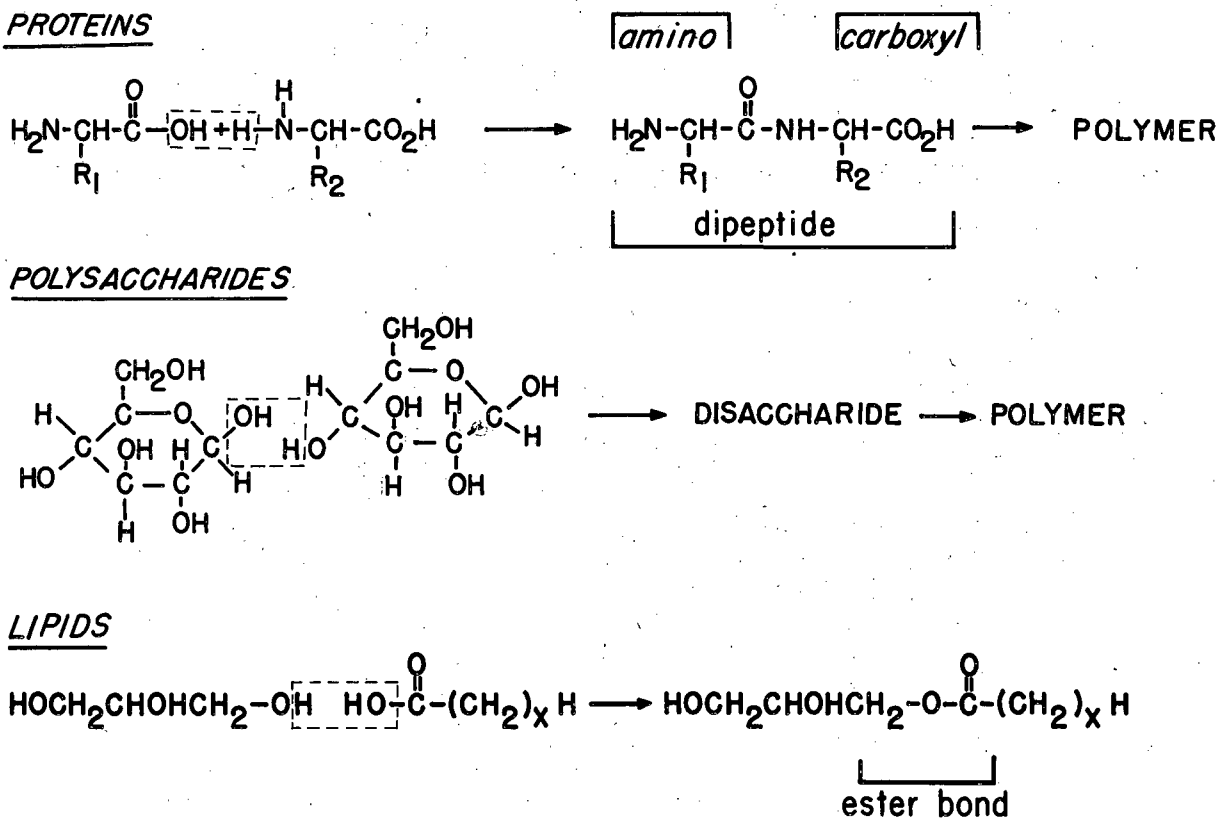

PROTEINS
amino
carboxyl
POLYMER
dipeptide
POLYSACCHARIDES
DISACCHARIDE
POLYMER
LIPIDS
ester bond
MUB-5261

Fig. 3

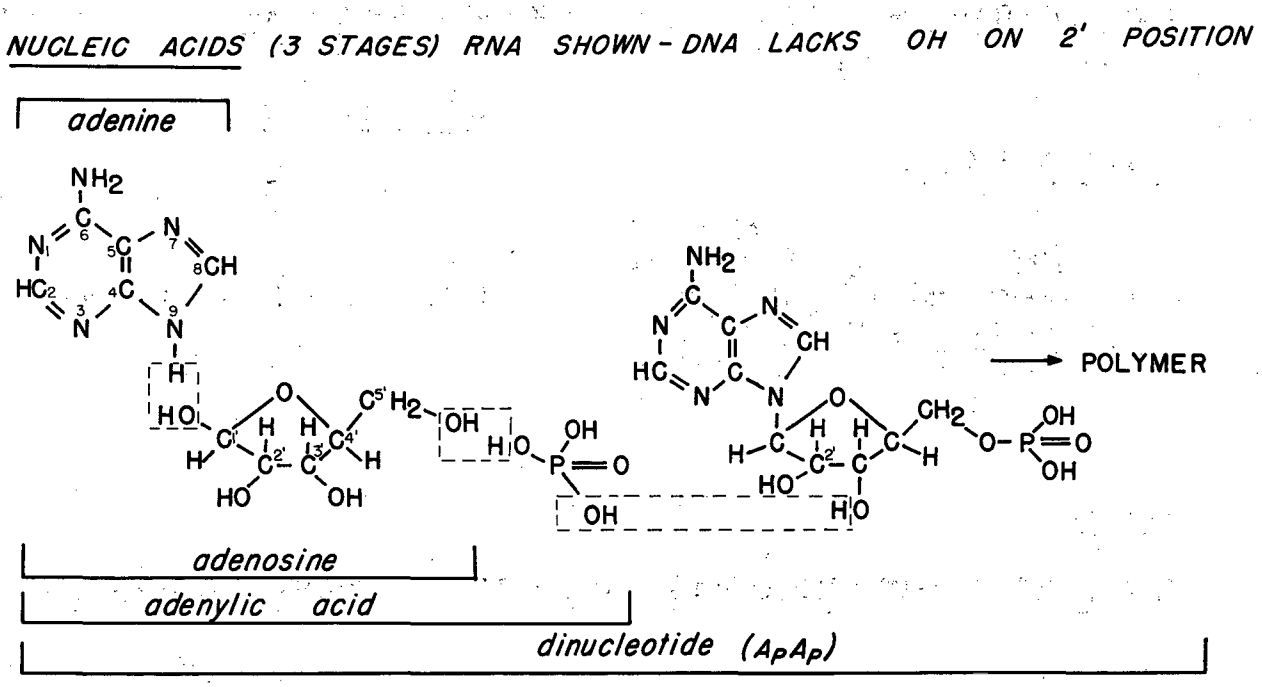
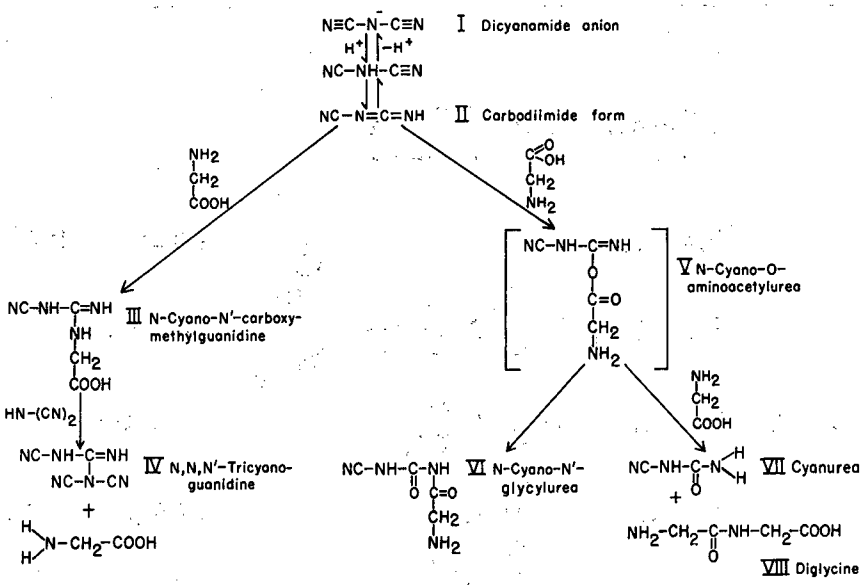
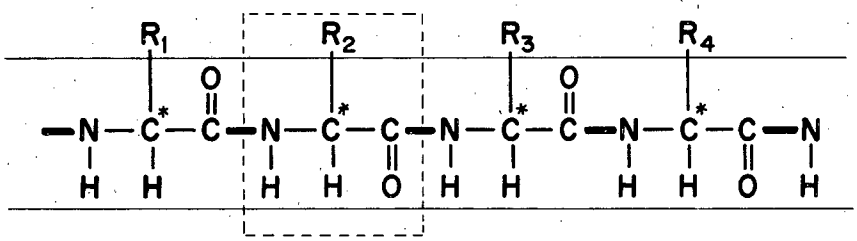

NUCLEIC ACIDS (3 STAGES) RNA SHOWN - DNA LACKS OH ON 2' POSITION
adenine
POLYMER
adenosine
adenylic acid
dinucleotide (ApAp)
MUB-5262

Fig. 4




MUB 13012

Fig. 5

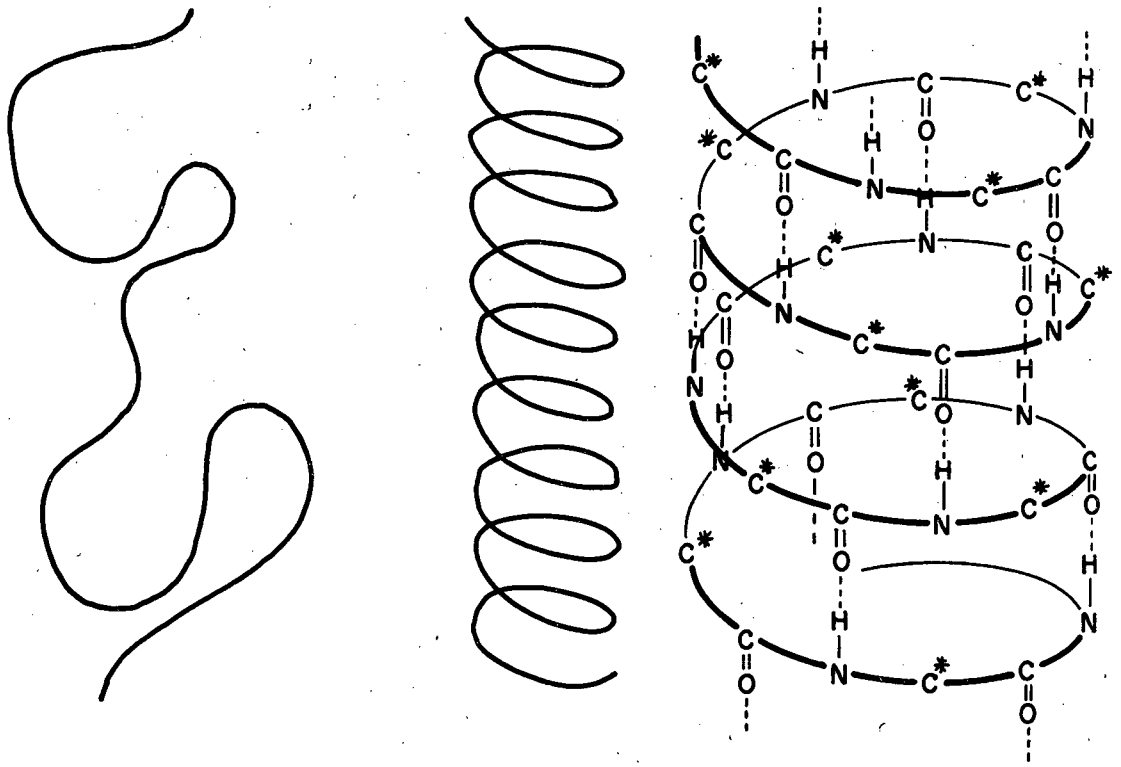



| NAME | EXAMPLES OF R | |
|---|---|---|
| ALANINE | $R_1 = -CH_3$ | (ALKYL-HYDROPHOBIC) |
| ARGININE | $R_2 = -CH_2\ CH_2\ CH_2-N(H)-C(=NH_2^{\oplus})-NH_2$ | (BASE) |
| GLUTAMIC ACID | $R_3 = -CH_2-CH_2-C(=O)-OH$ | (ACID) |
| TYROSINE | $R_4 = -CH_2-C_6H_4-OH$ | (AROMATIC) |
| HISTIDINE | $R_5 = -CH_2-C_3H_2N_2$ | (HETEROCYCLIC) |

PRIMARY PROTEIN STRUCTURE

MUB-7507

Fig. 6



RANDOM COIL α-HELIX

SECONDARY PROTEIN STRUCTURE

MUB-7506

Fig. 7

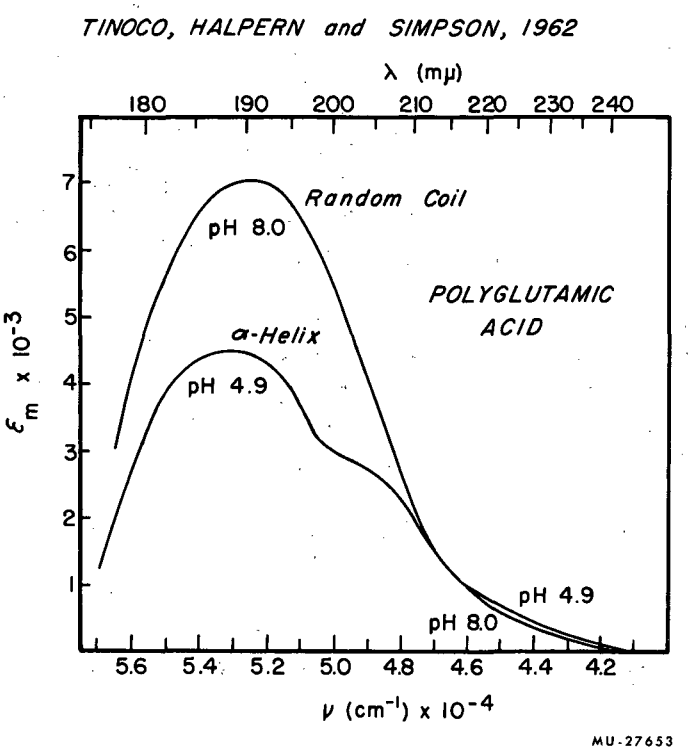
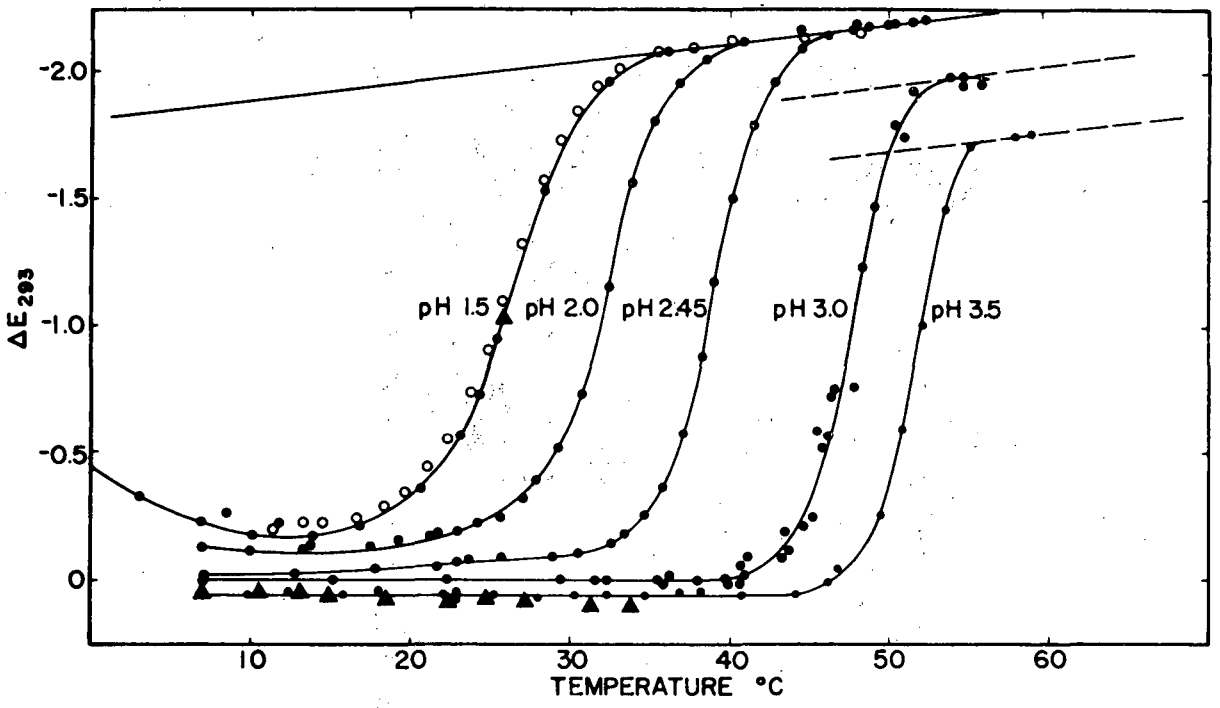

TINOCO, HALPERN and SIMPSON, 1962
λ (mμ)
180
190
200
210
220
230
240
Random Coil
pH 8.0
POLYGLUTAMIC ACID
α-Helix
pH 4.9
pH 4.9
pH 8.0
$\varepsilon_m$ x $10^{-3}$
7
6
5
4
3
2
1
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
ν ($cm^{-1}$) x $10^{-4}$
MU-27653

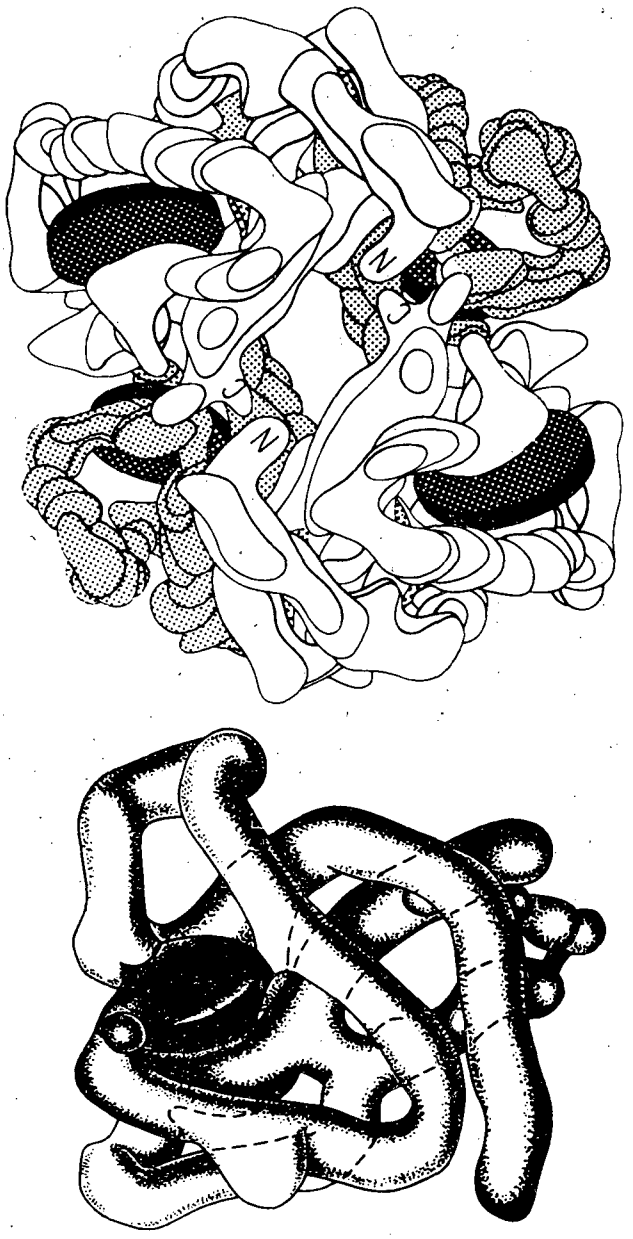
Fig. 8




**Change in Extinction Coefficient as a Function Temperature for α-Chymotrypsin at Various pH.** (R. Biltonen, 1965)

MUB-9867

Fig. 9




Myoglobin (after Kendrew) Hemoglobin (after Perutz)

MUB-12986

Fig. 10

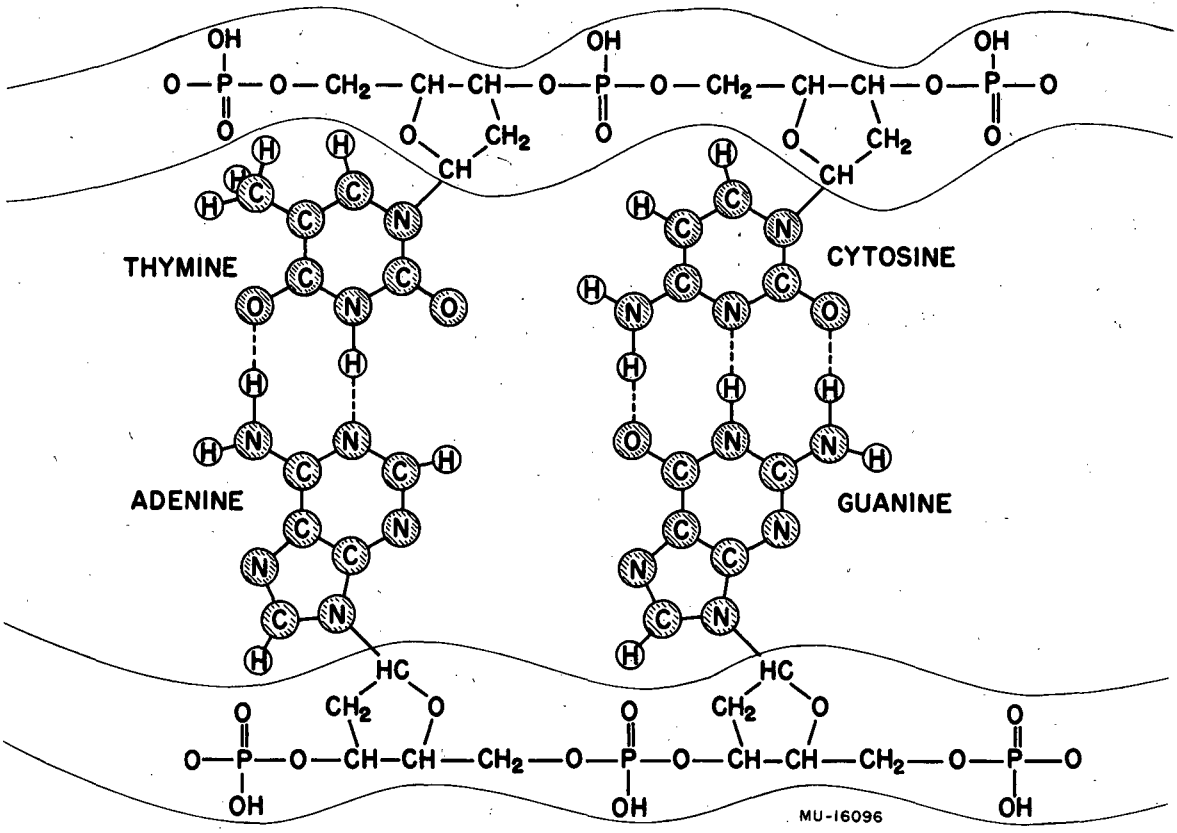



Fig. 11

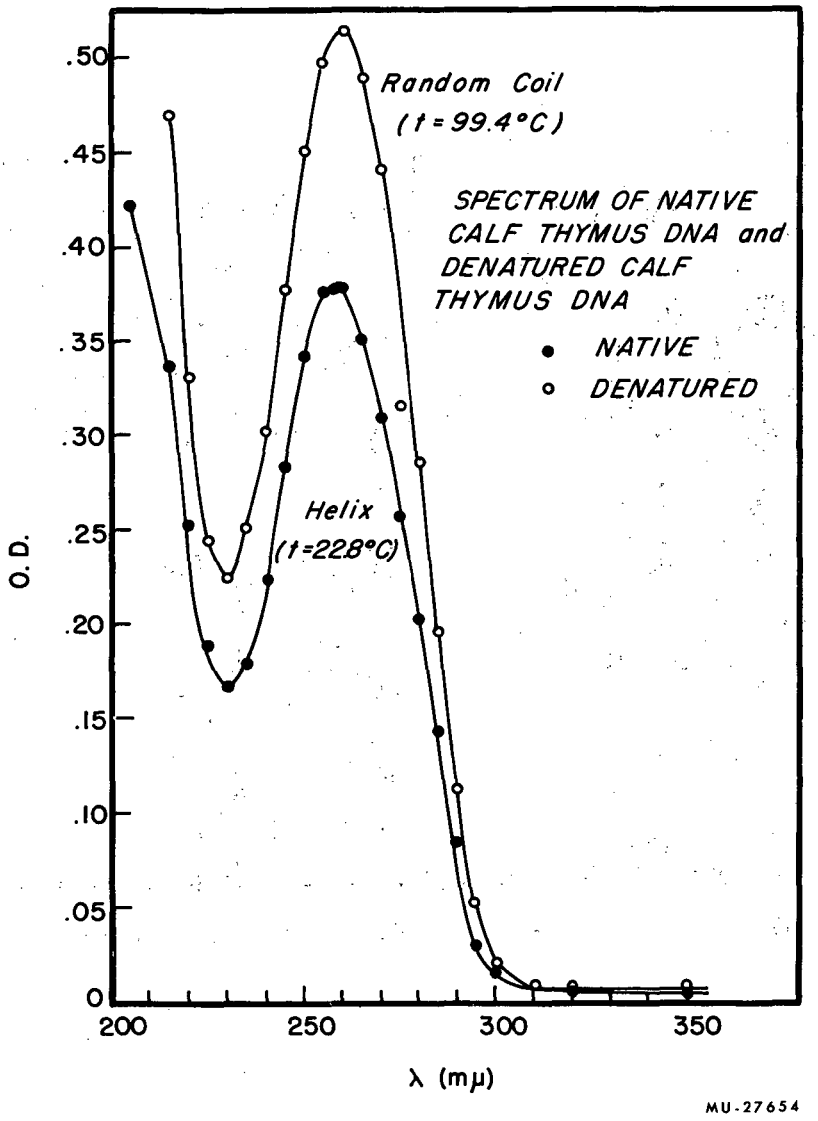
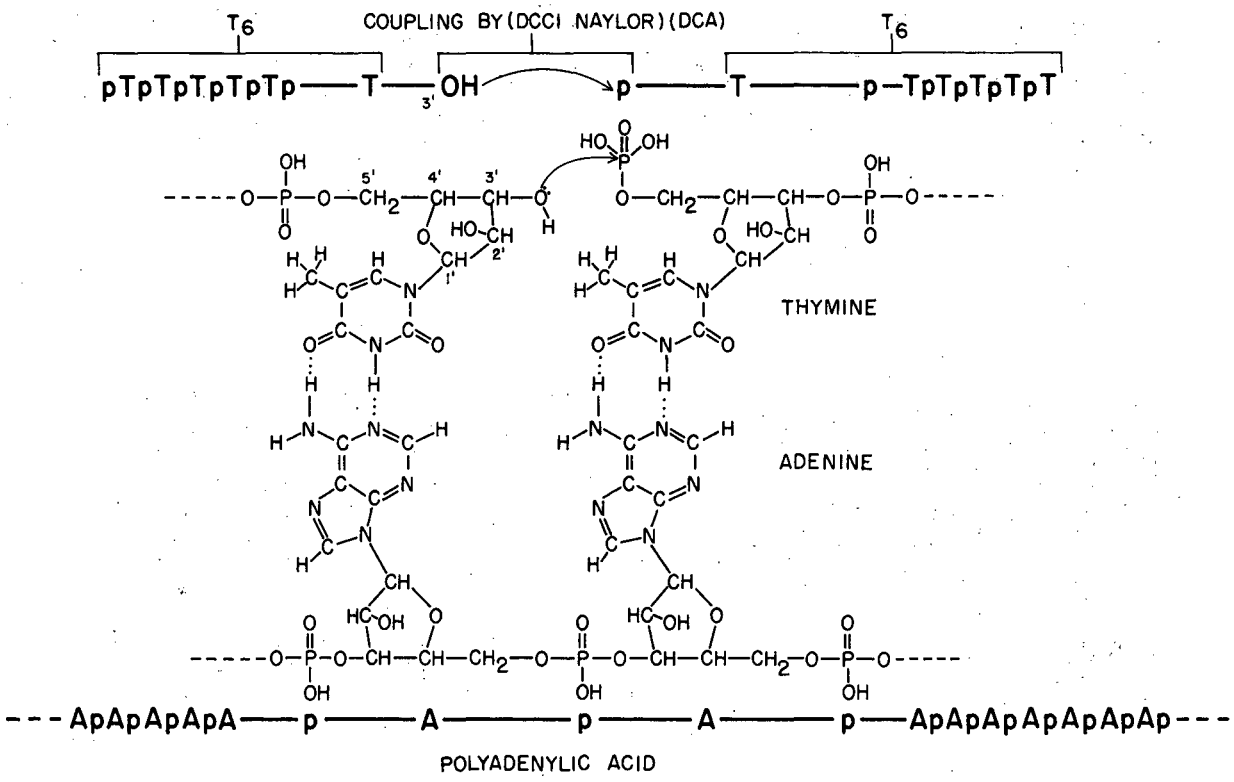

Random Coil
(t = 99.4°C)
SPECTRUM OF NATIVE
CALF THYMUS DNA and
DENATURED CALF
THYMUS DNA
NATIVE
DENATURED
Helix
(t=22.8°C)
O.D.
.50
.45
.40
.35
.30
.25
.20
.15
.10
.05
0
200
250
300
350
λ (mμ)
MU-27654

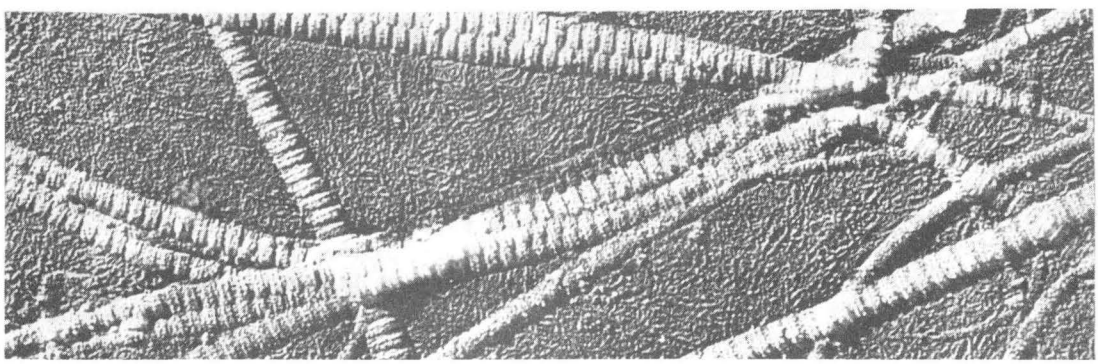
Fig. 12




MUB-10896

Fig. 13

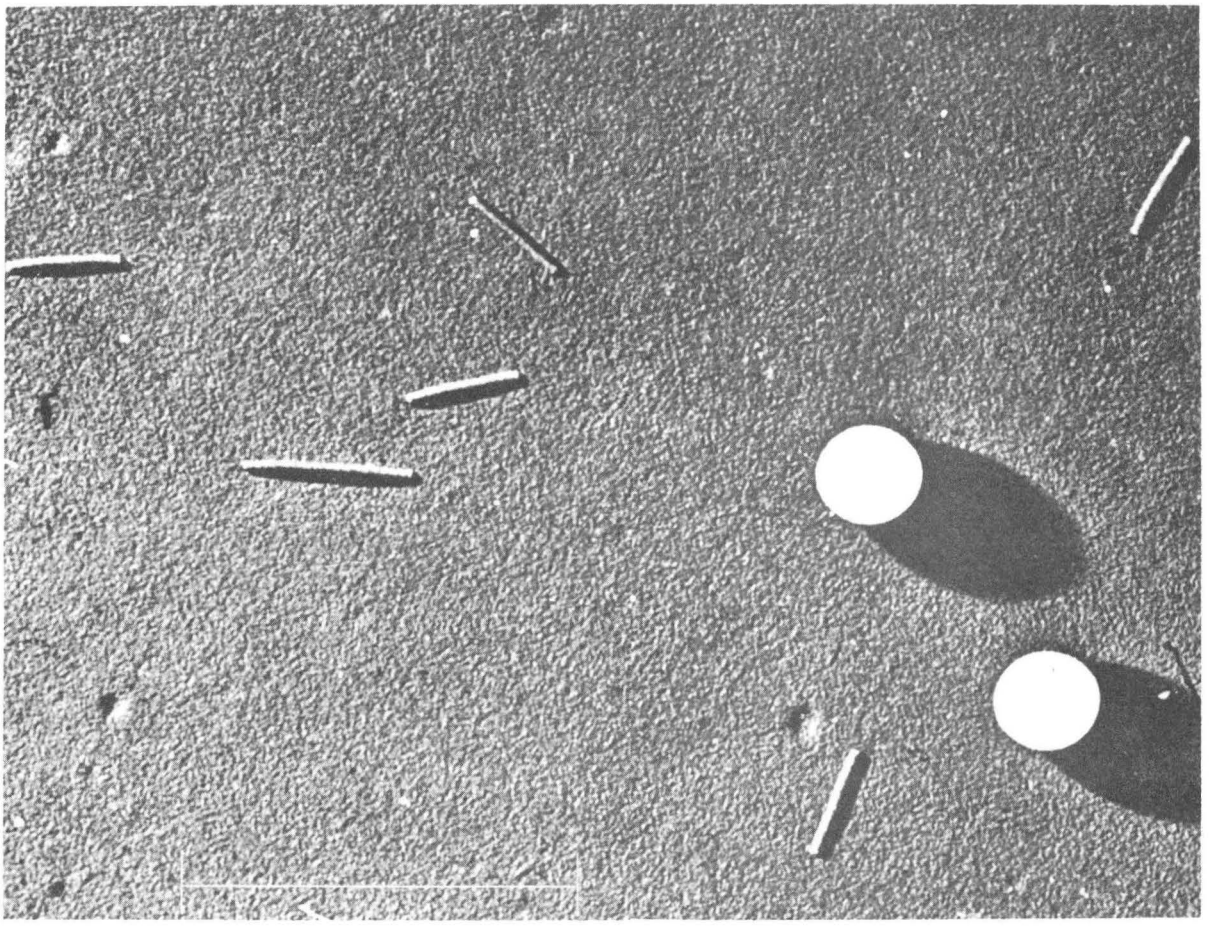
FILAMENTS OF COLLAGEN, a protein which is usually found in long fibrils, were dispersed by placing them in dilute acetic acid. This electron micrograph, which enlarges the filaments 75,000 times, was made by Jerome Gross of the Harvard Medical School.



FIBRILS OF COLLAGEN formed spontaneously out of filaments such as those shown above when 1 per cent of sodium chloride was added to the dilute acetic acid. These long fibrils are identical in appearance with those of collagen before dispersion.

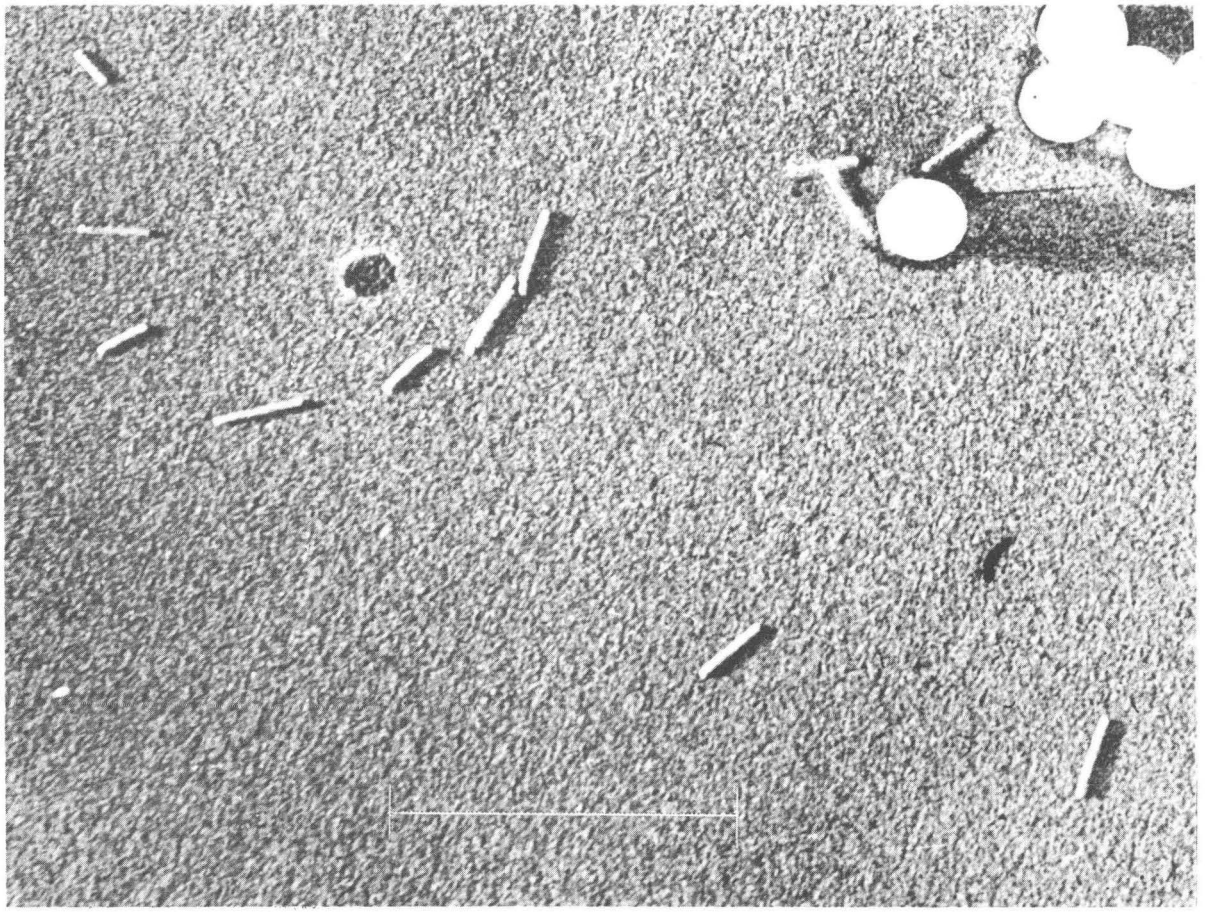
ZN-3215

Fig. 14



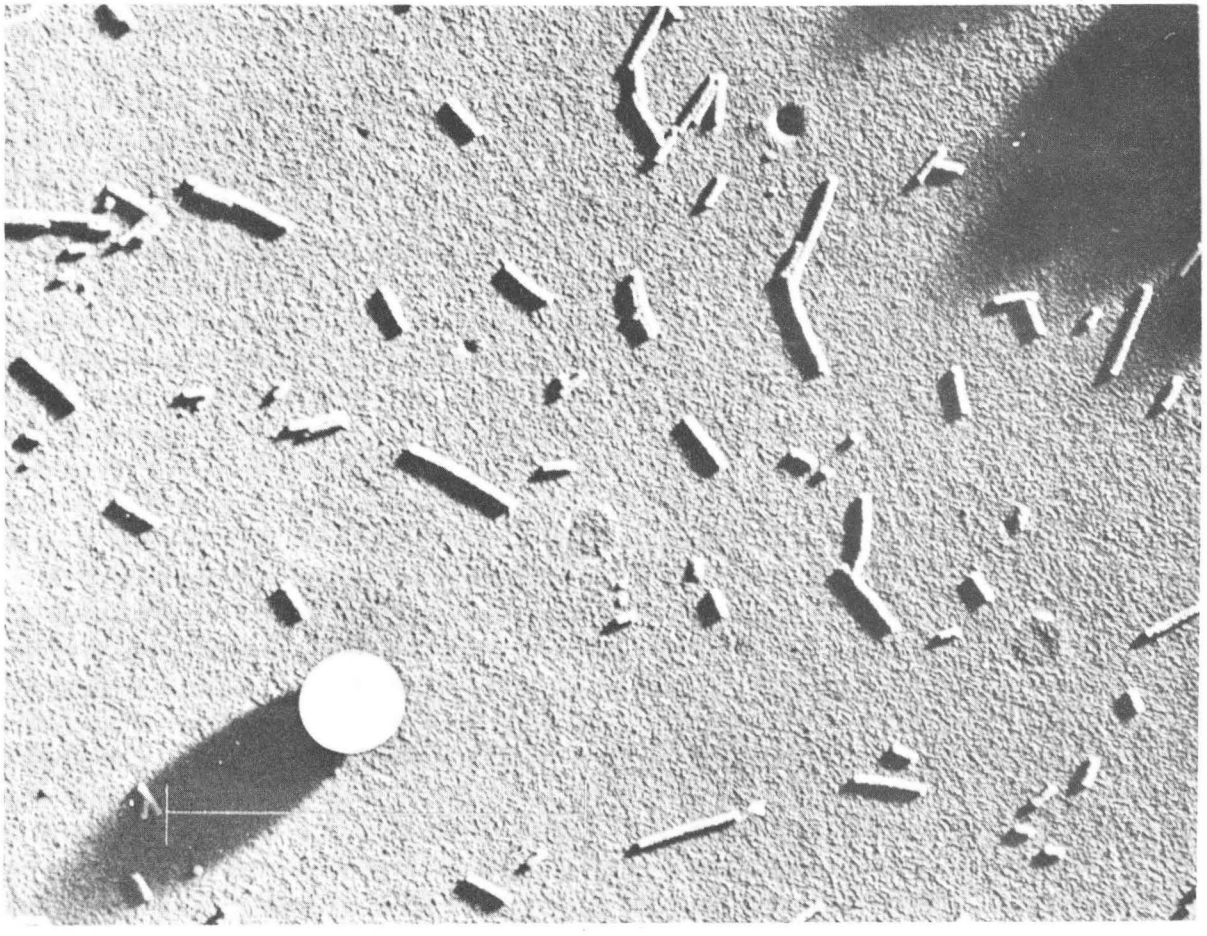
ZN-2289

Fig. 15



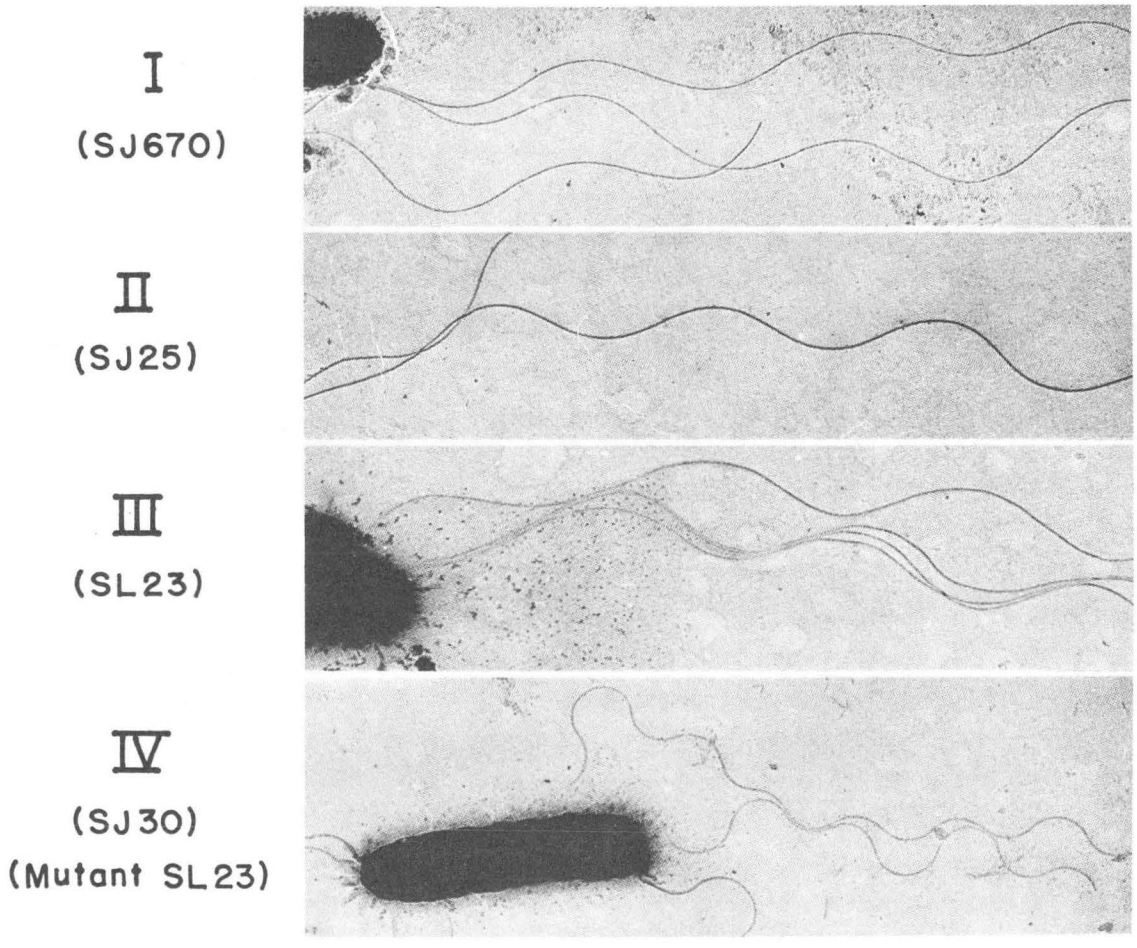
ZN-2287

Fig. 16



ZN-2288

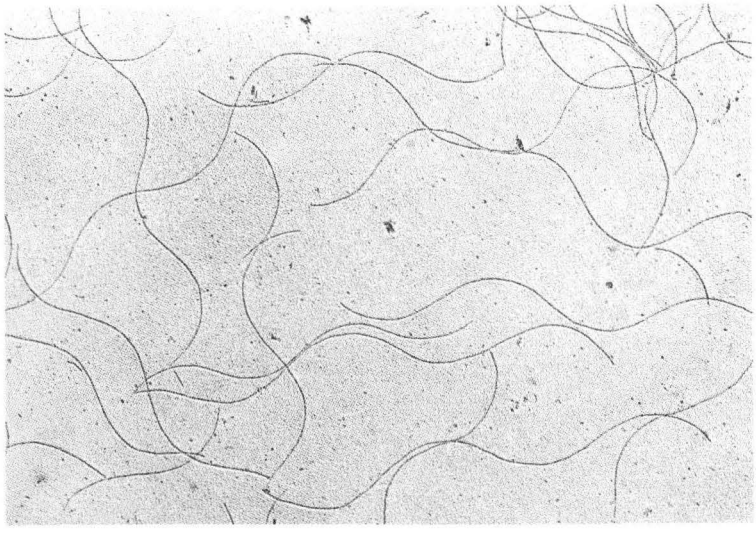
Fig. 17




FOUR KINDS OF FLAGELLA *SALMONELLA*
(Asakura, Eguchi, and Iino, 1966)

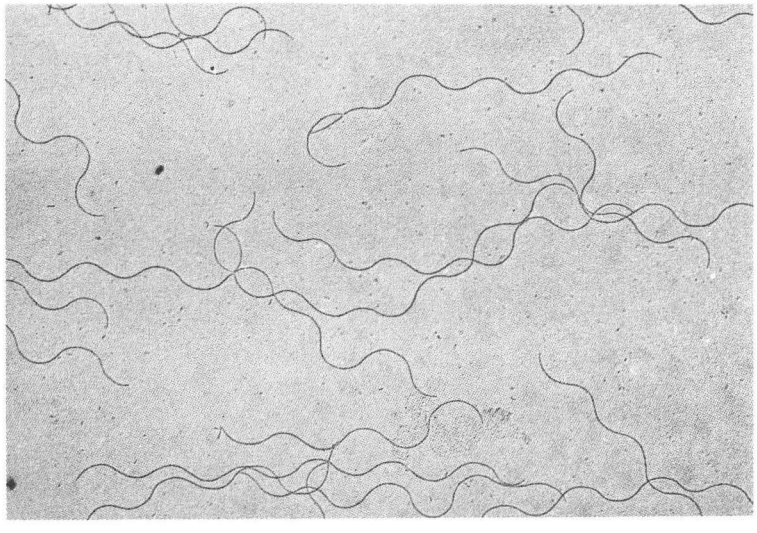
ZN-5854

Fig. 18



III (mono) / III (seed)

r = 20
6.0 mg/ml
0.1 M – NaCl
0.005 M – $CaCl_2$
pH 7.8
28°C – 5 hr.



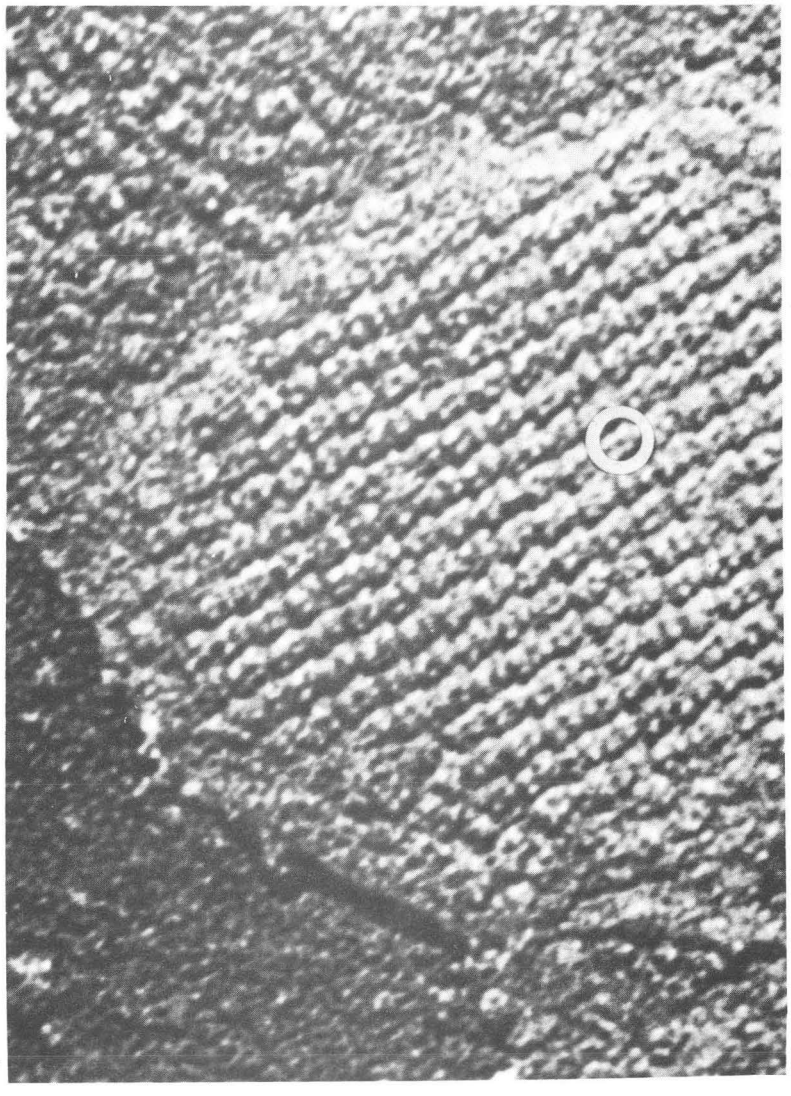
IV (mono) / III (seed)

r = 20
4.5 mg/ml
0.15 M – NaCl
pH 7.8
23°C – 3 hr.

RECONSTITUTED FLAGELLA
(Asakura, Eguchi, and Iino, 1966)
(X 15,000)

ZN-5855

Fig. 19



ZN-5021

Fig. 20

ZN-5856

Fig. 21

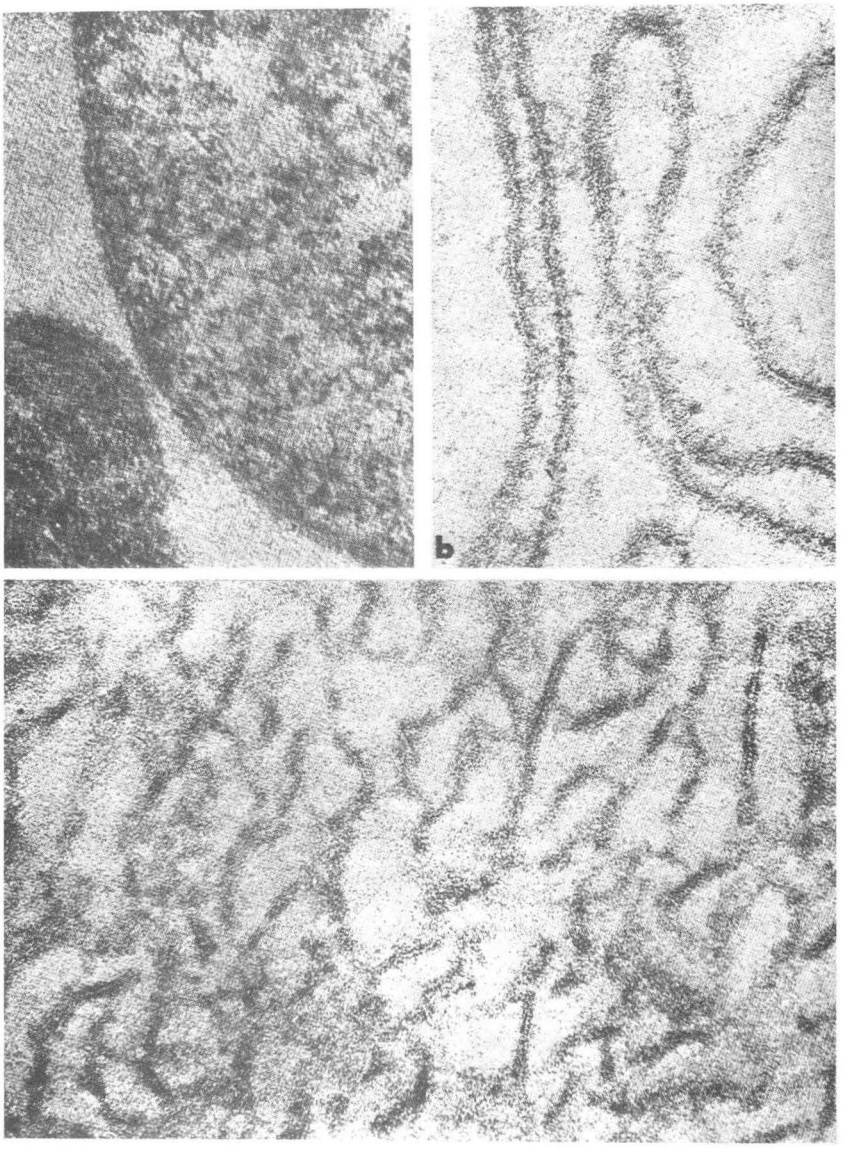
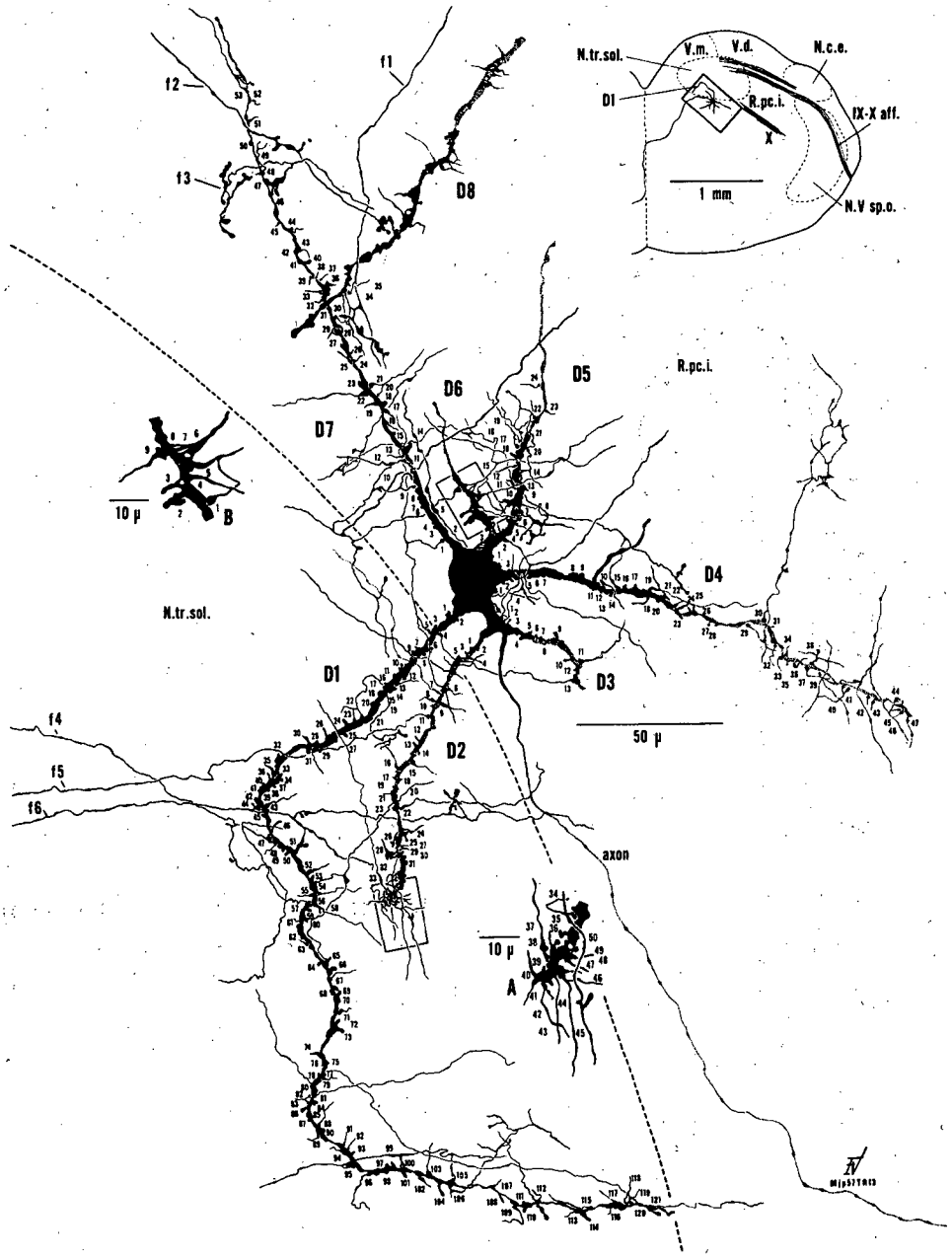



FIG. 3.—Electron micrographs of thin sections of: (*a*) cells of *Mycoplasma laidlawii*; (*b*) isolated membranes; (*c*) reaggregated membrane material. Picture magnification × 163,000.
[Razin et al., Proc. Nat. Acad. Sci. 54, 219 (1965).]

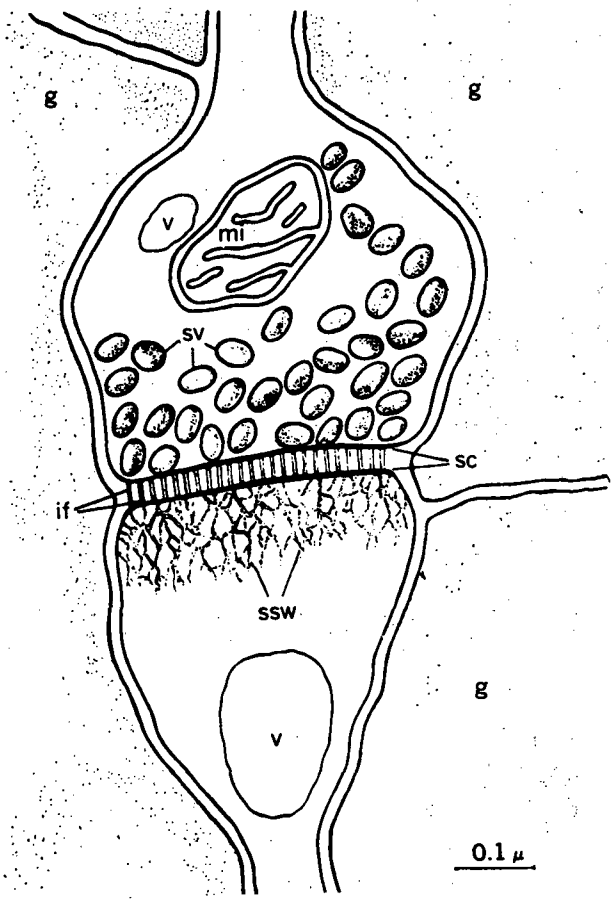
ZN-5857

Fig. 22




MUB-8258

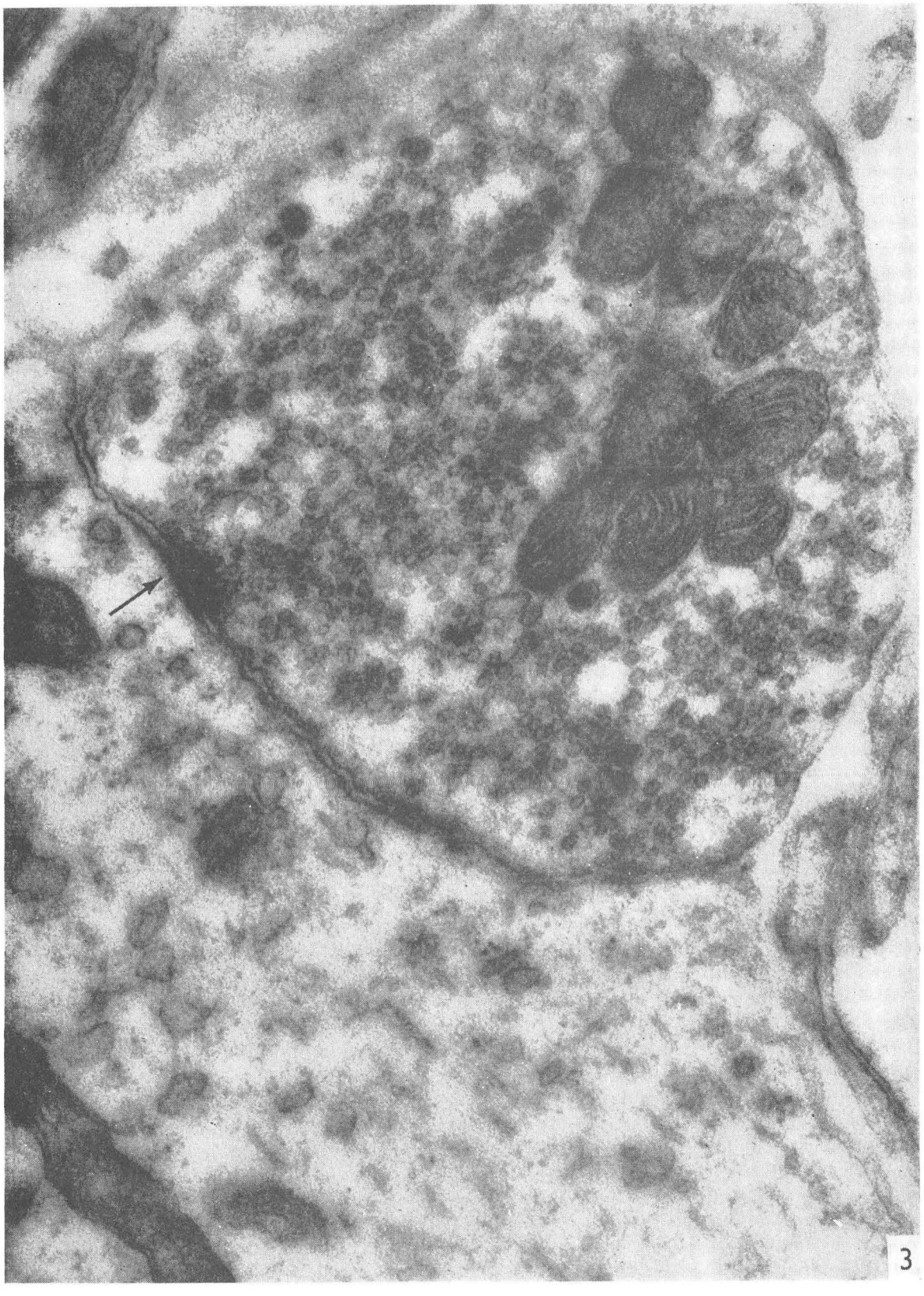
Fig. 23



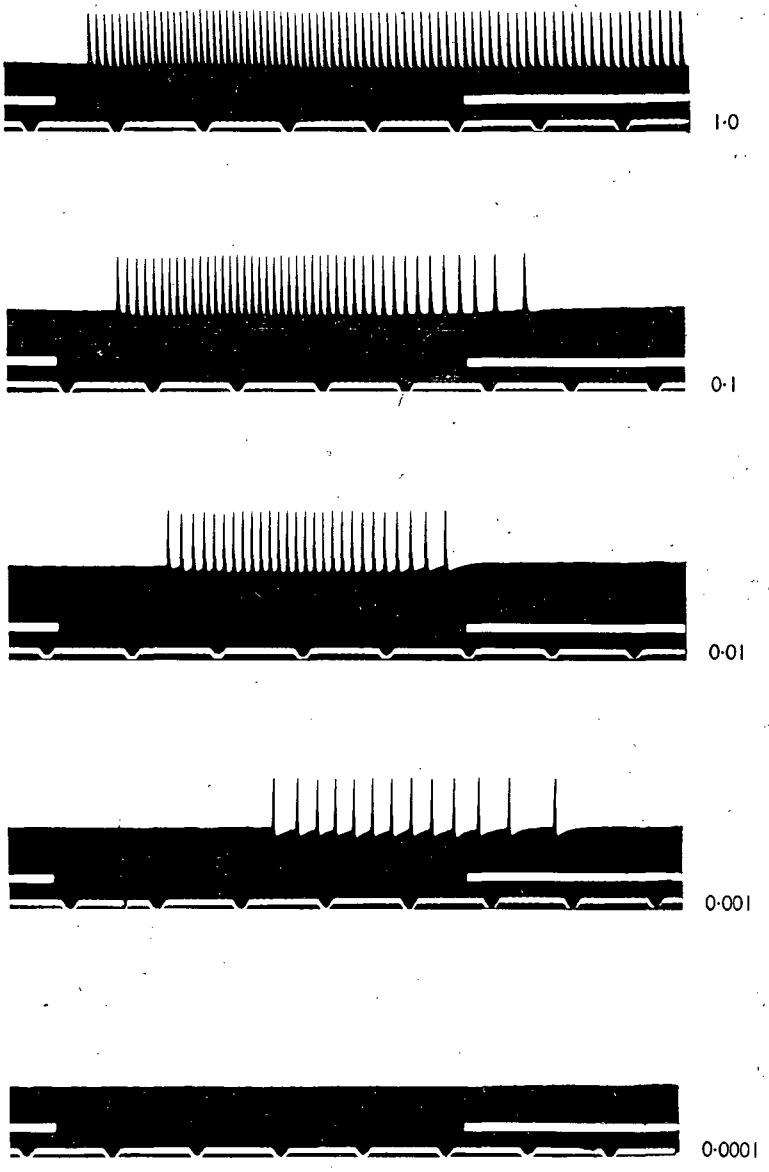

A common form of synapse in the mammalian brain, according to de Robertis (1962). The axonal (presynaptic) side above; the dendritic (postsynaptic) side below. *g*, glia; *if*, intersynaptic filaments; *mi*, mitochondria; *sc*, synaptic cleft; *ssw*, subsynaptic web; *sv*, synaptic vesicles; *v*, vesiculate body.

MUB 12985

Fig. 24

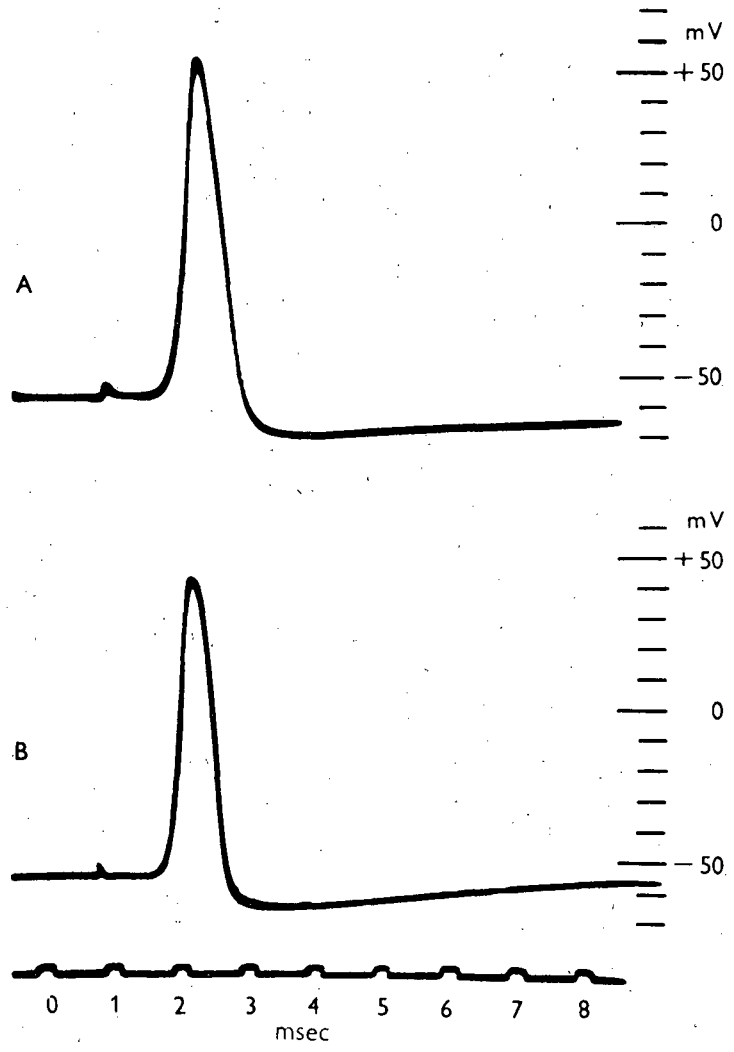



*Experimental Cell Research, Suppl.* 5 Fig. 25 Palay (1958)



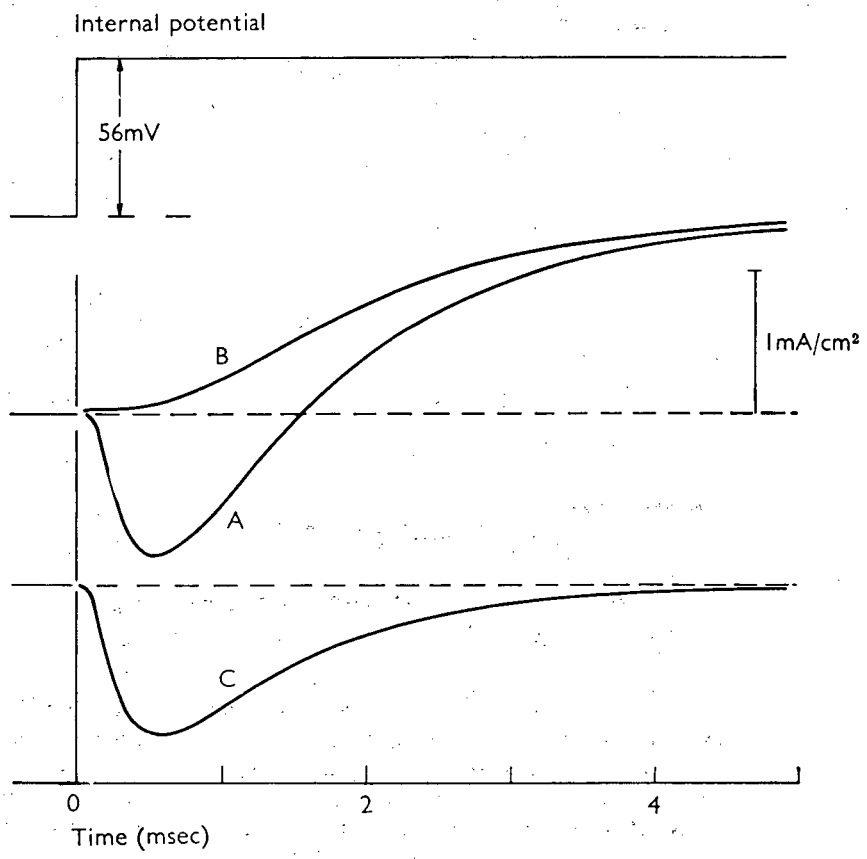

Impulses set up in optic nerve fibre of *Limulus* by one second flash of light with relative intensities shown at right. The lower white line marks 0·2 second intervals and the gap in the upper white line gives the period for which the eye was illuminated. (From Hartline, 1934.)

Fig. 26



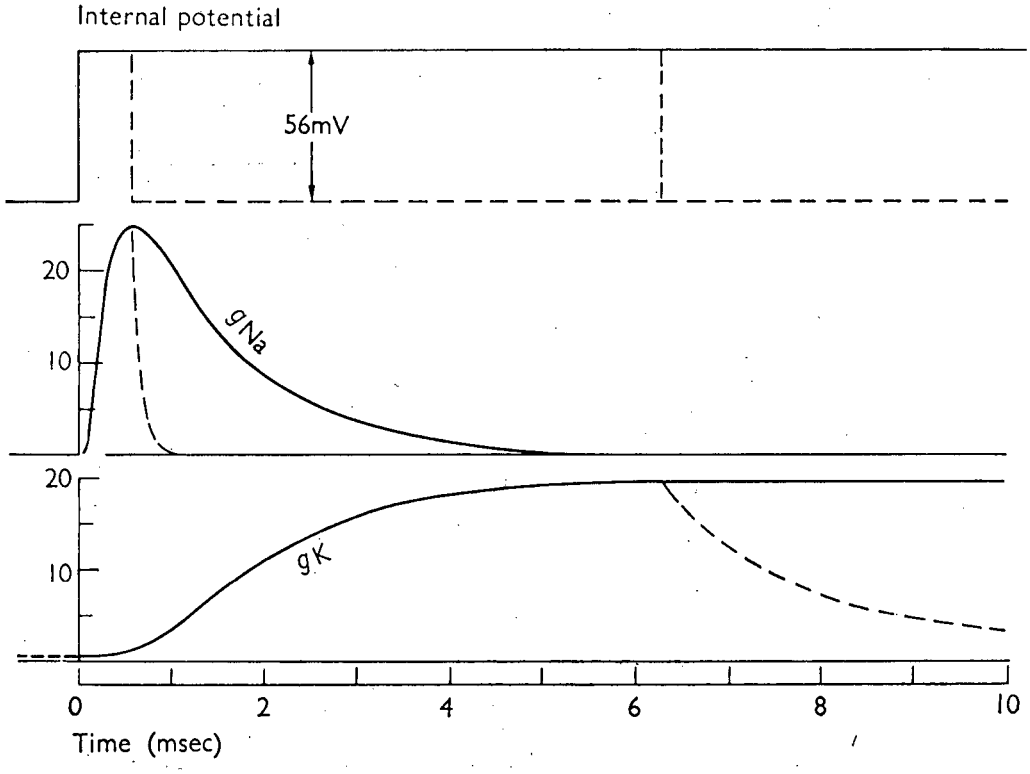

A, action potential recorded with internal electrode from extruded axon filled with potassium sulphate (16° C); B, action potential of an intact axon, with same amplification and time scale (18° C). The voltage scale gives the potential of the internal electrode relative to its potential in the external solution—with no correction for junction potential. (From Baker, Hodgkin & Shaw, 1961.)

Fig. 27



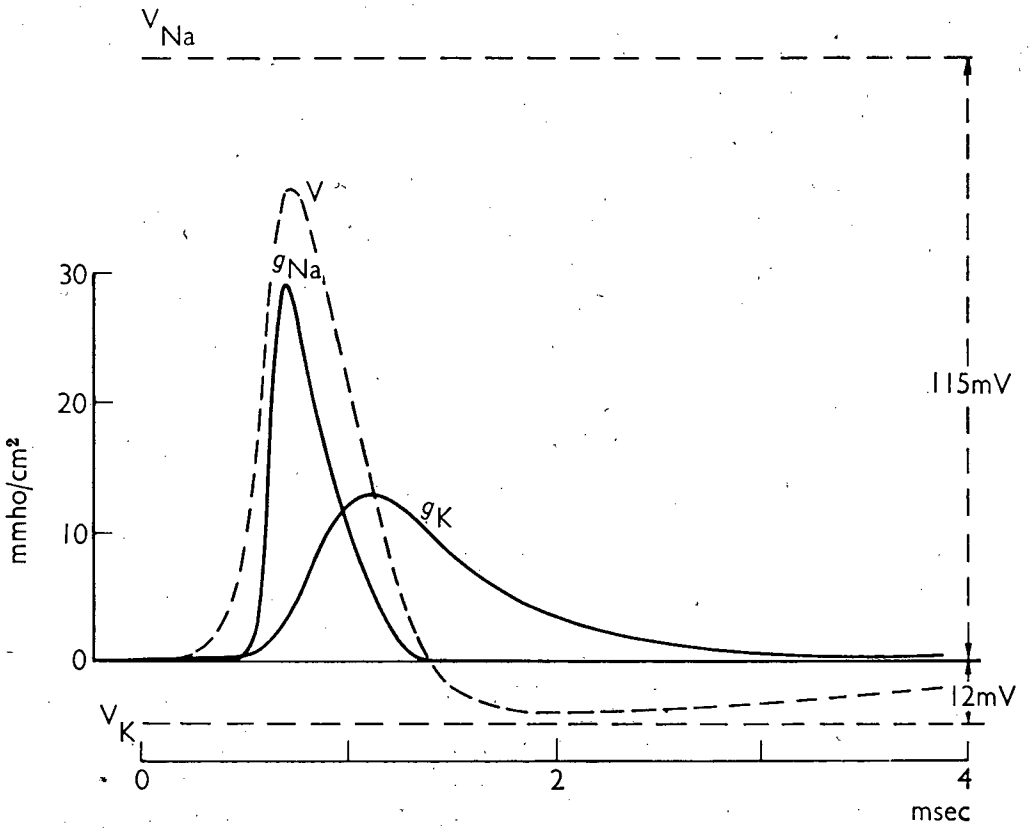

. Separation of membrane current into components carried by Na and K; outward current upwards. A, Current with axon in sea water $= I_{Na} + I_K$. B, Current with most of external Na replaced by choline $= I_K$. C, Difference between A and B $= I_{Na}$. Temperature 8·5° C. (From Hodgkin & Huxley, 1952a.)

Fig. 28



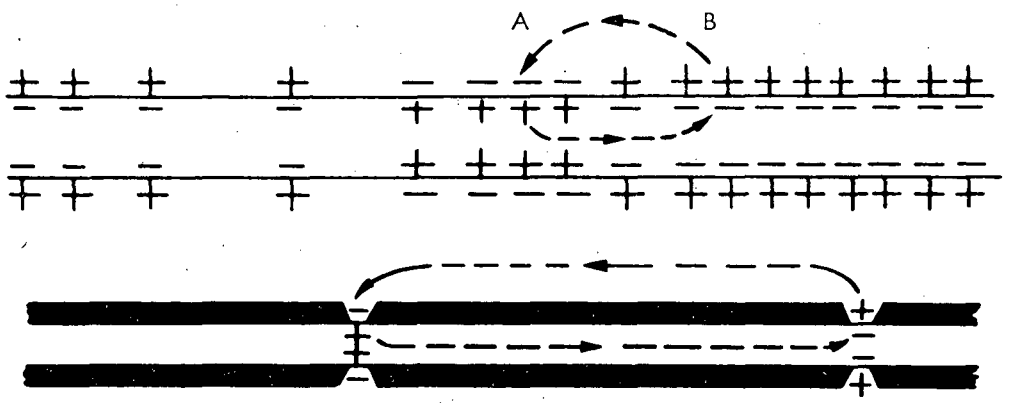

Time course of sodium conductance ($g_{Na}$) and potassium conductance ($g_{Na}$) associated with depolarization of 56m V; vertical scale in mmho/cm². The continuous curves, which were derived from those in Figure 27, are for a maintained depolarization; broken curves give the effect of repolarizing the membrane after 0·6 or 6·3 msec. (From Hodgkin, 1958, based on Hodgkin & Huxley, 1952a & b.)

Fig. 29




Theoretical solution for propagated action potential and conductances at 18·5° C. (From Hodgkin & Huxley, 1952d.) Total entry of sodium = 4·33 pmole/cm²; total exit of potassium = 4·26 pmole/cm².

Fig. 30




Diagrams illustrating the local circuit theory; the upper sketch represents an unmyelinated nerve fibre, the lower a myelinated nerve fibre.

Fig. 31

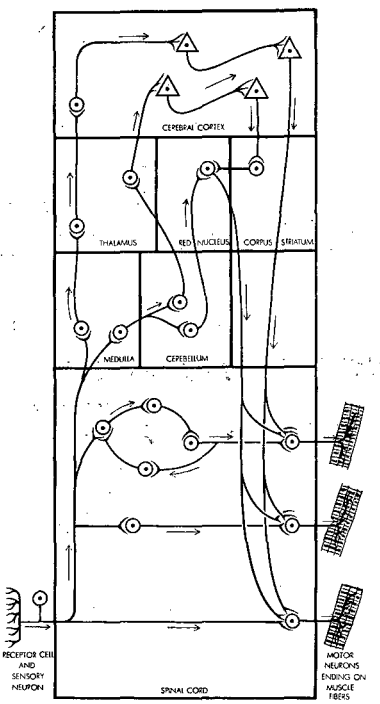

CEREBRAL CORTEX
THALAMUS
RED NUCLEUS
CORPUS STRIATUM
MEDULLA
CEREBELLUM
RECEPTOR CELL AND SENSORY NEURON
SPINAL CORD
MOTOR NEURONS ENDING ON MUSCLE FIBERS

Fig. 32