My title is taken from "thesis, antithesis, and synthesis". Scientific concepts grow in depth, HEGEL said, when a thesis is opposed by an antithesis and the differences are finally resolved by synthesis of the two originally opposed concepts. But there is always the danger that either the thesis or the antithesis may become a doctrine; so universally accepted that it becomes automatically the tool for explaining everything. And POPPER has said that a scientific theory differs from an unscientific theory in only one way: The proponent of an unscientific theory can explain every phenomenon in his field by his theory. He cites psychoanalysis. This view is common now among biochemists. MESELSON, a leader of the new nucleic acid chemists, once told me that all biology can now be explained in the terms of the chemistry of nucleic acid. In journalese, biochemistry has solved the "Secret of Life." But life is incredibly more complicated than the most sophisticated chemist can imagine. And I refuse to accept this doctrine. Complex phenomena are usually explained scientifically by simplification. But simplification versus complication implies a fundamental incompatibility. Introspective study always leads to the disclosure of more complexity than had previously been suspected. Simplification is only possible if a small, isolated, restricted field has been artificially (that is, scientifically) segregated and analyzed. Simplification is essentially the function of a limited outlook. But progress in scientific thought can only be achieved by such an artificial device. And this is the scientific dilemma. Biologists have always been forced to tolerate contradictions and disagreements because no theory broad enough to encompass the entire field has been proposed. Only a person with a limited outlook, or one who has discovered a universal law, can make broad generalizations. Seasoned geneticists with wide biological experience have always regarded the gene as devout as well-informed Christians regard the divinity of Christ. They do not find it too difficult to accept an invisible, unspecifiable God or a truly mystical Holy Spirit as divine. But a man, born of woman, who went through the normal developmental stages and came to a comparable end, poses a credibility problem. STURTEVANT and BEADLE wrote an outstanding textbook of genetics. But after it was published, they discovered that the gene meant something different to each of them. STURTEVANT, characteristically, did not go beyond the experimental facts and said there can be no doubt that certain regions of the chromosome, defined by crossing over, are concerned with certain specific functions. BEADLE said that he regarded the gene as a unit of function. MULLER was hopelessly romantic about the gene; MORGAN was more detached; he took a noncommittal course, exploiting the various manifestations of a single locus to prove the existence of the gene. But GOLDSCHMIDT said bluntly that the gene didn't exist. About the same time SCHROEDINGER, the mathematical physicist, wrote a small book entitled 'What Is Life?' He was romantically emotional but the only biologist who quoted SCHROEDINGER, to my knowledge, was MULLER. He found SCHROEDINGER'S views consistent with his own mystical belief that the gene is a Prime Mover. Many of the younger generation of biochemists have quoted SCHROEDINGER and have said furthermore that it was his book that led them to abandon their original fields of either physics or chemistry in order to study the gene. There was no
doubt in SCHROEDINGER's mind about either the reality or the particu­larity of the gene. I am reminded of FRITZ ZWICK's reaction to a paper written by BRIDGES concerning the size of the gene. BRIDGES set up a scale of different values beginning with the size of the electron, the molecule and so forth. ZWICKY said that only a person with no knowledge of physics would dare to say anything about the size of the electron. In this connection it is interesting that both SCHROEDINGER and EINSTEIN were profoundly disturbed about the theories of quantum mechanics formulated by the Logical Positivists who insisted that any reference to reality implied mysticism, and that any mathematical formulation that worked must be accepted. (I remember graduate stu­dents in Cal Tech saying that poor old EINSTEIN had lost his touch, he couldn't understand quantum mechanics.) EINSTEIN wrote SCHROEDINGER (this is an exchange of correspondence) "I think you have hit the nail on the head. The HEISENBERG-BOHR tranquilizing philosophy or religion is so delicately contrived that for the time being it provides a gentle pillow for the true believer from which he cannot very easily be aroused. So let him lie there. But this religion has so damned little effect on me that I cannot make head nor tail of it mathematically. My brain is also too worn out by this time." SCHROEDINGER answered, "It seems to me that the concept of probability is terribly mishandled these days. The quantum mechanics people act as if probabilistic statements were to be applied just to events whose reality is vague. The proper basis of reality is set aside as trivial by the positivists. The present quantum mechanics supplies no equivalent. It is not conscious of the problem at all. It passes it by with blithe disinter­est." And EINSTEIN responded "You are the only contemporary physicist besides LAUE who sees that one cannot get around the assump­tion of reality, if only one is honest. Most of them simply do not see what sort of risky game they are playing with reality. Reality as something independent of what is experimentally established. Only one of the tools of our trade remains - the field concept - but God knows whether this will stand firm. I think it is worthwhile to hold on to this, that is the continuum, as long as one has no really sound argument against it."

It is clear from the correspondence between EINSTEIN and SCHROEDINGER that both believed that faith in the continuum superseded any mathematical logical reality. But the gene was as real to SCHROEDINGER as the quantum was unreal.

KENNETH REXROTH in a review of Taoism described what he thought to be the difference between Western and Eastern thought: "Chinese thought implies that the beginning and end of knowledge are the same thing, as are intuitive apprehension of reality as a totality, before and behind the data of sense, or the construction of experience and reason. The Tao Te Ching insists over and over that this is both a personal and psychological and a social, moral and even political first principle. At the core of life is a tiny steady flame of contempla­tion. If this goes out the person perishes although the body and the brain may stumble on, and civilization goes rapidly to ruin. The source of life, the source of the order of nature, the source of knowledge, the source of social order are all identical. The imme­di ate comprehension of reality beyond being and not being, existence and essence, beginning and becoming are of prime importance. Contact with this reality is the only kind of power there is. Against that effortless power, all self-willed acts and violent attempts to rule self and/or natural processes are delusion and end only in disaster."

EINSTEIN's attitude is precisely that of the Chinese philosopher, that is, an intuitive apprehension in reality before and behind the data of sense, or the construction of experience and reason. Taoism is not contrary to Western epistemology. This same philosophy guided the greatest scientist of the Western world throughout his lifetime. When EINSTEIN's theory was contradicted by the experiments of a most
fond physicist, POINCARÉ was grateful that theoretical physics was at least free from Relativity. Einstein simply ignored the results.

LEW STADLER was a dedicated biologist. Although he gave little evidence in his published work of his deep and underlying interest in fundamentals, it was always clear to those of us who worked closely with him. He was always seeking for new approaches, asking for new outlooks and hoping to find unique and different ways of looking at questions. He was amazingly impatient and quick to disregard materials or ideas that did not seem fruitful, no matter how much energy or thought one had expended on them. He was interested in tetrad analysis of smuts and when my exhaustive survey of the problem indicated certain limitations with regard to smuts, his interest disappeared immediately. Jerry and I are deeply grateful for the year that he gave us to organize our lives and to work freely on the application tetrad-analysis to radiation-induced mutation. This study led to freedom from teaching and thirty years of study of yeast genetics. Today we are able to answer those questions Lew asked thirty years ago that could not be answered then, and would not be answerable now, without his help.

STADLER in a posthumous paper on the gene dealt with the differences of opinion on the nature of the gene. I have pointed out that every geneticist had some doubt about the gene. Even MORGAN (I might say especially MORGAN) described his uncertainties concerning biologically-effective mutants and experimental (or laboratory) mutations. Why do these differences of opinion exist? What is the basis of different attitudes? I believe they can only be understood by living through all the individual experiences inseparable from the individuals concerned and sharing the social, political and genetical backgrounds of these individuals. Men with a wealth of experience and a long and intense interest in a subject reach their conclusions by means that it is impossible for them to communicate or for others to understand. Every scientist's inferences are as completely individual as the person himself. Lew understood this and had the courage and the interest and the humanity to attempt comprise. But genetical concepts are now being based on the biochemical rather than the biological approach. These concepts have continued to pass through a long series of simplifications by restricting the analysis to narrower and narrower fields. It seems that at present with molecular analysis of viruses that the ultimate limit of simplification must have been reached. The proponents of the study of nucleic acids of viruses consider it to be a system that will explain not only genetics but all biology. The current concept that DNA is central to the problem of the living-state is dangerously near to becoming an indisputable doctrine. It could destroy or drastically diminish the opportunity for other philosophically more meaningful biological research. One united scientific community of logical positivists has reduced biology to the biochemistry of a specific molecule. But the biologists are still as divided, as confused, as unsatisfied, as uncertain and, generally, as silent as before. A sophisticated, divided elite can never compete with a united, unsophisticated community. I think the origin of the difficulty may be pinpointed to SCHROEDINGER's unfortunate book in which the most extremely oversimplified concept of the gene was presented to biochemists and physicists. This book inspired hundreds of them to quit what they were doing, mostly because of their guilt consciousness due to the atomic bomb, and turn to biology to forget their unhappiness.

The situation has progressed to its present state by the continued silence of the divided biologists. It is difficult to see how it could have been avoided. A simplicist approach is so appealing to the uninformed; the desire to conform is so general; the fear that one will be ridiculed, isolated, or even liquidated, if he does speak out, all lead to silent acceptance to what the most experienced and knowledgable biologists recognize as an absurdity. Biologists have surrendered their freedom to dissent, while biochemists, unacquainted with the basic
complexities take over. Of course, biologists have themselves to blame. They should have laid bare their uncertainties, but indoctrination, community-pressure and confusion prevented. There was a conflict between the geneticists as specialists and the environmentalists that forced the geneticists to close ranks and snub the opposition. On the defensive, geneticists did not say that genes were important components of the living state, but that they held the "Secret of Life." Now we are suffering the consequences. The biochemist speaks of the genes of viruses as "molecules." But the classical gene that recombinates at crossing over, during the pachytene of meiosis certainly does not exist in a virus. In summary, simplification has been over-simplified by those who have neither knowledge of nor concern with the philosophical problems that are basic to the consideration of the living state, by those who in EINSTEIN's words have lost touch with "reality as something independent of what is experimentally established."

LITERATURE CITED


GOLDSCHMIDT, R. B. 1955 Theoretical Genetics, University of California Press.


SCHROEDINGER, E. 1945 What is Life. Cambridge.


STURTEVANT, A. H. and G. W. BEADLE 1939 An Introduction to Genetics W. B. Saunders Co.
In these days when high school students of biology are taught about DNA, RNA and protein synthesis and when each month dozens of papers appear reporting results obtained with complicated and highly sophisticated techniques involving the use of very expensive equipment, it may not be amiss, and I hope you will be interested, in the facts and concepts which guided cellular biologists in earlier decades of cell research. I have worked with chromosomes and genes all of my scientific life and I propose to tell you in an anecdotal way about the problems in which I have had a great interest and of the tools with which we had to work.

I will tell you the reasons why I undertook a study of the chromosomes of mammals, in the early twenties, followed by an attempt to find the morphological sites of genes along the Drosophila chromosomes, and of the circumstances leading up to salivary gland chromosome work which localized certain genes to a single band of a salivary gland chromosome. Before World War II, I had been much intrigued by studies made, at this early period, trying to get light on how chromosomes and genes worked. As a result, when, in the mid-thirties, the molecular structure of proteins was demonstrated to be long chains of amino acids linked together by peptide bonds, and that which we call DNA and RNA are also long macromolecules of nucleotides, I sought to understand how these huge molecules behaved in the several mechanisms which are known to lay down, in the cytoplasm of oocytes, materials needed by the embryo during early cleavage stages. Then I want to tell you about some work on the royal jelly gland of the bee and will end up with some comments on the ultrastructure of cell organelles, on which I had worked earlier with a light microscope.

As I look backward over fifty years of work, I am impressed by the role which good fortune, luck, or call it what you will, plays in the selection of material which is favorable for your study and of the importance of being in the right place at the right time! And I include in the term "place", associations with stimulating and cooperative colleagues. In this respect I have been singularly fortunate, as you will see. I often tell graduate students that research is much like deer hunting. You have to be in the right place at the right time to see your prey and, of course, you must carry a loaded gun and know how to use it.

I will start my story with studies beginning in 1920. At that time the cytologist had as tools, a compound microscope, machines for cutting thin sections and a few common chemicals for the preservation of tissues and dyes that stained either basic or acid structures of cells.

By 1920 because of the work of McClung, and E. B. Wilson, and many
many others we knew that sex determining chromosomes occurred in insects and other invertebrates and from the work done by members of the Morgan School, especially DR. STURTEVANT, we knew that genes lie in linear order along chromosomes. But very little was known about the chromosomes of vertebrates. There was, of course, no good reason to doubt that mammals would show sex chromosomes, but no one had been able to demonstrate them. This was due in part to the use of histological fixatives which were inadequate for the preservation of small cells with high numbers of chromosomes, but mostly to the fact that successful fixation requires an instant and intimate contact of dividing cells with the fixative agents.

For a study of mammalian chromosomes I happened to be in the right place at the right time. For in our laboratory, the late CARL HARTMAN was making his pioneering studies in the development of the opossum and we had these animals in every cubby hole of our laboratory! As it turned out, I could not possibly have selected a more favorable material, for the opossum has a relatively low diploid number of chromosomes - 22 - and the sex chromosomes are, by far, the smallest elements in its genome. With an abundance of material available I was able to work out the entire story including the identification of the sex chromosomes in the polar bodies of opossum eggs. The opossum was the first mammal for which we knew the diploid number of chromosomes and the first time that the sex chromosomes had been identified in mammals.

From the opossum, I naturally turned to another mammal and that was man. Again I happened to be in the right place at the right time, for one of my former premedical students was practicing in a state mental institution in Austin where, for therapeutic reason, they occasionally castrated male individuals. This physician, knowing my interest in mammal chromosomes, made it possible for me to preserve, within thirty seconds or less after the blood supply was cut off, a human testis. From my study of the opossum I knew what to look for in primary spermatocytes of man and there I found an X-Y complex quite similar in morphology to the X-Y of the opossum. In order to make the evidence complete, it was desirable to know the diploid number of chromosomes in man and after months of searching I found a few spermatogonial metaphase plates in which I could count the chromosome number. Although in some metaphase plates I could find only 46 chromosomes my best cell showed 48 and I reported this as the true diploid number for man. But in the past decade, through a study of somatic divisions - tabu in the twenties - treated with colchicine derivatives it has been definitely established that man normally carries 46 chromosomes. The identification of the X-Y complex in the male has stood the test of time, and this was the main point of interest to me in making this study.

I do not need to tell my audience that for the past decade, people from all over the world have been studying the chromosomes of man, using tissue culture cells, in order to determine if deviations from the normal chromosomal pattern will explain certain obscure conditions, fortunately rare, which appear in man. There are today many examples that show this is true. The XXY sex complex results in the Klinefelter's syndrome. If a female lacks one X chromosome, as sometimes happens, this results in Turner's syndrome. Down's syndrome or a Mongolian idiot is produced when one of the smallest chromosomes is triplicated, and so on.

In the mid-twenties, my former colleague, the late H. J. MULLER made his discovery that X-rays produce gene mutations and bring about chromosome breakage, followed by chromosome rearrangements such as deletions, inversions and translocations. From his genetic analyses MULLER knew what marker genes had been separated by a given break. One day I found MULLER down on the floor with a pipette trying to recover some ovaries which he had spilled from a dish. As skillful as he was in genetic analysis, he didn't have great skill in handling such small
material. So I suggested to him, I think I caught him just at the right time, "Why don't you let me study those ovaries and tell you where the oogonial chromosomes have actually been broken?" Again, it was a case of being in the right place at the right time! MULLER furnished me with female Drosophila carrying a translocation and by examining oogonial metaphases I would determine how much of an exchange had taken place. In 1932 we published together a map of the X chromosome at metaphase in which it was shown that about 2/5ths of the proximal part of the X chromosome carried no known gene - or to put it another way, that the genetic crossover maps of the X in Drosophila consisting of some 70 units, lay entirely within the distal 3/5ths of the chromosome and that the remaining 2/5ths carried no known genes except for bobbed. We call this genetically inert area, following the terminology of HEITZ, heterochromatin.

The oogonial chromosomes of Drosophila are extremely small and error due to foreshortening or tilting, made it hazardous to estimate how much of the right arm of the third chromosome, for example, was really missing. For the more exact location of gene loci, larger chromosomes were needed. But where could one find such large elements in Drosophila?

After MULLER left the University of Texas for Russia, I continued to examine broken chromosomes and it happened that one day, while packing ovaries into a pupa case, I included a short fragment of a salivary gland. When sections were examined, I recognized salivary gland cells with their permanent spireme that BALBIANI had described about 1882. Perhaps, here were the large chromosomes that I had been looking for! But for the first larvae I dissected a white background was used, and I was unable to identify the transparent glands. So I reached over my laboratory table and stained the larval organs with aceto-carmine. Now it happens that the aceto-carmine (or acetic-orcein) is the only fixative stain suitable for the study of salivary gland chromosomes. Knowing that others had made smear preparations of salivary glands which were useless, I decided to try simply squashing the nuclei and there were five worm-like chromosome components spread out before me. I have often wondered what would have happened if I had used some other method of staining than aceto-carmine and the squash technique! No matter, whether it was pure chance, or good luck, I had what I had been looking for and after a year's work I published a paper showing the position of many genes along the X-chromosome.

Many of you are quite familiar with the salivary gland story. The worm-like structures represent the euchromatic areas of the Drosophila chromosomes. Very early in the development of these glands the two homologous chromosomes undergo a "somatic synapsis", due to a very strong attraction of homologous gene sites, and probably due to the same forces at work in meiosis. The two arms of the V-shaped II and III chromosomes rarely appear connected because their heterochromatic areas are not obvious in the chromocenter. The large diameter of salivary chromosomes is caused by the endomitotic divisions of the original two synapsed homologues. These endomitotic division cycles continue on until the time of pupation.

Salivary gland chromosomes have proved extremely useful, not only for the precise location of gene loci but for a precise study of the changes which have occurred during speciation. My colleagues, the late J. T. PATTERSON and the late WILSON STONE, and their students, have worked on a large number of species of Drosophila and they have found that the main changes which have occurred during speciation are so-called "centric fusions", with or without the loss of one centromere, and inversions either within or between the two arms of the large II and III chromosomes. Occasionally, due to unequal crossing over (possibly due to the presence of heterochromatic bands which occur all along salivary chromosomes) a doublet is formed, as at the "bar" locus. Other than centric fusions, translocations do not seem to play much of a role during speciation in Drosophila. Thus, in the hundreds of
species indigenous to the Hawaiian Islands, one can recognize the band patterns characteristic of all the chromosomes.

In locating gene loci, I used many of the chromosomal rearrangements analyzed by MULLER, and his former student WILSON STONE. Needless to say, I could not have located gene loci without suitable material furnished me by my colleagues and, at the time, I was associated with the only institution in the world, where stocks with rearranged chromosomes were available so that such precise locations of gene loci could have been made!

Salivary gland chromosomes are being widely employed for the solution of problems related to gene activation, by the German cytologists, BAUER, BEERMANN, and their students using the huge salivary chromosomes of Chironomus. Every month, it seems, papers appear involving the experimental use of chemical agents which affect active gene loci.

Some of you may wonder why I did not long continue to work with salivary chromosomes. The answer is simple. My training and my interests are those of a cytologist. I am not a Drosophila geneticist and having colleagues far better able than I to exploit the genetic areas opened up by salivary gland chromosomes, I turned to the problem of the ways genes work.

You well know that very significant discoveries about the structure of proteins and the nucleic acids were made in the mid-thirties. They both are huge macromolecules - proteins being a long chain of amino acids held together by peptide bonds and DNA was thought, at first, to be made up on a long chain of tetranucleotides. With these new concepts in mind, there arose in my mind (incidentally while I was deer hunting), this question: If proteins are as complex in structure as was thought and the DNA of chromatin consists of long macromolecules, how is it possible for a cleaving egg to synthesize de novo materials needed for cleavage divisions occurring at intervals of often less than an hour? Asked in this way, I put together in my mind diverse facts and it was quite clear to me, that the answer was that proteins and nucleic acids of cleavage were not synthesized de novo but were re-assembled from building blocks already present in the cytoplasm of oocytes. Anyone of my vintage knew that during oogenesis there were two main types of mechanisms at work; nurse cells, with the oocyte nucleus remaining relatively inactive, and oocytes in which the egg nucleus, itself, becomes very large and undergoes a complex series of morphological changes. Now let us follow the facts which made this idea clear to me.

In studying sections of ovaries, I had noted not only the large size of nurse-cell nuclei but also the apparent changes in the visible amount of basophilic material present. MULLER and I had often remarked on the occasional large masses of densely staining material present in nurse cell nuclei. The key to the problem was given by the earlier work of JACOBS, who, in 1925, had measured the volumes of nuclei of different sizes in the mouse liver and had found that the commonly occurring three sizes bore a volume relation of 1:2:4 from which he concluded that, in the mouse liver, there had been an inner division of the nuclear contents. And twelve years later GEITLER, working with larval cells of the water strider, Gerris, had shown that as these larval cells increased in size, the number of the heteropycnotic X chromosomes in the male also increased. From this basic observation, GEITLER showed that for nuclei of any given size, there were changes in chromosome configurations paralleling the changes seen during ordinary mitosis. He gave the term endomitosis to the 'inner divisions' of JACOBS.

By the mid-thirties, Feulgen's stain for DNA had come into use so it was possible to determine if the large masses of basophile material in nurse cells were made up of DNA.
A study of nurse cells, which I made with one of my students, MRS. REINDORP, quickly showed that as nurse cells increase in size, they undergo endomitotic divisions. In nuclei about 8µ in diameter, we found all the usual mitotic changes including a close approximation of a metaphase contraction. But no spindle was present, as nurse cells do not divide. Some nurse cell nuclei attain a diameter of 150µ, so we concluded that in these about 8 endomitotic cycles had taken place, which means that such a nurse cell contains the same amount of proteins and nucleic acid to be found in 128 diploid nuclei. Now there are 15 nurse cells in the egg follicle of Drosophila and these are absorbed into the oocyte cytoplasm where all traces of DNA disappear. In 1940, I wrote:

"The evidence, then indicates that in the cytoplasm of all eggs there are products of thousands of maternal chromosomes. Just in what form the constituent proteins and nucleoproteins exist is a matter for the biological chemist to determine. In the meantime, it seems reasonable to conclude that the rapid building up of the cleavage chromosomes is possible in segmenting eggs because the synthesis is more in the nature of a reassembling of already existing materials, such as nucleotides, etc., under the guidance of the active chromosomes, rather than the actual synthesis of the building blocks from relatively simple substances." Please note that I wrote this in 1940.

Now let me jump ahead and report to you that my colleague, DR. JOHN BIESELE and I have studied the fine structure of developing and mature nurse cells with an electron microscope and published our results in 1966. During each endomitotic cycle at prophase numerous nucleoli present in these polyploid cells undergo fragmentation and release into the nuclear sap, and eventually into the cytoplasm, myriads of ribosomes. Nurse cells are connected to the oocyte by protoplasmic bridges and the ribosomes - mostly in a polyribosome complex - enter the cytoplasm of the oocyte. So the nurse cell mechanism not only supplies to the egg's cytoplasm vast quantities of proteins and DNA but also ready made centers for protein synthesis, i.e. polyribosomes.

Having worked out the nurse cell story, it was natural to turn to a study of oocytes with large germinal vesicles and for this purpose, the common toad around Austin was selected for study. This work was done with one of my students, A. N. TAYLOR. As it turned out, the cytological mechanism involved in eggs with germinal vesicles, is different from that of nurse cells but the end result is the same. We reported that vast amounts of DNA, proteins, RNA and other substances are released during oogenesis into the cytoplasm of the toad oocyte.

Very briefly, during the early stages of meiosis, we found that in addition to the leptotene, pachytene and later stages of the chromosomes there are present, just under the nuclear wall, hundreds of extra-chromosomal DNA granules. Small nucleoli rich in DNA develop in association with one or more of these DNA granules and then both the nucleoli, and the chromatin granules disappear. At the same time, a halo of RNA formed in the cytoplasm of the oocyte. We interpreted this to mean, when the paper was written in 1942, that the DNA had been converted into RNA, as BRACHET had just described. Nowadays we would say that these DNA granules act as templates against which ribosomal RNA is synthesized.

The results which TAYLOR and I reported, in 1942, received little attention and in some quarters were greeted with extreme skepticism, quite understandably because, when the oocytes of urodeles were studied, no DNA granules were seen nor was any DNA found in the cytoplasm when chemical tests were applied. I may have been partially at fault in that I did not follow up this work but I was drafted to assume the helm of the University of Texas as President, and for 8 years I could do nothing else. But all's well that ends well and after twenty years, cytologists began to substantiate the findings of TAYLOR and myself, including McOREGOR who in 1964 actually studied the oocytes of a toad.
and confirmed our observations in great detail.

In the past decade, mitochondria have been shown to carry a built-in system of DNA, and when highly sophisticated chemical techniques revealed the presence of DNA in the cytoplasm, this has been interpreted as due to mitochondria. But in October of 1960, BRACHET with some of his co-workers showed that in the eggs of Xenopus, a toad, when the cytoplasm is first treated with a protease, and then examined with an electron microscope, two types of DNA molecules are clearly visible. There are short ring-shaped DNA molecules, doubtless derived from mitochondria, and ten times as numerous are long DNA molecules. It is thus clear that the reason we could not demonstrate DNA in earlier years in the cytoplasm is because it forms a complex with proteins and thus does not yield to methods usually used for the identification of DNA. So the evidence presented and correctly interpreted, from a cytological study of the nurse cells and of germinal vesicles in the toad, some 27 years ago, now rests on a solid biochemical basis!

At the present time, many workers interested in cell differentiation, are endeavoring to determine precisely how long the DNA deposited in the cytoplasm of ova lasts and when a net synthesis of new DNA begins. The evidence is well reviewed by a book by DAVIDSON, 1968, which appeared late last fall. In passing, let me point out to you, that the DNA introduced into the egg by the sperm at the time of fertilization, seems to play no initial role in development - up to about the time of gastrulation. It is the DNA derived from the female which holds sway. Let those who are interested in problems of maternal inheritance take note.

When it was clear to me, if not to others, how ready made precursors of proteins and DNA exist in the cytoplasm of ova, inevitably this question presented itself: In gland cells which secrete large amounts of protein along with quantities of DNA and RNA and many other things, are there similar mechanisms such as are found for oocytes? This question started one of the most interesting studies I have ever undertaken. It all began with the much too common agricultural Red Ants of Texas. Now it has been known for a century or more, that when you excavate the granaries of the agricultural ant, you will find that the embryos of grass seed are absent. The popular interpretation was, and is, that the ants remove the embryos from seed so the seeds will not sprout during a rainy spell. A moment’s consideration of the life history of these ants made it clear to me that seeds are the principal source of food for larvae and where could the ant find a richer source of proteins and nucleic acids than in the embryo lying between the cotyledons? Thus seed play the same role as the honey and bee bread of the honey bee.

Work was begun on the bee in 1943. At this time, CASPERSSON and BRACHET, independently, by the use of different methods, found that protein synthesis requires the presence of RNA.

By studying the way the royal jelly secreting cells develop in early pupal stages, as well as after the bee had emerged from the pupa, I hoped to be able to understand how the precursors needed for the secretion of the royal jelly were provided. In working on this problem, I used both the Feulgen reaction to identify DNA and the methyl green-pyronin stain technique used by BRACHET.

This is the story. In very young pupa the anlagen of the royal jelly gland consist of many little projections containing the definitive gland cell - as indicated by the presence of the intracellular duct - resting on a large polyploid nurse cell. Later the gland cell phagocytizes the polyploid nurse cell and then begins to undergo endomitotic cycles reaching a high polyploid condition. At the time of emergence, the royal jelly gland cells are still immature and non-functional. After feeding on the bee bread for a few days, the gland
cells begin to function and this keeps up as long as the worker consumes large quantities of bee bread—usually, under summer-time conditions this period lasts for about 6 days. In the winter time or under conditions of stress royal gland secretion may be greatly extended.

When the adult worker bee is about 11 days old it ceases to eat bee bread and assumes foraging duties. The gland cells lose nucleoli and most of their RNA and enter a period of inactivity. It is commonly believed by beekeepers that the royal jelly gland cells can be reactivated in an emergency, and I assume in this case that the worker resumes feeding on pollen.

As you all probably know, royal jelly is secreted by young worker bees and is the only food, aside from honey, that is fed a queen bee. Since a queen bee may lay as many as 1,000 eggs a day it goes without saying that she must have an adequate source—precursors—of both proteins and DNA. The royal jelly contains about 15% wet weight of proteins and while no one has demonstrated the presence of nucleotides in the jelly they must be present, masked perhaps, as in yolk in a DNA-protein complex for the queen bee, laying as she does hundreds of eggs a day, hasn't time to synthesize new deoxynucleotides.

When an electron microscope became available to me in 1964 with the cooperation of my colleague DR. BIESELE, we began to study the fine structure of the royal jelly cells, from an extremely early pupal stage. Through the work of PORTER, PALADE, and others it had been shown that the synthesis of proteins occurs in the tubules of the endoplasmic reticulum. An examination of functioning cells showed, as might be expected, the presence of a highly developed endoplasm complex of tubules. Our problem was to discover how this complex system was formed. We began with a stage when the definitive gland cell rested on its polyplid nurse cell. At this time the cytoplasm shows no endoplasmic tubules but polyribosomes are very abundant in the cytoplasm. Our problem was to discover how this complex system was formed. We began with a stage when the definitive gland cell rested on its polyplid nurse cell. At this time the cytoplasm shows no endoplasmic tubules but polyribosomes are very abundant in the cytoplasm. The anlage of the endoplasmic tubules are formed by the outpocketing of the outer wall of the nuclear envelope, as described by many workers. As the definitive gland cell undergoes endomitosis, we found stages comparable to the several phases of normal mitosis. At what corresponds to the prophase the numerous nuclei present undergo fragmentation releasing a myriad of ribosome-like bodies which pass out through the nuclear pore and provide the polyribosomes which take their places on the walls of the endoplasmic tubules.

Thus we have in royal jelly glands of the bee an interesting parallel to the formation of the cytoplasm of ova. In order for gland cells to function, the newly emerged adult must eat large quantities of bee bread, or pollen, which is extremely rich in proteins, nucleic acids and much else and the royal jelly continues to be secreted as long as the worker eats bee bread.

In discussing biological problems on which I have worked I have had two objectives in view. Nowadays, from generous research grants, it is possible for you to provide yourself with extremely expensive equipment—electron microscopes, ultracentrifuges and all the rest, equipment which no university could afford to purchase for you. This is as it should be, but I wanted to show you how much can be, and has been accomplished by the simple tools available in every biological laboratory. All that is needed is a keen eye, a lively imagination and an unlimited curiosity. If and when these government agency grants dry up, or are greatly reduced, it will still be possible to work with a minimum of equipment which every biological laboratory has at hand.

The second point is, as I see it, that you should select for your research broad biological problems and then apply such equipment and

---

1Since this was written I have found that in 1964 MARKO, PECHAN, and VITTEL, Nature, 202, 188-189 reported that royal jelly contains all 4 nucleotides of nucleic acid.
techniques as you may have at hand. From current literature, I get the impression that young people master some sophisticated technique such as labeling cellular structures with radioactive isotopes followed by autoradiography, DNA and RNA hybridization, ultracentrifugation in gradients and all the rest and then look around to see how they can use their acquired skills! From my experience I think you should first select and define some broad biological problem, select a suitable material upon which to work and use any available techniques for the solution of your problem. The most important thing is for you to have a biological and not a test tube approach.

And now let us turn from the past, look ahead and consider some of the unsolved problems. In spite of all we know about the chemistry of chromosomes, we still do not know very much about the physical make up of chromosomes. Do the gene strings, or chromonemata, on which the genes lie in linear order, have an axis of protein to which DNA, or gene loci are attached? A widely held concept is that a chromosome has an axis of DNA, a sort of super DNA molecule, or a series of DNA molecules held together by linker substances. The trouble with this concept is that we must assume that the gene string has a length of two or three meters, somehow packed into a nucleus some 10µm in diameter. In view of the genetic data I find it hard to believe this. And yet the work of CALLAN and of GALL on the lampbrush chromosomes of Triturus and their interpretation of the lampbrush loops backed up with much experimental evidence, indicates an overall extraordinary length of the chromonema involved. On the other hand, there may be a way out of this dilemma. Man, the frog, and many other vertebrates carry around 6 picograms of DNA per diploid cell. But all the urodeles have more than 30 picograms per diploid nucleus. Are we seeing in the lampbrush chromosomes of Triturus images in the loops due to the great excess of DNA present in diploid cells of salamanders? At the moment I think this a distinct possibility.

Another problem of wide spread interest concerns the number of chromonemata shown by dividing chromosomes. Most of the evidence supporting a multistranded structure stems from a study of somatic tissues. It must be remembered that the morphology of chromosomes reflects the functions carried out by the cell involved. In such highly differentiated cells only a few gene loci are active in the production of enzymes. If a diploid cell does not produce enough of a given enzyme then, obviously, there are two ways this need can be met. One would be to increase the number of diploid cells, or, alternatively, to increase the number of strands by endomitosis. The latter seems to be generally employed; for example there are a great number of chromonemata in the salivary gland chromosomes.

Germ cells have as their sole purpose populating the ovaries or testes with enough eggs or sperm to insure the survival of the species. In meiosis we usually see the single stranded condition, or, in a few cases two strands, and this is in accord with the genetic evidence.

LITERATURE CITED


From left to right: Dr. Anderson, Dr. Painter and Dr. Longley
Mrs. Painter at the reception in the Alumni Lounge.

Dr. Kimber (left), Dr. Longley (middle) and Dr. Neuffer (right)