BIOLOGICAL PATHWAYS

By Geminiano de Ocampo, M.D., Academician

The concept of biological pathways was formulated from clinical ophthalmological observations, experimental works and theoretical considerations. This presentation is a theoretical mental expedition. The concept maybe stated as follows: There are five intracellular pathways for biological processes. These are viability, development, differentiation, protection and proliferation. Metabolic processes along the metabolic pathways occur in all the biological pathways. The inward pathways start from the molecules in the outer cell wall while the outward pathways start from the genes and other intracellular organelles. These pathways convey biological inward, outward and intraorganelle messages upon which the biological processes depend. Many of the structural stations of these pathways have been identified. Their intermolecular connections however, are largely conjectural. The complete demonstration of the network of these pathways challenges biomedical research.

Clinical background. Corneal Viability — The questions are on corneal transplantation and deal with the desirability of the graft. How much of it is alive? How many of its cells are alive or dead? Are non-cellular membranes and stroma fibers alive or not?

Clinical Observations:

The sooner a graft is transplanted the more chances it has to take and remain alive. Direct transfer is best. At 4°C the graft remains suitable longer. It is most desirable when obtained within two hours after death. The more favorable the recipient eye is the more chances the graft will take and remain viable and clear. Better soil and good seed lead to more chances of survival and growth.

Self Renewal Concept of Biology. The essence of life and the living state that distinguishes the living from the non-living and the dead is self renewal. Self renewal is the common function of most life processes. The needs and the demands for self renewal determine the quantity and the quality of life processes.

The smallest living unit consists of organized molecules and the molecular complexes with the capability for self renewal. It may be called the biological unit or organelle. Examples are the gene, the mitochondriom, the cell membrane, ribosome, Golgi apparatus, mitotic apparatus, lysosome and the virus. Others are the extracellular membranes such as Descemet's membrane and Bowman's membrane.

The unit of renewal of the organelle maybe the atom, molecule, molecular complex or the whole organelle itself. On the control, speed, balance, nature and manner of self renewal depend the different biological phenomena and the various states of health.

Application of the concept. Self renewal serves to differentiate the living from the non-living. It is useful in relating observations about biological phenomena. It serves as a theoretical approach to a logical system of the cells and noncellular structures of a living organism. It is a broad unifying and basic principle in understanding diseases.

Using it as a common indicator and a unique dimension of biology it should be tested in investigation on the mechanism of life processes and the quantity, the form and the behaviour of living matter. Molecular tagging is the most useful tool of testing the concept.

Viability of corneal crafts. The focal point in corneal grafting as in all other biological transplantation is the graft's state of living and the crucial question is how can this living graft be kept alive in the recipient eye. We used in these experiments human cadavers, rabbits and monkeys.

Embryonation Experiments. I reversed the question how can one tell whether a cell is alive to how can one tell whether a cell is dead. A biological test is embryonation. If a corneal specimen is placed in the allantoin of a 9-day old chicken egg, after 48 hours all the dead cells in the epithelium, stroma and endothelium would disappear leaving only the living cells. By this procedure we could produce the acellular cornea. We made physiological, biochemical and physical tests on acellular cornea and obtained proofs that the acellular corneal fibers and membranes were alive but could not multiply. They are viable because they could exchange metabolites but like parasites they are dependent on the interspersed cells. They differ from artificial lifeless membranes. We made clinical and laboratory experiments using acellular cornea. The results support a different concept of viability of the cornea and other living tissues. We found incorporation studies which support the concept of the viability of the noncellular portion of the cornea. All these experiments and observations gave clinical, pathological, biochemical, physiological and biological evidence that the noncellular formed structures of the cornea are viable in the sense that viability means the capability to "survive with life" with or without the property to "propagate" life.

Death and the dead state. To understand further biological survival, viability and the living state, it is necessary to probe into the different aspects and tests of biological death and the dead state. We made experimental tests on cell death by the methylene

blue decolaration of conjunctival and endothelial cells and acridine orange test of the corneal endothelium.

Basic experiments on corneal necrosis. Many laboratory experiments gave the following conclusions: In corneal necrosis there is a massive and pathologic disintegration of the collagen framework in addition to a disorganization and disintegration of the ground substances as well as death and disintegration of the stroma cells. Corneal necrosis maybe produced by bacteria, chemicals, enzymes and physical agents like heat. Necrotizing substances are found in the toxins of p. aeruginosa and croton oil. Trypsin in dilute solutions affect only the ground substances. In higher concentration it further causes disintegration of the collagen fibers although this does not occur after freezing the cornea because of the alteration of the ground substances and probably also of the cement substances.

Collagen staining is lost in necrosis. The collagen fiber can exist in a necrotic state distinct from its native nennecrotic condition. Metachromasia is always absent in necrosis although it may disappear without necrosis. Changes in the ground substances and interfibrillar cement substance may cause softening or malacia and swelling without necrosis. Corneal opacities may be necrotic or not. Necrotic opacities may be delineated and demonstrated histologically and by histochemistry. Necrotic tissue may be debrided by enzymes (0.25% trypsin and fibrinolysin with DNAase) leaving the viable tissue intact.

A new concept of corneal viability. This may be stated as follows: All the formed and organized parts of the cornea, cells as well as the noncellular structures are viable. The only nonviable portion of the cornea is the amorphous and unorganized ground substances.

The collagen fibers and membranes of the cornea can exist in a native state as well as in a dead or necrotic condition. The viable native collagen fiber differ morphologically, histochemically, optically and biologically from necrotic collagen, reconstituted or extruded collagen strips, altered collagen catgut, gelatin or hydrolyzed collagen gelfilm.

In the preserved glycerinized cornea, the cells are dead but the collagen fiber and membranes are still viable. The acellular corneal graft used for keratoplasty can respire, dehydrate, incorporate S^{35} and remain transparent only by preserving its viability, unlike the unchanging and nonexchanging transparency of inert plastic or glass. Not only those structures that can grow or multiply or alive. The propagation of life is a different phenomenon from the maintenance of life or the ability to stay alive. The viable structures of the cornea show different degrees of vulnerability and what will preserve the life of the fibers may not

conserve that of the epithelium or the stromal cells or the endothelium. The endothelial cells are the most delicate and Descemet's membrane is the most hardy. It is therefore more conducive to meeting of minds and understanding if the cornea is considered not only as a single and whole viable structure but also to view it as composed of different viable parts with varying characteristics of viability.

Biological Unit. The quantum is for energy as the biological unit is for life. It deals with the how much of life and death at the subcellular levels and extracellular sites. The smallest unit of life is the biological unit. This is that which is capable of changing and exchanging its molecules and molecular complexes. While the cell may be the smallest unit that shows all the characteristics of life the biological unit is the tiniest unit that renew itself and possesses the most essential and basic property of living things, viability and self renewal. This is the organelle. Each organelle is composed of repeating organized subunits of organic with or without inorganic molecules of similar and/or different kinds. The biological subunit or molecular complexes compose the different formed structures of cells and noncellular structure. They make up the various particles and the organelles of the bacteria and the cell membranes. The free molecules of the tissue fluids and amorphous ground substances in between the formed structures, are not part of the biological subunits, but they serve as the reservoir which supply and receive the continuous exchange of molecules or molecular complexes.

Biological pathways and biological messengers. These are found in the cell. The inward pathways start from the outer surface of the cell wall and end at the genes while the outward pathways start at the genes and end at the inner surface of the cell wall. The terminals of the pathways are at the cell wall and the genes. The pathways form a network inside the nucleus across the nuclear membrane and in the messwork of the cytoplasm. They are collectively called smooth and rough endoplasmic tubules. They serve as the pathways to and from the cell wall and the nuclear wall. The secondary messengers that carry or transmit messages or molecules are those that carry biological messages from the cell wall to the nuclear wall. Some believe that prostaglandins could act as secondary messengers. There must be also some tertiary molecular messengers in the nucleus to carry biological messages from the nuclear messengers in the nucleus to carry biological messages from the nuclear membrane to the genes probably thru the supragenic molecules. I am not aware concrete observations and reports of this nature.

While the prostaglandins are considered inward secondary messengers the nature of their messages are still conjectural. If

they are connected with the inflammatory process as some think, they probably carry proinflammatory or prophlegestic messages. The corticoids are antiinflammatory or antiphlegistic. It is along this thinking that the existence of inflammatogenic genes is premised.

The concept of biological pathways attempts to suggest a framework for the existence of a structural basis for distinct biological activities in any cell. The discovery of the prostaglandins as the secondary biological messengers suggests the presence of primary biological messengers in the cell wall and membranes as well as tertiary messengers inside the cell nuclei.

Viability or survival pathway. This subserves the functions and needs for self renewal. It is the primary, initial, widest and constantly open biological pathway.

Survival is within and after one's life span. Viability refers to and concern only with life within the life span. In the biological organization of the organism those portions for survival are always active except perhaps in the state of suspended animation. Hence, there is always activity, however, minimal in the viability pathways. It can therefore be aptly called the primary biological pathway.

The other biological pathways subserve special functions other than mere survival. They are therefore designated secondary biological pathways within the cell and are open only when there are needs for self development, differentiation and specialized products, protection to self and other cells and proliferation. It is evident that more than one biological pathway could be open at anytime.

There are two kinds of metabolites — the primary metabolites which are essential for survival and the secondary metabolites needed for specialized functions. The latter travel through the secondary biological pathways. Some bacteria suspend differentiation products like toxins while they are proliferating or dividing.

The recent findings of biological messengers strengthen the assumption for the existence of intracellular biological pathways. These biological entities are postulated on the existence of molecular receptors many of which are now identified on the surface of the cell organelles. Transmitter molecules are produced at the receiver surface of the target structures. These intermediary transmitter molecules seem to have very short life span.

Biological development. This is a marvel of design and execution. Nature and Creator must be behind it. The factors for biological development are genetic and environmental. If there are (10^7) 10,000,000 genes in man these could theoretically be divided into 60% survival genes, 10% developmental genes, 10%

differentiative genes, 10% proliferative genes and 10% protective genes. The concept of biological development could be stated as follows. Full development of any biological unit from organelle to organism is the attainment of its nature structure, substance and function by the information from and control by developmental genes, the interaction with other biological pathways and the proper environmental factors. Completeness is premised on an adult design stored in the genome and built by the orderly, timely and sufficient supply of raw materials.

The new ideas are the postulation of the existence of adult design in the genome, developmental genes, development and proliferation of DNA themselves by nonmitotic replication and interaction with other genes and pathways.

This concept could be an approach to the search for the most effective vaccine and most helpful adjuvant as well as the most proper timing, combination and sequence of vaccination procedures.

Ocular developmental anomalies and biological pathways. These could be temporal, structural, functional, biochemical, biological and multidimensional. Glaucoma is shown by statistics to have polygenic inheritance. Defective genes responsible for developmental anomalies could be qualitative and/or quantitative in number of similar and dissimilar genes involved. The injury could be from ultraviolet light, gamma rays, some dyes like acridine or toxic molecules or drug and catabolic products, bacteria and virus.

Examples of developmental ocular anomalies are spatial (ectopia lentis, displaced or misdirected lashes, ectopia irides) temporal (persistent hyaloid artery), dacryostenosis, developmental and congenital glaucoma, ptosis, functional amblyopia, squint, ametropia, biochemical congenital night blindness, albinism, aniridia, iris coloboma, iridoremia, hypoplasia of the optic nerve; multidimensional (microphthalmos and anopthalmos). Corticoid glaucoma has a theoretical genetic basis. There are anomalies of development in myopia, congenital cataract, amblyopia and squinting, macular degeneration and underdevelopment of the fusion center.

The phenomenon of immune tolerance in undeveloped immune systems support the thesis of the development of the metabolic and biological pathways during the embryonic and fetal stages.

Embryonic antigens support the information theory of immunity, that is, there is a prestored information for the antibody in the genes of the antibody producing cells. This theory suggests that the genes have evolved in kind and number of the different species.

The development of the metabolic and biological pathways start from the inherited genes. The number of each kind of genes in the fetus, infant, adult or senile are not equal although their chromosomes may be similar. This could be due to differences in the number of copies of each genes. The immunological cells and the response to antigens by the fetus and neonates are different from that of the adult and the senile.

Biological differentiation. The kinds of differentiation are form, structure, substance and functions for survival or special functions. There must be different and proper qualitative and/or quantitative stimuli for the different biological functions along the different biological pathways. The prime purpose of differentiation from organelle to system is for specialization of function.

Examples of high specialization are the light receptors in the retina. These are specialized receptors for the different light waves in the rods and three kinds of genes in each cone as red genes, blue genes and green genes or three kinds of cones for red, blue and green.

Cultural media and differentiation. The problem we have met in the culture and sensitivity tests for bacteria had in one way contributed to the conceptualization of biological pathways. Some staphylococci do not develop pigment in a particular medium. The staphylococcic toxin may be absent in some media or may vary in amount and potency in other media. These observations suggested the existence in the genome of some mechanism with morphological basis. In other words there must be a genetic basis for differentiation and differentiation products.

Then we had some observations on the changing sensitivity of staphylococci to antibiotics. We observed over a period of five to ten years a definite decline in the sensitivity of conjunctival staphylococci to chloromycetin. After many more years this sensitivity returned. This is true with other antibiotics. This makes the choice of antibiotic a hit and miss affair without the aid of culture and sensitivity test. There are survival culture media and differentiative culture media for bacteria, virus and fungi or any plant and animal cell for that matter. All these must have a genetic basis in the differentiative and a proliferative biological pathways.

Differentiation like any biological phenomenon must have physical, chemical, morphological and molecular bases. The genes, paragenic molecules, gene complexes and genome segments that subserve differentiation must be physically proximate. Genomic mapping is advanced in lower forms of life but meager yet in man. This is a challenge to molecular biologists.

Other areas of investigation are the inhibition and promotion of differentiation, vaccine adjuvants, live and attenuated vaccines, relationship between pathogenicity and differentiative pathways,

virulence and differentiation, specificity or nonspecificity of the supragenic molecules, mutagenesis and resistance to antibiotics.

Protective pathway and subpathways. The concept of biological pathways assigns a portion or segment of the genome consisting of one or several chromosomes for the immunological system. The kinds of genes of the immunological genome must therefore be preformed or predetermined in a certain species in the fetal stage of development. However, it seems that the replicas or copies of each kind of the immune genes must be determined in the later stages of development of the organism. Hence while the immune genes in a single lymphocyte during the fetal state may be inherited and limited, the number of copies of the immune genes may be more or less in the child and the adult. Moreover the number and kind of lymphocytes may fluctuate depending on the need and the stimuli. Their proliferative activity also depends upon appropriate stimuli. On this concept the role of adjuvants for immunity vaccines could be based and sought for. For example, this could explain the role and usefulness of kock's adjuvants for cancer cell vaccine. All these could not only potentiate but even prolong the effectivity of our current bacterial vaccine for infectious diseases.

Theoretical consideration on immunity. It seems from studies that the almost total absence of trachoma among Filipinos may have some genetic element of natural immunity. The phenomenon of immunologically competent lymphocytes may be due to difference in the supragenic molecules and/or in the cell surface receptor molecules to antigens. The competency or incompetency of any lymphocytes to respond depends in the kind of antigens. The cell surface molecules, the recipients for antigen molecules may have variation. This may be the explanation for the effectivity of mixed vaccines. There must be a different lymphocyte population that respond to a different antigen. This may explain the differences in potencies and duration of vaccines according to their mode of preparation.

One basic question in immunology is where and how are the antigenic molecules produced. The concepts that may help clarify this are those of the mosaic molecular structure of the cell surface, the biological molecular messengers and supragenic repressor-derepressor molecular mechanism for inhibition and stimulation of genic activity. The concept of biological pathways will help clarify also the phenomenon of virulence and attenuated live vaccine.

Local immunity is closely related to that of inflammation. One way to the exploration of the problem of keloid is through the biological subpathways of immunity and inflammation.

Other problems that can be approached through the concept of biological pathways are: adjuvants to anticell vaccine like BCG

and levamisole, booster doses in immunotherapy of infectious diseases, suppression of immunity in corneal and organ transplantation, separation of inflammation from immunity, desensitization in immunotherapy, anticancer immunity and immunotherapy, fetal cell therapy and antirejection vaccine in tissue and organ transplantation.

A Theory of Inflammation. The inflammatory diseases are the most frequent of all human diseases. And yet inspite of countless observations, numerous experiments and voluminous literature on its manifestation, diagnosis and management there could be found such expression as "inflammation studies are largely descriptive." The problem is to find a unified hypothesis to account for much of the morphological data available without doing harm to physiologic evidence. In all inflammatory processes, agreement is on a few basic facts but there is disagreement on most of the critical issues. There is an overwhelming wealth of details but there is a conceptual confusion. In order to understand well inflammation it seems worthwhile to consider its theoretical aspects. We must pay attention to integration, a sort of intellectual synthesis with more emphasis on meaning and values than on mere facts. All these have induced this attempt at formulating a theory of inflammation. I took into account semantics, the definition, perspective, levels, phases, areas, cells, noncellular structures, basic observations, beneficial and deleterious effects and inflammatory cells. I also included biological postulates about primary and secondary metabolites, biological pathways, inflammatogenic agents, inflammatogenic injury and the existence of inflammatory genes for protection and repair. Then I formulated a theory of inflammation as follows: Inflammation is a network of reparative, protective and destructive physicochemical, physiological and biological reactions and interactions usually after inflammatogenic tissue injury. The inflammatory mechanism has genetic and environmental aspects.

The new ideas introduced are inflammatory genes, primary and secondary metabolites, biological pathways, inflammatogenic agents and injury and interaction. It seems that the inflammatogenes could elaborate inflammatory mediators without direct or indirect cell injury from changes or imbalance in the regulatory systemic or local neurohormonal mechanisms. These could be brought about by stresses which may be behind recurrent inflammation of obscure origin without evident inflammatogens.

The test of the theory is to demonstrate the existence of inflammatory genes responsible for early or late inflammatogens. These would necessitate demonstrating inflammatogenes similar to immunogenes, mutagenes, teratogenes and carcinogenes. Mapping of the chromosomal and extrachromosomal genes would also test

the theory. Another approach is the search for antiinflammatory agents against the various parts of the inflammatory mechanism.

Induced inflammation. Induction of inflammation is a form of therapy, for inflammatory and noninflammatory diseases in ophthalmology. Examples are inducing reactive inflammation for retinal detachment or retinal degeneration which may lead to detachment, thrombosis of retinal vein, ciliary ablation for intractable glaucoma, antiglaucoma filtering operation, tissue implant for torpid corneal ulcers, nonulcerative keratopathies and induction of fibrillogenesis.

A theory of carcinogenesis. If we must control cancer we have to understand it in all its aspects from a holistic perspective thru the biological pathways.

Fundamentally, carcinogenesis is a transformation of a cell in any stage of its life span. It consists essentially of transmissible imbalance of control of the proliferative and other biological pathways. It is brought about by endogenous and exogenous carcinogens acting directly or indirectly upon the proliferative control genes. These become the cancer genes which manifest abnormal primary and secondary metabolism.

The new ideas in this theory are: The concept of biological pathways, the concept of specialized segments of the genome for each biological pathway and the uninhibited proliferative control genes becoming the cancer genes.

During the early development of the blast cell the pathways for propagation are normally open but that for differentiation are still closed. At a later maturation stage the pathway for proliferation may be very active while that for differentiation may be minimally open. The diptheria bacilli do not elaborate diptheria toxin while it is proliferating. A mature brain cell and a rod or cone cell of the retina are highly differentiated but their proliferative pathways are closed.

In non-neoplastic growth, there is a temporary increase in positive derepressor supragenic molecules from injury and hormone, vitamin or mineral imbalance. These are found in inflammation, vitamin, mineral or trace metal deficiency or hormonal imbalance. Examples are Bitots spots in hypovitaminosis A, pterygium and inflammatory granuloma.

In neoplastic growth there is a permanent decrease or loss of negative repressor supragenic molecules brought about by carcinogens from internal environment or from external factors such as ingestants, inhalants, contactants, radiation or vibration and from genetic deficits.

Uses of the theory in the fight against cancer. The recognition of cancer cells by morphological means should be supplemented by physical, biochemical and biological methods.

Anticancer vaccines, vaccine adjuvants, cytacids and a cytostatics and restoration of balanced cell control should be continuously explored. Natural systematic vaccination could be attained through freezing and irradiation. Chemical and immunological detection of residual cancer cells after any form of therapy should be continuously investigated. The warhead principle of tagging the specific anticancer antibody should be pursued. The timing, the method and the extent of the surgery should be reevaluated in conjunction with the other modalities of therapy. The concept of biological pathways could serve as the framework for the all-out offensive against cancer.

A concept of biological degeneration. This is not a biological pathway but a deterioration occurring in any one or several biological pathways. It is a negative balance of self-renewal initially in the cell or noncellular structures. It may involve directly or indirectly any one or more of the metabolic and biological pathways. Metabolic disease must necessarily lead to degenerative diseases. Biological degeneration may also follow defective supply of raw material (nutritional) or inefficient degradation (uric acid) and elimination (neuropathy) or deficient detoxification (hepatopathy) of harmful intermediate and end metabolites.

Biological degeneration may be primary or genetic in origin and secondary or environmental in source. Defective elimination and degradation may be manifested by deposition of normal or abnormal intermediate or end metabolic products. This occurs in amyloid, lipsid proteinaceous, keratinoid, fibrinoid, hyaloid, osseous degenaration inside or outside the cells. Metaplasia like abnormal keratinization is degenerative. Genetic degeneration may be due to deficiency in number or kind of genes. Many diseases due to genetic defect in kind are well studies while those due to deficient number of genes are less known. Mascular degeneration of the juvenile, adult, or senile variety are most likely due to deficiency in number of genes. The secondary or environmental degeneration could be due to unhealthy internal environment or milieu interior from defective metabolism, elimination or transport. Abnormal external environment from radiation or ultrasonics, humidity, atmospheric pressure, oxygen deficiency or severe temperature could lead to secondary degeneration. Postinflammatory degeneration should be categorized under secondary degeration. The susceptibility of different cells and organelles to the external factors of degeneration is variable. Dystrophies are mainly nutritional degeneration. Vitamin deficiency and hormonal imbalance lead to secondary degeneration.

Molecular biology and biological pathways. Separation of the component molecules of the cells, nuclei, chromosomes, genes and

paragenic particles is the route of investigation on the different aspects of the biological pathways. I hope this would attract the interest of foreign and local molecular biologists. There is much literature on gene mapping in lower animals. There is, however, little work and much challenge for genetic molecular probing in man.

Application of the concept. Ophthalmology. I have communicated on biological pathways in ophthalmology.(1) (2) Among the areas where it could be useful are: Ocular hypovitaminosis A; vitreous hemorrhage, vitreous cells and vasculogenesis, diabetic retinopathy, glaucoma involving biological filters, fibroblastosis and cautery, preglaucoma, nonsurgical management of senile cataract, bullous keratopathy, corneal transplantation, uveitis, retinal detachment, maculapathy, retinitis, pigmentosa, squinting, optic atrophy, dacryosystitis, culture media and contact lenses.

Medicine. Among the areas where it may be helpful are organ transplantation, diabetes, hypertension, cardiopathy, infection, immune diseases, cancer, biological vaccines and degenerative diseases including senility.

Botany and Zoology. I hope the concept of biological pathways will stir some interest among plant and animal scientists.

References

1. de Ocampo, G., — Biological Pathways in Ophthalmology PJO 9:153-162, October-December, 1977
2. de Ocampo, G., Theoretical Bioophthalmology (Awaiting publication)

An extraordinary increase in information, knowledge and concepts concerning these pathways has occurred during the past two decades. Until the 1950's very little was known concerning either the cellular or molecular mechanisms involved in the antibody formation and cell-mediated immunity. Today, various scientific meetings, symposia and congresses dealing with the various aspects of these mechanisms and their applications to the broad area of biological science has tremendously increased the interest in these mechanisms.

Viability

The evidences presented by Dr. de Ocampo based on his clinical and experimental observations on acellular cornea has led him to the meaning of viability as "the capacity to 'survive with life with or without the property to' propagate life".

The graft versus host (GvH) reaction is influenced by the condition of the surrounding tissues.

What was the nature of the host tissue?

Which of the endocrine glands can be explored for biochemical and biological properties which may influence success of corneal grafts?

Biological pathways and biological messengers

While it is known that prostaglandins (PG) or sometimes known as macromolecules from mononuclear phagocytes of the series PGE_1 and PGE_2 are most often associated with inflammation, recent evidence has been presented that prostacyclin (PGI_2) may augment inflammatory reactions. (Robinson et al., 1970). However, prostaglandins may also inhibit immune-induced inflammation which is largely related to the stimulation of adenylate cyclase. They in some way function as those of corticosteroids, glucosteroids, etc.

Mononuclear cells in human peripheral blood produce a factor (MCF) that regularly stimulate the production of PGE and collagenase from resting ASC (adherent synovial cells). Robinson et al. (1979), in their in-vitro experiments indicate that rheumatoid inflammation involves interaction between the monocyte-macrophage, lymphocytes and synovial cells regulating the production of prostaglandins and other factors.

Did you observe such reactions in your experiments on corneal grafts?

Biological development

Can Dr. de Ocampo explain the theoretical divison of 10^7 (10,000,000) genes in man into 60% survival genes, 10% developmental genes, 10% differentiative genes, 10% proliferative genes and 10% protective genes?

Biological differentiation

Information on resistance to antibiotics support the concept that there is a genetic basis.

Are there mechanisms involved in the expression of a genetic trait?

Dr. de Ocampo has mentioned several developmental anomalies. He said that "corticoid glaucoma has a theoretical genetic basis." In his studies, what is the ratio of the expression of this ocular anomaly? What factors are involved?

Protective pathway and subpathways

Interferon was earlier known as an antiviral agent. It is also considered as an anti-cancer agent.

Can you explain the regulatory role of interferon regarding developmental and congenital glaucoma?

Dr. de Ocampo has enumerated the areas in opthalmology and medicine where the concept could be useful. He also hopes that the concept "will stir some interest among plant and animal scientists." These concepts have been explored by zoologists using many experimental animals (mice, rats, guinea pigs, dogs, cats, etc.), however, I cannot speak for the botanists.

Salvador Salceda, M.D., Discussant

Thank you Dr. Campos. My assignment scares me; I have no napoleonic complex intellectually.

As a student and an associate of Dr. de Ocampo in his numerous researches in ophthalmology, I have become aware of the many observational data cited so richly in his paper — serving as they do the foundation of the concept on Biological Pathways.

I share his anxiety and dissatisfaction over the lack of some unifying concept of the many morphological and descriptive observational data. As a researcher too, I welcome his efforts in formulating the Biological Pathways.

Discussing and commenting on the paper however, runs the risk of getting into details from which it is difficult to extricate.

I share the view that the smallest biological unit is not the cell nor is it the molecule — but something in between. An organelle is perhaps a prototype of what Dr. Ocampo believes as the smallest biological unit. Outside of the cell however, e.g., fibers and membranes, I am still at a loss what the biological unit is, considering that Dr. Ocampo believes that the formed non-cellular components of tissues are also alive and viable.

As to the five postulated biological pathways: *viability* being the most elemental and subserved by self-renewal, *developmental* for maturation, *differentiation* for specialization, *protective* for survival and *proliferation* for growth and multiplication, I have a few questions to ask.

1. Are there structures assigned to these pathways? If so what and where are they?
2. If these structures referred to by the author as "stations" how are the communication system brought about? How are messages sent to and from these stations?