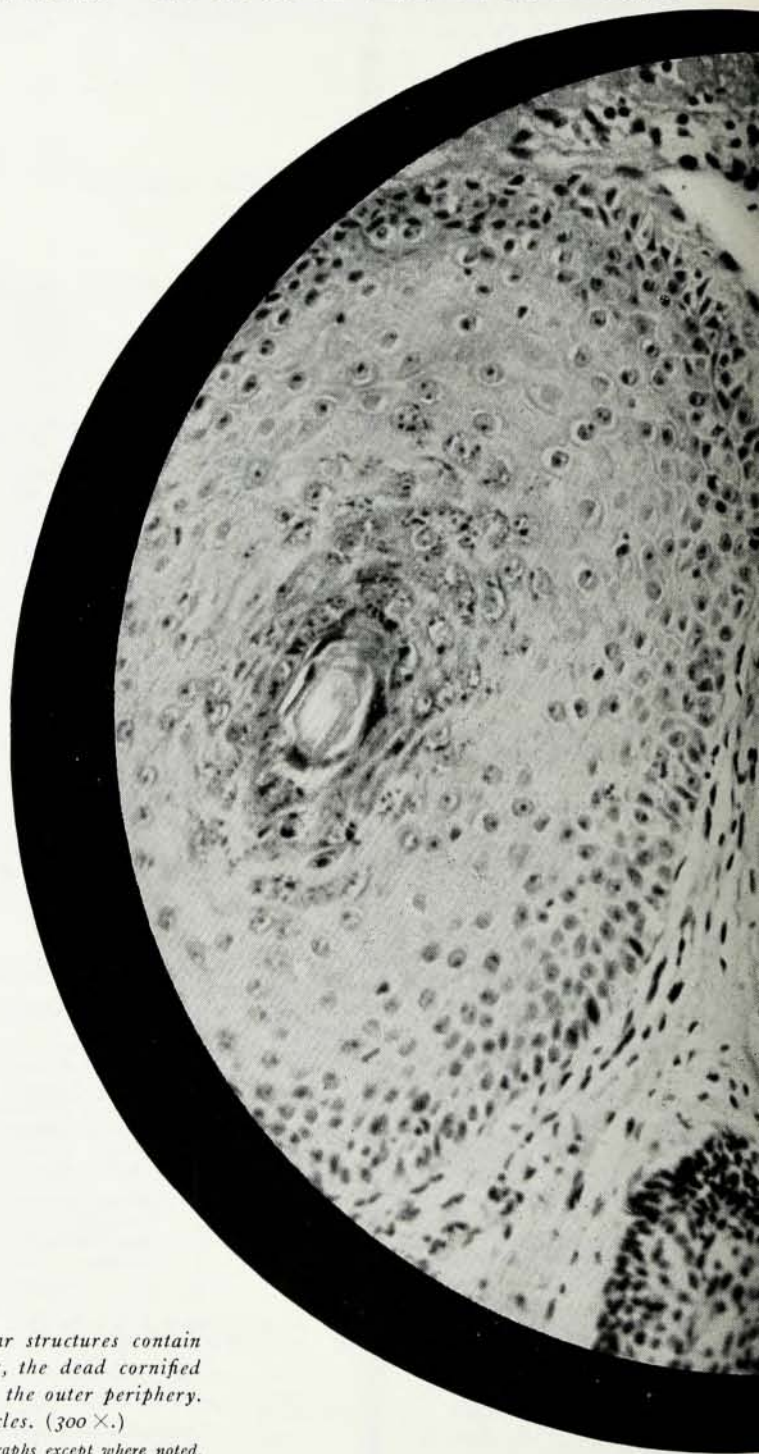


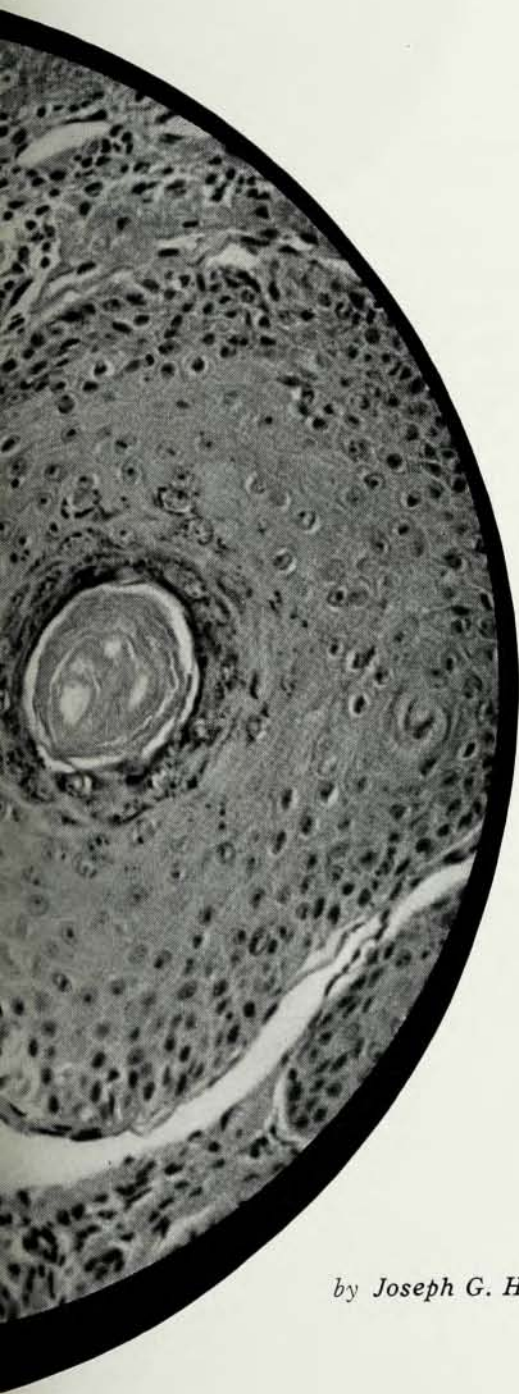
Having got over a first impulse to treat problems in biology as if they were problems in physics, the physicist has found many ways of applying the discipline of his thinking and the delicate capabilities of his instruments to the study of cancer.

CANCER BIOPHYSICS



Human pearl cell in skin cancer. The circular structures contain the strata of normal skin in concentric circles, the dead cornified cells being in the center, the germinal layer on the outer periphery. The cancerous cells seem to be growing in circles. (300 X.)

Roswell Park Memorial Institute provided all photographs except where noted.



Cancer brings about the cruelest irony known to man for in cancer the living conscious body can see itself growing wrong. In order to arrive at an understanding of this dread misdirected growth research workers in all the fundamental sciences have been called together to work in our cancer research institutions.

Of these scientists the physicist is confronted with the most difficult of fundamental problems. He may contribute techniques in the way of applied physics as a kind of highly refined engineering, but if he chooses to adopt the prefix bio and become a biophysicist he is promptly presented with a paradox. He has a discipline of thinking which is not suited to the work at hand. The word biophysics implies this. It is a ridiculous combination of incongruous extremes. Disciplined scientific thought has never taken more diverse forms than it has in the fundamental modes of thinking in biology and in physics.

Biophysics falsely ties biology to physics (particu-

by Joseph G. Hoffman

Joseph G. Hoffman is a professor of biophysics at the University of Buffalo's School of Medicine and is director of cancer research at the Roswell Park Memorial Institute, Buffalo, New York. A Cornell trained physicist, Dr. Hoffman has previously been on the staffs of the New York State Institute for the Study of Malignant Diseases, the National Bureau of Standards, and the Los Alamos Scientific Laboratory. His particular interest is in quantitative measures of tissue growth.

Looking from inside outward through outer strata of human skin. Through the hole can be seen the polygonal dead and cornified outer cells. The darker polygonal cells are in the stages of dying the genetic death. Here and there a nucleus is undergoing granulation process in which the genetic material is transformed into inert keratin.



larly atomic physics) on the assumption that biology is amenable to the application of known laws of physics. This makes the biophysicist's introduction to the cancer problem doubly frustrating: he can see the cells of the body grow wrong and he is conscious that the known laws of physics give little or no immediate insight into the understanding of living phenomena.

As a physicist he is confronted by a unique situation in the living cell. There are no carefully established theories on the behavior of energy in cells by which experiments can be designed. The physicist accustomed to the marvelous guidance, say, of quantum theory, or of Newton's laws, finds no such landmarks to give him ideas or directions in dealing with living matter.

First of all, he is told, all cells come from cells. This dictum applies also to bacteria and viruses. To the best of our knowledge living matter does not arise anew in our times. Cells have managed to survive through an immeasurable time interval. Secondly, he is told, the cell system seems to be capable of intelligence; it manages to do the right thing with great precision (the teleology of living systems, their direction toward a predetermined end). The cell seems to shape circumstances to its purposes.

Attempts at objective descriptions have got no further than interpreting this purposive behavior as being due to a complicated system of reactions occurring in a way which tends to preserve a system's equilibrium (Le Chatelier's principle). Lindeman has said that the living cell is a system which has and can maintain a very low entropy.

A Matter of Plumbing

About the first reaction of physicists to the living cell has been to construct a model which will live

up to the laws of quantum mechanics and thermodynamics. Aside from the fact that none of the models to date have been successful in imitating the cell, the basic rationale of this approach is open to question. The inference is that if a model could be constructed (even if only on paper) it would be made for the ultimate purpose of seeing if something like living matter could be created in our times.

Experience in biology and in physics thus presents the biophysicist with a contradiction which can be solved only by experimentation. The physical analysis must ultimately be corroborated by a synthesis in which, of course, the biologist and biochemist will have to collaborate closely. But even to suggest the possibility of synthesis at this time borders on fantasy. When the physicist attempts an analysis of living energetics he is tacitly assuming a distant and ambitious goal which has profound philosophical connotations. It recalls the old anecdote about Laplace. When Laplace presented his "Mécanique Céleste" to the Emperor Napoleon, he was asked what part God had in his astronomic system. Laplace is said to have replied, "I have no need for that hypothesis." It remains to be seen how many hypotheses biophysicists can dispense with.

From the standpoint of fundamental rationale, the biophysicist, now that the physicist has a bio prefixed, is in for a long period of soul-searching and doubt; but there are ways out of this uninspired state of affairs. First, the biologists have yet to make certain that very elemental forms of life cannot be created in our times. The discovery of the existence of crystalline viruses raises the possibility of creation under ordinary conditions. Secondly, there may be undiscovered properties of matter and energy as

they exist in the living state. At present, there are not even remote clues about such properties—solid state physics seems to be occupied with the relatively simpler substances of the inanimate state of matter.

For the time being, however, the biophysicist will have to go along with the biologist's concept of teleology according to which the living cell knows when and how to do the right thing. There will be no magnificent concepts analogous to the electromagnetic theory with its aesthetic completeness before him. His ideas will not be amenable to a quick check, as in modern nuclear physics, in a Geiger counter or cloud chamber. For a long time he will wind clocks, fix plumbing, and in general design fancy electronic gadgets in applied physics. In his spare time he can wonder if living cells react as they do by virtue of Le Chatelier's principle or if there may not after all be a vital force or a vital principle quite distinct from physical forces.

What is Cancer?

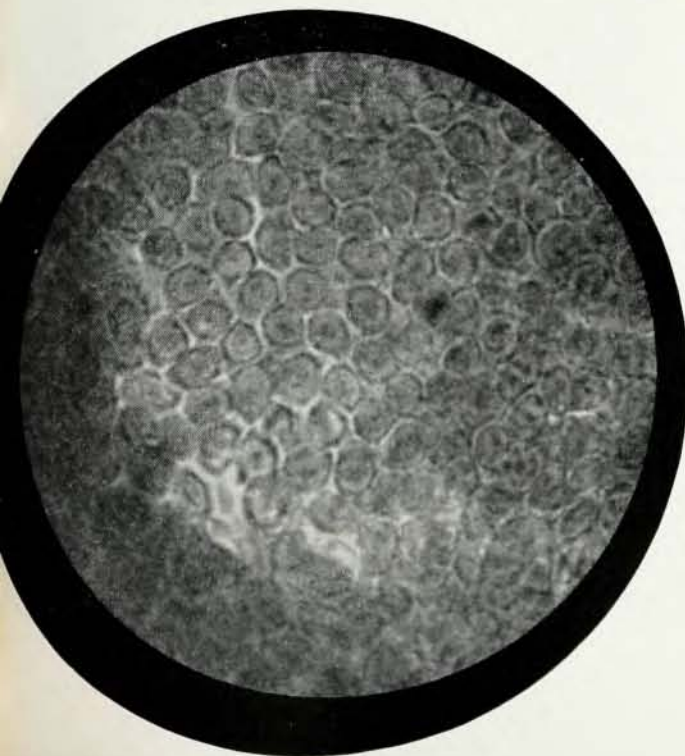
Even though the basic rationale of the biophysicists' subject matter leads to gloomy long range forecasts, the immediate problems which call for the special abilities of a trained physicist can be defined, particularly with reference to cancer research. These problems relate to obtaining more accurate

descriptions of what living cells can do physically. Specifically there are aspects of the creation of new cells and the orderly destruction of old cells which lend themselves to study by purely physical methods.

One of the current leading theories on the origin of cancer is that of the somatic mutation. According to this theory, any cell of the human body which is capable of reproducing itself can have its genetic system or its enzyme system perturbed in such a manner as to cause the cell and its offspring to grow wrong. This idea carries with it the inference that cells which normally would no longer reproduce themselves may start dividing into daughter cells which are not needed or wanted in the orderly tissue.

Another leading theory on cancer assumes that many cancers are due to a virus which lives parasitically on body cells. The virus gnaws at the gene system, or at the enzyme system of the cell, thereby causing the cell to reproduce wildly. The virus may be considered as being a particular agent producing a somatic mutation. The general theory of somatic mutations assumes that chemicals, heat, ionizing radiations, and mechanical irritations as well as viruses can produce the genetic perturbation.

No one has yet seen and identified a mutated cell in any mammal. The somatic mutation hypothesis is an extrapolation from the observed mutations seen in fruit flies, or in bacteria, or in bacteriophage. On the other hand, there are tumors in experimental animals for which the virus agent has been isolated and tested. Only in a speculative sense can either



Looking from inside outward through innermost stratum of human skin. The cells are densely packed, and are almost entirely nucleus with little surrounding cytoplasm. This is the germinal layer of the skin from which the other outer skin cells stem (690×).

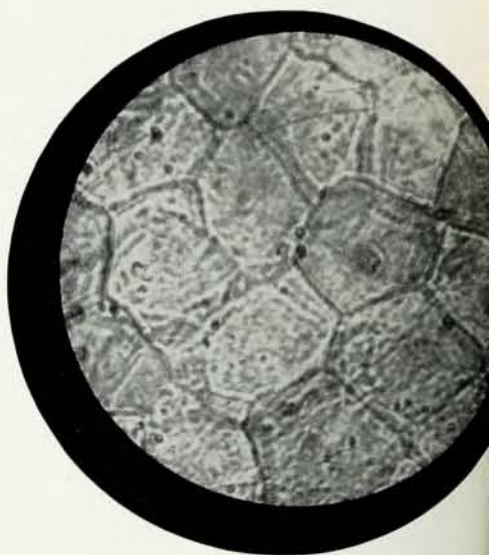
theory be applied to explain human cancer.

Of interest to the physicist is the phenomenal memory which cells appear to have for cancer-inducing stimuli. In inanimate systems this memory is called hysteresis. Laboratory experiments show that, for periods up to two years after the application of a cancer-stimulating chemical, skin cells of rabbits will remember the stimulus and behave as if there had been no lapse of time. In addition to remembering the first cancer-inducing stimulus, the cells tend to acquire a new kind of sensitivity. Although no tumors are elicited by the first application of the

cells live in skin under normal conditions. If cells live only six months in the skin layer and are sloughed off, then the memory of the tissue to cancer-inducing agents might be assumed to reside in the underlying tissue somewhere. Or, the memory may be imparted to the daughter cells in the process of division.

In analyzing the length of life of a cell in human skin, the physicist can find a familiar concept: the amounts of radioactive members present in equilibrium in a radioactive decay series. In skin, the cells are the changing elements and undergo a

Phase contrast photomicrograph of the outer cornified layer of human skin. These cells are thin polygonal sheets. Here and there are seen faint outlines of the "hole" left by the nuclear material. The cells are held to one another by a film of oil.



chemical, cells have become sensitive to agents which by themselves have never been known to produce tumors. Thus simple wounding or the application of an irritant like turpentine will elicit tumors from skin which was treated twelve months previously with a cancer-inducing chemical. The explanation of these effects must await the disclosure of the exact item in the tissue which can retain the memory of the cancer-inducing agent.

Life of a Cell

By way of contribution, the physicist can use radioisotopes to look for the part of the tissue which has this memory. More directly yet, he can contribute to an understanding of how long the tissue

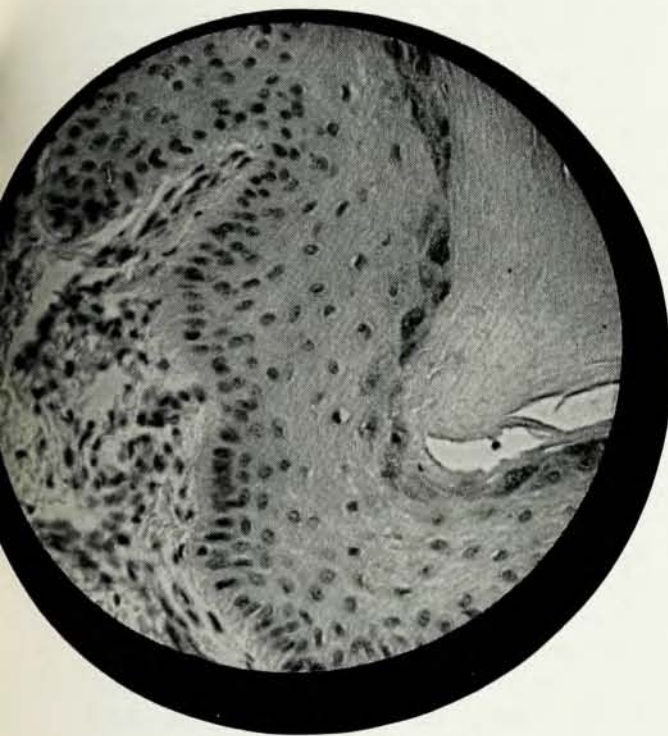
series of transformations as they move outward toward cornification, death, and sloughing away. Since the cells undergo large changes in shape and size in the process, the establishment of the equilibrium number of cells present in each stage requires several special techniques for preparation of the skin to permit analysis under the microscope. The cells arise in the innermost, basal, or germinal layer of the skin and depart as dead cornified cells on the outside layer. Here can be seen the beginning and end of a series with the equilibrium numbers of cells visible and countable. The dynamics of such a system lead to the type of analysis with which the physicist is trained to deal.

Other such dynamic systems in the human body are the sequence of cells in the bone marrow that

lead to the circulating blood platelet formation or to red blood cell formation. The physicist can contribute to the analysis of the transients which appear in disturbed equilibrium conditions among the members of the blood population. This is particularly true in studying the various leukemias which can be regarded as a wide open subject as far as the transient states of cell populations of the bone marrow and circulating blood are concerned. It should be noted that this kind of study of cell populations in leukemia is quite aside and distinct from any therapeutic studies.

servation under which valid observations can be made on living systems. The experimental data show that the gene system can make an exact duplicate of itself and that the reduplication can occur many powers-of-ten times over without a mistake. In addition to being able to reproduce itself precisely, the gene system carries in it the machinery for reproducing the special accessories that grow on certain cells, such as cilia, or secretory granules, or hemoglobin, or hard horny material, as the case may be.

Not only is the physicist needed for his ideas on the reduplication process, he is needed also for his



Transverse section through human skin from ball of the foot. The upper right shows a homogeneous mass made up of hundreds of layers of dead cornified cells. The columnar line of cells is the germinal layer. How long does it take for cells to grow from the inner germinal layer to the outer cornified layer?

Reduplication

The physicist is needed in cancer research for the fundamental concepts which he can contribute concerning the molecular mechanisms involved in the cell's gene system, even though he may never be able to study them directly. If cancer arises from a perturbation in this system, we should know about its physical properties within the limitations of ob-

ideas on the process by which the duplicate genes are passed on to daughter cells. The two sets of genes existing in each human tissue cell are copied and, by a remarkable set of forces, the copies are pushed (or are they pulled?) away from the originals. The origin, nature, and range of these forces are little understood at present. In addition to the forces which push apart the two sets of copies from the original genes in body cells, there also remain to be described the forces which push apart the two sets

Microtome section cut parallel to human skin surface. Dark areas are islands of cells produced as the knife cut through skin corrugations. The cells are in the final dying stages and tend to cluster their nuclei in twos and threes. In between the cell islands are flaky strips of dead cornified cells.



of genes in the precursor of germ cells. The germ cells have but one set of genes so that when two germ cells unite the resultant cell has only two sets of genes, one from the mother and the other from the father. The cell system is able to count the genes it possesses and to make certain that its progeny receive the correct number of genes.

The ability of a cell to count its genes and share them properly with its posterity gives cogent support to the argument that cells have teleological powers. This ability appears to become lost in some cancers, a fact which was discovered by Boveri early in the study of tumors. The reduplication and counting of genes goes awry. Experimental data indicate that the long memory for cancer-inducing agents, and the loss of the reduplication process, and the loss of the counting ability of cells hang together. Perhaps the single crystals which are genes have been cracked or chipped or merely slightly poisoned in the cancer state. In any case, direct examination of chromosomes under the microscope indicates that a very small number of atoms, as numbers of atoms go, has been affected in the change from a normal to the malignant state.

Genetic Death

The ability of the genetic system to reproduce itself exactly and count its genes is, however, no more important than its ability to destroy itself at the proper moment. It has been proposed that cancer arises when cells lose the ability to undergo an orderly death. Such a death can properly be called a genetic death because the gene system has arranged to destroy itself. Tissue is highly organized and in it exists a precision machinery for dying at the right time and in the right way. If only one cell in one hundred million fails to follow the orderly process of dying, cells soon accumulate in a tumor and get in the way of their normal neighbors.

Our quantitative understanding of the genetic death has come from the physical technique of selec-

tive absorption of ultraviolet in nucleic acids which are constituents of genetic material. Caspersen in Sweden has developed the method and has shown, for example, that the genetic material of the immediate precursor of the red blood cell disappears simultaneously with the appearance of hemoglobin. When the genetic material has vanished, the cell is a full fledged red cell incapable of further reproduction. It is then ready for its special job of transporting oxygen.

The formation of calcium deposits in dentine cells of the rat incisor has been found to occur with the destruction of the genetic material. Another common example occurs in skin cells; the dispersal and disappearance of the genetic material can be seen by special chemical staining. In this case, the cell becomes hard and cornified and, as in the blood and dentine cells, incapable of reproduction.

The physicist can contribute further to our understanding of the genetic death by means of selective infrared absorption. Infrared studies are more desirable than the ultraviolet because of the higher selectivity of the infrared bands. Moreover, ultraviolet has an undesirable photolytic effect on materials being studied. Infrared studies have received further impetus from another contribution from physics, namely, the reflection type microscope. Thompson and Barer at Oxford have shown the reflection type microscope to be a very promising instrument for biological analyses in the infrared, especially where only microscopic samples are available.

Another source of information about this all important genetic death of somatic cells will be electron microscopy. The first and obvious method is to micro-section cells in which it is known that the genetic material is being transformed (as, for example, in the so-called granular cells of human skin) and look for molecular structures. The next

step is to study the cornified cells of skin; in these cells can be seen the "hole" where the nucleus and its genes were once located. Preliminary results show a distinct difference between the molecular patterns in the "hole" and the surrounding cytoplasmic areas into which the genetic material was seen to disperse. Such molecular patterns have already been shown by physicists in nonreproducing parts of living organisms. Information about molecular patterns has come almost exclusively from the electron microscope which can be regarded as an indispensable tool for the biophysicist.

Cell Turnover

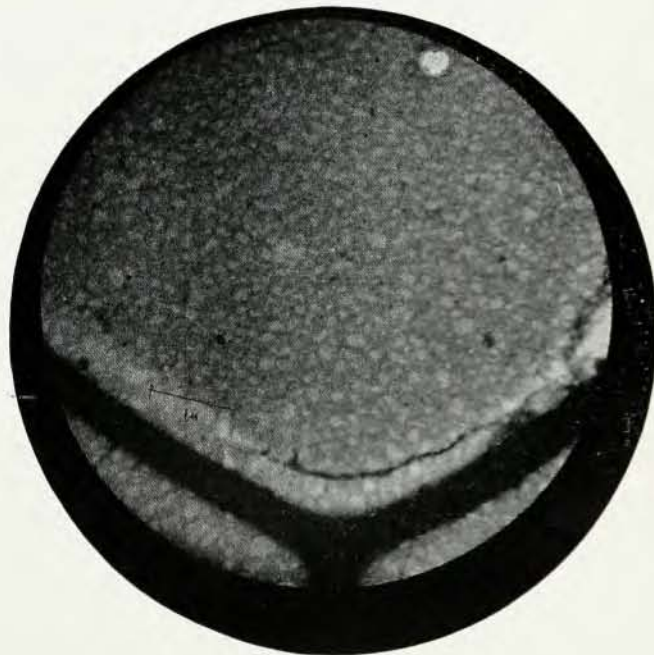
The concept of genetically-determined death of cells leads to questions concerning the quantities of cells which pass from the body and the rates of turnover. Following are figures which indicate orders of magnitude. The skin area of the human body is one and a half square meters; there are ten thousand cornified cells per square millimeter which are renewed every ten days. Thus a billion dead skin cells are sloughed away mechanically each day from the human body, and correspondingly a billion divisions occur each day to make replacement cells. In the circulating blood stream there are four and six-tenths liters of blood; each cubic millimeter of blood has five million red cells; each red cell stays in circulation one hundred and twenty days. There are then two hundred billion red cells lost each day which have to be replaced by cell divisions in the bone marrow cells. Another interesting cell in the blood stream is the lymphocyte, which lives about fifteen hours. The body contains about fourteen

billion lymphocytes, and these are replaced at a rate of twenty-two billion per day.

The enumeration of cell turnover in the entire body is of great interest to the biophysicist because of the insight it can provide into the underlying mechanisms of birth and death of cells. The data available vary widely in their accuracy, but one can estimate that about five hundred billion cells per day are lost by an average human being. This figure represents the number of cell divisions necessary to give replacement cells. It also gives an indirect measure of precision with which the cell reduplication process takes place. In ten years of adult life about a quadrillion cell divisions have presumably occurred.

Figures of this kind offer a springboard from which to plunge into speculation. For instance, bacteria produce a spontaneous, viable mutation once in every hundred million divisions. Lacking any other data, we assume that human cells might give rise to the same spontaneous mutation rate and find that five thousand viable mutants are produced every day or a total of ten million in ten years. At the same time one can expect that nonviable mutants are about one hundred times more frequent than the viables. It is probable that viable mutants are produced also by such external agents as the action of heat and ionizing radiations and chemicals whose actions are not measured by frequency of cell division. It is a wonder cancer occurs no more than it does!

Electronphotomicrograph of human red blood cell. The heavy black line marks margin between three contiguous red cells. The granular area is the film of coagulated blood serum adhering to the plastic replica of the red cell. This represents a new technique for studying coagulated serum in cancer cases.





Bovine collagen fibers under the electron microscope. The periodic structure shows spacing of about 660 Å. Courtesy Jerome Gross and Jour. Exp. Med. 88: 555-568, 1948.

The study of the rate of turnover of cells points to an extremely precise mechanism underlying the molecular rearrangements in the cell during reduplication. In addition to the submicroscope precision, there is an equally precise balance among the cell populations in the human body. Also, the counting of the possible wild mutant cells has a practical background. Clinicians have long known that a malignant tumor may recur after five, ten, or fifteen years in another part of the body even though the primary tumor had been completely excised. One explanation for this type of recurrence is that a few individual cells from the primary tumor went into the circulating blood stream and finally lodged elsewhere in the body. It requires no stretching of the facts to suppose that one of the many possible viable mutants and its progeny will ultimately flourish as a tumor. This is not to say, however, that all cancer arises from mutants.

Aside from the practical aspect of the behavior of cancer, the enumeration of cell populations and their rates of turnover in, say, the human body, is a unique type of observation which will give information about the entire organism as well as about the individual cells. This kind of observation is to be contrasted with that needed for the application of atomic physics. As Delbrück has pointed out, the data needed for describing the atomic system of the living cell can only be gotten at the expense of altering and destroying the system. Concerning the limitations on the study of living systems, we can do no better than to quote Delbrück: "Instead of aiming at the whole of the phenomena exhibited by the living cell we now expect to find natural limits and, thereby, implicitly, new virgin territories, on which laws may hold which are independent of those of physics, by virtue of the fact that they relate to phenomena whose appearance is conditioned on *not* making observations of the type needed for applying atomic physics."

There will be but one way for the physicist to learn which kinds of observations can be made on living matter, and that is to work with cells and

animals. After he has learned the nature of the indeterminacy surrounding normal living things he will be better equipped to study the cancer problem.

Directions for Research

A final word on speculative matters. What would a biophysicist do if he had a billion dollars to carry out experiments? Ask any scientist this question and you find out what is bothering him.

The biophysicist would like to know the quantity of heat exchanged as a cell dies, be it a cell of a cold or warm blooded animal. This involves a few million cells and a microcalorimeter and some chemical which can cause a quick end to respiration. In addition to the absolute amounts of heat exchange, it would be good to know the rate of exchange. Another experiment would involve having cells live in a high magnetic field, say up to thirty thousand gauss. At some field strength Zeeman splitting must occur. How long could cells live without drastic changes in such high magnetic fields? The effect would probably be not unlike that in cells living in the presence of high levels of ionizing radiations.

In another direction, the biophysicist would like to know the spectral distribution of electromagnetic radiation inside living tissue. In humans we know there is heat, but is there a broad spectrum characteristic of a black body or a series of lines or bands? And what kind of spectrum exists in the tissues of the cold blooded animals like fish?

Last but not least is the question of enzyme action. Many workers believe cancer is basically a malfunctioning of enzyme systems. How does an enzyme cause a specific chemical reaction to go? Does it bend and buckle molecules by electrostatic means till they are reactive? Or does it simply impart the extra energy needed by a complex collision process? This question takes the biophysicist into the borderland science of chemical physics, another way of saying that the biophysicist will always find himself wandering in fields other than his own.