An Overview of Fifty Years in Cancer Research: Autobiographical Essay

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The year 1975 marks a half-century during which I was continuously engaged in biological research activities, chiefly in the cancer field. The reports of these research efforts are scattered throughout many journals and many years. It seems appropriate, therefore, to write a brief description of these efforts in chronological fashion, hoping that it will be of interest and utility, both inspirational and practical, to others. In 1918, at the age of 21, I matriculated at 2 universities in Prague, Czechoslovakia (the Karl University and the German University). Students were then allowed to choose their subjects at each university, and equal credits were granted. In 1923 I obtained my D.Sc. (Rerum Naturalium) degree magna cum laude in natural sciences, majoring in chemistry and physics. One year prior to this, I accepted a research position at the newly organized Masaryk University in Brno, Czechoslovakia. After 2 years at the Department of Experimental Pathology, headed by William Laufberger, I joined the Institute of Physiology, which was headed by E. Baback, Rector of the University and a former associate of Pflüger.

Since my early student years my major interest has been the structure and function of living organisms. My studies evolved from problems of metamorphosis of tadpoles and the nutritional status of frogs during hibernation to the metabolism of normal and neoplastic mammalian cells in relation to their ultrastructural characteristics, problems of immunity in neoplasia, and the role of viruses in oncogenesis. Not content only with inquiries into the mystery of normal and abnormal growth, I have also searched for ways to modify or control cancer.

Prior to entering cancer research, I investigated carbohydrate metabolism in frogs, i.e., from the larva stage into mature frogs. These investigations required that I develop a method that would permit determination of glycogen in small amounts of tissue, and I worked out a procedure that required only 0.3 to 0.5 g of liver tissue (8). These studies revealed for the first time that tadpoles store glycogen in small amounts of tissue, and I worked out a procedure that required only 0.3 to 0.5 g of liver tissue (8). These studies revealed for the first time that tadpoles store glycogen in the liver during ontogenesis until metamorphosis and that mature frogs use glycogen to survive during hibernation (9, 10).

Carbohydrate Metabolism

The significant findings of Otto Warburg on the high glycolytic activity of malignant tissues, and my earlier studies on carbohydrate utilization during ontogenesis and in

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I found that in 5 types of tumors the glycogen content diminished dramatically with eventual exhaustion as the tumor increased in size. The loss of glycogen was also reflected in the liver of tumor-bearing animals. If the animals were kept on a high-carbohydrate diet, their tumor proliferated more rapidly and they died sooner than those kept on a regular diet (11).

Studies on the pH of Tumor Tissue and of Blood of Tumor-bearing Hosts

In view of the high glycolytic activity of tumor tissue, it was expected that its pH would be on the acid side. I devised a then new potentiometric method to determine the pH values directly with a platinum or glass electrode; and contrary to what had been found in vitro, the pH in tumors was found to be alkaline (pH 7.4 to 7.6), whereas the pH of the muscle of the same animal was 6.8. It appeared that the lactic acid produced by the tumor tissue in vivo, due to the high glycolytic activity, was well buffered. However, when tumor-bearing mice carrying Ehrlich mouse carcinoma and chickens carrying Rous sarcoma were placed on a high-carbohydrate diet with supplemental injections of glucose every 2 hr, the high glycolytic activity led to a shift from alkaline to acid.

These results pointed to the fact that the tumors are sufficiently rich in buffers so that lactic acid is immediately neutralized. The results also explained why the blood pH, even in advanced cancer, remains alkaline (12). These results were substantiated by a number of investigators (50, 51, 58) and led to the development of a so-called "acidotic" treatment by Fischer-Waseles (7). As a result of interest in this research, I was awarded a 2-year fellowship in 1931 to continue cancer research in the United States, where I have remained ever since. Unfortunately, the acidic therapy subsequently proved to be a failure (13, 14).

During my years at the Masaryk University, I also studied medicine for 3 years and was granted the degree of M.U.C. (Medicine Universal Candidate) by the Medical School. Since my interest was in basic research, I did not pursue an M.D. degree, but instead I took courses in tissue pathology and electron microscopy.

Search for Abnormal Blood Constituents in Cancer Patients

While working under a fellowship in the Department of...
Biochemistry at Harvard Medical School, I carried out an elaborate investigation of chemical constituents in the blood of cancer patients. It was a disappointing study since nothing specific was revealed that could possibly serve for diagnosis of cancer (15).

As time passed, I worked more and more with tumor tissues, and as I became interested in the living cell, I went to the Rockefeller Foundation in New York City for assistance and advice. There I was referred to Robert Chambers, Professor of Biology at New York University, Washington Square College. He welcomed me and, despite limited space and means, he and Gladys Cameron, who had worked for 20 years with Alexis Carrel, graciously helped me to acquire the technical procedures of tissue culture using chick embryo tissues.

Tissue Culture Experiments

In order to cultivate tumor tissues, I designed a medium consisting of blood serum and extracts from embryos of mice of the same strain from which the tumor had arisen. After having succeeded in establishing cultures of mouse mammary carcinoma 63, I attempted to cultivate human tumors. No antibiotics being available, it was necessary to obtain human tumor tissue aseptically. Fortunately, the late Carl Eggers, at Lenox Hill Hospital, and Howard Taylor, at Bellevue Hospital, were most cooperative in supplying me with human material. After many trials and errors, I succeeded in obtaining good epithelial growth without fibroblasts in cultures of a metastatic nodule of a human breast cancer. I recall vividly how the late Robert Chambers was delighted to see, for the first time, a growth of human epithelial cells in culture. Later I was able to obtain good growth in cultures of a human myxosarcoma. Having established several tumors, human and animal, in vitro, I attempted again to find an agent that would inhibit growth of malignant cells without toxic effect on normal cells.

Search for Antibodies against Neoplastic Cells

To develop immunity against cancer cells had been the dream of Ehrlich and others. Jensen in 1903 injected rabbits with large quantities of mouse tumor cells and treated tumor-bearing mice with the antiserum that he claimed caused regression of the tumors. Lumsden (52) had also reported that serum of mice "immunized" with carcinoma 63 killed the malignant cells in culture without damaging normal cells.

I set up experiments along lines similar to those of Lumsden, and I found that medium supplemented with sheep serum supported the growth of human and animal cells. I therefore decided to use sheep for developing a possible anticancer serum.

Sheep were given repeated injections of either plasma or carcinoma cells from patients with breast cancer, and the antiserum was added to cultures of various human normal or neoplastic tissue. An intriguing growth inhibition was noted (16), without toxicity, on growth in vitro of various tumor and normal tissues. In investigating further (17), however, I found that no effect of this experimental sheep serum on oxygen consumption and respiratory quotient of mouse Sarcoma 180 and normal mouse kidney cells was found in vitro, using the Warburg manometric technique; so the mystery remains unsolved.

Radiobiological Studies

The failure of efforts with chemical and immunological agents for therapeutic purposes led me to undertake experiments on the effects of ionizing radiation on neoplastic and normal tissue cells. Since there was no radiation equipment at the Washington Square College, I sought cooperation at the Department of Radiation Therapy, Bellevue Hospital, which was affiliated with the College of Medicine of New York University. Ira Kaplan then Director of the Radiation Therapy Department at Bellevue Hospital and Director of the Cancer Division of the Department of Hospitals of the City of New York, became interested and arranged for the use of the radiation apparatus at Bellevue for my research. Laboratory space and animal quarters were provided for me in a building belonging to New York University Medical School. I organized and equipped the laboratory, office, and animal quarters with funds obtained from research grants and with my own money.

A survey of the literature revealed limited knowledge of the biological responses of mammalian tissues to various sources of ionizing radiation, or of specific doses required to inhibit proliferation. Even such basic factors as lethal and sublethal doses for various types of mammalian tissues had not been definitely determined. Therefore, in my initial experiments I undertook to determine the radiation doses that would prevent growth or destroy tissue cells in vitro. In order to simulate conditions used in clinical radiation therapy, I decided to irradiate small pieces of about 2 to 3 mm of freshly excised tissue instead of growing cells in in vitro cultures. This procedure proved later to be a lucky strike.

I decided to use mouse embryo kidney for my first experiment on radiation effects. Whereas unirradiated kidney cultures showed luxurious outgrowth of a mixed-cell population, only a limited outgrowth, related to the dose applied, was noted in the irradiated cultures. With gradual increase of X-ray dose, lymphocytes, leukocytes, macrophages and, finally, fibroblasts were eliminated without affecting the growth of epithelial cells. Identical observations were made on cultures of human embryo kidney and tongue. Thus, the selective response of various mammalian cell types to ionizing radiation was determined (18). These observations were later substantiated by a number of other investigators, including Chambers and Cameron (5). By using small tissue explants, I also determined the equivalent roentgen value of X-rays for gamma rays from radium (18).

Effects of Radiation on Metabolism in vitro

Having established techniques for determining the effect of ionizing radiation on growth of normal cells in vitro, I undertook experiments to determine the effect of radiation on metabolic processes. Kidneys from 3- to 4-month-old mice were removed and small explants were exposed to X-irradiation. No appreciable effect on oxygen consumption
and respiratory quotients was observed with doses of X-rays that had prevented the in vitro growth of mouse kidney cells. Even a dose of 100,000 R of X-rays failed to have a discernible effect. Thus, it was revealed for the first time that less radiation is needed to prevent cell growth than to affect metabolic processes. However, appreciable effects on such processes were noted when the kidney tissue was exposed to large doses of irradiation from other sources, such as radium and radon (19, 39). These studies were then extended to neoplastic tissues.

**Radiation Effects on Tumor Explants Grown in Vivo and in Vitro**

Mouse Sarcoma 180 tumor fragments were irradiated in vitro and portions were bioassayed for ability to grow in vivo and in vitro. It was found that much less radiation was required to prevent the tumor implants from growing in vivo than was needed to produce the same effect on explants in cultures (20). The reason for this difference is still not adequately explained.

A further significant finding from this study was that the irradiated, attenuated Sarcoma 180 tumor implants that failed to produce tumors immunized the host against subsequent viable tumor implants. Tumor grafts irradiated with a large dose of X-rays, enough to prevent tumor cell growth in vitro, failed to “immunize” the host. Thus, it became evident that, in order to preserve the capacity of the tumor implant to induce a resistant state in a heterozygous host, it should be exposed to no more than a threshold or “attenuating” radiation dose (21).

**Radiation Studies in Isologous Host-Tumor Systems**

The experiments, which showed that resistance against neoplastic growth could be induced by inoculating radiation-attenuated tumor into heterozygous hosts, were then extended to isologous host-tumor systems. The late Halsey Bagg of Memorial Hospital kindly supplied a mammary adenocarcinoma designated 755, propagated in isogenic mice of the C57 “Y” inbred strain. This tumor had arisen in the mammary glands of a female of this strain and killed 100% of recipient mice. Two significant observations resulted from this study. (a) The radiation-attenuated 755 tumor grafts failed to immunize the isogenic recipient hosts. Likewise, mice in which tumors had regressed after effective in vivo X-irradiation failed to mount an immune response to subsequent implantation of freshly viable tumor grafts. However, a prolonged latent period was noted in the reimplanted mice. Whether this prolongation could be attributed to a weak or “subliminal” antigenic effect of the regressed tumor cells or to the older age of the recipient hosts at the time of reimplantation could not be ascertained. (b) Radiation caused an alteration in hair color. Gray hair, instead of the natural black of the C57BL mice, appeared on the X-ray entrance site where the tumor was situated and on the opposite side where the X-rays exited. Whereas the ability of the follicles to produce new hair on the epilated area was recovered, the ability to produce black pigment was lost. Thus, a selective response of a cell component to a specific radiation dose was revealed. Further, 3-mm-thick lead shielding that was then accepted as an efficient shield for normal tissue proved to be inadequate (22). This observation led to the increase of lead thickness as a protective measure for normal tissue.

Since Bagg could supply only a limited number of the inbred C57 “Y” mice, the experiments were continued with inbred mice of the C3H strain and a mammary tumor that had arisen spontaneously in one of them. Contrary to expectations, the C3H mice did not give as uniform a response to irradiated tumor implants as did the C57 “Y” mice. The question arose as to whether all C3H mice were truly isogenic.

At the 19th Annual Meeting of the American Association for Cancer Research, I met the late J. J. Bittner, with whom I discussed the results I had obtained with the C3H mice. Bittner kindly sent a number of his own highly inbred line of C3H (high-cancer) mice, and these were carefully inbred by brother and sister mating. When a sufficient number of mice were available and a mammary tumor had developed spontaneously in a breeding female, experiments were begun. Those mice in which irradiated tumor grafts failed to produce tumors were reimplanted with viable tumor grafts. All reimplanted mice produced tumors. It was also shown that irradiated tumor implants failed to prevent the spontaneous development of mammary tumors that usually occurred in almost 100% of breeding females. Experiments along this line were also performed on mice of the inbred DBA/212 line, kindly supplied by T. S. Hauschka. Over 90% of breeding females of this line produced mammary tumors and were later shown to carry the characteristic MTV (43). By the method of implanting radiation-attenuated tumor grafts, it was found that about 5% of the mice were not isogenic to the mammary tumor that arose in a female of this strain and that had been carried by serial transplants in mice of this line (29). This method was also useful in detecting isogenicity of host rat tumor systems (23, 24).

In a study on mice with bilateral mammary tumors, one tumor was X-irradiated, while the tumor on the other side was lead shielded. The irradiated tumor regressed completely, whereas the unirradiated one continued to grow (28). This demonstration definitely reversed the prevailing opinion, which had been based on observations of heterologous host-tumor systems, that there is no need to irradiate all cancer nodules in the same host. The experiments also showed that tumors grown in heterologous hosts required less radiation dosage to induce total regression than did tumors grown in isogenic hosts, thus demonstrating a close genetic relationship between the tumor cell and the cells of the immune defense system of the host. Ionizing radiation failed to alter the “genetic” makeup of the tumor cells so as to render them “foreign” to the isogenic host. As a consequence, the isogenic host-tumor system became a prerequisite in experimental cancer research, particularly when prospective therapeutic agents such as radiation, chemical, or other forms were to be evaluated. The significance of these findings was stressed in an excellent review paper by Scott (61).
A. Goldfeder

Studies on Tumors in Isoologous Hosts Growing at Different Rates

By continuous brother-sister mating of DBA/212 mice and by selection of mammary tumors arising spontaneously in breeding females of this strain, I was able to establish tumors that grew in 100% of the grafted mice of both sexes. Although morphologically identical, some grew slowly and others grew at a faster rate. It was gratifying to have Potter of the McArdle Laboratory point out the value of using such a system in experimental cancer research at the International Cancer Congress held in London in 1958. I demonstrated that 2 analogous mouse mammary adenocarcinomas, both growing in isogenic hosts of the Bittner C3H or of the Hauschka DBA/212 mice, respectively, differed markedly in growth rate, metabolic activity, and radiosensitivity. A definite relationship was found among architectural composition, degree of differentiation, cellular arrangement, extent of mitoses, metabolic activity, and response to ionizing radiation. For the first time I demonstrated that a tumor can be totally destroyed by a given dose of irradiation and that the treated mouse can live out a normal life-span. This was made possible by lead shielding, which effectively protected normal tissues of the treated mouse. Even a dose as large as 12,000 R delivered to the tumors in situ did not sterilize reproductive organs of treated mice, and mating of cured males with cured females produced litters. As a result, I suggested that exposing multiple small areas, as I had done in animal experiments, could be applied clinically to avoid skin damage (26, 27). This method, permitting larger doses of radiation, proved effective in radiation therapy.

Experiments with Radioactive Tracers

Until World War II, little was known of the basic phenomena of radiation biology. During and since World War II, new areas of radiation research developed. Studies began on elucidation of mechanisms involved in radiation death, carcinogenesis, aging, etc. Furthermore, ionizing radiation became a powerful analytical tool in the form of radioactive tracers in metabolic studies and in chemical reactions.

In order to familiarize myself with the application of these new tools, I took a course in isotopes, the first time such a course was given, at Oak Ridge National Laboratories in 1948. To my great surprise, I was elected Valedictorian of the class, an honor that was most gratifying.

Metabolic studies involving static and dynamic levels of phosphorylated intermediates in the glycolytic cycle of adenocarcinomas of different growth rates were initiated. While it had been shown that the rate of O2 uptake and aerobic glycolysis was about 3 times higher in the fast-growing dbrB tumor than in the slower growing C3H tumor (19), in this study the pattern of glycolytic cycle components corresponded to that of normal differentiated tissues, with the exception that the lactic acid level was higher in the tumor tissues. Among the tumors, lactic acid was higher in the slow-growing C3H than in the fast-growing dbrB mammary tumors. Further, the fast-growing tumor (dbrB) showed significantly higher levels of glucose 1-phosphate and ATP and a lower level of inorganic phosphorus than did the C3H tumor. These findings indicated that a higher level of energy was available for vital function in the dbrB tumor and that its intermediary metabolism proceeded at a faster rate. Thus, it became evident that 2 mouse mammary adenocarcinomas, although of the same morphological classification, differed significantly in their metabolic activity. These findings are relevant to the timing of radiation or drug therapy (35).

Results of studies using 32P incorporation in tumors indicated that the phosphorus in the ATP fraction of the fast-growing dbrB tumor was more completely equilibrated with the inorganic phosphorus at the end of 1 hr than was that of the slow-growing C3H tumor, indicating further a more rapid metabolic turnover in the rapidly proliferating dbrB tumor (1).

Protein Biosynthesis Using [14C]Leucine

In later studies it was shown that protein synthesis also proceeds at a faster rate in the rapid-growing tumor, than in the slow-growing one, indicating again the importance of knowing the metabolic characteristics of neoplasms, particularly when therapy with various agents is concerned. Another significant finding was the observation that a dose of 3000 rads resulted in an increase of protein biosynthesis at 1 hr after irradiation, indicating release of ribosomes and change in permeability of the cell-boundary membranes (55). This was later documented by electron microscopic observations (30, 45).

Kinetic Studies of Nucleic Acid Synthesis of Tumors of Different Growth Rates

As soon as [3H]thymidine became available, I undertook a study of the life cycle of the cells of 2 tumors. This was the 1st determination of kinetics and the various phases of the mitotic cycle of 2 morphologically and biologically diverse, but genetically isologous tumors, a spindle-cell and an epithelial cell tumor. These tumors differed in the G1, S, and G2 phases. The longer S time of the spindle-cell tumor was rather surprising, since it had been presumed that cells of faster growth rate would synthesize DNA at a faster rate. Furthermore, the opinion then prevailed among investigators that the S time was constant for various cell types. However, these experiments revealed that the integrity of the cytoplasmic organelles and the ploidy of the cells influenced the time needed for the cells to traverse the S phase of the cell cycle (31, 46).

To explore further the role of intracellular organelles played in cellular function, we undertook the investigation of the status of nucleoli in tumors of different growth rates. Three morphologically different types of nucleoli were detected in tumor cells: dense, trabeculate, and ring shaped (59).

Kinetic studies, using [3H]thymidine, revealed that tumor cells with dense nucleoli are in an active state of proliferation, whereas cells with trabeculate and ring-shaped nucleoli are in a resting state. These criteria proved helpful in quantitative evaluation of cells with respect to their proliferation phase in a mixed-cell population of tumors (60).
Experiments on Whole-Body Radiation

New phases of experimentation on whole-body irradiation were initiated involving radiation protection, radiation effects on internal organs, and radiation carcinogenesis. A procedure was devised to irradiate 25 mice whole-body simultaneously. The effect of added vitamins in the diet was tested as a protective measure. Irradiated mice on the vitamin diet survived a longer period of time than those on regular diet (37).

Based on observations that the spleen was the most radiosensitive organ and that protection of the spleen during radiation provided much longer survival, further experiments were performed to study the protective effect of spleen extracts (49). Casimir Funk, the discoverer of vitamins, became interested in the problem and he prepared a number of extracts from young calf spleens. These preparations somewhat enhanced the survival of X-irradiated mice of 3 strains: DBA, C57BL, and Albino mice. The most effective protection was obtained by injecting isogenic bone marrow cells (36).

Radiation Effects on Internal Organs of Whole-Body Irradiated Mice

The topographical distribution of alkaline phosphatase in several organs of whole-body X-irradiated and unirradiated mice was studied. Although cytological changes occurred, particularly in the duodenum and lymphoid elements of the spleen, the usual topographical distribution of alkaline phosphatase persisted in the kidneys and liver. Alkaline phosphatase activity persisted in the columnar cells of the villi and in the mucosal layers of the intestines although the architecture of these cell types was disrupted. Of significance was the fact that intestinal epithelium proved to be more radiosensitive than the kidney cells, as indicated by the release of this enzyme (54). The release of enzymes through or from membranes has become a significant criterion for cellular radiosensitivity.

ATPase Activity of Nuclei and Mitochondria of Irradiated Tumor Cells

The results of alkaline phosphatase studies indicated the need for more information about selective radiation effects on cell membrane enzyme systems. With Leo A. Miller, a biochemist who joined my laboratory, the distribution and activity of ATPase were studied. The localization of ATPase in mitochondria was already known but its association with the nucleus was uncertain. A procedure was worked out to isolate nuclei in pure form from mouse liver, and the activity of ATPase of nuclei and mitochondria isolated from a mouse mammary adenocarcinoma and spindle-cell tumor was compared with mouse liver. Three significant observations evolved: (a) ATPase was localized at the nuclear membrane; (b) a greater mitochondrial ATPase activity of epithelial tumor than that of spindle-cell tumor was attributed to the greater number of mitochondria in epithelial tumor cells as revealed electron microscopically; and (c) a greater nuclear ATPase activity in the spindle-cell tumor than that of the epithelial tumor was attributed to the larger size of the spindle-cell tumor nuclei. A correlation was also found between the integrity and quantity of mitochondria and ATPase activity (53). On X-irradiation of nuclei and mitochondria of slow-growing epithelial and rapid-growing spindle-cell tumor, an initial increase followed by a decrease in ATPase activity was noted as soon as 10 min after irradiation of both tumor types, which was radiation-dose and time-dependent. The initial increase was explained by the release of this enzyme from the boundary membranes of the nuclei and mitochondria (45).

Catalase Activity of Irradiated Tumors

We were then led to investigate the distribution and extent of catalase activity of tumors because it had been found by DeDuve et al. (6) that the activity of this enzyme is membrane bound and is localized within the mitochondrial fraction.

The catalase activity of the slow-growing epithelial tumor (DBAH) was about 7 times greater than that of the fast-growing spindle-cell tumor (DBAG). While the highest activity in the epithelial tumor was found in the mitochondrial fraction, the cell sap of the spindle-cell tumor contained the greatest activity. No activity was found in the nuclear fractions of either tumor. Within 15 min postirradiation, a shift in distribution of catalase activity from the mitochondrial to the cell-sap fraction occurred in the epithelial tumor. Thus, the higher levels of catalase activity in the cell-sap fraction of the irradiated tumors indicated leakage of the enzyme from the boundary membrane of the catalase-containing particle associated with the mitochondrial fraction. It was evident that the membrane system enveloping the enzyme was more radiosensitive than the catalase activity itself (30, 47).

Oxidative Phosphorylation of Tumors Varying in Growth Rate

The significant difference in mitochondrial catalase activity between the DBAH epithelial cell and the DBAG spindle-cell tumors, both isogenic to the same host, led us to investigate the "status" of the mitochondrial oxidative capacity of these 2 tumors. It soon became apparent that in order to obtain a sufficient amount of mitochondria for analysis, a larger amount of tumor tissue was required from the spindle-cell tumor than from the epithelial tumor. In some instances, there was oxygen uptake by DBAG tumor mitochondria but no phosphorylation, indicating the inferior quality of the DBAG tumor mitochondria. These results confirmed the significant difference between the mitochondria of these 2 types of tumors (30).

In further studies, the values for ATP, glucose 6-phosphate, and cytochrome oxidase paralleled those obtained for ATPase (46).

Mitochondrial Protein and RNA Synthesis in X-irradiated Mouse Livers

The effects of irradiation on mitochondrial protein and RNA synthesis were studied in vivo, using [14C]leucine and
[H]uridine as tracers. Higher levels of protein and RNA synthesis were found in X-irradiated liver mitochondria than in nonirradiated controls. This increase was explained by the intracellular increased transport of RNA and protein precursors due to change in permeability of the boundary membranes (55).

In recent studies on the time-course of effects of irradiation on protein biosynthesis in mouse livers in vivo, it was noted that as soon as 10 min after 2000 rads, protein biosynthesis was about 50% above that for normal controls and reached control level within 4 hr. The enhanced protein biosynthesis after X-irradiation was reflected in the increase in ribosomal population released from the membranes and in the increase in concentration of acid-soluble precursors in the intracellular pool of the X-irradiated liver cells due to permeability changes (56, 57).

Electron Microscope Studies

The experiments on ATPase activity, distribution of catalase, and P:O values all indicated the possibility that the quantity and functional activity of the spindle-cell tumor mitochondria might be inferior to those of the epithelial tumor. I discussed this problem with George Palade of the Rockefeller Institute, who had published an extensive paper on mitochondria, and he kindly offered to investigate the morphological and structural status of mitochondria of both tumor types. He found that the mitochondria of the epithelial tumor cells were more numerous and contained more internal membranes (cristae) than those of the spindle-cell tumor cells, in which the mitochondria were relatively few in number and contained few cristae, and some of which appeared deformed and “swollen.” These findings proved to be significant not only for explaining the results of the ATPase, catalase, and P:O studies but also for explaining the difference in cellular radiosensitivity seen in later experiments. The need to examine the ultrastructures of neoplastic cells and their response to ionizing radiation led me to take a course in electron microscopy, organized in the Cell Biology Department of the Rockefeller Institute, by Porter and Palade for members of the Rockefeller Institute. I was, in fact, the only outsider allowed to take the course. Since then, I have been privileged to continue to use one of the electron microscopes at that institute for my research.

Cellular Radiosensitivity at the Subcellular Level. This phase of my research revealed that cells, either normal or neoplastic, that are equipped with well-differentiated cytoplasmic organelles are more radiosensitive than cells having poorly differentiated cytoplasm. These observations also explained my previous tissue culture findings that showed lymphocytes to be most radiosensitive and renal epithelium most radiation resistant. Electron microscopic studies showed that lymphocytes had a narrow rim of cytoplasm, few mitochondria, and a small number of ribosomes, whereas renal epithelial cells had an abundance of mitochondria, ribosomes, etc. This finding also proved true for neoplastic cells of corresponding origin. For example, nephromas, liver tumors, mammary epithelial tumors, and other tumors of epithelial origin showing well-preserved cytoplasmic organelles are more radiosensitive than lymphoma cells.

Electron microscopic studies of the spindle-cell tumor also pointed to the role of the membrane system in cellular radiosensitivity. Dilation of the bordering membranes and of the endoplasmic reticulum membranes that formed cisternae in some cases indicated radiation-induced changes in the permeability of the cellular membrane system. These findings explained the reason for the release of enzymes from or through the membranes of irradiated cells as previously mentioned (30, 45, 53–56) and also explained the release of ribosomes from membranes of X-irradiated cells as indicated by increase in ribosomal protein biosynthesis (57). The results of these experiments provided documentation for the “enzyme release” theory proposed by Bacqué and Alexander (2) as one of the possible mechanisms of radiation-induced cell injury.

Studies on Oncogenic Viruses. While taking the course in electron microscopy at the Rockefeller Institute, each participant was required to perform microscopic work on some biological material of his own interest. I chose a mammary tumor that had developed spontaneously in a breeding female of the DBA/212 strain of mice. I wondered whether or not these tumors were of viral origin. At that time, a paper by Bernhard (4) listed the various strains of mice that carry the MTV. No mention was made of the DBA strain. This gave me the impetus to test the possibility of detecting MTV in the mammary tumors of the DBA/212 mice, which were subsequently found in abundance (43).

Oncogenic Viruses in Mice of a Tumor-resistant Strain

In my earlier experiments dealing with spleen preparations as protection against radiation injury, I used albino mice obtained from a dealer. These albino mice proved to be tame and easy to handle. Therefore, I decided to inbreed them and set up a colony in 1953. Since the dealer had no information about the origin of these mice, I designated them “X.” The X-strain mice have been used in large numbers for whole-body irradiation experiments. It became apparent that the mice that survived radiation, and some that lived out a normal life-span did not develop neoplasms. These mice were also of interest in that no malignant tumors, neither mammary nor lymphoid, developed spontaneously among the breeders. This was in contrast to the DBA/212 strain, the breeding females of which developed mammary tumors with great frequency. Failure of the irradiated X-mice to produce neoplasms was noteworthy in view of the many published papers reporting neoplasms in irradiated animals. In fact, studies on radiation carcinogenesis became a common effort in the cancer field after World War II.

When immature X/Gf mice were exposed to X-rays, known chemical carcinogens, or combinations of X-rays and chemical carcinogens, only 3 to 4% of a total of 1023 mice developed a mammary tumor, lymphoma, or other internal neoplasm. Of these rare neoplasms, MTV particles were noted in only 1 mammary tumor that had developed in a female treated with urethan alone (40) and in another mammary tumor that had developed in a female treated with X-ray plus...
Transmission of MTV to Newborn X/Gf Mice by Foster Nursing

It has been well established that transmitted MTV induces tumors in foster-nursed mice of low susceptibility to this virus. Since the X/Gf mice are naturally resistant to spontaneous development of mammary tumors, it was of interest to test their response to exogenous MTV. Newborn X/Gf mice were foster-nursed on DBA/212 mothers of the high-cancer line mentioned previously (43). About 40% of the 1st generation (F1) foster-nursed X/Gf breeding females developed mammary tumors at ages ranging from 12 to 18 months. A drastic decrease occurred in the incidence of mammary tumors in ensuing generations. Among the F1 generation, only 1 breeding female produced a mammary tumor, but none of the F1 generation did. The presence of the MTV in the foster-nursed progeny was later documented electron microscopically (34, 42) and at a molecular level (48).

Cessation of mammary tumor development in the foster-nursed X/Gf mice was ascribed to their high level of natural antibodies against MTV (3).

One of the mammary tumors induced by foster-nursing has been propagated by serial transplants in isogenic X/Gf mice. Electron microscopically, the transplanted tumors have shown the characteristic MTV continuously for 8 years to date. This finding lends support to the "proto-virus theory" of Temin (64), suggesting the continuous replication of the virus by the initially infected cells and their daughter cells. Thus, the foster-nursing experiment demonstrated (a) the infectivity of the MTV, even in tumor-resistant mice, and (b) the significance of the immune competence of the infected host in countering viral invasion (34, 42).

The X/Gf mice also proved to be resistant to polyoma virus (44), known to produce a variety of neoplasms in mice and in other animal species. They were resistant to Friend leukaemia virus when adult; newborn X/Gf mice proved to be partially susceptible (38).

Search for Factors That May Be Responsible for Tumor Resistance in X/Gf Mice

Since the X/Gf mice were exceptionally free of cancers, studies were undertaken to determine what factors might be responsible for resistance to neoplasia. It was found that these mice possess a high immune competence (62), high splenic phagocytic activity (63), and appreciable levels of circulating antibodies against mouse MV (3). No leukemias or lymphomas have ever developed spontaneously in the X/Gf mice, which offers a model system for the study of resistance to oncogenesis.

Chemical Carcinogenesis

Since the isolation of the potent carcinogenic chemicals from tar experiments, chemical carcinogenesis became one of the most active phases in experimental cancer research. When p-dimethylaminoazobenzene (known commercially as "butter yellow") proved to be a potent liver carcinogen in rats, it was a challenge to search for a compound that would counteract its carcinogenicity. It occurred to me that spleen tissue might be worthwhile to test because of its resistance to spontaneous development of solid tumors and to metastasis. An experiment was set up with 1 group of rats placed on a standard rice diet mixed with 0.06% carcinogen and a 2nd group on the carcinogenic diet to which was added 10% dried calf spleen. In all rats on the carcinogenic diet only, liver cirrhosis occurred, tumors appeared within 170 days, and all were dead by 236 days. Of those rats on the carcinogen plus dried spleen diet, no cirrhosis occurred and only 8% developed tumors after 170 days. Of the remainder, about 50% had hepatomas at 300 to 400 days (25). Tissues of other organs, or proteins and amino acids added to the carcinogenic diet, were also found to reduce the carcinogenicity of this chemical; others later showed that riboflavin exerted a protective effect on liver carcinogenesis.

Retrospective Remarks

During my early years at high school, when the teacher placed on the blackboard several problems of choice, I always selected the one that appeared most complicated. My classmates wondered why I had not chosen the simpler problems. My reply was: "It seems to me more interesting to solve a complicated problem rather than a simple one." This attitude has prevailed throughout my life and is evidenced by my choice of research on the cancer problem and radiobiology, both being among the most complicated fields of endeavor.

As I look over the sequence of my research activities, the diversity of the experimental approaches were the lamps I used to shed some light on the mechanisms involved in the alterations in the structure and function of neoplastic cells. When prospects of continuing productive experimentation along a particular line appeared futile, I terminated the work and directed my efforts toward more promising investigations. Although my experiments on immunological control of cancer were unsuccessful, they pointed to the close genetic relationship of the neoplastic cell to its host of origin and indicated the importance of using isogenic host-tumor systems in experimental therapy. Similarly, I did not pursue the use of chemicals as curative agents because of their lack of selectivity, but these studies led me to discover the effectiveness of ionizing radiation. I succeeded in demonstrating that neoplasms can be cured by using proper radiation doses and efficient shielding of normal tissues. I also demonstrated that tumors even of the same morphological classification might differ in their response to radiation and that each tumor should be treated individually according to its biological characteristics. This knowledge is a prerequisite for effective radiation therapy.

Through the years, my research efforts have been concerned with structure and function of normal and neoplastic cells. In pursuing these studies, I have used biochemical, biophysical, and cytological techniques on tissue cultures, tadpoles, frogs, mice, rats, and sheep. Experiments were
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performed on whole animals, tissues, whole cells, and intracellular organelles. I have constantly focused my efforts on the mechanism(s) of cellular proliferation as a means of influencing or countering neoplastic growth. I have always believed that an understanding of the basic mechanisms of normal and abnormal growth is necessary for effective control.

In retrospect, I now understand what the late Carl Sternberg meant when he told me in my student days that he would not hesitate to begin again with research on neoplastic growth. If I were to start my scientific career again, I would not hesitate to begin again with research on neoplastic growth.

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