SUMMARY OF THE MEETING

In these last two days we have had our minds stimulated by facts and ideas, skillfully collated and presented by research workers for various countries. On your behalf, I would like to thank Dr. T. Sugimura, Dr. H. Tanooka and their colleagues, not only for the marvelous hospitality, but also for the successful manner in which this symposium has been organized and run. We will all go away with fond memories, new information and, no doubt, new ideas. On behalf of the organizing committee, I would like to thank the speakers who have done us proud in presenting their work with clarity and conveying the joy of research.

One of the great values of symposia like this one is the exchange of views of researchers with quite different perspectives. This symposium has dealt with views based on work at every level of biological organization, from the molecule to the level of the human being.

What did we hear at this symposium about radiation carcinogenesis? The spectrum of research in carcinogenesis is broad, and increasingly the approach is led by the developing technologies. This brief summary cannot do justice to all the papers, but hopefully will remind you of areas that you would like to learn more about when you go home.

The opening address was given, appropriately, by S. Kondo (Kinki Univ.). He discussed possible models that might apply to the multi-event process of radiation carcinogenesis. He suggested that dose responses for acute and chronic myeloid leukemia in the atomic bomb survivors differed, the latter having a threshold. In the case of acute myeloid leukemia, mutations of proto-oncogenes in hematopoietic stem cells could explain the response. The presence of a threshold response in chronic myeloid leukemia suggested that radiation acted on the stage operationally known as progression.

Kondo thought that high doses of radiation caused a persistent disturbance in the cell renewal system. The implication was that radiation induced some cancers only at high doses...
above a threshold value by killing a critical number of stem cells. He proposed that the opening statement to the Symposium should be that carcinogenesis was the problem not just of a single cell but also of the society of cells.

**Human Cancer after Exposure to Radiation**

The session on radiogenic human cancer was opened by the doyen of the study of human radiation leukemogenesis, Dr. Ichimaru (Atomic Disease Institute, Nagasaki). His contention of not only a threshold dose for chronic myeloid leukemia but also for an even larger threshold for acute myeloid leukemia is at odds with the analyses made by most biostatisticians. Dr. Ichimaru and his colleagues have not embraced the new dosimetry, DS86, which has altered the apparent dose-response relationship. Ichimaru thought the chronic myeloid leukemia was the most characteristic radiogenic leukemia and the incidence was higher in Hiroshima than Nagasaki. This city difference remains unexplained. Is it related to differences in (1) the de novo incidence of chronic myeloid leukemia between the cities (this is not considered to be significant), (2) the age distribution, (3) other constituent factors such as viral sequences, or (4) the neutron dose, which is higher than is suggested by DS86? Perhaps a fifth possibility, which is still unidentified, should be included.

It was suggested that slopes of the plots of relative risk as a function of dose increased in the following order, in terms of the types of leukemia: acute myeloid leukemia, acute lymphocytic leukemia, and chronic myelogenous leukemia.

Van Kaick (Heidelberg, FRG) and Mori (Chiba) discussed the late effects of thorotrast. There are a number of examples of iatrogenic cancers that have occurred, sadly, in sufficient numbers to make them valuable populations for epidemiological study. The cancers caused by the contrast medium thorotrast are an example. The results of the follow-up of German and Japanese patients have many similarities. Liver cancer predominates, with an incidence of over 400 cases in the German series of 2326 patients. In about 75% of the cases of the Japanese study, the injections were associated with diagnosis of injuries incurred in World War II. In one subsample, 56 liver cancers were found out of 262 cases inspected. Van Kaick noted that brain tumors occurred as early as 3 years after carotid artery injections, leukemias after 5 years with 1.7:1 male-female ratio. One out of ten of the leukemias was chronic myeloid leukemia; lymphocytic leukemia was not increased but multiple myeloma was. A striking finding in the Japanese series was that about 23% of the cases had two to four different types of malignancies.

In neither series is there evidence of excess of small-cell lung cancer. The most frequent liver tumors were carcinomas but about 35% of the tumors were hemangiosarcoma, a rare tumor. Cirrhosis appears to be found more frequently in the Japanese series.

The effects of levels of radiation that are in the range of environmental doses are well nigh impossible to study as the size of the populations required is enormous. Attempts have been made to study people exposed for their lifetime to doses from background radiation that are in excess of the average background radiation level, but such studies are fraught with difficulties. Some years ago in the U.S., Frigerio and Stone examined the relationship of altitude, and therefore radiation level, to cancer mortality, and despite all the pitfalls in
such studies a negative correlation was suggested. Dr. Chen (Beijing) described the study of very large populations in two areas in China in which the background radiation differs by a factor of about 3. The mortality rate for all cancers was reported to be lower in the higher background radiation area than in the control area. This finding is nectar to the proponents of hormesis. The populations were carefully matched, but taking account of all factors, some of which may not even be suspected, is a testing task. As Chen said, "more information is needed."

Dr. Izumi (Nagasaki Univ.) reported on the effects on the thyroid of residents in the Nishiyama district of Nagasaki who were protected by the mountains from direct effects but exposed to the fallout from the atomic bomb. This population was compared to survivors of the direct radiation. Thyroid nodules were found in 4.9% of the residents who were thought to be exposed to the fallout radiation and in 4.2% of those exposed directly to the atomic bomb radiation. These incidences can be compared to 1.6% in the control population. The study indicated a marked sex and age dependency. In those exposed to the atomic bomb radiation, there was a significant increase in the incidence of nodules in females but only in those aged less than 20 years at the time of exposure. There was no increase in the incidence in males. The estimated maximum dose to the thyroid from fallout $^{137}$Cs was 0.12–0.24 Gy. The question was raised about what level of fallout had occurred with atomic bomb tests, in particular the Chinese tests, and whether this had contributed to the total dose. The answer was not known.

Developmental Stage-Dependency

Dr. Shimizu (RERF) reviewed the findings of Life Span Study of the Radiation Effects Research Foundation for the 1950–1985 period, based on DS86. Increases in cancer of breast, lung, esophagus, stomach, colon, ovary, bladder, and thyroid and an increase in multiple myeloma were found. No excess has been detected yet in cancer of the rectum, gallbladder, pancreas, prostate, or uterus or in malignant lymphoma. In the sample of 42,000 persons exposed to 0.1 Gy or greater, there has been an excess of 80 leukemias and 260 other cancers, a ratio of solid cancers to leukemia of 3.25 : 1. This ratio will continue to increase because the excess in leukemia is now very small but a majority of the atomic bomb survivors are still alive (62% in 1985). The UNSCEAR risk estimate of total cancers in 1977 was based on a prediction that the final ratio of solid cancers to leukemias would be 5 : 1. Those exposed when young are now reaching the age at which cancer incidence increases in the unexposed population, and the excess of some solid cancers occurs only when the age at which the incidence of the same types of cancer starts to rise in the unexposed population is attained. This clearly demonstrates that initial events persist and that age-dependent host factors determine expression of the initial events. Thus, while the initial events are essential, it is the host and exogenous factors that influence expression of the cancer potential.

The risk of cancer in children exposed in utero or between 0–9 years of age appears to be comparable. For example, the relative risk at 1 Gy is 3.77 for all cancers in those exposed in utero and 3.97 for those exposed between 0 and 9 years of age.
Nomura's (Osaka Univ.) experimental work on paternal exposure and cancer in the progeny of mice preceded by quite some years Gardner et al.'s findings at Sellafield. Nomura's findings were that irradiation of spermatogonia increased the incidence of lung tumors in the F1 generation of ICR mice but did not increase leukemia. However, doses in the range of 0.36–5.04 Gy to spermatids and spermatozoa increased the incidence of leukemia two- to threefold, whereas in two other strains a five- to tenfold increase in leukemia was found in the F1 generation after exposure of spermatogonia to 5.04 Gy of X rays.

The striking difference in the experimental results from the findings that have been considered to be mechanistically similar in humans is that the doubling dose is about 40 times greater in mouse than man.

Sasaki (NIRS, Chiba) reported the results of a study of B6C3F1 mice irradiated at 17 days gestation, at birth, and at 7, 35, 105, 240 or 365 days of age. The most sensitive age for life shortening was in the early postnatal period. The study showed that each tumor type has an individual age dependency. Fetal and neonatal mice were not sensitive to the induction of myeloid leukemia or Harderian gland tumors but the susceptibility for induction of lung, liver, and pituitary tumors, for example, was high in late fetal development.

Benjamin (Colorado State Univ.) recounted the experience with the dogs irradiated at 8, 28 or 55 days postcoitus and 2, 70 and 365 days postpartum. The doses used were 0.16 and 0.83 Gy. Surprisingly the unexposed control dogs had no mammary or other malignant tumors, but tumors, including malignancies, were found in dogs irradiated in the perinatal period. Tumors appeared within 4 years postirradiation and more frequently in females. Benjamin suggested that perhaps the fact that during embryogenesis several proto-oncogenes are expressed might influence radioresistance to tumor induction in the early stages of embryogenesis.

**Experimental Radiation Carcinogenesis**

The first paper of the session on experimental carcinogenesis was by Ullrich (Univ. of Texas at Galveston). The in vitro mammary cell system is being used to study the progressive changes in tumorigenesis. The frequency of an altered phenotype, characterized by the ability to culture the cells, is high after irradiation. While 1.0 Gy induces 20% incidence of mammary tumors in the BALB/c mice, injections of cells isolated from mammary glands exposed to 1.0 Gy into cleared fat pads will cause neoplasms in every fat pad. Clifton and Gould (Univ. of Wisconsin) have reported similar results. These workers also found that chemical carcinogens induce fewer initially altered cells but that more cells go on to express the cancer potential than after irradiation. Ullrich reported that over-expression of c-myc was detected in the mammary cells. An early change detected by a monoclonal antibody was a mutant of P53, which suggests the importance of suppressor genes. Although not tested for in the early cell passages, Rb mRNA expression was lost in late passages. Using an assay developed by Folkman, the angiogenic activity was assessed after exposure to gamma rays and neutrons. Angiogenic activity was found early and more frequently after neutrons than gamma irradiation.

Covelli (ENEA, CRE Casaccia) discussed dose-response relationships, especially for
high-LET radiation. Quadratic and linear dose-response curves were considered the best fits to the initial slopes of the curves for myeloid leukemia and liver tumors after exposure to low- and high-LET radiation, respectively. If this is the case, RBE values must be very high at very low doses and would not show any limiting value, a finding that is different from those at Argonne National Laboratory and Oak Ridge National Laboratory. In the case of ovarian tumors no simple model fits the data and there is a threshold response. The response is consistent with the mechanism suggested many years ago, namely that the radiation, even at very low doses, kills the sensitive oocytes, upsetting the ovary-pituitary hormonal feedback, leading to increased and protracted gonadotrophin secretion that in turn results in tumors of the follicular cells. In the liver a very marked age dependency for susceptibility was found. The susceptibility decreased from the highest, when the fetus was irradiated, to the lowest, at 9 months of age, the oldest age tested. The results suggested that RBE for liver tumor induction also decreased with age.

Seed (Argonne National Laboratory) described the stages in the development of myeloproliferative disease in beagle dogs. It is well nigh impossible to induce excess myeloid leukemia in dogs with single doses. Dogs are radiosensitive and have a low LD_{50}, and at the highest doses at which the animals survive the induction rate of leukemia is low so that excess leukemia could be determined only with very large sample sizes. However, at low dose rates, 0.17 Gy/day and less, but at high total doses, >12 Gy, a high incidence of myeloproliferative disease occurs. There is a four-stage sequence in the development of the disease: (1) suppression (bone marrow damage); (2) recovery with compensatory increase in proliferation; (3) accommodation; and (4) preleukemic transition. An interesting characteristic is the crisis when in some animals the increasing aplastic anemia leads to death while others recover and become candidates for leukemia with a probability of about 0.5. Accompanying this recovery, and perhaps the reason it occurs, is the development of increased radioresistance of the bone marrow cells. This adaptation is reflected in the appearance of a shoulder in the cell survival curve for the granulocyte-monocyte stem cells, an increase in D_{0} and in split-dose recovery. These results suggest an acquired increase in the ability to repair the radiation-induced damage. It would be interesting to know if the analogy of these preleukemia changes with the changes that occur in \textit{in vitro} cell transformation as cells go through crisis and change from a cell strain to a cell line can be extended to the changes from a diploid to an aneuploid state that occurs in cells \textit{in vitro}. Unfortunately, dog chromosomes and aberrations in them are difficult to analyze. However, a nonrandom lesion has been found in chromosome 1, and the percentage of cells showing this lesion, which appears before the crisis, increases at a rate consistent with some power function. This might signify clonal expansion. Clearly at some stage after the crisis the cells acquire autocrine function and autonomy. If the irradiation is stopped before this stage, leukemia does not appear to develop.

Yokoro (RERF, Nagasaki) and his colleagues have compared the carcinogenic effects of tritiated water with the radiation of other qualities. Tritiated water was administered by a single interperitoneal injection, and if the dose was 20 mCi/mouse or less, the mice survived long enough to get tumors in many tissues. There was a striking lack of dose dependence for
tumor induction between 3.75 mCi and 15 mCi. When the mice were given 0.25 mCi/ml continuously in drinking water, the tumor incidence was 70% (0.05 mCi/ml), 77% (0.25 mCi/ml) and 83% (0.1 mCi/ml) with a preponderance of T-cell lymphomas of the thymus. In the highest concentration the ratio of T-cell lymphomas to the total tumors decreased as the concentration was lowered. Dr. Yokoro recounted some of the findings with other radiation qualities. His experiments demonstrated unequivocally that irradiation initiated rat mammary cells that remained dormant until stimulated to grow by hormones. This finding predated but predicted what has since been found in humans.

*Cellular Aspects in Radiation Carcinogenesis*

Clifton (Univ. of Wisconsin, Madison) discussed the cells of origin of radiogenic thyroid cancer. Clifton developed an *in vivo-in vitro* method for studying the induction of thyroid cancer some years ago in which follicular cells are dispersed and injected into fat pads where they reform into functional thyroid follicles. Transplantation assays indicate that about 1% of the monodispersed follicle cells are clonogens. Initiation of these clonogens by radiation is a common event, although overt cancer is not. The expression of the initiated cells is influenced by the number of normal cells in the grafts. The more normal cells there are in the graft the lower the frequency of the development of cancers. The question is whether this suppression of the expression of the oncogenic potential is due to cell-cell interactions or a hormonal feedback. It appears that both operate and when sufficient cells are grafted the thyroid-pituitary axis can be reestablished. The conclusion was that the thyroid clonogens have many of the characteristics of stem cells and must be presumed to be the cells at risk for cancer induction.

The cells at risk and the development of thymic lymphoma was discussed by Muto (NIRS Chiba). In an elegant study thymocytes from B10 Thy-1.1 mice, 1 month after irradiation, were sorted into four subpopulations using anti-CD4 and anti-CD8 monoclonal antibodies. When these separate cell populations were injected into the thymus, the subpopulation that gave rise to thymic lymphomas could be identified from the presence of the markers. Two other markers, the J11d and TL-2 antigens, which are not expressed in normal thymocytes, appear in the cells of animals exposed to the fractionated irradiation. The results indicated that the cells at risk for the induction of thymic lymphoma were not the undifferentiated stem cell but committed cells with proliferative capacity. Sado (Chiba), with whom Dr. Muto works, discussed the results of their experiments which are concerned with the complex interactions between cells that originate in the bone marrow and with those of thymus. In the experiments using bone marrow transplantation, B10 Thy 1 congenic mice were exposed to the classical four weekly exposures of 1.5 Gy. When the irradiated B10 mice were grafted with bone marrow cells from normal B10 donor mice, tumor development was suppressed because the transformation of the irradiated thymocytes to prelymphoma cells was suppressed. The results of a number of different experiments suggest that the radiation-induced depletion of thymocytes is followed by repopulation of the depleted thymus with T-cell precursors that are more radioresistant and are part of the residual thymic cell population. The prelymphoma cells arise from the regenerating thymic cells,
and it appears that this involves an arrest in differentiation and a loss of growth control before they proceed to form thymic lymphomas.

Kamiya (Hiroshima Univ.) and colleagues have used the rat mammary cell transplantation system to assay epithelial clonogens. When cells are grafted into the host two types of cell colonies may form, alveolar and ductal. The cells of the ductal colonies retain clonogenic ability whereas the alveolar cells, which differentiate to produce milk, do not. Hormonal manipulation was used to influence differentiation. For example, a pituitary and an estrogen-releasing tube implanted into the spleen plus adrenalectomy result in a sevenfold increase in DNA in the mammary gland and about a fivefold increase in clonogens. When such mammary glands were irradiated, carcinomas increased linearly with dose with shorter latent periods and a significantly higher cancer incidence than in rats irradiated but without the hormonal manipulation to increase the number of clonogens.

Radiation Carcinogenesis in the Skin

Tanooka (National Cancer Center Research Institute, Tokyo) reported some of his experiments on the carcinogenic effects of beta rays from $^{90}$Sr/$^{90}$Y. The resistance of the mouse skin to tumor induction is well shown by the lack of tumors after single doses up to 170 Gy. The presence of initiated cells could be demonstrated by the application of 4-nitroquinolone 1-oxide on the skin. Multiple exposures, three times a week to doses in the range of 2.5–11.8 Gy per fraction, caused 100% incidence of skin tumors and also some bone tumors. As the dose per fraction was decreased, the latent period increased and the tumor incidence decreased with only one squamous cell carcinoma and one osteosarcoma with lifetime exposures to 0.75 Gy per fraction. The results suggested a threshold-like dose response. Interestingly, TPA did not promote tumors exposed to multiple doses of beta rays. It was established that the induced skin tumors were monoclonal in origin.

Radiation induction of skin cancers was also the subject of Burns' (New York University) talk. It has become dogma that carcinogenesis is a multistage process. If that is the case in radiation carcinogenesis, how many stages are there? Burns noted that the incidence of squamous and basal cell carcinomas is consistent with two events or a quadratic response; based on split-dose experiments, there is a capability for repair.

Burns suggested that a dose-response relationship independent of elapsed time can be formulated if the tumor yield can be separated into a product of two functions, one of dose and one of time, and one can be expressed as $Y(D,E) = F(D)g(t)$ where $Y(D,t)$ is the cancer yield per animal, $D$ is dose, and $t$ is the elapsed time after exposure. The experimental data for tumors in irradiated rat skin are considered to be a power function of the rats' life-span. The Weibull is one such power function that is consistent with the multistage theory of carcinogenesis. Based on the linear-quadratic model $F(D) = CLD + BD^2$, where $L$ is linear energy transfer, $D$ is dose, and $C$ and $B$ are constants, the dose response becomes predominantly linear as doses are increasingly lowered and as the LET is increased. It should be noted that when different tissues are compared other factors, probably target size and number, must be taken into account. There was evidence that c-myc is amplified and expressed in carcinomas but not in the one sarcoma tested. K-ras was also activated, which involves
a point mutation, but the probability that this change is radiation-induced must be small. There is the question in skin, just as in the case of other tissues, of what changes are primary and which are secondary.

Sadamori (Nagasaki Univ.) examined the atomic bomb survivors recorded in the Scientific Data Center of Atomic Bomb Disaster at Nagasaki University School of Medicine. One hundred and fifty cases with skin cancer were identified in the 65,268 survivors. The ratio of basal cell carcinomas to squamous cell carcinomas is about 1.6, a much lower ratio than the ratio found in other irradiated and unexposed populations. Distance from the hypocenter was used as a surrogate for absorbed dose, and a significant inverse correlation between the incidence of skin cancer and the distance from the hypocenter was found. Hopefully the incidence of skin cancers as a function of dose will be determined for all the atomic bomb survivors.

The next talk by Bowden (Univ. of Arizona) concentrated on the molecular changes in the cells of radiation-induced skin cancer. In the context of the operational division of skin carcinogenesis into initiation, promotion, and progression, Bowden discussed the effects of ionizing radiation. He suggested that ionizing radiation was a weak initiating agent in the production of malignant squamous carcinomas. DNA extracted from different types of tumors initiated by X rays and promoted with TPA shows dominant transforming activity. Unlike chemical carcinogenesis of the skin, the ras family is not involved. In squamous cell carcinomas three different transforming genes were identified. The question is whether any of these changes are due to direct action of the radiation. Radiation-induced progression was studied using a line of cells capable of forming papillomas. Irradiation of the benign cell line induced a malignant cell that showed high levels of transcripts of c-fos, c-jun, transin, metallothionein II A genes and stromelysin, which degrades the basement membrane. The increased level of stromelysin is related to the action of the stromelysin promoter. These studies suggest that by astute development of cell systems it should be possible to unravel the molecular changes in the carcinogenic process.

Tumor Suppressor Genes and Oncogenes

Niwa’s (Hiroshima Univ.) study was about the oncogene changes in radiation-induced thymic lymphomas. In these tumors c-myc was expressed at a high level but the gene showed no rearrangement. However, a loss of certain loci was found using a mini satellite probe. Terada (National Cancer Center Research Institute) was concerned with the attribution of the various genetic alterations. The genes in which changes occur differ in different types of human cancer, although some changes are common to some types of cancer. It is becoming clear that loss of heterozygosity at a number of loci is a common feature of human cancers, for example, P53 and Rb genes, and the remaining alleles show mutations and thus a loss of the normal gene products of these suppressor genes. It is probably reasonable to believe that the number of suppressor genes that will be identified will increase. The importance of the sequence of the changes in suppressor genes and oncogenes is recognized but the sequence is not known.

Kamada (Hiroshima Univ.) presented results obtained from the cytogenetic examination
of 75 radiation-related leukemias. Molecular studies have not revealed association with point mutations of N and K ras genes or in rearrangements of the bcr gene and the leukemias.

Sasaki's (Kyoto Univ.) talk dealt with tumor suppressor genes and, in particular, the question of paternal imprinting. The latter possibility stems from the observation that in sporadic nonfamilial osteosarcoma and the osteosarcomas after radiotherapy most of the initial somatic mutations were assigned on the copy of the Rb gene derived from the father. It is hoped that the work will reveal the mechanisms of the bias toward the paternal origin in the germinal and somatic mutation of tumor suppressor genes.

Nettesheim (NIEHS) discussed the role of the growth factors, TGFs α and β, in the regulation of proliferation of normal and transformed rat tracheal epithelial cells. TGF α is a polypeptide that is structurally related to epidermal growth factor (EGF) and binds to the EGF receptor. TGF α is also secreted, but in small quantities, by normal tracheal epithelial cells. It appears that neoplastically transformed epithelial cells become less dependent on such factors as insulin, EGF and pituitary extracts and that growth is influenced by the autocrine TGF α. This change in the factors controlling cell proliferation is a fairly early change. TGF β, which is secreted in a latent form and must be activated, plays a different role and is important in the cell-cell interactions that are important in controlling cell population.

It is now generally accepted that carcinogenesis involves a number of so-called stages, and as we have heard, the molecular changes involved are being detected. There appear to be three major rate-limiting steps in the induction of cancer by radiation: 1) the events that lead to altered control of proliferation; this change has proven very difficult to induce in human cells in vitro; 2) the development of autonomy of the altered cells; and 3) angiogenesis without which the growth and spread of a cancer cannot occur. The precise changes associated with these three steps in the development of cancer are not known, but we have heard about some of the changes that must be involved; however, many questions remain. Radiation appears to induce deletions more frequently than point mutations; does this suggest a greater likelihood that changes in suppressor genes are involved rather than point mutations? How is the expression of the initial events induced by radiation suppressed for so long, sometimes more than thirty years? What determines the eventual expression of the initial events and the cascade of events that lead to overt cancer? The various cell tissue and whole-animal systems that are being developed and that were discussed by the speakers together with the studies of exposed human populations will provide ways by which to unravel these complexities of cancer induced by radiation.