Meetings

Cancer Research: U.S.–Japan Cooperative Science Program

A wide range of biological and biochemical problems are involved in research of experimental hepatomas. Recent American research has been directed mainly toward the "minimal-deviation" type of hepatomas; it is believed that a clearer understanding of cancer in rat liver might be gained by comparisons of these hepatomas with normal liver. Japanese oncologic research has been developed on the basis of the maximum-deviation type of experimental hepatomas, such as found in the Yoshida ascites hepatomas. Many investigators believe that the etiology could be clarified by comparing the hepatomas with the normal liver because it was believed the latter type of hepatomas have more typical characteristics of cancer cells. Both concepts were discussed at a symposium on cancer research, held at Kyoto University, Kyoto, Japan, 4–5 November 1965, under the auspices of the U.S.–Japan Cooperative Science Program.

A large series of transplantable, experimental rat hepatomas possess different growth rates, from rapidly to slowly growing tumors, that may be considered as representative of many degrees of progression of cancer (H. P. Morris, National Institutes of Health). Such hepatomas were induced in inbred strains of rats by aromatic amines, for the most part related to fluorene amines. Many of these hepatomas have been characterized biochemically as having "minimal deviations" from normal liver. Morris proposed that the "minimal-deviation" type hepatomas provide an extremely valuable tool in determining the essential from nonessential changes in the oncogenic processes by comparing them with more rapidly growing hepatomas and with normal liver. He referred to the work of Siperstein on the lack of feedback control in cholesterol biosynthesis from mevalonate observed in all hepatomas of hepatoma-bearing animals and man on diets high in cholesterol. S. Takayama (Cancer Institute, Tokyo) developed similarly well differentiated and transplantable hepatomas in Donryu strain rats with diethylnitrosamine and in TCR strain mice with fluorene derivatives. The conversion of well differentiated to more poorly differentiated types was noted by Takayama during serial transplantation.

As a pathohistologist T. Yoshida (Cancer Institute, Tokyo) attempted to explain the cancer at the cellular level rather than on the level of the whole tumor. He defined cancer as emancipation of cells from the surrounding tissues. H. Satoh (Tohoku University, Sendai) reported on the survival time of host animals inoculated with a line of Yoshida ascites sarcoma; such time was inversely correlated to the cell numbers administered intraperitoneally. He proposed that the cell number with a lethal dose of 50 in the host was a quantitative indication of the malignancy and virulence of ascites tumors. In further discussions it was pointed out that age, sex, and homogeneity of recipient host greatly influenced the growth rate as well as transplantability.

Extensive morphological studies were made on Morris rat hepatomas by M. D. Reuber (National Institutes of Health). Based on histological and histochemical findings, these rat hepatomas were classified into four categories: (i) highly differentiated hepatomas that grossly resembled normal liver, where the cells grew in solid sheets and were larger than normal hepatic cells; (ii) well differentiated hepatomas that appeared grossly as soft and tan, brown, or liver-colored and histologically that resembled normal liver with cords two to several cells wide; (iii) poorly differentiated hepatomas or undifferentiated carcinomas which were grossly firm, gray or white, coarsely lobulated and histologically showed cells growing in crowded sheets with complete loss of pattern and lining cells; and (iv) cholangiohepatomas that were well differentiated hepatomas with focal areas of adenocarcinomas. He pointed out also the correlation of these morphological characteristics with the growth rate and with some of the enzyme patterns. S. Odashima (Sasaki Institute, Tokyo) then discussed the attempts of ascitic conversion of Morris' transplantable solid hepatoma lines. He obtained four ascites types of hepatomas out of 15 different hepatoma lines tested and pointed out that only poorly differentiated hepatomas or undifferentiated carcinomas were converted to ascites, but none of the well differentiated ones were convertible.

Cells studied by electron microscopy and cytochemistry reveal that malignant cells, such as Novikoff hepatoma cells, were characterized by a profusion of microvilli on the cell surface with a high level of activity of nucleoside triphosphatase and a low level of activity of nucleoside diphosphatase (A. B. Novikoff, Albert Einstein College of Medicine, New York). The size of the nucleus was large and the nucleoli were multiple and large. The mitochondria were small and few in number in this very rapidly growing malignant hepatoma. Little endoplasmic reticulum was found in the cells of Novikoff ascites hepatoma, but numerous ribosomes, most of which were not attached to the membrane, were noted. Lysozomes were small and widely distributed in the ascites hepatoma cell. The Golgi apparatus lay adjacent to the nucleus. In contrast, the slowly growing hepatoma 5123 cells had large mitochondria, considerably more endoplasmic reticulum, more of its ribosomes on the membrane, a somewhat smaller nucleolar mass, and a smaller ratio of nucleus to cytoplasm. The Gogli apparatus was very large. In short, cells of slowly growing tumors did not show features associated with the more malignant cells, although there were many gradations in between. However, most, if not all, of the cytological characteristics generally associated with the more malignant tumors might reflect the rapid division of these cells. It was concluded, therefore, that nothing was seen to be characteristic of malignancy as far as the cytological evidence is concerned. Little difference in fine structures of hepatoma 5123 was found after several years of transplantation.

An analysis of the chromosomal pat-

terns of 24 transplantable ascites hepatomas was obtained from the primary hepatomas induced by azo-dye feeding (H. Isaka, Sasaki Institute, Tokyo). Every ascites hepatoma had its own chromosomal pattern and no hepatoma had the modal chromosome number of 42. Most of them had the numbers between the hypodiploid and hypotetraploid. In addition, the chromosome number was not related to either growth rate, transplantability, growth pattern, or drug susceptibility. However, various abnormal types of chromosomes appeared occasionally in the ascites hepatoma cells.

Using five different lines of rat liver ascites hepatomas and a series of Yoshida sarcomas, H. Satoh (Sasaki Institute, Tokyo) reported that serial passage in animals or storage at −80°C resulted in alterations of transplantability, growth rate, growth pattern, chromosomal constitution, and drug susceptibility of these ascites cells.

S. Weinhouse (Temple University, Philadelphia) opened the biochemistry program of the conference. He reported on glycolysis, respiration, and enzyme deletions in a series of chemically induced transplantable rat hepatomas. A clear-cut relationship exists between growth rate and glycolytic activity in which the phosphorylation reaction catalyzed by hexokinase was the rate-limiting step. The tumors with low glycolytic rates and low hexokinase activity grew slowly, conversely those with high glycolytic capability and high hexokinase activity grew rapidly, with many gradations in between. Slowly growing hepatomas, with a few exceptions, had little or no glucokinase activity and the enzyme was completely unresponsive to glucose or insulin, whereas this enzyme activity in normal liver was greatly enhanced by feeding of glucose. Weinhouse found no close correlation between growth rate and activities of fructokinase, fructose-1,6-diphosphatase, and glyceroaldehyde kinase. However, an inverse relationship was found between growth rate and oxidative activity toward fatty acids. The rapidly growing tumors oxidized fatty acids slowly and readily produced ketone bodies at rates somewhat less than those of normal liver. Slowly growing tumors oxidized little or no fatty acids and produced no ketone bodies. H. Satoh and S. Tsuiki (Research Institute, Tohoku University, Sendai) reported on a particular line of rapidly growing ascites hepatoma which accumulated an enormous amount of glycogen in an early stage of transplantation. S. Tsuiki studied the glucose metabolism from suspensions of hepatoma cells but did not obtain any enzymic evidence to account for the marked increase of glycogen. However, glycogen was produced from glucose added even in the presence of amytal or under anaerobic conditions.

A deviation in the sugar metabolism of host liver occurs after subcutaneous implantation of Walker's carcinoma cells or intraperitoneal implantation of Yoshida hepatoma AH 130 (M. Suda, Osaka University, Osaka). Two isozymes of pyruvate kinase, antigenically and electrophoretically distinguishable, were isolated. One (M type) was found in every tissue and organ, but the other (L type) occurred only in the liver and kidney. The L-type enzyme markedly increased after administering insulin or a high carbohydrate diet; the M type increased slightly under these conditions. After the tumor transplantation, the M type in host liver increased several-fold, but the L type tended to decrease and no longer responded to carbohydrate feeding. When the solid tumor was extirpated, these enzymic activities went back to the normal levels. The M type was also predominant in the regenerating liver and fetal liver. Studies showed that in the host liver the hexokinase activity increased two to three times while the glucokinase activity decreased to about one third of that of normal liver. Similar findings were observed in the liver of a rat connected by parabiosis to a tumor-bearing rat.

T. Sugimura (National Cancer Center Research Institute, Tokyo) reported that rat muscle had a glucose-ATP phosphotransferase with K_m for glucose of $10^{-4}M$ in addition to hexokinase (K_m, $10^{-6}M$). Although the normal liver had glucokinase (K_m, $10^{-2}M$) and hexokinase, some ascites hepatomas contained the enzyme with K_m of 10^{-4} and hexokinase but no glucokinase. He described also two types of aldolases, muscle type and liver type. The ratio of activities of these enzymes with fructose-1,6-diphosphate, and fructose-1-phosphate as substrates were 60 and 1 for muscle- and liver-type enzyme, respectively. In the Yoshida ascites hepatomas so far tested, the ratio fell between 40 and 60, whereas the "minimal-deviation" type hepatomas had a ratio of 3 to 6. The studies were extended further to several microsomal enzymes and phospholipids in the hepatoma cells.

The results of extensive studies on the biochemical pattern of a spectrum of Morris hepatomas with different growth rates were presented by G. Weber (Indiana University School of Medicine, Indianapolis). He noted that in carbohydrate and lipid metabolism, the anabolic pathway decreased, whereas the catabolic pathway increased along with the increase in growth rate of tumors. In contrast, in the metabolism of amino acid, DNA, and RNA the synthetic pathway increased but the catabolic pathway decreased directly with increasing growth rate, indicating that the biochemical pattern in general reflected the rapid growth and division of these cells. In addition, Weber found with an increase in growth rate a gradual decline in the overall metabolic pathway and key enzyme systems to regulatory control. The failure of response to certain RNA metabolism patterns and amino acid levels and to hormonal regulation was also evident. Similar biochemical patterns of gradual alterations related to the growth rate were not observed in embryonic or regenerating livers. Weber reported a close parallel in uptake of radioactive DNA and rate of growth, as obtained by Morris, by determining the frequency of transfers in transplantable hepatomas.

Y. Nishizuka and O. Hayaishi (Department of Medical Chemistry, Kyoto University, Kyoto) explained the complete biosynthetic pathways of NAD from tryptophan and niacin. They indicated that almost all the enzyme activities in these pathways were lower in "minimal-deviation" hepatomas than in the normal liver. In addition, the activity of an enzyme which removes the phosphate of niacin ribonucleotide, a precursor to NAD, increased several-fold, thus resulting in the decrease of NAD synthesized in the hepatoma cells.

T. Ono (Cancer Institute, Tokyo) examined the enzyme patterns of 53 Yoshida ascites hepatomas and 16 "minimal-deviation" solid Morris hepatomas. The most rapidly growing Yoshida ascites hepatomas still retained a trace of the enzyme pattern observed in the parenchymal cells of normal liver. Although activities of several enzymes correlated either negatively or positively to the growth rate, some enzymes, such as deoxycytidylate deami-

nase and ornithine transcarbamylase, showed no correlation with the growth rate or histological type. From a survey of isozyme patterns of several enzymes he tried to quantitate malignancy of these experimental hepatomas; he studied several isozymes including lactate dehydrogenase, glucose-6-phosphate dehydrogenase, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, malate dehydrogenase, and α-glyceroaldehyde dehydrogenase. Ono showed that the isozyme pattern of the LDH (lactic dehydrogenase) of all ascites hepatomas tested retained essentially the normal pattern of rat liver, and that the variation among the 53 Yoshida strains was caused in part to a gradation of differentiation. The "minimal-deviation" type hepatomas exhibited the well differentiated pattern similar to that of normal adult liver. Although one minor peak of the glucose-6-phosphate dehydrogenase isozyme was deleted in a few cases no abnormal isozyme pattern was observed. Ono proposed three biochemical parameters which could possibly be used to quantitate malignancy of experimental hepatomas: (i) enzyme activities which correlated either positively or negatively to the growth rate (glucose-6-phosphatase, and glutamate dehydrogenase), (ii) independence of inducible or suppressible enzymes from host control (tryptophan pyrrolase and glucose-6-phosphate dehydrogenase), and (iii) systemic effect of hepatomas on the host liver enzymes, such as catalase activity.

In his closing remarks W. Nakahara (National Cancer Center Research Institute, Tokyo) referred to the historical background of his early studies on the catalase activity in tumor-bearing animals. He emphasized that extensive studies on the cell membrane might be desirable for better understanding of malignancy, because less adhesiveness and invasiveness were common during the oncogenic processes.

The symposium clearly established that a continuous spectrum of hepatomas revealing different growth rates represented many degrees of progression of cancer cells and that more studies were vital. The clinical symptoms or signs, growth rate, transplantability, and others, used in the past must now include both the biochemical and biological approaches. These newer approaches have now opened up new frontiers of cancer research. The etiology of cancer, however, is still far beyond our present knowledge. Rather, all findings presented so far appear to be reflected from or due to adaption to the rapid division of cancer cells. No evidence was presented indicating that the primary event of oncogenic processes is from somatic mutation. It is still unknown whether or not all alterations so far observed in cancer cells are due to a common but single alteration in the enzyme-synthesizing systems or to multiple combinations of alterations in the regulatory mechanism for individual enzyme-synthesizing systems. Disturbance or unbalance might be present at higher levels of regulatory mechanisms of cellular activities, such as differentiation, development, or growth rather than a single metabolic step.

The proceedings of this symposium will be published shortly in the Japanese journal of cancer research, *GANN*, edited by the Japanese Cancer Society.

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Swine in Biomedical Research

In recent years swine have been used increasingly in biomedical research. To explore the basis for and extent of this use, as well as to provide a firm basis for the future use of swine in biomedical research, an international symposium was held at the Pacific Northwest Laboratory, Richland, Washington, 19–21 July 1965.

Several papers reviewed our knowledge of swine genetics and reproduction and current research in these areas. D. F. Cox (Iowa State University) reviewed swine genetics, noting that domestic swine may provide some unique opportunities for research because of the vast array of genetic variation both between and within the various breeds. He pointed out that, with the exception of information on the inheritance of blood antigens of swine, our knowledge for this species of the genetics of inherited traits that are controlled by a few genetic factors is limited. Expanding on this latter point, J. Moustgaard and M. Hesselholt (Royal Veterinary and Agricultural College, Copenhagen) reported that the presence or absence of antigenic factors on the surface of swine erythrocytes is controlled by alleles belonging to 14 chromosomal loci. Fourteen blood-group systems have been established and designated by letters.

R. A. McFeely (University of Pennsylvania) presented recent work on swine cytogenetics. The pig appears particularly well suited for studies of cytogenetics because it has 38 chromosomes, which can be paired and grouped as readily as human chromosomes.

D. Smidt and associates (University of Göttingen) have transferred the eggs from miniature swine sows to other miniature swine sows and reciprocally between miniature and Landrace sows. Embryo implantation rate was 25 percent, and about one-half of these were carried to term. The size of the sow influenced the birth weight, and the weight differential persisted until the offspring were about 6 weeks old.

Birthe Palludan (Royal Veterinary and Agricultural College, Copenhagen) presented an interesting, comprehensive review of studies on the teratological effects of vitamin A in swine with avitaminosis and hypervitaminosis. A number of malformations were observed, the most frequent being microphthalmia.

Several papers described dental and skeletal research. E. B. Jump and M. E. Weaver (University of Oregon), who pioneered in the use of miniature swine in dental research, described some advantages and limitations of this species. They noted that the pig masticates with both incision and trituration and is unique among the common laboratory animals in having a long period of deciduous and transitional dentition. The length of this period permits experimental studies on many dental problems afflicting children. Preliminary studies demonstrate the suitability of miniature swine for clinical experiments in orthodontics, periodontics, restorative dentistry, and the pathology and therapeutics of the tooth pulp. An interesting but unexplained difference between man and swine is that swine produce large amounts of dental calculus without experiencing the periodontal disorders associated with dental calculosis in man.

F. A. Spurrell, W. J. L. Felts, and L. A. Baudin (University of Minnesota) presented data on the development of osteons in swine and man and