Meetings

Mammary Tumor Virus in Mice

Mammary tumors were once considered the exclusive problem of the geneticist. Now, however, the problem is approached by the most advanced techniques of many disciplines. At an informal working conference on the mouse mammary tumor virus held at Inverness, California, 28–30 October 1964, this progress was outlined by C. C. Little (Jackson Laboratory, Bar Harbor, Maine), who was instrumental in establishing most of the mouse strains used in present-day mammary-tumor and leukemia research and in whose laboratory the carcinogenic agent in milk was discovered almost 30 years ago.

Several methods of isolating and studying particles associated with the mammary tumor agent were reported at the meeting. The data in general showed correlation of specific particles (B particles) with tumorigenic activity, but there are also subviral elements and interfering substances (including viruses) which, unless excluded, prevent a perfect quantitative correlation. G. Miroff (Rockefeller Institute) reported the presence of B particles in the milk of his tumor-susceptible lines (C3H, A, RIII, BALB/CfA) and their complete absence in his tumor-free, agent-free lines (C3Hf, Af, BALB/C). In the susceptible lines, however, differences among strains in both the morphology and stability of the B particles were found by Miroff and B. Magdoff-Fairchild (Rockefeller Institute). The existence of a factor in milk that destroys virus particles, which varies greatly from strain to strain, being most potent in BALB/CfA and weakest in RIII, was indicated. Studies on fractions from cesium chloride density equilibrium gradients gave poor correlation of B particles with bioactivity and indicated the presence of an inhibitor which has a high molecular weight. L. Dmochowski and J. Sykes (M. D. Anderson Hospital and Tumor Institute, Houston, Texas) reported that virus particles and tumor-inducing activity from milks of strains of both high (RIII, A, C3H) and low (Af, C3Hf, C57Bl) cancer-susceptibility were distributed throughout preformed gradients of sucrose or cesium chloride, even after 72 hours of centrifugation at 105,000*g*, but that when potassium tartrate or citrate gradients were used a sharply defined zone having a density of 1.17 to 1.19 g/cm^3 contained the virus particles and the tumor-inducing activity. Although this virus particle band was present in all milks except that of C57Bl/6/J/Dm (tumor incidence, 0.01 percent), it was weaker in milks from strains of mice of low susceptibility to mammary tumors: milk from another family of C57Bl (C57Bl/Dm) mice having a mammary tumor incidence of 38 percent showed a weaker, but definite, virus-particle band. D. H. Moore (Rockefeller Institute) reported correlation of bioactivity with concentration of B particles isolated by elecrophoresis and by preformed density gradient velocity sedimentation; unlike most crude preparations, isolated particles can be titrated in assay mice.

Chemical studies on viral particles isolated from the milk of RIII mice by velocity gradient centrifugation in the chemically inert synthetic polysaccharide Ficoll, which has a high molecular weight, were described by M. J. Lyons (Rockefeller Institute). The particles contained 27 percent lipid and 0.8 percent RNA (3.7×10^6 molecular-weight units); experiments pointed to the essentially single-stranded character of the nucleic acid. The average mononucleotide composition of the viral RNA in moles percent was: uridylic acid, 28.9; guanylic acid, 30.2; CMP cytidylic acid, 21.6; and adenylic acid, 19.3. The particles are highly tumorigenic when inoculated into C57Bl assay mice. The purity and structural integrity of the particles were attested by electron microscopic examination. Evidence of interfering factors was observed by Moore in both C3Hf tumors and in RIII milk. Addition of C3Hf tumor extracts to RIII milk destroys the activity of the milk, and the addition of RIII milk dialysate to RIII milk either destroys or reduces the milk's bioactivity, while the dialyzed milk causes an unusually high incidence of tumors in C57Bl mice.

Evidence that the mammary tumor virus is an essential factor in mammary tumorigenesis—that various hormonal stimulations, such as excessive breeding, injection of hormones, or transplantation of pituitaries in the absence of the virus, fail to produce tumors in highly susceptible genetic strains—was presented by Miroff and Moore. Susceptible, agent-free mice failed to develop tumors after receiving many hypophyseal isografts subcutaneously or four isografts inside the kidney capsule. When agent-bearing male hybrids, $(C3H \times A)F_1 \times C3H$, were castrated and injected continuously to the limit of tolerance with diethylstilbestrol, 90 percent developed mammary tumors within 11 months; agent-free hybrids similarly treated developed no tumors.

Attempts to employ tissue cultures in viral assays and for virus production were reported by E. Y. Lasfargues (Columbia University College of Physicians and Surgeons). He found that mammary tissues from virgin mice can be maintained in vitro longer than highly differentiated tissues from lactating mice; this span depends on the degree of differentiation. Furthermore, all mouse tissues appear to have a life of only 3 to 4 months. The milk agent can transform mammary cells from immature animals in such a way that the life of the cells in vitro is indefinitely prolonged. Concomitantly, giant multinucleated cells and nuclear alterations appear. In organ cultures of agent-free mammary tissues, an organized alveolar type of hyperplasia is induced by electrophoretic and centrifugal density-gradient fractions containing purified viral B particles; this response of the tissues is specific but too weak to be used as an assay method.

To explain the failure of viral particles from C3Hf milk or tumor extracts to show infectivity, D. R. Pitelka (University of California, Berkeley) introduced the hypothesis that C3H mice carry two morphologically indistinguishable mammary tumor viruses. One is a strong virus readily transmitted by way of the milk or tumor extracts;

the other is a relatively weak virus transmitted at conception by either parent, causing few tumors but many hyperplastic alveolar nodules late in life. Ordinarily this second virus, called nodule-inducing virus (NIV), is overshadowed by the strong virus which causes many tumors relatively early in life; when animals are deprived of the strong virus by foster-nursing, the second virus becomes evident. In the two mouse strains used in these experiments, the distribution of the viruses is as follows: C3H mice carry both; C3Hf carry only NIV; BALB/C fostered on C3H milk carry only the strong, milk-transmitted virus (MTV); reciprocal hybrids of BALB/C and C3Hf carry NIV; BALB/C carry neither.

H. A. Bern (University of California, Berkeley) reported on studies designed to test the transferrence of NIV by tissue transplantation. When normal mammary ducts from BALB/C females were grafted into gland-free fat pads of young, NIV-bearing hybrids (BALB/C × C3H), nodules containing the virus particles eventually developed in the transplanted tissues. These nodules, when retransplanted into young, virus-free BALB/C hosts, produced particle-bearing outgrowths and eventually some tumors. The results suggest that NIV can be transmitted from the original hybrid to transplanted BALB/C mammary tissue, and from transplants of this tissue to BALB/C hosts. Evidence was presented by K. B. DeOme (University of California, Berkeley) that the two viruses interfere with each other. BALB/C mice which do not carry either virus are susceptible to MTV at all ages, but the incidence of infection decreases with increasing age. C3Hf mice which harbor NIV are susceptible to MTV only up to 18 days of age. Susceptibility of transplanted tissues was also tested. Mammary tissues from BALB/C mice of all ages are equally susceptible to infection with MTV, although transplants from C3Hf mice aged 18 days or older are completely refractory to MTV infection.

S. Nandi (University of California, Berkeley) reported on MTV activity in blood and mammary tissues of C3H and BALB/C (designated C^+) mice fostered on milk from C3H mice; his recently developed noduligenic test was used to detect MTV. Viral activity was found in the blood of C^+ mice at all ages but was not detectable in mammary tissues before 3 weeks of age. When tested in 3-week-old C3Hf hosts, MTV was rarely detected in either blood or mammary tissues of C3H donors of any age or physiological state. Blood from C3H mice proved to be a poor source of MTV, even when tested in highly susceptible C hosts. These results suggest that the presence in C3H mice and the absence in C^+ mice of an agent which interferes with the activity of MTV may account for the observed difference in MTV activity of these two strains under Nandi's experimental conditions. The interfering agent is probably NIV.

P. D. Blair and D. W. Weiss (University of California, Berkeley) gave evidence that MTV, NIV, mammary tumors, and hyperplastic nodules all induce an immunologic response in isogenous mice. Animals of virus-bearing strains which come in contact with the virus very early in life, however, may become tolerant to the viral antigens and therefore may not respond immunologically to them. The antigens of MTV are almost identical to those of NIV, although differences are detectable. Blair and Weiss also reported some progress in detecting the presence of viral antigen by hemagglutination tests and immunodiffusion techniques.

A wide variation in tumor incidence and in mean age of tumor onset in families of RIII and C3H mice was reported by Andervont. Different families of presumably agent-free C3H mice given RIII virus by foster-nursing vary in their tumor incidence from 3 percent at a mean age of 20 months (weak virus?) to 44 percent at a mean age of 15 months (stronger virus?). The agent in RIII is less active than that in C3H, and its activity does not increase during passage through C3H mice by the natural route of transmission. Families of presumably agent-free RIII mice given C3H agent by fostering show a tumor incidence of 99 percent at an average age of 6.4 months in breeders and an incidence of 94 percent at an average age of 8.1 months in virgins in one family, and an incidence of 8 percent at an average age of 17.8 months in breeders in another family.

Inhibition of the mammary tumor virus by leukemia virus in RIII mice was reported by F. Squartini (University of Perugia). The RIII strain has a tendency to lose MTV, and the tumors have a low malignancy and growth rate. Both RIII mice and BALB/C mice fostered on RIII milk demonstrate the presence of a virus which causes lymphatic leukemia; the leukemia virus is in unstable equilibrium with the MTV and, depending on circumstances, one or the other may prevail. Like other leukemia viruses, this one is transmitted to embryos *in utero* and to newborns by way of the milk, together with the MTV. In the supposedly virus-free strain BALB/C, with low susceptibility to mammary tumors and leukemia, there seems, however, to be a strong association between sporadic cases of mammary tumor and lymphatic leukemia; there appears to be some synergistic action between the two viruses.

W. E. Heston (National Cancer Institute) presented evidence against an inhibitor's being responsible for the medium incidence of mammary tumors in the C3HfB line (fostered on C57Bl, which is assumed to harbor an inhibitor). A comparable strain started by fostering C3H on BALB/C mice (the latter are not considered to harbor an inhibitor) has the same tumor incidence as C3HfB. Thus it is concluded that the incidence does not depend upon the foster mothers but is inherent in the C3H line. Breeding females of strain A mice fostered on C57Bl have a much lower incidence than the fostered C3H line.

W. S. Murray (Jackson Laboratory) reported a study of seven reciprocal crosses made up from combinations of three strains which develop mammary carcinoma and two which do not. Crossing C57Bl mothers with C3H fathers, for example, results in about the same tumor incidence as has been obtained by foster-nursing C3H on C57Bl mice, whereas the reciprocal cross (C3H mother, C57Bl father) gives a tumor incidence of 100 percent. When the mother is of a strain with low susceptibility to cancer or of an agent-free strain, most of the tumors are of type "C," fibroid, or hemangiomatous. Murray advanced the concept that almost all inbred stocks of mice carry the MTV, each stock having reached equilibrium between the physiology produced by the genes it carries and the success or failure of these physiologies in propagating or suppressing the virus.

A new inbred mouse strain, designated GR, in which breeding females have a high incidence of mammary tumors that are highly responsive to hormones, was described by O. Mühlbock (Netherlands Cancer Institute). Mice taken by Caesarean section and fostered on the tumor-free C57Bl strain show the same high incidence as the unfostered GR stock. Nevertheless, the

presence of an MTV was demonstrated by fostering tumor-free hybrids, $(O_{20} \times DBAf)_{F1}$, on the GR stock; the hybrids then had high incidence of tumors. By reciprocally crossbreeding the GR and C57Bl strains, it was shown that a GR agent is transmitted as well by the male as by the female.

The likelihood of a mouse's having breast cancer is proportional to the number of mammary glands permitted to develop. This was concluded by A. Dux (Netherlands Cancer Institute) from experiments in which she removed from one to nine of the mammary glands from young females. Dux also reported that the genetic influence on tumorigenesis is located specifically in the mammary gland. She transplanted mammary glands from C3H susceptible and O_{20} resistant females into completely mammectomized hybrids (C3H $\times$ O_{20} agent-bearing and $O_{20} \times$ C3H supposedly agent-free). With C3H glands in agent-bearing hosts, tumor incidence was 100 percent at a mean tumor age of 233 days; with O_{20} glands in agent-bearing hosts, it was 95 percent at a mean age of 323 days; with C3H glands in agent-free hosts, it was 85 percent at a mean age of 492 days; and with O_{20} glands in agent-free hosts, it was 11 percent at a mean age of 511 days.

Methods for obtaining large quantities of milk from mice were reported by W. F. Feller (National Cancer Institute). Fourteen days after birth, the young are taken away from the mother 4 hours before she is milked, and she is injected intraperitoneally with 100 milliunits of oxytocin before gentle suction (10 cm-Hg) is applied to moistened nipples; many mothers yield 1 ml per milking. The young are returned to her immediately. She can then be milked once daily for 5 days.

The mass of data presented permitted far-reaching conclusions to be drawn. The MTV (the B particle) can initiate mammary carcinoma and represents the milk agent responsible for most of the spontaneous mammary tumors of mice. It is a lipid-rich RNA virus resembling the myxoviruses. A second virus, a nodule-inducing virus, morphologically indistinguishable from the MTV, is responsible for the few tumors that appear late in life in some strains (particularly C3H) which have been kept free of MTV by foster-nursing. The nodule-inducing virus is transmitted by either parent only at conception. Both viruses are under hormonal and genetic control in the host, the development of tumors being dependent on both factors, but neither hormones nor heredity ordinarily initiate a mammary adenocarcinoma without the presence of a virus. Many so-called virus-free mice in which tumors have appeared under certain conditions are now believed to be virus-bearing. Most, but not all, strains and families of mice are carriers of one or both (or possibly other) related viruses, and as such can respond to hormonal stimulation by the development of tumors.

Subviral infectious units and viral inhibitors are in evidence. Nodule-inducing virus and also leukemia virus can interfere with the action of MTV, possibly through an immunological response; such responses to these viruses have now been demonstrated.

The meeting was made possible by a grant to the Rockefeller Institute by the Lilla Babbitt Hyde Foundation of New York.

DAN H. MOORE

Rockefeller Institute, New York City

Paleopathology

A symposium on human paleopathology was held in Washington on 14 January 1965 under the auspices of the Subcommittee on Geographic Pathology, National Academy of Sciences–National Research Council.

The chairman, Saul Jarcho (Mt. Sinai Hospital, New York), summarized the historical development and present condition of human paleopathology in the United States. The great age was the period approximately between 1900 and 1935, represented by the work of Hrdlička, Moodie, Hooton, and H. U. Williams. In contrast, the last 30 years have seen no major synthetic work and no major discovery. In the United States at the present time, Jarcho continued, paleopathology functions as a province of physical anthropology in which much of the work is done by a few general pathologists, osteopathologists, orthopedic surgeons, and roentgenologists, most of whom have had no formal training in anthropology. Because most contemporary pathologists have avoided paleopathology, the new methods used in their laboratories have not been applied to prehistoric tissues. Jarcho said that urgent current needs are (i) establishment of a registry for examination and central recording of prehistoric human remains and for applying the new analytical methods, and (ii) the extension of medical indexing to include paleopathology, thereby overcoming the inaccessibility of the literature. These innovations would serve as a basis for the reanimation of human paleopathology in the United States.

Temkin (Johns Hopkins) stated that the interest in paleopathology displayed by medical historians had sometimes produced uneasiness in archeologists: an example was Sudhoff's research on the history of syphilis, especially his work on the famous edict of Maximilian I in 1495, research that was challenged by the more accurate work of Haustein. Temkin felt that the best place for paleopathology in the academic structure has not yet been determined. Long (Pedlar Mills, Va.) agreed that paleopathology has been slighted in medical schools and that the same is true of comparative pathology. The dry Southwest and the moister river valleys elsewhere in the United States are capable of yielding valuable information on the past incidence of such diseases as tuberculosis, arteriosclerosis, and stone. Bayne-Jones (Washington, D.C.), after presenting family reminiscences of his grandfather, Joseph Jones, who exhumed syphilitic skeletons in the southeastern United States and thereby provoked prolonged controversy, emphasized the importance of the epidemiologic viewpoint in paleopathology. It would be especially important, he felt, to gain information on diseases which developed simultaneously in widely separated regions. Cassedy (NIH) spoke of the close relation between research in paleopathology and research into modern health; both are included in the mandate of the Public Health Service. NIH has supported a wide range of laboratory research in pathology, believing that historical studies have a definite value in the understanding of disease; paleopathology is an appropriate field for government support. The History of Life Sciences Study Section has made a formal statement of its interest in the field.

T. D. Stewart (Smithsonian Institution) referred to the Smithsonian Institution's long interest in paleopathology. He expressed disillusionment with much that has been published in the literature, citing several examples. Hooton's work on Pecos Pueblo has great merits and some defects; it is one of the few studies of prehistoric