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Studies of Fibroblastic Cells Cultivated from Bone Marrow of Leukemic and Non-Leukemic Patients.* (26420)

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Technics employed for *in vitro* preparation of human bone marrow cell cultures have been reviewed by Osgood(1), Reisner(2) and Berman(3). The present report describes a procedure for cultivating cells from leukemic and normal bone marrows on feeder layers of human amnion cells. Employing this technic, the susceptibility of normal and leukemic bone marrow cell cultures to certain viruses was investigated. The fluorescent antibody staining technic was also applied in attempts to detect a specific antigen in leukemic bone marrow cell cultures and efforts were made to isolate a cytopathogenic agent from leukemic marrow aspirates.

Materials and methods. Sternal bone marrow aspirates were withdrawn aseptically in 1-3 ml amounts from patients undergoing open cardiac surgery and from children with acute leukemia. The latter specimens were obtained during the course of diagnostic and follow-up aspirations performed at the Tumor Therapy Clinic of Children's Hospital Medical Center. To each ml of aspirated material was added 0.0025 mg of sterile heparin(4). Aspirates were suspended in bovine amniotic fluid (BAF) medium(5) containing 20% horse serum which had been inactivated for a half hour at 56°C. Nucleated cell counts were performed on the final suspensions.

Primary monolayer cultures of trypsinized human amnion cells (HA) were prepared in 150 × 16 mm test tubes as previously described(5). The monolayers were nourished with BAF medium containing 5% inactivated horse serum. In some experiments 5-15-day-old HA monolayers were x-irradiated with 3,500 r. They were exposed for 12 minutes and 40 seconds at a distance of 40 cm from a Siemens x-ray tube operating at 250 KV and 15 ma, filtered with 1 mm of aluminum. The dose rate was 277 r/min. X-irradiation was of sufficient intensity to arrest cellular multiplication(6). Cell viability, however, appeared to be unaffected as judged by morphologic appearance and acid production.

Viruses. The behavior of the following viruses was studied in bone marrow cell cultures:

Measles, Edmonston strain, 28th human kidney cell passage(7).

Mumps, Enders strain, 47th chick amniotic sac passage.

Sindbis, Strain AR339, 10th chick embryo cell passage(8).

Poliovirus Type I, Brunhilde strain, passed 1× in human uterine and 2× in human amnion cell cultures.

Herpes simplex, L strain, passed 5× in chick embryo amniotic sac and 3× in chick embryo cell cultures(9).

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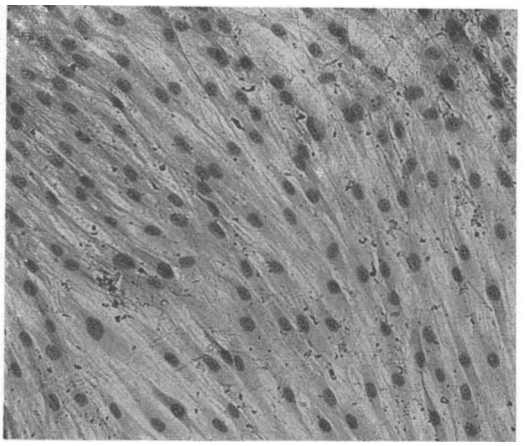

FIG. 1. Monolayer of fibroblastic cells of human bone marrow origin. The monolayer obscures underlying feeder layer of human amnion cells. H & E. × 115.

Influenza virus A/Formosa/3-13-57.

Experimental. Bone marrow cell culture technics. Bone marrow aspirates in 1-3 ml amounts were suspended directly in BAF medium containing 20% inactivated horse serum and 1 ml aliquots were dispensed into tubes containing x-irradiated and non-irradiated HA monolayers. Total nucleated cell counts of these preparations averaged 110 million cells, and each aspiration yielded an average of 60 cell culture tubes. Centrifugation of bone marrow aspirates and use of the resulting supernatant fluid and buffy coat resulted in a reduced yield of nucleated cells. Tubes were incubated in a slanting position at 37°C. Culture fluids were changed initially after 2-3 days and thereafter every 3-4 days until fibroblastic cells developed (Fig. 1). The term "fibroblastic" is used only in a descriptive sense. When confluent fibroblastic sheets became established, BAF medium containing 5% inactivated horse serum was used and tubes could be incubated at 32°C. Cellular metabolism was reduced at the lower temperature and culture fluids needed to be changed less frequently. This method of cultivating uncentrifuged bone marrow cells appeared consistently reproducible.

A total of 157 centrifuged and uncentrifuged bone marrow specimens were cultivated. Fibroblastic sheets developed from 61 (72%) aspirations obtained from 85 patients free of hematologic disorders and from 43 (60%) withdrawn from 72 leukemic subjects.

Fibroblastic growth developed equally well on x-irradiated and non-irradiated HA monolayers. The fibroblastic sheets remained in good condition when maintained at 32°C for 3-4 months. Attempts to grow bone marrow cells directly on glass were infrequently successful. Although a single cell culture of fetal bone marrow on feeder layers was subcultured 17 times, repeated efforts to establish a permanent strain of these fibroblastic cells failed.

The development of both normal and leukemic bone marrow cell cultures was characterized by 3 cytological phases similar to those described by Berman(3) and Woodliff (10). In the first phase myeloid cells were morphologically distinguishable. During the second phase large, round monocytoid cells predominated, many of which became bipolar. The third phase was marked by the appearance of sheets of fibroblastic cells which covered and obscured the underlying human amnion. The duration of the different phases varied and overlapped. Generally, sheets of fibroblastic cells formed in 10-14 days. When fibroblastic sheets did develop on glass, the same 3 phases were observed. Cells of epithelial morphology did not appear in our cultures.

Attempted differentiation between normal and leukemic bone marrow cell cultures. There were no apparent differences in morphology, rate of metabolism, or type of degeneration. The indirect fluorescent antibody technic(11) was used unsuccessfully in an effort to detect a specific antigen in fibroblastic tissue cultures derived from leukemic marrows. Leukemic serum, obtained from children in relapse and during the remissive stage of the disease, and labelled horse anti-human gamma globulin§ were consecutively layered on normal and leukemic bone mar-

§ Obtained from Sylvania Chemical Co., Orange, N. J. and adsorbed with both human and mouse liver powder to remove non-specific staining material.

row cell cultures. No specific fluorescence was observed.

Effect of viruses on bone marrow cells. Experiments were performed to determine whether there were differences in viral susceptibility between the cultures derived from normal and lukemic aspirates. Both normal and leukemic marrow cell cultures supported the propagation of poliomyelitis, measles and herpes simplex viruses. Polio and herpes simplex viruses caused cytopathic changes in the fibroblastic cells which were observable under low power microscopic inspection. Measles virus chronically infected both types of marrow cell cultures; although no cytopathic lesions were seen, typical inclusion bodies(7) were observed after hematoxylin and eosin staining. This chronic infection persisted for 4 months and was confirmed by demonstration of infectivity of the tissue culture fluids for primary human amnion cell cultures in which typical cytopathic changes were observed(5). Neither normal or leukemic marrow cultures supported the growth of egg adapted mumps virus. Only normal marrow cell cultures were inoculated with Sindbis and influenza viruses. Sindbis virus was cytopathogenic for these cultures. Influenza virus was not cytopathogenic for normal bone marrow cell cultures, but after inoculation such cultures hemadsorbed both guinea pig and chicken erythrocytes. The hemadsorption technic of Vogel and Shelokov(12) was used in these studies.

Attempts to demonstrate a transmissible agent in leukemic cells. Efforts were made to isolate a cytopathogenic agent from aspirates obtained from leukemic patients. Primary human amnion, monkey kidney (rhesus), chick embryo cell and normal bone marrow cell cultures were inoculated with untreated leukemic aspirates and with ground fibroblastic cells and supernatant fluid obtained from leukemic cell cultures. Cytopathic changes were not observed.

Discussion. The origin of the fibroblastic cell in bone marrow cell cultures remains obscure. It has not been determined whether these cells are of hemic origin or derive from the connective tissue. If hemic in origin they may represent the transformation of more than one type of bone marrow cell.

We have not determined why HA feeder layers so readily promote the development of bone marrow cells. Attempts to explore this problem were made by suspending and maintaining bone marrow aspirates in various proportions of stock BAF medium and BAF medium that had nourished HA monolayers ("conditioned" medium). However, the use of "conditioned" medium did not facilitate development of fibroblastic sheets on plain glass. Three explanations are suggested to account for the beneficial effects of a feeder layer. First, the bone marrow cells may adhere to the HA cells with greater ease than they do to glass. However, unsuccessful efforts to cultivate bone marrow cells on glass were characterized by an arrest at the round cell stage and not by failure of the cells to attach to glass. Second, autotoxic metabolites formed by the bone marrow cells may be removed by the HA cells. Third, the feeder layer may elaborate metabolites which are beneficial for survival and multiplication of certain cells found in the bone marrow but which either do not accumulate in effective concentration in the medium or are extremely labile at 37°C.

Summary. The use of a feeder layer of x-irradiated or non-irradiated primary human amnion monolayers in cultivation of leukemic and normal human bone marrow aspirates results in establishment of primary, human fibroblastic cell cultures. Comparison of normal and leukemic cultures failed to reveal differences in morphology, type of degeneration, rate of metabolism or viral susceptibility. A cytopathogenic agent was not recovered from leukemic aspirates and use of the fluorescent antibody technic failed to reveal a specific antigen capable of reacting with leukemic sera in leukemic bone marrow cell cultures. Cultures of cells from both leukemic and normal bone marrow supported multiplication of polio, herpes simplex and measles virus. Normal marrow cell cultures supported multiplication of Sindbis and influenza A virus, but not of an egg adapted strain of mumps virus. The reproducible cul-

tivation of cells obtained from human bone marrow provides an accessible source of primary human fibroblastic cell culture that may prove useful in both microbiological and hematological experimentation.

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Occurrence of Antibody in Human Vaginal Mucus.* (26421)

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The occurrence of specific antibody activity outside the tissues proper and often contained in mucous secretions has been described in a number of mammalian species. Pierce has reviewed critically much of the relevant material(1). He refers to such antibody as mucoantibody, implying only that the activity is found to occur admixed with mucoprotein. Such antibody found in saline extracts of feces, and presumably occurring free in the bowel, has been called coproantibody.

The evidence suggests that such antibody in the upper respiratory tract is closely related to, and possibly derived from, circulating antibody. In other instances, notably in the case of antibody found in feces, vaginal secretions, and urine, antibody response following active immunization by the parenteral route appears to differ significantly from serum antibody response in that the activity appears and reaches a peak earlier than serum antibody, and disappears while serum antibody persists. In contrast to serum antibody, such antibody is probably not cumulative, and the observed titers may reflect significantly the rate of antibody formation. Quantitative studies have supported this view(1,2,3), and it is inferred that the antibody is formed locally.

The occurrence of muco-antibody in areas subject to an essentially local infection without invasion of the deeper tissues provides a rational basis for an effective antimicrobial prophylactic immunity, and may also be a factor in the normal microbial flora of such areas. In the first instance, immunity to experimental enteric cholera in the guinea pig (4) and rabbit(5), and similar Shigella infections in the guinea pig(6) and mouse(7) appears to be a function of the presence of antibody in the bowel. Similarly, immunity to Trichomonas infection of the bovine vagina is also associated with presence of antibody in the vaginal mucus(1). In the second, the occurrence of coproagglutinins to coliforms and enteric pathogens in man(8,9, 10) as well as in experimental animals is suggestive in connection with the observed succession of immunologic types of coliform bacilli in man(11).

In addition to the immune response demonstrable in bovine vaginal mucus to Trichomonas infection, antibody to homologous sperm has been found in the rabbit following

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