* The work with bacteria was carried out by Cowie and Roberts at the Carnegie Institution of Washington, and the experiments with mice were done at the National Institutes of Health by Gelderman and Lincoln.

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FURTHER OBSERVATIONS OF THE LYMPHOMAS OF AFRICAN CHILDREN

BY GILBERT DALLDORF, FERNANDA BERGAMINI, AND PATRICIA FROST

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH, NEW YORK, NEW YORK

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During 1963, cytopathogenic, filtrable agents were frequently encountered in cultures of embryonic human kidney cells following their inoculation with supernatant fluids from primary human amnion cultures which had previously been exposed to extracts of tumors and other specimens from East African children with malignant lymphomas of the kind described by Burkitt and others.[1] The results seemed noteworthy because they suggested an intimate association between the agents and the disease, and also because of the nature of the isolations which involved two phenomena, an initial induction of a peculiar spindling and twisting of the amnion cells (Fig. 1) and subsequently destructive changes in kidney cells inoculated with fluid from such altered amnion cultures. The direct inoculation of embryonic kidney cells with extracts of tumors or bone marrow never caused cytopathogenic effects nor did the isolated, transmissible agents have the capacity to induce the amnion lesions. Nevertheless, the two effects were closely associated and clearly related to the specimens.[2]

The cytopathogenic agents were later cultivated on protein-rich media and found to have the characteristics of mycoplasma.[3] They failed to induce tumors in a variety of animals, and serologic tests in which they served as antigen gave suggestive but inconclusive evidence of a relationship to the disease. The mycoplasma also failed to induce the amnion lesions caused by the specimens.

The following year five additional East African patients were investigated. A

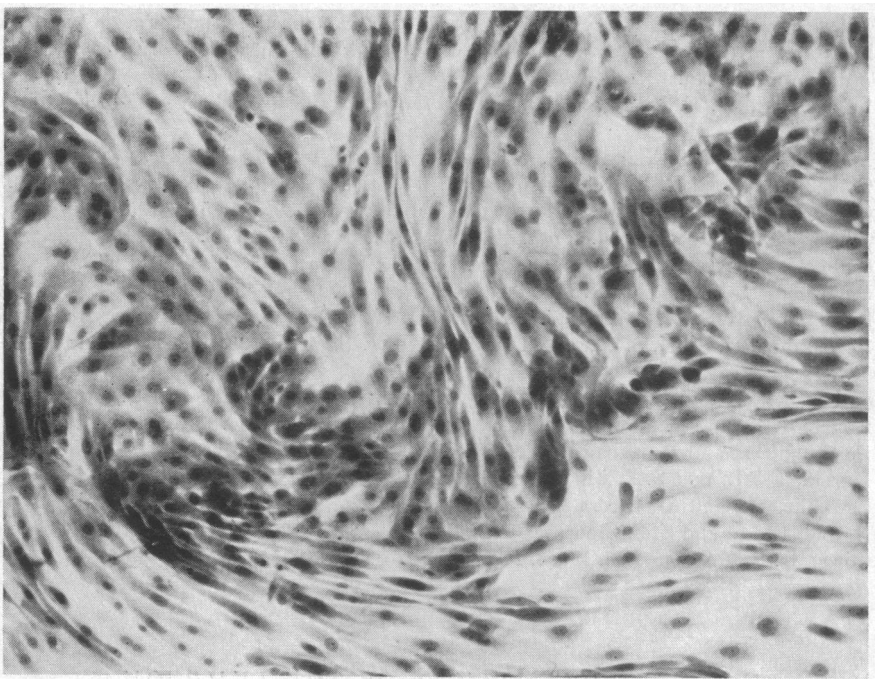

FIG. 1.—Metamorphosis of human amnion cells following treatment with extracts of tumors or bone marrow (×100, H-E).

bone marrow aspirate from a child whose tumor was in remission and who had received no treatment for several months induced characteristic changes in amnion cells as did the extract of tumor from a second child whose treatment (methotrexate) had been inaugurated within the week. Blood clots and marrow samples from the others, who were then under intensive chemotherapy, were inactive.

Five additional patients were studied in South Africa. Tumor was accessible in only one. Extracts of it repeatedly induced lesions in cultivated amnion cells. Three of five bone marrow specimens were also active as were two of five blood clots, each of these representing an individual patient (Table 1). The supernatant fluids from none of the altered amnion cell cultures in 1964 were cytopathogenic for embryonic kidney cells, and mycoplasma were not isolated.

The morphologic effects in the amnion cells appeared identical in both years, and the altered amnion cell cultures in 1964, as in 1963, were demonstrably resistant to infection with certain arboviruses. In 1963 Sindbis virus had been used. Bunyamwera virus was substituted in 1964.

Efforts were made during 1964 to collect and test suitable control specimens. Three dissimilar tumors of children available in Kenya were inactive in amnion cell cultures, as were 11 samples of bone marrow and 13 blood clots collected from young patients with other diseases. Two fecal specimens, one from a child with Hodgkin's disease, and the other a retinoblastoma, were active. Fecal specimens from children with malignant lymphomas also have frequently induced morphological alterations and resistance to challenge in amnion cell cultures. A number have yielded enteric viruses of various kinds. Interfering activity in fecal speci-

TABLE 1

Patient	Clinical summary	Amnion Effect: Tumor	Marrow	Blood clot	Stool
	Nairobi, 1963				
Fa 4-yr girl	Jaw tumor of 2 months' duration. No treatment when initial samples were collected. Marrow specimens collected after therapy were inactive.	NT	+	−	+
Ok 7-yr boy	Jaw tumor of relatively small size. Untreated when initial samples were collected.	+	?	+	+
Ma 3-yr boy	Jaw tumor. Duration uncertain. No treatment when initial samples were collected.	+	NT	NT	+
Mu 8-yr boy	Jaw tumor of large size. Untreated when first samples were collected.	+	NT	NT	−
Sa 5-yr boy	Jaw tumor originally. Samples collected during prolonged therapy.	NT	NT	NT	+
Su 11-yr boy	Jaw tumor. Course of cytoxan before admission.	NT	NT	NT	+
Gi 7-yr boy	Superficial nodes initially involved. Jaw tumor appeared during hospitalization.	NT	NT	−	−
Mi 7-yr boy	Abdominal and cervical tumors. Two brief courses of cytoxan before samples taken.	NT	−	−	Enterovirus
	Nairobi, 1964				
Kit 9-yr girl	An abdominal tumor, probably ovarian. Cytoxan therapy during preceding 6 months. Treatment interrupted before specimens were collected.	NT	+	−	−
Kin 6-yr boy	Jaw tumor. 2-month cytoxan therapy.	−	−	−	+
Ta 10-yr boy	Jaw tumor treated with two courses of cytoxan.	−	−	−	−
Th 13-yr boy	Jaw tumor. Specimens collected 6 days after treatment begun.	+	−	−	+
Mb 5-yr girl	Jaw tumor. Manitol myleran treatment.	−	−	−	NT
	Johannesburg, 1964				
De 6-yr boy	Retroperitoneal tumors with cervical and inguinal nodes involved. Specimens collected 10 days after methotrexate treatment was begun.	NT	+	+	Reovirus
Jo 7-yr boy	Tumor of left tibia. Kidney mass (nephrectomy). Tumor of femur. Left axillary and inguinal glands enlarged. Presence of tumor in them verified histologically. Treated.	NT	−	−	Reovirus
Do 7-yr girl	Tumor of tibia of 5-months' duration. Radiologic evidence of tumors of right shoulder, pelvis, and thoracic spine. Small ovarian tumor. No treatment.	+	+	−	−
To 6-yr boy	An abdominal tumor involving the ileum.	NT	−	+	NT
Gu 3-yr girl	Enlarged cervical glands. Typical histologically. Massive bone marrow involvement. Specimen taken 24 hr after 6 MP and Medrol given.	NT	+	−	NT

NT indicates no specimen or not tested; inadequate or contaminated.

mens is presumably less significant than that of tumor and marrow specimens and should be discounted until more fully investigated.

The changes in the amnion cell cultures closely resemble those that follow treatment with interferon.[4] In Gresser's experiments the effect was reversible while ours persisted throughout the life of the cultures, suggesting the presence of an enduring, interferon-inducing infection. Thus far, we have failed to transfer infection to various cell lines or to animals, although suggestive lesions have been observed in the latter. The variability of the amnion cell response, evidently dependent on the condition of the cells, and the limited quantity of the more active specimens have been additional obstacles to a methodical study of the phenomenon.

The production of interferon is known to be induced by a wide and growing variety of agents and the presence of an interferon provides no clue to the identity of the responsible factor or, in the present case, to the sameness of the factor in the several active preparations. The evidence presently available is therefore limited to the association of the amnion effect and the resistance of such altered cells to superinfection, with the cardinal lesions of the disease and the absence of activity in control specimens. The activity of fecal specimens, conversely, seems of much less significance. It is noteworthy that cultivated lymphoma cells have been shown by the Henles to be resistant to infection by vesicular stomatitis virus and to transfer the resistance to amnion cells.[5] Dr. Henle has not, however, observed alterations in the appearance of his amnion cells.[6]

Stewart *et al.* described focal, hyperplastic lesions in amnion cell cultures treated with homogenates of leukemic bone marrow but did not test for interference.[7] Rapid growth is a characteristic of our altered amnion cells as well.

The source of the mycoplasma remains unknown. All of the patients from whom mycoplasma were isolated had tumors of the jaws and mycoplasma can frequently be recovered from oral and genital tissues. Possibly, mycoplasma plays a part in determining the common localization of these lymphomas in the jaws and ovaries. The vagina of the immature girl is an indifferent barrier to peritoneal infection which would affect the ovaries. The association of mycoplasma with lymphatic malignancies of other types is also well known[3] and is currently being energetically investigated.

The South African patients were clinically noteworthy. None of the five had presented with a tumor of the jaws or ovaries, the hallmark of the disease in central Africa. In two, the initial tumor was of a long bone. This is infrequently seen in Kenya[8] and Uganda.[1] Still more remarkable was the character of the disease in a 3-year-old white girl ("Gu") whose solitary tumor was restricted to a cervical lymph node. The histologic appearance of the tumor was typical (Fig. 2) of the African lymphomas. The child's bone marrow was massively invaded by tumor cells. Following death, 10 months later, the marrow of the vertebrae and pelvic bones as well was completely destroyed by tumor. Other organs were diffusely infiltrated by tumor cells in widely scattered foci. Only two small (1 cm in diameter) solid tumors were found, both in the liver.

A 7-year-old girl, with a histologically typical tumor of the tibia, also had extensive involvement of her bone marrow. Three sites were sampled early in the illness, and in each the normal marrow was replaced by lymphoblasts. Microscopic examination of bone marrow was made in two other patients. In one, the cellular pattern was not remarkable. The other showed the attrition associated with chemotherapy.

The clinical, anatomical, and radiological features of four of our Johannesburg subjects have been reported in detail by Schmaman, Gampel, and Luntz[9] who noted the differences between skeletal lesions of their patients and those Cockshott had found characteristic of the disease in Nigeria.[10]

The African lymphomas appear to be reciprocally related to childhood leukemia.[8] Lymphatic malignancies of young children which are usually leukemic in North America are characteristically tumorous in central Africa. Both forms seem to occur in southern Africa in approximately equal numbers, and South Africa could be considered an intermediate.[11] Under the circumstances, combination or mixed forms of the disease might be expected and the atypia of our sample, the absence of jaw tumors, and the frequency of long bone and marrow involvement, may be an example of this. Quite characteristic cases do occur in South Africa. Gluckman described three from the Transvaal.[12]

Materials and Methods.—Great care was taken promptly to freeze the specimens which were thereafter stored at −70°C. The homogenates were usually prepared as 20 per cent suspensions in Hanks balanced salt solution; they were centrifuged at 2500 rpm for 30 min in a refrigerated centrifuge and the supernatant fluids tested for sterility in thioglycollate, BHI, and Sabouraud's broth, and on blood agar. When necessary, antibiotics were added (1000 units penicillin, 1000 μg

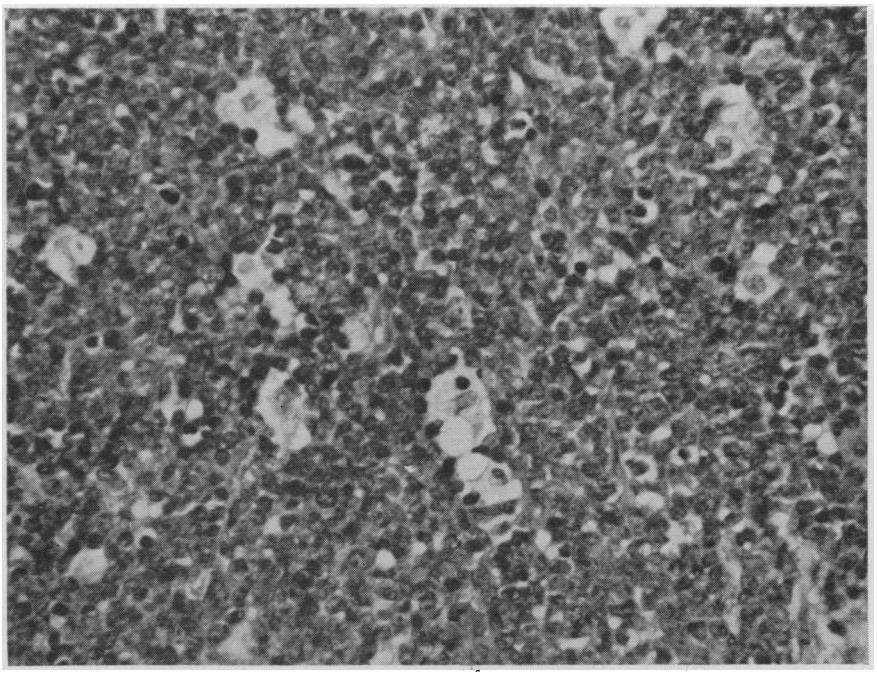

FIG. 2.—The microscopic appearance of the lymphoma removed from patient "Gu" (×400, H-E).

streptomycin per ml). Such supernates were inoculated in 0.1-ml amounts into tube cultures or 0.3-ml amounts into plastic (Falcon) flasks with maintenance medium which was replaced after 24 hr and at 4-day intervals thereafter.

The primary amnion cultures were maintained with either LY, M150, Eagle's basal, or Bodian's medium supplemented with 5 per cent fetal calf serum, and antibiotics (100 units penicillin and 100 μg streptomycin per ml).

The interference tests were routinely carried out with the 167H strain of Sindbis virus provided by Dr. John Enders. The virus was passaged in chicken fibroblasts, the harvested fluids clarified by centrifugation, and aliquots stored at −70°C. Once the morphologic changes were well developed, usually between the 8th and 15th days, the amnion tube cultures were rinsed twice with maintenance medium and challenged with 100–1000 $TCID_{50}$ of virus. Thirty min after adding the virus suspension, 1.5 ml of maintenance medium was applied. The outcome was recorded after complete destruction of the control cultures, usually the third day.

These studies would have been impossible without the help of colleagues in Africa. The authors are greatly obliged to Peter Clifford of Nairobi, Geoffrey Falkson and W. J. Pepler of Pretoria, and James Gear and members of the staff of the South African Institute for Medical Research, Johannesburg. The 1964 specimens were initially tested while the authors were guests of that laboratory.

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*CELL GROWTH AND THE INITIATION OF TRANSFORMATION BY SV40**

BY GEORGE J. TODARO AND HOWARD GREEN

DEPARTMENT OF PATHOLOGY, NEW YORK UNIVERSITY SCHOOL OF MEDICINE

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Although the sequence of events involved in the multiplication of viruses in mammalian cells has been extensively studied, the mechanism by which certain of these viruses are able to disrupt cellular growth control is not at all understood. The two processes appear to be quite independent, since oncogenesis may occur in cells which do not support viral replication. This is true of the interaction between the mouse cell line 3T3 and the oncogenic virus SV40; the virus multiplies very little, if at all, but is capable of transforming a large fraction of the population,[1] destroying cellular sensitivity to contact inhibition.[2] The very high contact sensitivity of the line and the absence of a complicating cytocidal effect make it possible to examine the transforming function of the virus on nongrowing as well as growing cells. The experiments to be described here show that the virus cannot initiate transformation in a strictly nongrowing population. The fixation of the transformed state as a heritable cellular property requires one cell generation after infection, while the expression of the transformed state requires several more generations. Cells which are not permitted to grow through a cell division cycle subsequent to infection are altered neither in their genotype nor in their phenotype.

Materials and Methods.—Fibroblast line 3T3, a spontaneously established cell line of mouse embryo origin,[3] was used between the 105th and 200th cell generation. Cells were infected with SV40 strain 776, grown on Rhesus monkey kidney cultures[4] and kindly supplied by Dr. John Easton (National Institutes of Health). The transformation assay using SV40 and cell line 3T3 has been described in detail.[5, 6] The transformed colonies may be scored, after confluence is attained, against a uniform background of untransformed cells.

Results.—(1) *Cell growth and the expression of the transformed state:* The transformation of 3T3 by SV40 has usually been studied by infecting a population of cells and inoculating them on the following day at high dilution into a number