between the solar cycle (indicated by relative sunspot number) and zodiacal light brightness.

The photometers on OSO-5 were directed at a constant ecliptic longitude ($90° \pm 4°$) but various ecliptic latitudes, generally within 35° of the ecliptic, although excursions were made to both the north and south ecliptic poles. Measurements made at the same ecliptic latitudes (in both hemispheres) but at different times over the 4-year period failed to show differences in brightness or polarization that were greater than the limits of accuracy of the determinations ($\pm$ 10 percent, in general) as shown in Fig. 2. No evidence for the influence of Comet Encke has been obtained. Nor has any north-south asymmetry been measured, although the rocket observations of Wolstencroft and Rose (*6*) had suggested that the brightness of the south pole exceeded that of the north pole by about 15 percent (at the time of observation), which they interpreted as possibly indicative of the motion of the earth with respect to the invariant plane.

Any temporal changes in the brightness and polarization of zodiacal light appear to be below the accuracy limit of the OSO-5 experiment. However, the next generation of satellite-borne zodiacal light probes may yield information on any changes of lesser magnitude (*10, 26*).

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Evidence for Fetal Antigen in Human Sarcoma

Abstract. *A common antigen (S_2), initially thought to be uniquely associated with human sarcomas, has been found to be widely distributed in patients with other tumors as well. Absorption studies with human embryonic tissues suggest that S_2 may be a fetal antigen. The presence of antibody to S_2 in patients with tumors and in their relatives implies a propensity in these individuals for cellular dedifferentiation which may be a prerequisite for malignant transformation.*

A diffuse cytoplasmic antigen, specific to human sarcomas, has been described by Morton and Malmgren (*1*) and Priori *et al.* (*2*) in acetone-fixed tumor cells tested by an indirect immunofluorescence technique. Antibody to this antigen has been demonstrated in a high percentage of serums from patients with various histologic types of sarcomas, their family members, and close associates. In contrast, few serums from normal individuals have been found to react against the same antigen. Type C virus particles were also reported by both groups in cells said to be positive for the sarcoma antigen (*2, 3*). These observations have been cited as indirect evidence for an infectious, possibly viral etiology of human sarcomas. We have identified an antigen in human sarcomas which closely resembles and probably is identical to the antigen described by Morton and Malmgren and by Priori *et al.* An extensive effort to characterize the antigen and determine its distribution among patients with cancer has been undertaken. Results to date indicate that while the earlier serologic findings can be confirmed, neither the antigen nor antibody directed against it has as limited a distribution as the first reports suggested. This diminishes the likelihood that the antigen is associated with an etiologic agent responsible for a specific type of cancer.

Monolayer cell cultures were maintained in Eagle's minimum essential medium with 10 percent fetal calf serum. For testing, cultures were trypsinized and the cells were placed on glass slides with eight antigen wells. The slides were incubated at 37°C for 24 to 48 hours to permit cells to attach and spread, and the cells then were fixed with acetone. Indirect immunofluorescence tests were carried out after the method of Goldman (*4*). Details of this procedure have been published elsewhere (*5*).

Initially we had considerable difficulty in demonstrating this antigen, since it is often manifest only as a low-intensity, diffuse fluorescence. However, in a comparison of the reactivity of serums from sarcoma patients with that of normal serums when applied to the same antigen on adjacent wells of a single slide, significant differences became apparent. A positive reaction is seen in Fig. 1A. The majority of cells show a diffuse cytoplasmic fluorescence with perinuclear accentuation. Normal serums persistently failed to elicit any cytoplasmic fluorescence; instead, they often produced a nuclear fluorescence (Fig. 1B), while the saline controls showed no fluorescence at all. The nuclear fluorescence is thought to be due to nonspecific absorption of serum globulins to the fixed nuclei. The brightest cytoplasmic fluorescence was seen in cells derived from benign giant cell tumors of bone.

An earlier report by Giraldo *et al.* (*6*) indicated that another antigen was present in cultured sarcoma cells characterized morphologically by a punctate cytoplasmic distribution. This has subsequently been referred to as

the S_1 antigen (*7*). The diffuse cytoplasmic antigen now described is, therefore, designated the S_2 antigen.

To assure uniformity of the test antigen, a large number of slides were prepared from the first two passages of a single giant cell tumor culture. Antigen was seldom detectable in cultured cells after the third passage. Slides were read without prior knowledge of the nature of the serums tested, and a positive serum and saline control were always included on each slide.

Patients' serums were all collected from persons admitted to Memorial Hospital, New York, or their relatives, with diagnosis confirmed by review of tumor tissue pathology. Age- and sex-matched control serums came from blood donors at the New York Blood Center.

For absorption tests, cells grown in bulk were suspended in an equal volume of phosphate-buffered saline, freeze-thawed three times (− 190°C to 37°C), and sonicated (Byosonic, 10 seconds per milliliter). This preparation was then used for absorption. End titer of a positive reference serum was determined on the test cell line (Riv). A dilution of two and a half tubes above the end titer was used in absorption tests. The reference serum, diluted to one-third of the absorption dilution, was placed in a microfuge tube (Beckman, New Brunswick, N.J.) and mixed with 2 volumes of the sonicated antigen. After being kept on ice for 3 hours, the mixture was centrifuged for 15 seconds (Beckman 152 microfuge). The supernatant was removed and two further twofold dilutions were made. All three dilutions were then tested for immunofluorescence activity. Absorption tests were repeated three times, and 50 percent human albumin solution was used as a control.

Antibody to the S_2 antigen was detected in the serums of 72 percent of a group of patients with a variety of sarcomas (Table 1). Nearly a third of family members also had antibody to the antigen, while only 10 percent of serums from normal persons were reactive. The antibody was, however, not restricted to patients with sarcoma. As seen in Table 1, serums from patients with carcinoma of breast, lung, ovary, and gastrointestinal tract also frequently demonstrated anti-S_2 reactivity. Interestingly, few patients with leukemias and other lymphoproliferative malignancies had detectable levels of anti-S_2 antibody. Of seven positive serums in the control group, four were derived from donors under age 10 (three under age 5). The remaining three donors were over age 20. Antibody titers usually varied from 1 : 20 to 1 : 80. No correlation between titers of antibody and histology could be made. When serum specimens from seven patients with sarcoma were tested before and for several months after surgery, no changes in titer were found.

An established fetal lung fibroblast line, Wi-38, was used as a control for the giant cell tumor line. Repeated tests with serums known to have anti-S_2 activity did not reveal any cytoplasmic antigen in cells of this line.

With a panel of nine positive and nine negative reference serums, additional lines were tested for S_2 antigen by immunofluorescence. Table 2 indicates that in addition to sarcoma (Fig. 1C) and benign giant cell tumor lines (Fig. 1A), cultures of embryonic tissue (Fig. 1D) and normal skin, both of tumor-bearing and normal individuals

Table 1. Prevalence of antibody against S_2 antigen.

Diagnosis	Number of serums tested	Number of serums positive	Percent positive
Sarcomas	64	46	72
Family members	20	6	30
Carcinomas			
Breast	15	8	53
Lung	15	6	40
Ovary	15	5	33
Gastrointestinal tract	15	3	20
Melanoma	15	3	20
Lymphoproliferative malignancy			
Hodgkin's disease	15	2	13
Acute myelogenous leukemia	15	2	13
Acute lymphocytic leukemia	15	1	7
Non-Hodgkin's lymphomas	15	1	7
Autoimmune disease	9	1	11
Normal donors	65	7	11

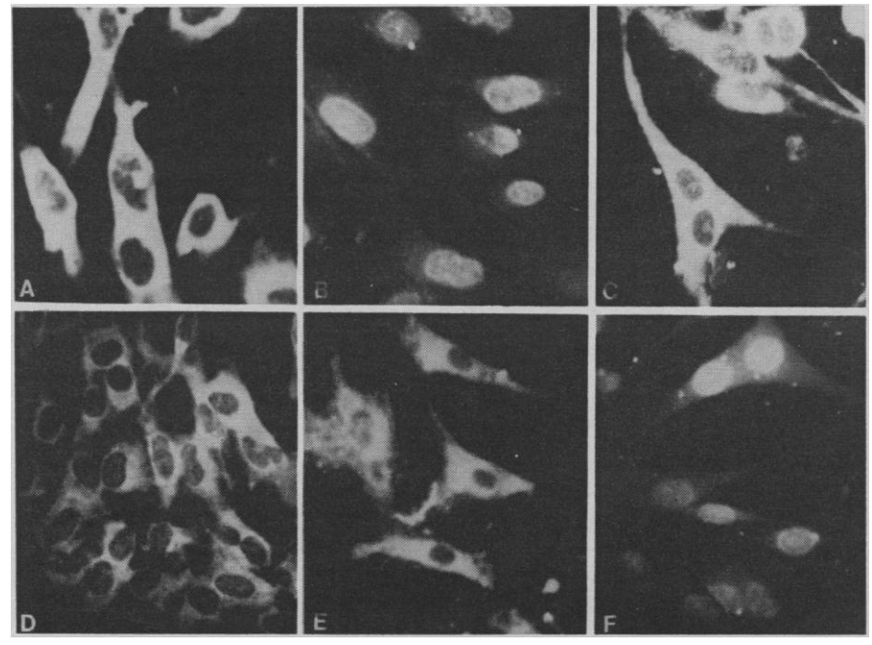

Fig. 1. Immunofluorescent photomicrographs demonstrate diffuse cytoplasmic fluorescence in cell lines Riv (A), Cas (C), E-2 (D), and Be (E). For illustrative purposes bright reactions have been selected; more frequently fainter fluorescence is observed, but its pattern of distribution remains the same. Figure 1B shows a negative reaction with normal serums on the cell line Riv. Nonspecific nuclear fluorescence is seen. Figure 1F demonstrates positive absorption test on the cell line Riv. The same serum that gave the positive reaction in (A) failed to elicit any cytoplasmic fluorescence after being absorbed with an antigen extract for the cell line E-2. The absorbed serum produced nuclear fluorescence (× 360).

Table 2. Distribution of S_2 antigen. Symbols: +, positive; −, negative.

Cell line	Passage level	Histological diagnosis	Cell type	Result
Riv	1–5	Giant cell tumor of bone	Predominantly fibroblastic	+
Cor	1	Giant cell tumor of bone	Predominantly fibroblastic	+
Koo	1–2	Osteosarcoma	Predominantly fibroblastic	+
Grn	5	Osteosarcoma	Predominantly fibroblastic	+
Cas	4	Osteosarcoma	Predominantly fibroblastic	+
Ri	1–5	Liposarcoma	Predominantly fibroblastic	−
Min	1	Fibrosarcoma	Predominantly fibroblastic	−
Wi-38	25–32	Embryonal lung	Predominantly fibroblastic	−
E-2	1	Whole human embryo (8 to 10 weeks)	Mixed	+
E-3	1	Whole human embryo (10 to 12 weeks)	Mixed	+
Riv-Sk	1–3	Normal skin from patient	Fibroblastic	+
Cas-Sk	2–4	Normal skin from patient	Fibroblastic	+
Be	3	Normal skin from healthy donor	Mixed	+
Sm	1	Carcinoma of pancreas	Fibroblastic	−
SK-OV 1	14	Carcinoma of ovary	Epithelial	−
SK-LU 1	20	Carcinoma of lung	Epithelial	−
FL	9	Normal amnion	Epithelial	−

(Fig. 1E), were also found to contain S_2 antigen. The antigen could not be identified, however, in all sarcoma lines and was absent in the three carcinoma-derived cell culture lines tested.

Antigenic identity of the demonstrable antigens in various lines was determined by immunofluorescent absorption technique. A typical positive absorption study is shown in Fig. 1E. A strong positive reaction is seen in Fig. 1A with a test serum applied to the Riv reference cell line. The same serum, after absorption with an antigen extract of the embryonal line, E-2, failed to produce any cytoplasmic fluorescence (Fig. 1F) on the same reference cell line. Interestingly, the absorbed serum, which originally did not show any nuclear fluorescence, produced some nuclear fluorescence. This phenomenon was routinely observed with absorption study. Other antigen extracts from sarcoma cell lines Grn, Cas, and Cor, and from lines prepared from normal skin of patients with sarcoma, Cas-Sk and Riv-Sk, completely absorbed activity of the same reference serums when tested on the Riv cell line. Antigen extracts from the cell lines SK-L1 (acute myelomonocytic leukemia in adult), SK-L7 (acute myelomonocytic childhood leukemia), and LN (normal lymph node from a hemophiliac) failed to absorb the reactivity of the same serums. Reactivity was also not diminished by absorption with sheep red blood cells or human blood group substances A and B. The Riv cell line and two other positive lines were tested for mycoplasma contamination and were found to be free of mycoplasma at the time of the study.

Electron microscopy did not reveal any type C particle in three positive lines.

The S_2 antigen resembles the sarcoma-specific antigen of Morton (*1*) and Priori (*2*) in its morphology, its presence in sarcoma and benign giant cell tumor, and in the frequency with which antibody to this antigen is found among patients with sarcoma and their relatives in contrast to a control population. The present study indicates, however, that while the S_2 antigen can be readily associated with sarcomas, the same antigen can also be linked to a variety of carcinomas, since patients with these tumors share the finding of a high prevalence of anti-S_2 antibody. Because it is unlikely that a single virus would be responsible for such a broad range of neoplasms, S_2 is a poor candidate for an oncogenic virus–associated agent.

The presence of S_2 antigen in embryonic tissue suggests that we may be dealing with a fetal antigen. Its occurrence in adult normal skin is consistent with the findings of Stonehill and Bendich (*8*) that fetal antigens that are found in mouse tumors also occur in adult skin of mice. It is noteworthy that carcinoembryonic antigen (*9*), another example of fetal antigen, has similarly been shown to be present in lower concentration in noncancerous colonic mucosa (*10*), and circulating carcinoembryonic antigen has also been reported in serums from patients with inflammatory bowel disease (*11*) and alcoholic liver disease (*12*). These observations suggest that antigens that are expressed in fetal tissues and certain types of tumors perhaps represent a heterogeneous antigenic system which at times is detectable in a more limited fashion in some specialized adult tissues as well. As is postulated for other fetal antigens which are detected in patients with cancer, S_2 may represent the product of cell derepression. In this context, occurrence of anti-S_2 antibody in family members of sarcoma patients suggests a familial tendency to derepression, which in some but not in all affected persons, perhaps with the assistance of an oncogenic agent, may result in malignant cell growth. If so, antigens such as S_2 may prove to be useful in the development of screening tests for the identification of individuals at high risk for the development of tumors.

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