between systemic sarcomas and intracranial meningiomas and gliomas.

Approximately 30 percent (4 out of 12) of blood donor serums used as controls gave positive staining reactions against meningioma-derived cell cultures. This incidence is similar to that observed for the antibody directed against the S-antigen (*5*). Therefore, the presence of immunofluorescence antibody in human serums cannot be considered, at present, to be diagnostic of the presence of a meningioma or other brain tumor. Such antibody may result from experience with a relatively common antigen which is preferentially localized in neoplastic cells but may be completely unrelated to the oncogenic process. This hypothesis would also explain why the immunofluorescence reaction was not entirely specific for cell cultures derived from meningiomas. Further definition of this immunologic relationship by other techniques, such as complement-fixation or cytotoxicity, may indicate more specific relationships than are demonstrated here.

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Human Sarcomas Contain RNA Related to the RNA of a Mouse Leukemia Virus

Abstract. *Labeled DNA complementary to the RNA of the Rauscher leukemia virus was hybridized with RNA from the polysome fraction of human sarcomas. Eighteen out of 25 specimens contained RNA possessing homology to the RNA of the mouse leukemia virus but not to that of the unrelated viruses causing mammary tumors in mice or myeloblastosis in chickens. Further, no normal adult or fetal tissues showed significant amounts of RNA specific to mouse leukemia virus. It appears that human sarcomas contain RNA sequences homologous to those found in an agent related to a virus known to cause sarcomas in mice.*

We have recently developed (*1*) means to detect virus-specific RNA in the polysome fraction of fresh and frozen tumors. Use of this technique revealed (*2*) that 67 percent of 29 human malignant breast tumors contained RNA that could hybridize with DNA complementary to the RNA of the mouse mammary tumor virus (MMTV). None of 30 control preparations from normal or benign breast tissues contained detectable quantities of this type of RNA.

Breast carcinoma RNA samples that hybridize with MMTV-DNA showed no ability to hybridize with DNA complementary to an unrelated (*3*) murine leukemia agent, Rauscher leukemia virus (RLV). When we turned our attention to human leukemias, we found (*4*) that 89 percent of 27 leukemic patients contained, within their white blood cells, RNA homologous to that of RLV but not to that of MMTV.

The unrelatedness of virus-specific RNA found in human breast tumors and the virus-specific RNA found in human leukemic cells is precisely what one would expect from the accumulated experience with the corresponding animal oncogenic viruses. In animal systems all naturally occurring sarcoma viruses (from chicken, mouse, cat, and so forth) are defective (*5*) and require related leukemogenic virus as helpers. If the human situation is similar, one would predict that, like the leukemias, human sarcomas would also contain RNA that can hybridize to DNA homologous to the RNA of RLV.

We have now found that 68 percent of human sarcomas do contain RNA that can hybridize with DNA homologous to the RNA of RLV. Again, as with the leukemic cells, the sarcoma RNA does not hybridize with DNA homologous to RNA of either MMTV or of the unrelated avian myeloblastosis virus (AMV).

Figure 1 shows representative density profiles (in Cs_2SO_4 gradients) of the products of annealing reactions between RLV [^{3}H]DNA and polysomal RNA (pRNA) preparations isolated from four different human sarcomas. These results (from 1 to 6 percent hybridization of the input RLV-DNA) are comparable to those obtained if

Table 1. Results of hybridization between RLV [^{3}H]DNA and pRNA isolated from human sarcomas. Between 200 and 500 μg of pRNA from each sample were hybridized to 2000 count/min RLV [^{3}H]DNA, and the reactions were analyzed by Cs_2SO_4 density centrifugation. The amount of DNA banding in the RNA region of the gradient (between densities 1.62 and 1.68 g/ml) was then determined. The results are expressed as the radioactivity (count/10 min) (corrected for background) banding in the RNA region for each sample tested and as multiples of σ, the operational standard deviation (see legend to Fig. 3). The annealing reaction is considered positive only if the number of counts per 10 minutes per RNA region is greater than 3σ. Twenty-five sarcomas tested, 68 percent positive.

Diagnosis	RNA (count/10 min)	(Count/10 min) per σ	Reaction
	Fibrosarcoma		
(1448)	363	6.87	+
(1444)	427	8.08	+
(MV)	403	7.63	+
(1455)	96	1.81	−
	Osteogenic sarcoma		
(1415)	433	8.20	+
(PH)	220	4.16	+
(23–2)	253	4.79	+
(23–5)	908	17.20	+
(1468)	173	3.27	+
(1443)	95	1.79	−
	Osteolytic sarcoma		
(1464)	42	0.795	−
	Liposarcoma		
(1438)	333	6.30	+
(1441)	449	8.50	+
(1458)	252	4.77	+
(1450)	454	8.59	+
(1442)	94	1.78	−
	Leiomyosarcoma		
(1418)	297	5.62	+
(1449)	214	4.05	+
(1447)	157	2.97	−
(1463)	145	2.75	−
(1446)	46	0.87	−
	Neurofibrosarcoma		
(1437)	313	5.92	+
(1456)	182	3.44	+
	Rhabdomyosarcoma		
(21–43)	531	10.05	+
(1459)	99	1.87	−

RLV-DNA is hybridized to RNA from animal and human leukemic cells (*4*). The density of these complexes implies that the RNA is much larger than the DNA and determines the density of the DNA-RNA hybrid structure.

The positive responses with the sarcoma pRNA's are in contrast with the lack of reaction with pRNA from normal adult and fetal tissues (Fig. 2A is a typical result). In none of these does one find significant amounts of RLV [^{3}H]DNA in the RNA density region of the gradient.

Figure 2B shows the effect of varying the concentration of osteogenic sarcoma pRNA and normal striated muscle pRNA on the amount of hybridization with RLV [^{3}H]DNA. The percentage of [^{3}H]DNA complexed to the RNA is determined by isopycnic separation in Cs_2SO_4 gradients. No detectable reaction is observed with pRNA from normal striated muscle up to a concentration of 6.4 mg/ml. However, pRNA from an osteogenic sarcoma shows no evidence of saturation at 6.8 mg/ml at which about 2.5 percent of the RLV [^{3}H]DNA is complexed. Four sarcomas were obtained in amounts sufficient to permit such concentration studies, and similar results were obtained.

Because it is impractical to present the Cs_2SO_4 gradient profiles of every sample examined, a more convenient recording of our findings is used. After correction for background counts, the sum of the tritium counts in the RNA density region (between densities 1.62 and 1.68 g/ml) was used to measure the amount of DNA complexed to RNA. To achieve the accuracy desired, 10-minute counts (count/10 min) were taken with each sample. We adopted the convention that hybridization results were negative if the number of counts per 10 minutes in the RNA region of the gradient was less than three standard deviations greater than the background mean.

Table 1 lists the sarcoma samples tested for RNA complementary to RLV-DNA; results are recorded as the total number of counts per 10 minutes (corrected for background) in the RNA density region and as multiples of the mean background standard deviation. Figure 3 presents a pictorial summary of the reactions observed with sarcoma (Table 1) and control tissues.

Of the 25 sarcomas examined, 68 percent gave positive results with values that insure the reality of the RNA hybridization peak, with a probability bet-

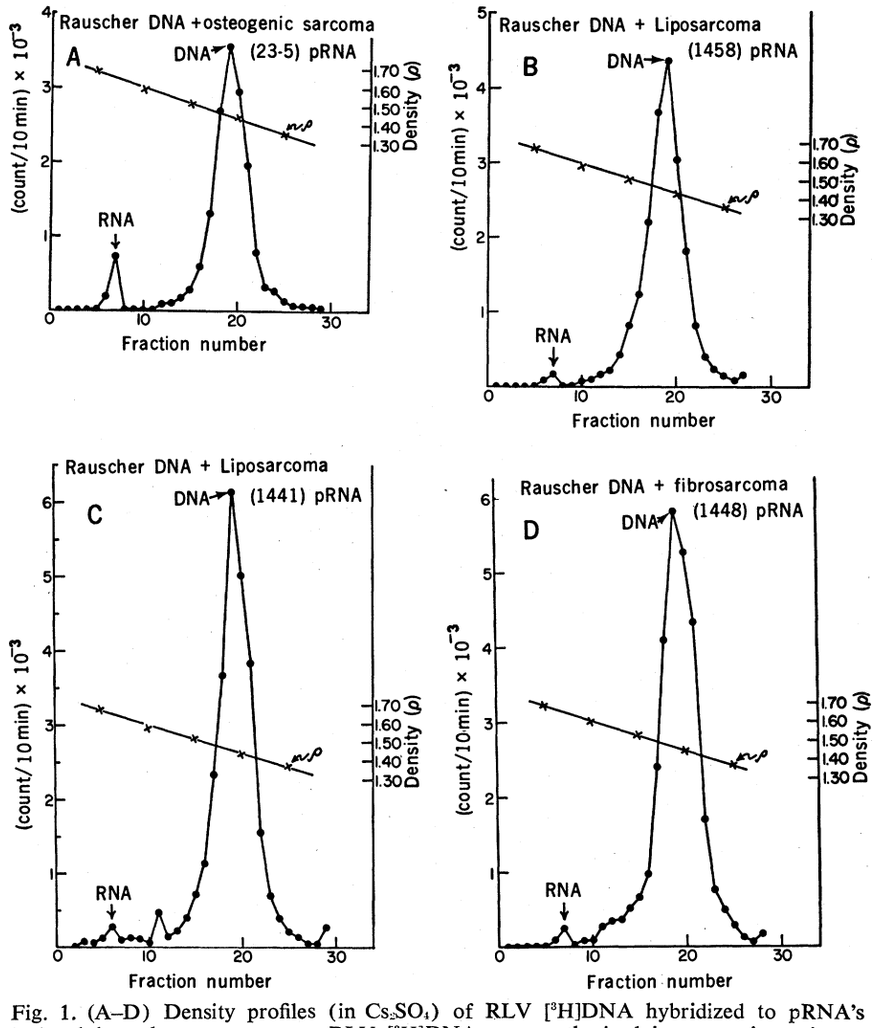

Fig. 1. (A–D) Density profiles (in Cs_2SO_4) of RLV [^{3}H]DNA hybridized to pRNA's isolated from human sarcomas. RLV [^{3}H]DNA was synthesized in a reaction mixture of 1 ml containing detergent-disrupted virus (100 μg of virus protein) purified from RLV-infected mouse plasma (*9*), 50 μmole of tris(hydroxymethyl)aminomethane (tris), *p*H 8.3, 40 μmole of KCl, 6 μmole of $MgCl_2$, 2.5 moles of dithiothreitol, 0.00125 percent NP-40 detergent (Shell), 100 μmole each of deoxyguanosine triphosphate, deoxyadenosine triphosphate, and deoxycytosine, and 5×10^4 pmole of [^{3}H]thymidine triphosphate at 8000 count/min per pmole. The reaction mixture was incubated at 37°C for 180 minutes, then brought to 5 percent sodium dodecyl sulfate (SDS) and extracted with an equal volume of a mixture of phenol and cresol. The RLV [^{3}H]DNA product was purified by Sephadex G-50 chromatography and then treated with 0.5*M* NaOH at 43°C for 24 hours. In studies such as those described here, it must be shown that the radioactive DNA product bands solely in the DNA density region of a Cs_2SO_4 gradient and that it hybridizes with homologous RNA and not to cellular RNA. The fraction containing RNA in our study is derived from cytoplasmic monosomes and polysomes. Suitable care must be exercised to insure the complete removal of protein contaminants. The pRNA was isolated from frozen human sarcoma tissue by disruption in a Silverson homogenizer at 4°C in two volumes of 5 percent sucrose in TNM buffer at (0.01*M* tris, *p*H 7.4, 0.15*M* NaCl, 0.002*M* $MgCl_2$). The suspension was centrifuged at 15,000*g* for 30 minutes at 0°C. The supernatant was then layered on 20 ml of 25 percent sucrose in TNM and centrifuged for 180 minutes at 180,000*g* in a Spinco 60 Ti rotor. The pellet was resuspended in TNM buffer with 0.5 percent SDS, and the RNA was extracted twice with an equal volume of a mixture of phenol and cresol (*p*H 8.0). Nucleic acid was precipitated from the aqueous phase by addition of two volumes of ethanol and one-tenth volume 4*M* LiCl. The pRNA was redissolved in 50 percent formamide and 50 percent 0.005*M* ethylenediaminetetraacetate (EDTA). The [^{3}H]DNA (2000 count/min per reaction) was incubated at 80°C for 15 minutes in 75 percent formamide to denature the DNA. After quick chilling to 0°C, 200 to 500 μg of sarcoma pRNA was added, and the annealing mixtures were brought to 0.4*M* NaCl, 50 percent formamide in a total volume of 60 μl. After incubation for 18 hours at 37°C, the reaction mixtures were added to 5.4 ml of 0.005*M* EDTA, mixed with an equal volume of saturated Cs_2SO_4 to yield a starting density of 1.52 g/ml and centrifuged at 44,000 rev/min in a Spinco 50 Ti rotor for 60 hours at 20°C. Fractions (0.4 ml) were collected from the bottom of the tube and assayed for radioactivity precipitable by trichloroacetic acid.

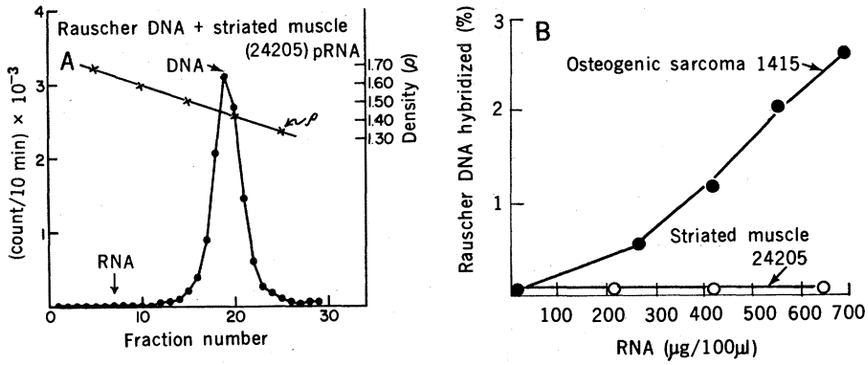

Fig. 2. (A) Density centrifugation profile (in Cs_2SO_4) of RLV [^{3}H]DNA after annealing to pRNA from human striated muscle. The pRNA input was 400 μg per 60 μl of hybridization reaction mixture. (B) Comparison of annealing reactions at varying RNA concentrations between RLV [^{3}H]DNA and pRNA's from osteogenic sarcoma (1415) and human striated muscle (204205). The individual annealing reactions were analyzed by Cs_2SO_4 density centrifugation and the percentage of DNA hybridized was determined by the [^{3}H]DNA (count/10 min), after correction for background, banding in the RNA region (between densities 1.62 and 1.68 g/ml) of the gradients.

ter than .999. In contrast, none of the pRNA preparations from 34 normal adult and fetal tissues gave a reaction that could be designated unambiguously as positive.

The fact that 68 percent of the sarcoma pRNA's yielded positive hybridizations with RLV-DNA, whereas none of the 34 control tissues exhibited this type of response, argues for the specificity of the annealing reactions. Further support for this conclusion can be obtained by the use of a [^{3}H]DNA complementary to the RNA of MMTV or to that of the AMV. We have shown (*3*) that RLV-DNA and the homologous viral RNA do not cross-hybridize significantly with the corresponding nucleic acids of either MMTV or AMV. If the annealing reaction is specific, one would not expect a sarcoma pRNA, positive for a reaction with RLV-DNA, to show the ability to hybridize either with MMTV-DNA or AMV-DNA. Table 2 shows that these expectations are realized. Of the six sarcoma pRNA's giving a positive reaction with RLV-DNA, none responded either to MMTV-DNA or to AMV-DNA.

The data described here provide no measure of the degree of homology between the RLV-DNA and the RNA detected in human sarcoma cells. To settle this issue will require the synthesis of RLV-DNA in amounts adequate to permit comparative saturation curves of labeled RLV-RNA and the relevant RNA strands from the sarcomas. The fact that hybrids are found in the RNA region of the density gradient implies that the RNA is much larger than the DNA product. However, experiments must now be designed and performed which will determine how large this RNA is, how much viral sequence it contains, and whether the viral information is covalently linked to normal cellular message. The latter point is particularly relevant to whether the viral information is integrated into the genome of the cancer cell.

Purified messenger RNA fractions will be required to make the quantitative aspects of the hybridization assays more certain. In particular, it is apparent that a negative outcome in a hybridization test cannot be accepted as proof for absence of the relevant RNA. From the data presented, one can conclude that the probability of finding RNA homologous to RNA from RLV is much greater in human sarcomas than in normal tissues. Indeed, if the provirus (*6*) or oncogene hypotheses (*7*) are valid, some part of this information might be found with more sensitive tests in presumably normal cells derived from fetal tissues or phytohemagglutinin-stimulated lymphocytes, both of which have been reported (*8*) to possess the group-specific antigen of the mammalian leukemogenic viruses.

Although our experiments do not constitute definitive proof of a viral etiology of human sarcomas, they provide rather compelling evidence for the presence in these human tumors of RNA similar to that found in an agent known to be related to a virus that causes sarcomas in an experimental animal.

In many ways the most noteworthy features of the experiments described emerge when they are compared with our studies of human breast cancer (*2*)

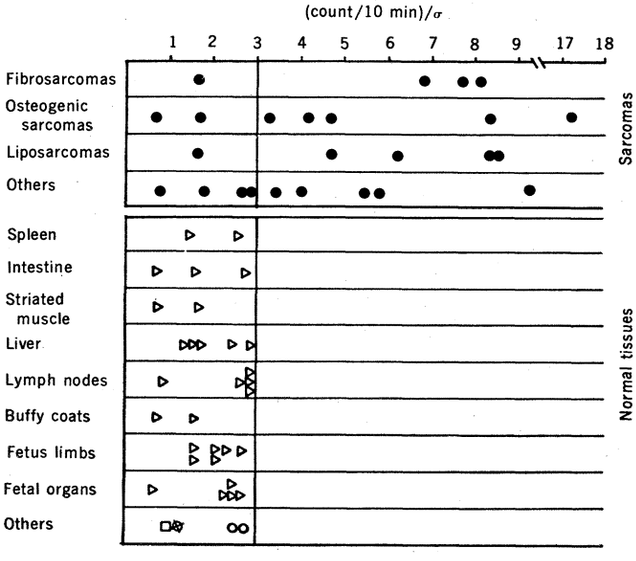

Fig. 3. Results of hybridization reactions with RLV [^{3}H]DNA and pRNA's from human sarcomas and normal human cells. The pRNA's were isolated from sarcoma specimens (fibrosarcomas, osteogenic sarcomas, liposarcomas, leiomyosarcomas, neurofibrosarcomas, and rhabdomyosarcomas), normal adult tissues (spleen, intestine, striated muscle, liver, lymph nodes, and white blood cells), fetal tissues (limbs, liver, and lungs), placenta (⊗), NC37 cells (□), and phytohemagglutinin-stimulated lymphocytes (○). The amount of [^{3}H]DNA (count/10 min) corrected for background banding in the RNA density region (between densities 1.62 and 1.68 g/ml) was determined for each reaction. An operational mean and standard deviation (σ) were determined of each counter by the total number of counts per 10 minutes of three fraction tubes (numbers 2, 3, 4) in the consistently negative region of each of 50 gradients. The number of counts per 10 minutes corrected for background banding in the RNA region of the gradient was then divided by the appropriate operational standard deviation. Any reaction with count/10 min in the RNA region less than 3σ is considered negative; thus we are 99.9 percent confident of the existence of the pRNA-DNA hybridization peak in those reactions retained as positive (greater than 3σ).

Table 2. Results of hybridization of pRNA isolated from human sarcomas with MMTV [^{3}H]DNA and AMV[^{3}H]DNA. No positive reactions occurred. Method and data analysis are the same as in Table 1.

Product DNA	Diagnosis	RNA (count/ 10 min)	(Count/ 10 min) per σ
MMTV	Liposarcoma (1458)	0	0
MMTV	Osteogenic sarcoma (23–2)	20	0.38
AMV	Osteogenic sarcoma (23–5)	75	1.42
AMV	Fibrosarcoma (1448)	146	2.76
AMV	Liposarcoma (1441)	85	1.61
AMV	Liposarcoma (1458)	144	2.73

and human leukemias (*4*). RNA from human breast carcinoma is unrelated to the RNA of the RLV but is homologous to the RNA of the virus that causes breast tumors in mice (*2*). On the other hand, RNA from human leukemic cells is unrelated to that of the breast tumor-inducing virus, but instead shows a unique homology to the virus that causes leukemia in mice. Our studies demonstrate that human sarcomas contain RNA's that show the same sort of relatedness as do the RNA's from human leukemias; their RNA is homologous to an animal leukemogenic agent but not to that of an animal mammary tumor virus. Each of these investigations lends support to the specificity and meaningfulness of the positive responses observed in the others. Finally, the specificity pattern observed with the RNA's from human neoplasias and the viral DNA's mirrors precisely what has been observed with the analogous diseases in the animal models. Whatever these results may ultimately mean for a viral etiology of human cancer they do suggest a remarkable similarity in the specific virus-related information found in corresponding tumors of mice and men.

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8 December 1971

Temperature Regulation in the Bumblebee Bombus vagans: A Field Study

Abstract. Bombus vagans *forages for nectar at 5°C in shade and at 31°C in sunshine. The production of heat while the bumblebee is on flowers, at ambient temperatures below 24°C, helps to maintain a thoracic temperature that is near the minimum for flight between flowers. However, at ambient temperatures above 24°C the thoracic temperature is no longer regulated at 32° to 33°C and rises.*

Bumblebees occur in boreal and arctic regions where diurnal temperatures fluctuate and overcast skies may prevail for the entire day. They rely for food nearly exclusively on nectar and pollen throughout their life cycle and use a portion of their caloric intake to regulate nest temperature (*1*). Food must be constantly available during brood rearing. The fact that bumblebees are able to forage at low ambient temperatures (T_A), at which no other bees are able to fly (*2*), should, in part, compensate for the small reserves of food in the nest [see (*3*)].

Like some other insects, *Bombus vagans* F. Smith has a relatively high thoracic temperature (T_{Th}) before initiating flight. Warm-up before flight in bumblebees (*4*), as in honeybees (*5*), involves rapid contractions (shivering) of the flight muscles. A layer of hair covering the body reduces the rate of heat loss by nearly one half (*6*) and thus reduces the energetic cost of maintaining a large difference between T_{Th} and T_A. However, to my knowledge, regulation of thoracic temperature has not previously been demonstrated in bumblebees.

Bumblebees land on each flower they visit and could either cease heat production and cool down or continue expending energy and maintain a high T_{Th} during these intervals. I report here that the workers of *B. vagans* (mean body weight, 120 mg) regulate their thoracic temperature when foraging in the field from *Epilobium angustifolium* L. (fireweed). The thoracic temperature is maintained at about 32° to 33°C by "warm-up" after the bees land on the flowers.

All measurements were made in the field near Farmington, Maine, during July and August, 1970 and 1971. The bees were grasped from the flowers between gloved thumb and forefinger, and a thermistor (*7*) was then quickly inserted from the ventral side to the approximate center of the thorax and into the abdomen from the posterior. Temperatures were read to the nearest 0.2°C from a telethermometer (Yellow Springs) within 4 seconds after the bee was grasped. Nectar volumes were measured in 2-μl calibrated capillary tubes, and sugar concentrations were determined with a pocket refractometer (Bellingham and Stanley). Ambient temperatures were measured to the nearest degree Celsius in the shade near the flowers.

Few *B. vagans* were foraging at 5°C (the coldest observed dawn), but many were active in shade at 15°C and in noon sunshine at 31°C. The mean T_{Th} of bees in shade at a T_A of 9° to 24°C was 32° to 33°C (Fig. 1). This T_{Th}, being slightly lower than that of bumblebees in continuous flight at these T_A's (*8*), is about 3° to 4°C above the minimum T_{Th} and at least 9° to 10°C below the maximum T_{Th} at which flight is possible (*9*). The conspicuous independence of T_{Th} from T_A implies that T_{Th} is "regulated." However, at a T_A of 26° to 31°C T_{Th} increased nearly directly with T_A, an indication of a lack of regulation of T_{Th} during foraging at this upper range of T_A where measurements were made. Stabilization of T_{Th} at high T_A, which would indicate temperature regulation through heat loss [see (*10*)] or reduction of heat production [see (*11*)], was not observed as T_{Th} rose from 33°C (Fig. 1) and approached 38°C (in shade) and 40°C (in sunshine).

The difference between T_{Th} and T_A,