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Virus-Like Particles in Human Leukemic Plasma.* (28856)

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Among the problems complicating study of the possible relation between viruses and human neoplasia are the lack of adequate techniques for detecting and isolating small numbers of virus particles from human tumors(1,2), and the inability to carry out transmission experiments in man comparable to those performed in animals(3). Even if a tumor is viral in origin, the level of virus in the tissues or in blood may be expected to be variable(4). Experimentally, injection of cell-free extracts from primary animal tumors generally results in a low incidence of disease transmission even in newborn, inbred animals. High yields of infectious particles that may infect other strains or species are often obtained only after repeated passage(5,6). In other instances, transplantable tumors, clearly produced originally by viral infection, have been shown to contain no(7) or at most a few infectious particles. A number of reports describing virus-like particles in human tumor material have been made(8-13; E. Frei, J. B. Moloney, A. J. Dalton, pers. comm.)

It appeared of interest to approach the problem of detecting oncogenic viruses in two steps, first to develop methods for isolating very small numbers of particles having the physical properties of viruses from human plasma or tissues, and second, to determine the possible infectivity of such particles in a variety of cell types grown in tissue culture and in several adult and newborn animals using more than one route of injection.

In this report 2 types of particles from human leukemic plasma are described, one of which resembles a previously described murine leukemia virus in morphology(18, density 1.18, T. E. O'Connor, R. F. Zeigel, and F. J. Rauscher, personal communication) but which differs from the murine virus in density. While the possibility that the particles are (a) viral and (b) causal remains to be examined, the correlation of distinct morphological entities with a pathological state is considered significant.

Methods. Whole-heparinized blood was collected and stored at 5°C until used. Cells and platelets were removed by centrifugation for 1 hour at 2700 rpm in a swinging-bucket rotor (International PR-2 centrifuge with head No. 855, 5281 $\times$ *g* at R_{min}, 11,239 $\times$ *g*

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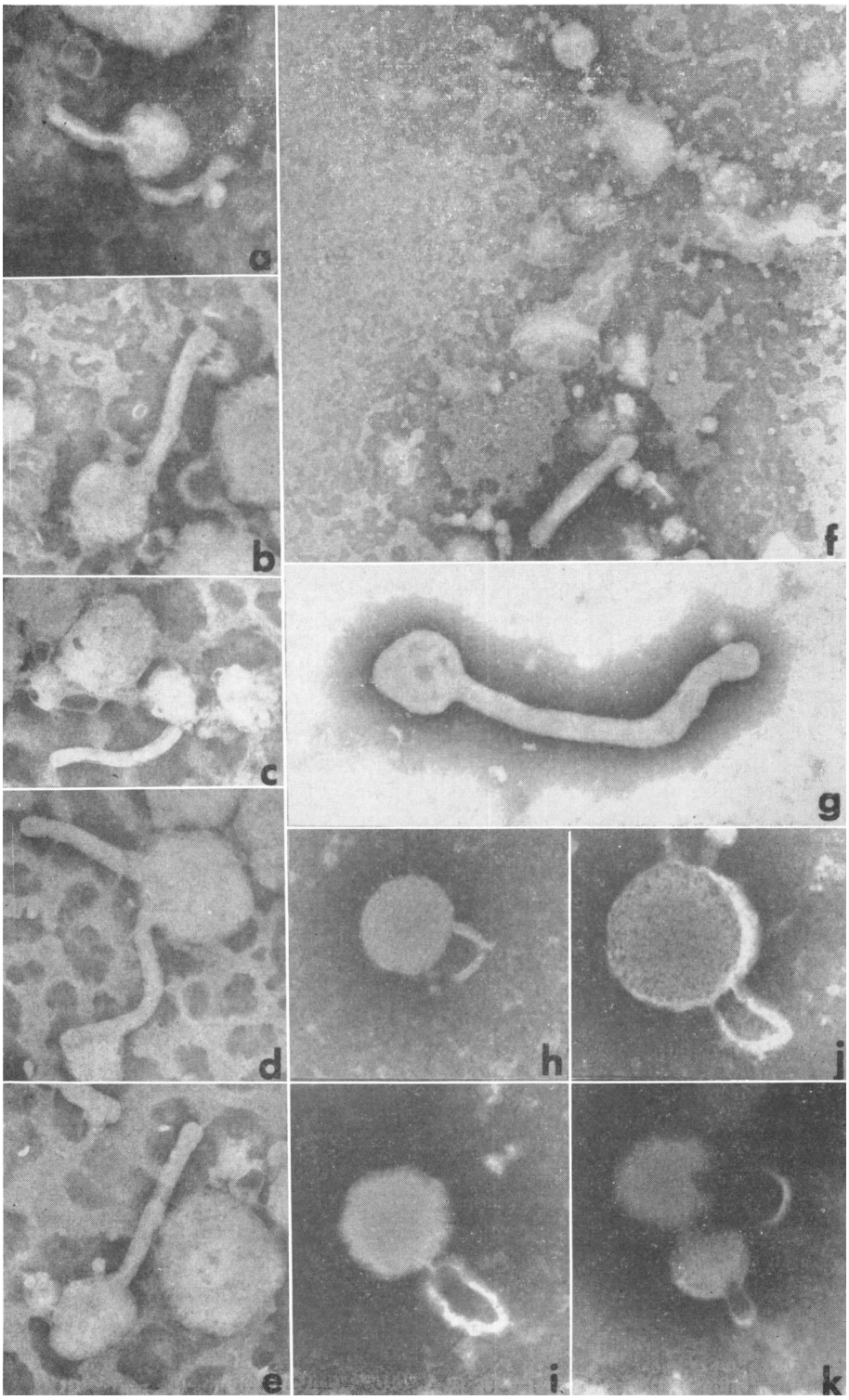

FIG. 1. Virus-like particles observed in human leukemic plasma (a-e, g-k) and particulate material from normal plasma (f). Unfixed preparations negative-stained, 1% phosphotungstic

at R_{max}). The supernatant was carefully removed, placed in Spinco No. 40 rotor centrifuge tubes, and spun for 1 hour at 40,000 rpm (144,880 $\times$ g at R_{max}). If a sample did not completely fill the tube, the additional volume was made up with 0.15 M sodium citrate (pH 6.8). The supernatant was withdrawn and the pellet resuspended in 10 ml of sodium citrate buffer. This suspension was used for initial electron microscopy.

To separate the particles in question from contaminating material, equilibrium centrifugation was used. A number of gradients were used in orienting experiments to determine the proper densities. For the majority of the studies gradients were made by layering consecutively 11 ml each of CsCl (pH 6.7) having densities of 1.3054, 1.2424, and 1.1854 in that order in Spinco No. 30 rotor tubes. A 3 ml portion of the resuspended pellet was layered on top of the gradient, and the tubes spun at 25,000 rpm for 6-8 hours in the No. 30 rotor. The mathematical basis for this method of equilibrium centrifugation and the automated system for recovering the gradient and continuously recording refractive index and absorbance using a 0.2 cm light path flow cell are described elsewhere(15). The system used here has a wavelength-shifting device to record at 260 and 280 mμ. The refractometer was calibrated with CsCl solutions of known refractive index. Tables listing physical density as a function of refractive index have been compiled by a computer using the equation of Ifft, Voet, and Vinograd(16).

The fractions recovered from the CsCl equilibrium centrifugation were dialyzed against 0.15 M sodium citrate for at least 1 hour. The samples were removed, and the dialysis sacs washed once with buffer. The sample and buffer wash were combined and centrifuged 1 hour in the Spinco No. 40 rotor. The supernatant was carefully decanted and the pellet resuspended in the one or 2 drops of buffer remaining in the tube. An aliquot of this unfixed sample was placed directly onto a grid covered with a carbon film and negatively stained with 1% phosphotungstate at pH 7.0(17).

Results. Two types of virus-like particles were observed. One of these resembled the Rauscher virus(18,19) and was not observed in material which had not been banded in CsCl or when part of the extensive background had not been removed with brief ether treatment (Fig. 1 a-e). Particles of similar morphology were recovered without ether extraction at a density level of 1.25 after banding in a CsCl gradient (Fig. 1 g). The head diameter was 200–300 mμ and the tail length varied widely. Double-tailed particles frequently were seen. Twenty-two particles were found in 54 electron micrographic examinations of samples from 8 patients. For convenience, these have been designated "R" type particles. None was observed in 55 examinations of similarly prepared samples from 18 controls (Fig. 1 f).

The second type of particle observed, called the "Q" type, was hexagonal in outline. It has an envelope surrounding an electron-dense center and a short narrow tail (Fig 1 h-k). Head diameter averaged 140 to 180 mμ, and the intact tail width was 20 mμ. The particle did not resemble varicella (20) or herpes virus(21) sometimes found in the blood of leukemic patients. "Q"-type particles were observed 50 times in 54 examinations of leukemic blood from 8 patients, and 5 times in 55 examinations of blood from 18 control subjects. All particles seen in control preparations were from the same person. It has been suggested that the "Q" type particles could be specific granules from platelets. The data in Table I describe the nonleukemic individuals tested. The occurrence of "R" and "Q" particles in relation to diagnosed leukemia is shown in Table II. Of the patients studied, all but one had received radiation or chemotherapy. Few particles of

acid, pH 7.0. a-g, $\times$ 40,000; h-k, $\times$ 80,000. a-e. "R" type particles from second pellet (40,000 rpm), following ether extraction. Leukemic No. 7. f. Particulate material from normal plasma. No virus-like particles found having density of those observed in leukemics. Nonleukemic No. 1. g. "R" particle after banding isopycnically in CsCl, density 1.25. Leukemic No. 5. h-k. "Q" type particles from second pellet (40,000 rpm). Leukemic No. 7.

either type were observed in the untreated patient.

Evidence for a third, morphologically different, particle has been obtained at a density of 1.25 from the plasma of a patient with lymphosarcoma. These results will be reported elsewhere. No "R" or "Q" particles were observed in this individual.

TABLE I. Non-Leukemic Blood Samples Examined.

Non-leukemic No.*	Sex	Age	Known therapy—comments
1	♂	42	
2	♂	43	
3	♀	49	
4	♂	37	5 possible "Q" particles
5	♂	44	
6	♀	40	
7	♂	22	
8	♂	22	
9	♂	45	
10	♂	38	
11	♂	30	
12	♂	54	
13	♂	52	
14	♂	24	
15	♂	46	
16	♂	34	Polycythemia vera; out-patient
17	♀	38	Hodgkin's disease; x-irradiation; in-patient
18	♀	42	Breast tumor; subsequently removed; irradiation therapy; out-patient, previously in
19	♀	61	Lymphosarcoma; x-irradiation therapy; out-, previously in-patient. Third type of particle observed. See text.

Summary: Avg age = 40.2, 14 ♂, 5 ♀.

* Samples No. 1-15 were supplied by ORNL Medical Division; 16-19 by ORINS Medical Division.

Optical density and refractive index recordings from a leukemic sample and a control are shown in Fig. 2. The position of virus-like particles in the gradient is indicated on the chart. The peak shown in the normal chart did not contain virus-like particles when examined by electron microscopy. Three of the 5 suspect particles were found in a banded control at a density of 1.25. Although the recordings obtained are different for each kind of leukemia examined, further studies are required owing to the small number of samples. The patients were not in close contact with each other, nor with the one positive control, suggesting that the particles are not indigenous to the hospital area.

Discussion. In this study we were concerned with the correlation between incidence of leukemia and the presence of particles having the morphology and density of virus particles. Two distinct types of particles, designated R and Q, have been demonstrated. All leukemic plasma from patients examined in this study was found to contain one or both of these entities. No data on infectivity of these particles are available. No evidence for a causal relation therefore exists, and the particles observed, if viral, may be passengers which find leukemic cells or individuals suitable hosts. It should be noted,

TABLE II. Leukemic Blood Samples Examined.*

Leukemic No.	Sex	Age	Status	Diagnosis	Therapy	Particles observed
1	♀	58	Out	CL	0	†
2	♀	42	Out, in	"	X Ch	R
3	♂	63	In	"	X	†
4	♀	51	"	AG	X	Q
5‡	♂	54	Out, in	CL	X Ch	R
6	♀	15 D	In	AL	Ch	R Q
7	♂	36	"	CG	X Ch	R Q
8	♀	77	Out, in	SG	Ch	Q
9	♀	61	" "	CG	X Ch	R
10	♂	66	In	"	0	R

Summary: Avg age = 52.3, 4 ♂, 6 ♀

* Abbreviations: A = acute; C = chronic; Ch = chemotherapy; D = deceased; G = granulocytic; In = in patient; L = lymphocytic; 0 = no treatment; Out = out patient only; Out, in = out patient, previously in; S = subacute; X = X-irradiated.

† Samples 1 and 3 were neither ether treated nor banded in CsCl. Re-runs on new samples are not complete.

‡ At time the sample was drawn, patient had chicken pox and shingles.

however, that negative bioassays are not conclusive, since many viruses are species-, strain-, and cell-specific, and definitive experiments completely parallel to those done in animals connot be done in humans.

While considerable variation in the R type particles has been seen, no particles intermdiate in morphology between R and Q types have been observed, suggesting that the particles are indeed distinct entities. The variability of these particles parallels that observed in Rauscher virus studies.

Out of the 3 patients with chronic granulocytic leukemia examined, 2 who had received radiation and chemotherapy had a much higher incidence of particles than the third. The third sample, from a patient who had received no treatment, had only a few particles. The possibility that radiation and chemotherapy in man may cause the release of virus particles by a mechanism analogous to that observed in lysogenic phage exposed to ionizing radiation(22,23) or chemical mutagens(24) deserves consideration. Alternately or concomitantly the reduction in immunological response following radiation treatment may also play a role in the increased number of particles.

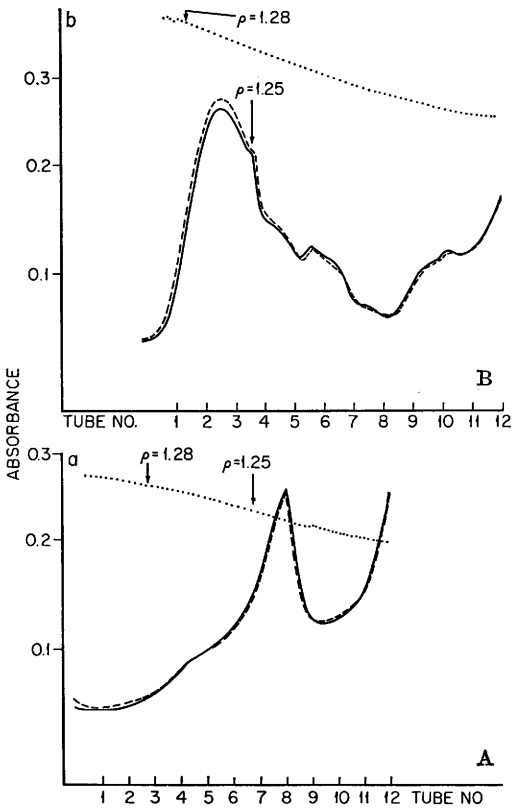

FIG. 2. Recording of absorbance at 260 (dashed line) and 280 mμ (solid line) and refractive index in CsCl gradients (dotted line) used for particle separation. A, (See text for details) non-leukemic No. 4; B, chronic lymphocytic leukemia, leukemic No. 5; virus-like particles observed at a density of 1.25. Note absence of peak at this density in control sample.

The particulate contamination observed in the second pellet (40,000 rpm) with leukemic plasma was morphologically distinct from that observed in the controls. The contaminating particles, which were destroyed by brief ether treatment, may be bits of cytoplasm shed by leukemic cells or, especially in the controls, may be large lipoprotein aggregates.

Virus-like forms were observed only after the extraneous particles were removed by either ether treatment or banding in CsCl. Unless the high level of contamination is reduced, there is very little chance of observing virus-like particles.

The particles described were isolated from 10 ml of blood samples using fairly simple techniques that involve both pelleting and isopycnic banding. Since pelleting is known to inactivate many viruses, methods for isolating particles having a narrow range of sedimentation coefficients(25) and densities (15) from large volumes of blood or from tissues without pelleting have been developed. The application of these systems to the problem of scanning tumors and plasma for viruses will be described elsewhere.

Summary. Differential and zonal ultracentrifugation followed by brief ether exposure or by isopycnic banding in CsCl, has yielded virus-like particles from the plasma of 8 leukemic patients. In electron microscopic examinations, two morphologically different particles were observed, designated "R" and "Q." In 18 controls, no "R" particles were found, but in one control "Q"-like particles were observed. The density of the particles is the same in all instances.

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Nephrotoxic Serum Nephritis in Thymectomized Rats.* (28857)

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Injection of serum from rabbits immunized with homogenates of rat kidney (NTS) promptly induces a nephritis in the rat. Apparent fixation of rabbit γ-globulin as well as rat complement within glomerular capillary walls may be demonstrated by immunofluoresence. Following the acute injury, progression to a chronic nephritis not unlike that observed in humans may be noted. Development of the latter has been presumed to result from either a reaction between host's antibodies formed in lieu of the heterologous antibody fixed in the kidney(1) or damaged renal tissue induced by the initial injury(2). In an attempt to gain some insight into the pathogenesis of the chronic nephritis resulting from injections of NTS, particularly that attributed to the role of host antibody production in this process, it was considered worthwhile to observe the clinical, biochemical, morphologic and immunohistochemical features of NTS nephritis in rats thymectomized at birth. This procedure has been observed to suppress the development of autoallergic encephalomyelitis and tuberculin sensitivity, delay the rejection of skin homografts and inhibit the production of antibodies of the precipitating and hemagglutinating type(3).

Materials and methods. One ml of NTS was intravenously injected into 26 two-month-old male and female Long-Evans rats which had been thymectomized at birth and weighed 150-180 g. Twenty intact rats of similar strain, age and weight received a comparable dose of the same NTS. Eight controls received 1 ml of normal rabbit serum (NRS).

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