Unusual Particles in Human Plasma From Leukemia and Lymphosarcoma [1]

W. W. HARRIS, N. G. ANDERSON, T. W. BARTLETT, ELIZABETH L. RUTENBERG, L. L. McCAULEY, *and* R. M. KNISELEY, *Biology Division, Oak Ridge National Laboratory,[2] Biophysical Separations Laboratory, Oak Ridge Gaseous Diffusion Plant,[2] and Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee*

SUMMARY

Plasma of 2 patients out of 255 with either leukemia or lymphosarcoma yielded large numbers of 10 and 25 mμ particles in phosphotungstate negatively stained preparations. For 1 of the 2, repeated sampling over a period of months showed continuing high particle incidence until death. The particles were unlike any found in a survey of over 300 human plasma samples.—Nat Cancer Inst Monogr 21: 389–394, 1966.

VIRUS-LIKE PARTICLES in human leukemic plasma, bone marrow, and lymph nodes have been reported by numerous investigators (*1–9*). After initial observations on particles in human plasma (*6*) in this laboratory, a survey of over 300 human plasma samples was undertaken. The total sample for statistical analyses was 302, of which there were 141 leukemias, 114 lymphomas, and 47 miscellaneous controls including hospital patients and normals. There were 55 chronic lymphocytic leukemias and 24 lymphocytic lymphosarcomas within the total sample. Eleven different physicians and institutions participated. Results of the large survey will be reported elsewhere.

However, during the survey, plasma of 2 patients, now deceased, showed unique particles in high concentrations which are discussed here. Diagnosing physicians reported the first, patient A, to be chronic lymphocytic leukemia, and the second, B, to be lymphocytic lymphosarcoma.

MATERIALS AND METHODS

Patient A was under treatment in a Nashville, Tennessee, hospital, and B was a patient at the Oak Ridge Institute for Nuclear Studies, Medical Division. Sample collection was under the supervision of Dr. R. M.

[1] This research performed under the Joint National Institutes of Health-Atomic Energy Commission Zonal Centrifuge Development Program which is supported by the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, and the U.S. Atomic Energy Commission.

[2] Operated for the U.S. Atomic Energy Commission by the Nuclear Division of Union Carbide Corporation.

794–527—66——45

Kniseley of the latter hospital. He supplied the following data pertaining to the two cases discussed here.

Patient A: F N2-T6212163
Diagnosis: F820 AMA SNDO[3]
Age: 44
Date onset: 3/60
Date sample: 2/64
Status: Outpatient
Local radiotherapy over 1 year before
No total body radiotherapy
Steroid therapy completed within 1 year of sample
Alkylating agents—on treatment at time of sample
No cytotoxic agents
No known virus infection

Patient B: F 111242
Diagnosis: 830[3]
Age: 41
Date onset: 1/61
Date sample: 3/64, 8/64
Status: Inpatient
No surgery or total body radiation
Radiotherapy, local over 1 year prior
Steroid treatment—on treatment at time of samples
Alkylating agents—completed over 1 year before
Antimetabolites—none
Cytotoxic agents—none
Virus infection—unknown or unclassified organism

Sample Preparations

In patient A, plasma prepared by the originating institution from whole heparinized blood was forwarded packed in ice; plasma of patient B was prepared in this laboratory from whole heparinized blood. The clarification procedure was that of Burger *et al.* (*6*).

Plasma volume of each was 4 to 6 ml, prepared as follows: The plasma was placed into a Spinco No. 40 rotor tube and the tube filled with sodium citrate 0.15 M *p*H 6.7. The sample was centrifuged for 1 hour at 38,000 rpm in the Model L centrifuge. The brake was not used. The pellet was resuspended in 2 ml 0.15 M *p*H 6.7 sodium citrate buffer, transferred to a No. 30 rotor tube, brought to 27 ml with 0.15 M sodium citrate, and the tube was capped. With a Pasteur pipette, 10 ml of $\rho = 1.66$ cesium chloride solution was added to the bottom of the tube and centrifuged for 2 hours at 24,000 rpm. The tube was photographed and the band corresponding to $\rho = 1.24$ was withdrawn with a probe admitted through the tube cap and pumped through a gradient analyzer which plotted the refractive index of the solution. The band at $\rho = 1.24$ was transferred to a No. 40 rotor, made to volume with 0.05 M *p*H 7.0 sodium citrate, and centrifuged 1 hour at 38,000 rpm. The pellet was used for electron microscopy.

[3] Standard Nomenclature of Diseases and Operations, American Medical Association, Fifth Edition.

Negative Staining

The pellet was resuspended in a drop of 0.15 M sodium citrate. An aliquot of this sample was placed directly onto a Formvar-carbon coated specimen grid and stained with 1 percent phosphotungstate (PTA), *p*H 7.0.

RESULTS

Particulate spherical objects were observed in negatively stained electron micrographs of plasma of the 2 patients after differential centrifugation and banding. In each sample particle size distribution was bimodal, one of 10 mμ diameter and the other 25 mμ, as shown by arrows in figure 1. The larger particles are shown at higher magnification in figure 2. No regular internal structure was observed at higher magnifications in either PTA negatively stained or uranyl acetate positively stained preparations. Rodlike structures, figure 2, were also observed in the two cases.

DISCUSSION

Had these particles been observed only once in each of these 2 of the 302 patients examined, some preparative artifact would have been suspected, as indeed it was until samples from patient B taken over a period of several months repeatedly showed the same pattern.

Interaction of heparin and citrate was ruled out indirectly by the fact that plasma of patient B was processed within an hour after withdrawal, while that of patient A was in transit for several hours, and also by the fact that over 200 other plasma samples which did not show these particles were in transit for varying times, even up to 1 day or so. A direct test in which whole heparinized blood was allowed to stand overnight did not produce the particles.

The biochemical nature of the particles is not known. The fact of recovery in a density gradient of 1.24 eliminates heavy particles such as ferritin (*10*) or glycogen (*11–13*), nor do the particles have the proper morphology for either of these compounds. The largest of these ether-insensitive particles (25 mμ) is smaller by almost a factor of 4 than the smallest mycoplasma elementary bodies reported by Hummeler *et al.* (*14*) for mycoplasma isolated from human leukemia.

Whether the particles described here are related to cell debris, virus, or mycoplasma is incidental to the fact that physical particles of unusual homogeneity of size and high incidence were observed in plasma of 2 patients of nearly the same age at onset of disease, the same sex, and who had received treatment for a leukemoid condition. These results suggest a possible source of particles for physical and biological study.

REFERENCES

(*1*) DMOCHOWSKI, L., and GRAY, C. E.: Electron microscopy of tumors of known and suspected viral etiology. Texas Rep Biol Med 15: 704–753, 1957.

(*2*) ———: Studies on submicroscopic structure of leukemias of known or suspected viral etiology. Blood 13: 1017–1042, 1958.

(*3*) BEARD, J. W.: Virus tumors in cancer research. *In* Stern Symposium. New York, Feb. 24–25, 1958.

(*4*) BRAUNSTEINER, H., FELLINGER, K., and PAKESCH, F.: On the occurrence of virus-like bodies in human leukemia. Blood 15: 476–479, 1960.

(*5*) ALMEIDA, J. D., HASSELBACK, R. C., and HAM, A. W.: Virus-like particles in blood of two acute leukemia patients. Science 142: 1487–1489, 1963.

(*6*) BURGER, C. L., HARRIS, W. W., ANDERSON, N. G., BARTLETT, T. W., and KNISELEY, R. M.: Virus-like particles in human leukemic plasma. Proc Soc Exp Biol Med 115: 151–156, 1964.

(*7*) PORTER, G. H. III, DALTON, A. J., MOLONEY, J. B., and MITCHELL, E. Z.: Association of electron-dense particles with acute human leukemia. J Nat Cancer Inst 33: 547–556, 1964.

(*8*) SMITH, K. O., BENYESH-MELNICK, M., and FERNBACH, D. J.: Studies on human leukemia. II. Structure and quantitation of myxovirus-like particles associated with human leukemia. J Nat Cancer Inst 33: 557–570, 1964.

(*9*) BENYESH-MELNICK, M., SMITH, K. O., and FERNBACH, D. J.: Studies on human leukemia. III. Electron microscopic findings in children with acute leukemia and in children with infectious mononucleosis. J Nat Cancer Inst 33: 571–579, 1964.

(*10*) FARRANT, J. L.: An electron microscopic study of ferritin. Biochim Biophys Acta 13: 569–576, 1954.

(*11*) DROCHMANS, P.: Morphologie du glycogen. Etude au microscope électronique de colorations négatives du glycogene particulaire. J Ultrastruct Res 6: 141–163, 1962.

(*12*) BARBER, A. A., HARRIS, W. W., and ANDERSON, N. G.: Isolation of native glycogen by combined rate-zonal and isopycnic centrifugation. Nat Cancer Inst Monogr 21: 285–302, 1966.

(*13*) BARBER, A. A., HARRIS, W. W., and PADILLA, G. M.: Studies of native glycogen isolated from synchronized *Tetrahymena pyriformis* (HSM). J Cell Biol. 27: 281–292, 1965.

(*14*) HUMMELER, K., TOMASSINI, N., and HAYFLICH, L.: Ultrastructure of a mycoplasma (Negroni) isolated from human leukemia. J Bact 90: 517–523, 1965.

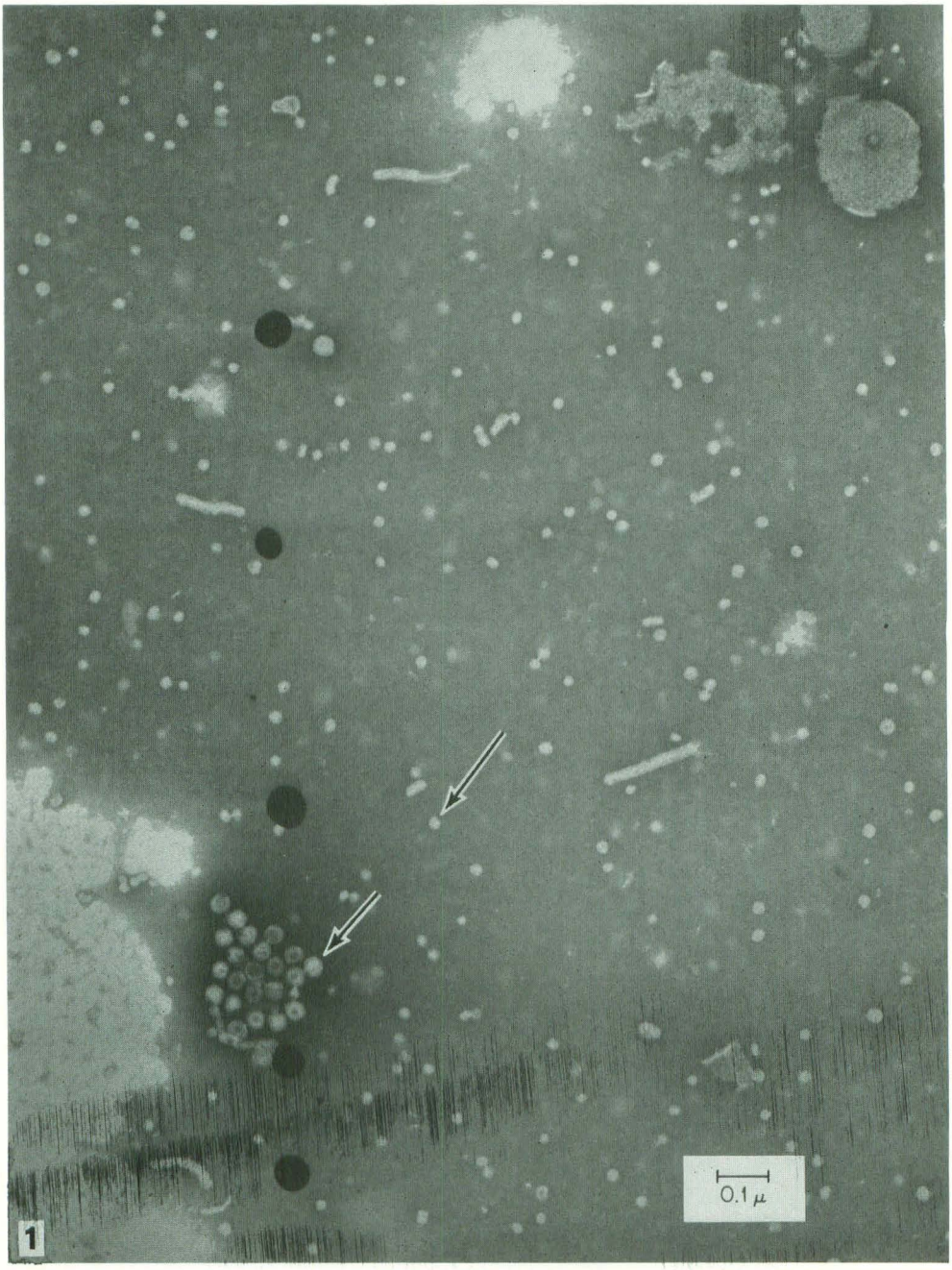

FIGURE 1.—Typical field showing 10 mμ and 25 mμ particles found in plasma of both patients A and B. Phosphotungstate negative stain. × 80,000

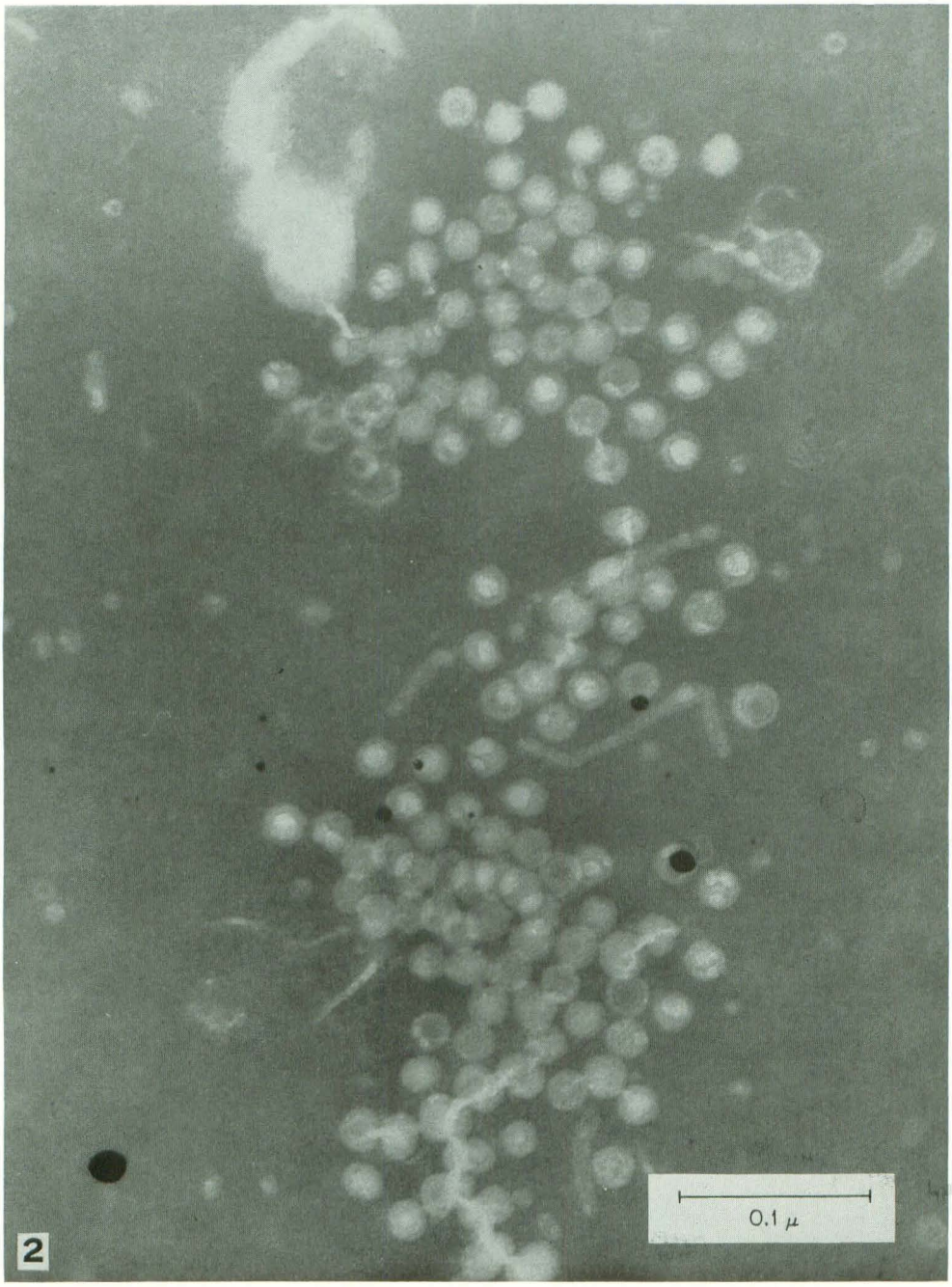

FIGURE 2.—25 mμ particles from plasma of patient with lymphocytic lymphosarcoma. Phosphotungstate negative stain. × 300,000