Studies on the Murine L-4946 Ascitic Leukemia in Swiss Albino Mice[1]

HUMPHREY[2] has presented evidence that the murine L-4946 neoplastic state may be horizontally transmitted by cell-free preparations. Though this tumor arose spontaneously in AKr mice, evidence from several laboratories[3,4] supports the contention that it is distinct from the SCHOOLMAN-SCHWARTZ murine system[5]. Pathologically, this entity is characterized by an ascitic fluid containing large numbers of mononuclear cells and virtually no involvement of spleen, liver or thymus. Utilizing techniques of differential centrifugation, it has been possible to isolate a fraction at $9,000 \times g$ which is responsible for the induction of ascites and a second component containing virus-like particles which sediments at $100,000 \times g$ and is biologically inactive. Some physical characteristics of these 2 fractions, as well as their interrelationship to the production of lymphosarcoma, will be discussed.

Materials and methods. The infectivity of various preparations was adjudged by the appearance of ascitic fluid and of anemia in the experimental animal. The virulence of cell-free preparations was defined as the time by which 50% of the animals had expired.

Ascitic fluid was differentially centrifuged. Pellets obtained at $1,500 \times g$; $9,000 \times g$; $15,000 \times g$; $30,000 \times g$ and $130,000 \times g$ were assayed for biological infectivity. The $130,000 \times g$ supernatant was also assayed. The fractions obtained by the above procedures were assayed for RNA and DNA content[2]. Sensitivity to storage at low temperatures (4°C → −30°C) and sensitivity to ether extraction.

Electron microscopy was carried out on specimens using an Hitachi-Perkin-Elmer electron microscope[6,7].

Results. Microscopic analysis of ascitic fluid revealed mononuclear cells, irregular in size and shape, containing vacuoles and having globular excrescences at the surface of the cell membrane suggestive of budding or pinocytic vesicles.

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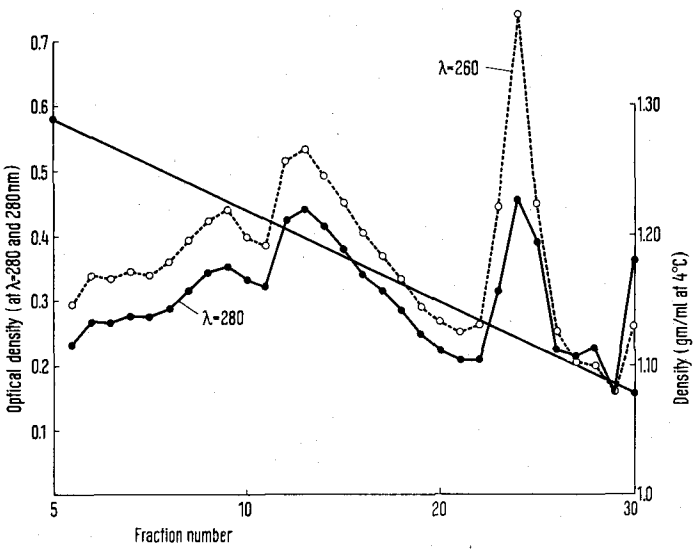


Fig. 1. Sucrose gradient ultracentrifugation of $130,000 \times g$ pellet. Resuspended $130,000 \times g$ pellet applied on a 20–60% sucrose gradient centrifuged at $105,000 \times g$ for 12 h at 4°C in 3 → place swinging angle IEC preparative ultracentrifuge. Each fraction (plotted on the abscissa) represents 25 drops; ordinate represents optical density (O.D.) at 260 and 280 nm and sucrose density.

Virulence of L-4946 ascitic fluid preparations

	No. of leukemic mice	No. infected (%)	Day of 50% mortality	DNA[a] (μg/ml)	RNA[a] (μg/ml)
Whole Ascitic					
(1) Whole Ascitic Fluid	38/40	95	20	1.93	1.33
(2) Pellet No. 2 ($9,000 \times g$)	24/30	80	27	0.002	0.178
(3) Pellet No. 3 ($15,000 \times g$)	6/20	30	–	0.0	0.0
(4) Pellet No. 4 ($30,000 \times g$)	1/25	4	–	0.0	0.0
(5) Pellet No. 5 ($130,000 \times g$)	1/55	1.8	–	0.0	1.05
(6) Final supernatant	0/15	0	–	0.0	0

Results of differential centrifugation of whole ascitic fluid. Data expressed as 50% mortality and as number of animals that developed leukemia. [a]Concentration in mg/ml; each pellet suspended to original volume. Mean value of 3 determinations with a variation of less than 15%.

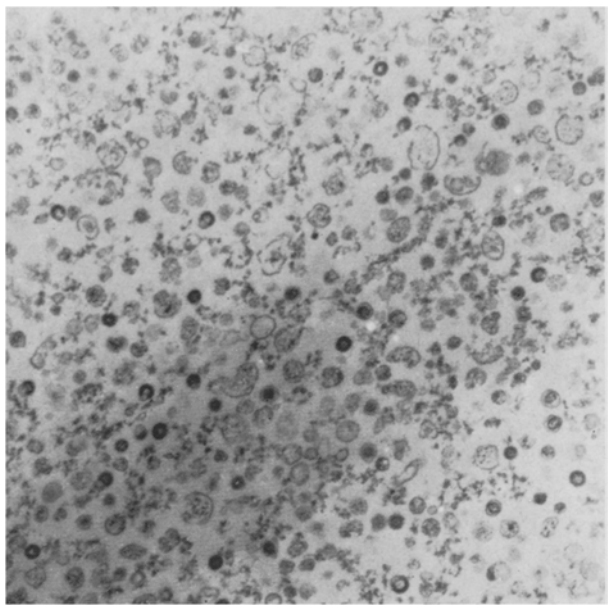
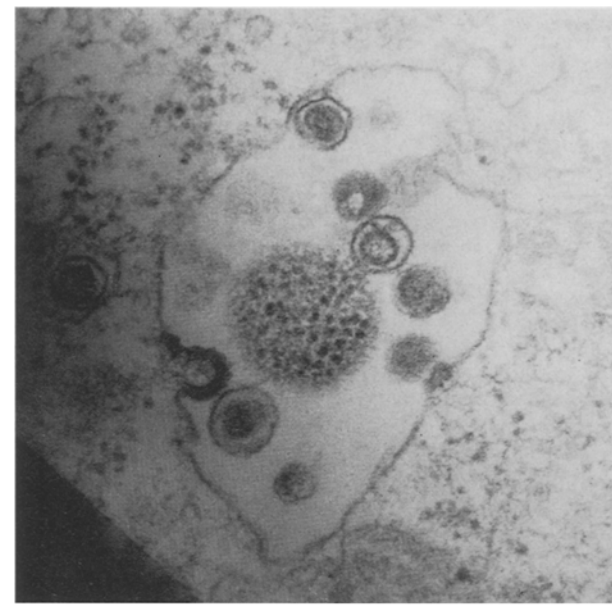


Fig. 2. Electron-microscopic morphology of 130,000 × *g* pellet.



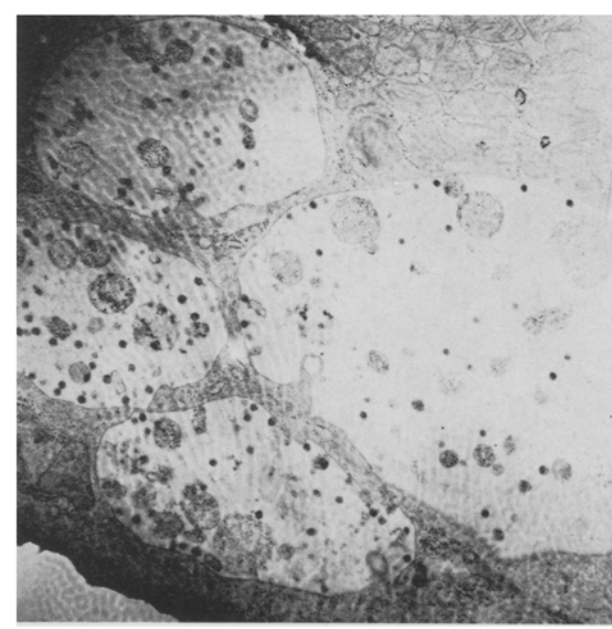


Fig. 3A and B. Electron-microscopic morphology of intracellular particles.

Ascitic fluid devoid of cells prepared by differential centrifugation had infective capacity limited to the resuspended 9,000 × *g* pellet, as is shown in the Table. No tumorogenic activity was noted in other fractions. The neoplastic activity in the 9,000 × *g* pellet is not due to the presence of ascites cells (Table). The Table indicates that tumorogenic activity correlates with the presence of RNA in a particulate fraction that sediments at 9,000 × *g*. This fraction contains particles approximately the size of platelets. Extraction with ether or storage at 4°C for longer than 24 h results in loss of oncogenic activity.

The 130,000 × *g* pellet (pellet No. 5) was applied to 20–60% sucrose gradient. The absorption profile at 260 and 280 nm is shown in Figure 1. The calculated density of the peak containing virus particles is 1.123 (Figure 1), and contains only RNA (Table). This fraction has not resulted in the development of ascites for as long as 6 months after inoculation.

Electron microscopic examination of the 130,000 × *g* pellet revealed hexagonal particles which occasionally were noted to have tails (Figure 2). This was demonstrable by both negative staining and epon-fixation methods. Examination of epon-fixed whole cells verifies the intracellular presence of such particles (Figures 3A and B). These particles arise by budding off from vacuolar membranes or from larger particles localized within vacuoles.

The larger packets in vacuolar lumens measure approximately 2,000–3,000 Å, their size varying from cell to cell or within different regions of a given cell. The particles seen on electron microscopy of the 130,000 × *g* pellet vary in diameter from 600–1,000 Å (Figures 3A and B).

Animals infected with the 130,000 × *g* pellet that did not develop ascitic leukemia were subsequently immune to infection with whole (cellular) ascitic fluid.

Discussion. Earlier attempts[2,3] at the isolation of an infectious virus or particle which would induce the L-4946 murine leukemia were unsuccessful.

The discrepancy between these results and those of HUMPHREY regarding the infectivity of the 130,000 × *g* pellet is not readily explainable at present. The possibility of inactivation occuring during the preparation of our 130,000 × *g* pellet is acknowledged. It is also possible that this preparation either contains 'incomplete particles' or that a 'helper virus' or substrance is destroyed during preparation. None of these possibilities may be distinguished on the basis of the present data. The data presented here and that of HUMPHREY indicate that this disease is transmissible by oncogenic subcellular component.

Zusammenfassung. Virologische Untersuchungen einer Mäuseleukämie, die sich durch eine «subzelluläre onkogene Komponente» übertragen lässt.

O. M. RENNERT

Departments of Pediatrics and Biochemistry, Institutional Division of Genetics, Endocrinology and Metabolism, University of Florida College of Medicine, Gainesville (Florida 32601, USA), 5 July 1971.