that they were not undernourished as the animals studied by CARLINI and MASUR[3,4]. Therefore, we believe that the strong stress suffered by the rats undergoing morphine withdrawal is the factor associated with the THC capacity to induce aggressiveness in these animals.

Résumé. Les rats soumis à la morphine ont été traités pendant la phase d'abstinence avec des injections i.p. d'amphétamine et (−) Δ^9-*trans*-tétrahydrocannabinol. Ces deux drogues provoquèrent une conduite agressive chez ces animaux. La durée du traitement préalable avec la morphine et le temps d'abstinence précédant l'injection des drogues furent des facteurs importants dans l'apparition de l'agressivité. Le Δ^9-THC fut à cet effet plus puissant que l'amphétamine.

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The L-4946 Leukemia in the CF[1] Mouse

The viral nature of some murine leukemias has been well established[1,2]. Yet the viral passage of some mouse leukemias has not been accomplished. This paper will present data to suggest that another murine leukemia (L-4946) may be passed by subcellular material. An extract containing RNA and protein which will induce an abdominal lymphosarcoma in the adult CF1 mouse has been isolated from L-4946 ascitic fluid.

Material. The ascitic form of this leukemia[3] in the CF1 mouse was maintained by intraperitoneal injection of 0.5 ml of leukemia ascitic fluid, each mouse receiving approximately 10^6 cells. Leukemogenic activity was defined as death from leukemia within 40 days of inoculation in 2-month-old adult female CF1 mice, mortality as the ratio of the number of mice who died of leukemia within 40 days to the total number of mice inoculated. Time of 50% mortality is defined as the day by which half of all injected mice in a specific group had died.

Methods and Results. A $12{,}000 \times g$ supernatant of the ascites fluid was found to be leukemogenic. The ascitic fluid of leukemic mice was centrigufed twice at $12{,}000 \times g$ for 10 min and the second supernatant used for experiments or further purification.

Seven strains of mice were inoculated with either ascitic cell or the $12{,}000 \times g$ supernatant. Inoculation of 1×10^5 ascitic cells caused death in all 7 strains of mice. Four strains (CF1, AKr, C3H and A) were susceptible to the $12{,}000 \times g$ supernatant. The CBA/2 and C5F Black did not die or accumulate ascitic fluid during the period of experimental observation (60 days).

Five successive 40-min centrifugations were performed on the ascitic fluid and the resulting supernatants; each pellet was obtained after increasing the centrifugal force until the last pellet and the final supernatant were obtained at $125{,}000 \times g$. Leukemogenic activity was found in all pellets and the final supernatant. The apparent heterogeneity of the leukemogenic agent, implied by the presence of activity in all fractions, could be explained by adsorption of the agent to various constituents of the ascites fluid, entrapment of activity in the sediments, or presence of an inhibitor.

The leukemogenic activity of the $12{,}000 \times g$ supernatant was precipitated by either ethanol or ammonium sulfate. Both ethanol and ammonium sulfate precipitated the leukemogenic activity in an insoluble form which was assumed to contain fibrin or fibrinogen. Plasmin was found to remove large quantities of an inert protein material from the precipitate; however, the final leukemogenic precipitate remained insoluble in saline.

Four enzymes (trypsin, DNase, RNase and lysozyme) were used to study the chemical properties of the $12.000 \times g$ supernatant and subcellular extract. The leukemogenic activity of 1.0 ml of the $12{,}000 \times g$ supernatant was not decreased after incubation with 0.1 mg of trypsin, DNase, RNase or lysozyme at 37°C for 1 h (Table I). However, when the plasmin-insoluble precipitate was incubated under these same conditions, the leukemogenic agent was sensitive to trypsin, and possibly to RNase. The resistance of the $12.000 \times g$ supernatant activity to trypsin may have been due to substrate competition between the oncogenic agent and the high concentration of plasma proteins in the supernatant.

Chemical characterization. The plasmin-insoluble precipitate was the most highly purified preparation of the leukemogenic agent. Table II presents data comparing this preparation to the $12{,}000 \times g$ supernatant from which it was isolated. Protein determination of the $12{,}000 \times g$ supernatant indicated that each mouse received 3.2 mg of protein. The composition of the plasmin-insoluble precipitate was 94% protein and 6% nucleic acid. This nucleic acid was RNA.

The saline supernatants obtained after washing L-4946 ascitic cells with saline in preparation for cell dilution experiments were found to be leukemogenic. The origin of this leukemogenic activity was thought to be the elution of adsorbed subcellular material from cell surfaces and/or the rupture of fragile cells.

The leukemogenic activity of the saline supernatant was found to be similar to that of the $12{,}000 \times g$ supernatant. Centrifugation of the saline supernatant at $125{,}000 \times g$ for 1 h yielded a leukemogenic supernatant.

The saline supernatant was also subjected to ethanol precipitation by the same technique that was described for the $12{,}000 \times g$ supernatant, except that the ethanol concentration was increased from 7 to 15% in order to obtain precipitatable material. The first precipitate at 15% ethanol contained 2 components – one saline-soluble, the other saline-insoluble. Both precipitated components were leukemogenic, killing all recipient mice.

Discussion. As previously reported[4], the leukemogenic activity of the $12{,}000 \times g$ supernatant is inactivated by

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Table I. A comparison of the stability of the 12,000 × *g* supernatant and plasmin-insoluble percipitate to 4 enzymes

Enzyme	A) 12,000 × *g* supernatant		B) Plasmin-insoluble precipitate		C) Plasmin-insoluble precipitate	
	Mortality	Day of 50% mortality	Mortality	Day of 50% mortality	Mortality	Day of 50% mortality
Incubation control (no enzyme)	7/10	22	9/10	20	10/10	15
DNase	8/10	19	9/10	18	9/10	20
RNase	7/10	20	3/10	—	10/10	20
Trypsin	10/10	19	2/10	—	2/10	—
Lysozyme	10/10	20	9/10	17	9/10	23

A 12,000 × *g* supernatant (A) and 2 separate preparations of plasmin-insoluble precipitate (B and C) were incubated with 4 enzymes. One ml aliquots were incubated at 37 °C with 0.1 mg of enzyme. Incubation time was 60 min for A and B and 75 min for C. Each incubation aliquot was equally divided among 10 mice.

freezing, incubation at 60°C for 3 min, and filtration through bacterial retaining filters. These characteristics cast some doubt on the non-cellular nature of 12,000 × *g* supernatant. Since FRUTH has demonstrated that one leukemic cell can probably transmit leukemia in the mouse, the criteria for any non-cellular preparation must be rigorus.

Although the 12,000 × *g* supernatant did not satisfy non-cellular criteria, the non-cellular transmission of the L-4946 leukemia in the CF1 mouse is suggested by stability of the subcellular extract in distilled water and the presence of protein and RNA but no DNA in the subcellular extract.

There is a significant difference between the centrifugation studies reported in this article and those reported by RENNERT[5]. In the CF1 mouse, leukemogenic activity could still be detected in a 125,000 × *g* supernatant as well as in the 30,000 × *g*, 60,000 × *g*, and 90,000 × *g* pellets. In the Swiss albino mouse, RENNERT found no leukemogenic activity in a 133,000 × *g* supernatant and little leukomegenic activity in the 30,000 × *g* and 133,000 × *g* pellets.

The observed difference could be explained by a difference in the minimal number of viral particles required to cause infectivity in the CF1 and Swiss albino mice. Centrifugal force of greater than 100,000 × *g* would certainly sediment the vast majority of viruses of the size RENNERT has described. Even though the percent viri remaining in these high-speed supernatants would be very small, the number of viri in a supernatant could still be large enough to infect a susceptible mouse strain, but not another, less susceptible strain. Extracted RNA from L-4946 ascites cells, the 12,000 × *g* supernatant or the plasma-insoluble precipitate were never leukemogenic in the CF1 mouse[3, 6–8].

Zusammenfassung. Mittels Ultrazentrifugation von infektiöser Ascitesflüssigkeit bei 125.000 × *g* und einem gereinigten zellfreien Extrakt konnte ein RNS-Virus als infektiöses Agens und Ursache der Leukämie und eines Lymphosarkoms nachgewiesen werden. Einzelne Mäusestämme erwiesen sich als resistent.

G. B. HUMPHREY[9, 10]

Table II. The protein and nucleic acid content of the 12,000 × *g* supernatant and plasmin-insoluble precipitate

Preparation	Mortality	Day of 50% mortality	Quantity per mouse	
			Protein (μg)	RNA (μg)
12,000 × *g* supernatant	8/10	22	3,200	–
Plasmin-insoluble precipitate	8/10	22	40	2.5

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The Decrease in the Concentration of Organic Material in the Course of Formation of the Enamel Matrix

Enamel maturation consists in the transformation of the previously secreted young enamel, rich in organic material and water and poor in calcium salts, into the highly calcified mature enamel. Briefly, there is a removal of organic material and water and addition of new calcium salts which crystalize in apatites. The loss of organic material has been shown by radioautographic[1–6] and biochemical studies[7–10]. Once the young matrix is