COOH-terminal tyrosine was observed in all three murine γA proteins studied, including one in which monomer and polymer forms were isolated and analyzed separately where both had a COOH-terminal tyrosine. Six of seven human α-chains had a COOH-terminal tyrosine. As mentioned above, one preparation completely lacked tyrosine, for unknown reasons, although it is possible that this represents a real heterogeneity within the γA class and is a manifestation of an α-chain subclass (*7*).

Table 4 summarizes the data and shows, for comparative purposes, the COOH-terminal sequence of γG heavy chains and κ- and λ-chains. The two most striking findings are (i) the very close relationship between the α- and μ-chains, compared with that of the γ-chain and (ii) when the COOH-terminal pentapeptide of the μ-chain is compared to κ- and λ-chains and a deletion is inserted for the sake of showing maximum homology, it is seen that the μ-chain pentapeptide more closely resembles that of the λ-chain than that of the κ-chain. These homologies are consistent with the hypothesis previously mentioned, that both heavy and light chains evolved from a common ancestral gene.

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1. Fc fragment refers to the crystallizable fragment obtained after papain digestion of γ-globulin. All the nomenclature used is that which was recommended as a result of a conference on human immunoglobulins sponsored by the World Health Organization [*Bull. World Health Organ.* **30**, 447 (1964)].
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Leukemogenic Activity of Ether-Extracted Rauscher Leukemia Virus

Abstract. *After rapid multiple extractions of mouse plasma virus with ether, the aqueous solution contained viral nucleoids that were infectious when inoculated intracranially into newborn BALB/c mice. The infectivity associated with the ether extract was not neutralized by the specific antibody prepared against the whole virus. No intact virus has been seen in these preparations. Treatment with ether completely removed the virus envelope from the particle and produced an apparently homogeneous preparation of viral nucleoids. After the extractions with ether, leukemogenic activity was inactivated by exposure to ribonuclease. The leukemogenic activity of the many-passaged Rauscher virus that has been propagated in tissue culture and that has low infectivity was also retained, and, in two experiments in which material was inoculated intracranially into mice, this activity appeared to have been enhanced by multiple extractions with ether.*

This study was undertaken as an attempt to extract biologically active ribonucleic acid (RNA) from Rauscher leukemia virus. RNA was extracted by various modifications of the phenol procedure of Gierer and Schramm (*1*), as well as by the ether-treatment technique of Notkins (*2*). The RNA prepared by either hot or cold phenol techniques was noninfectious, since all newborn and weanling BALB/c mice inoculated (subcutaneously, intramuscularly, intravenously, intraperitoneally, and intracranially) with each such preparation failed to develop the Rauscher leukemia syndrome during observation periods ranging from 9 to 14 months. However, newborn BALB/c mice developed the typical disease after intracranial inoculation with the aqueous phase of ether-treated Rauscher virus (*3*). This report presents some of the biological properties, after treatment of intact virus with ether, of the "infectious nucleoids" of mouse plasma virus and of virus cultivated and apparently attenuated in vitro (*4*). Morphological features of the preparations are also discussed.

Plasma was collected from viremic BALB/c mice, and the virus was concentrated tenfold by differential centrifugation similar to the method described by Moloney (*5*). The virus pellet was resuspended in 0.01*M* phosphate-buffered saline (PBS) containing $10^{-4}M$ ethylenediaminetetraacetic acid (EDTA), at *p*H 7.2, and 2 percent fetal calf serum, and was stored at −120°F (−84°C).

The procedure for ether extraction, similar to that described by Notkins (*2*), was as follows. Equal amounts of concentrated plasma virus and freshly chilled ethyl ether were mixed for 2 minutes in a Vortex mixer. The phases were separated by centrifugation at 2000 rev/min for 5 minutes in a refrigerated centrifuge. The interphase layer, probably containing insoluble lipoproteins, was then removed and discarded. This procedure was repeated, usually five or six times, until the interphase between the aqueous and ether layers cleared. Fresh ether was added after each extraction. Residual ether was removed by bubbling nitrogen slowly through the chilled aqueous layer.

Approximately 80 percent of the original volume was recovered after multiple extractions with ether and ex-

Table 1. Bioassay of nontreated and ether-extracted Rauscher plasma virus after intracranial inoculation into newborn BALB/c mice, each mouse receiving 0.02 ml of inoculum. PBS, phosphate-buffered saline.

Inoculum (mouse plasma virus)	Palpation		Death		Average wt. of spleen (mg)
	P/T*	Mean time (days)	D/T*	Mean time (days)	
Intact virus + PBS (1:1)	10/10	28	3/10	34	1170
Intact virus + ribonuclease (1:1)†	10/10	28	2/10	37	965
Ether-extracted virus (undiluted)	15/18	33	4/18	29	1281
Ether-extracted virus + PBS (1:1)	8/14	43	4/14	42	530
Ether-extracted virus + ribonuclease (1:1)†	0/10		0/10		96

* Number with palpable spleens or number dead over total number inoculated. † Final concentration of ribonuclease was 100 μg/ml.

posures to nitrogen. The undiluted aqueous phase was considered equivalent to the tenfold concentration of virus with which we started. Newborn BALB/c mice were each inoculated intracranially (IC) with 0.02 ml of the undiluted solution from the aqueous phase. Control mice were also inoculated IC with either intact or ether-treated virus that had been incubated with equal amounts of either PBS or ribonuclease (final concentration, 100 μg/ml) in a water bath at 37°C for 30 minutes.

Preparations of intact virus, incubated with either PBS or ribonuclease, produced the disease in 100 percent of the inoculated animals (Table 1). Significant difference in the mean times to splenomegaly, as determined by spleen palpation, or death were not obtained in either case. Leukemogenic activity of the mouse plasma virus was retained after rapid multiple extractions with ether but was completely inactivated when subsequently exposed to ribonuclease. After treating this virus with ether, 83 percent of newborn BALB/c mice that were inoculated IC with the undiluted aqueous-phase solution developed the Rauscher leukemia syndrome within 30 to 40 days.

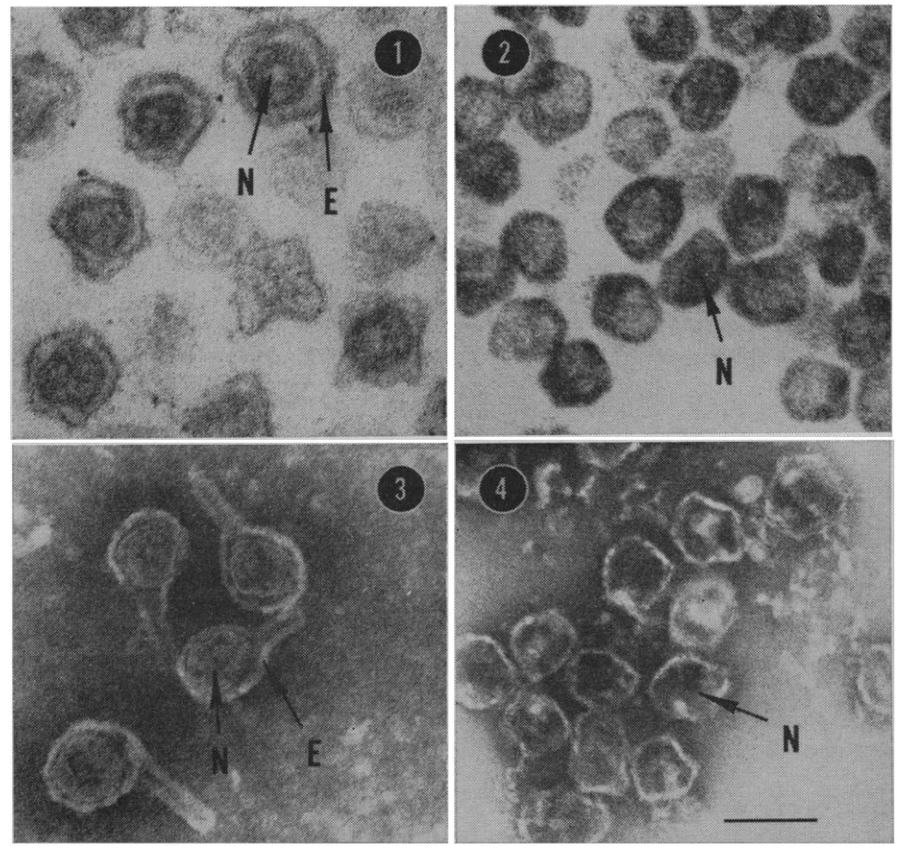
Although some leukemogenic activity of the Rauscher mouse plasma virus was retained after extractions with cold ether, the infectivity titer dropped considerably. Intact mouse plasma virus had an infectivity titer of $> 10^{4.5}$ per 0.02 ml when titrated by IC inoculation into newborn BALB/c mice. However, the infectivity titer of the ether-extracted virus dropped to $< 10^{1.0}$ per 0.02 ml when assayed in newborn BALB/c mice by IC inoculation, a loss of over 99 percent of the virus activity. Newborn mice, inoculated IC with a 1:10 dilution of this extracted material, did not develop palpable spleens during an observation period of 6 months.

In order to determine whether the infectivity of the ether extract was due to residual intact virus or to the virus nucleoids, virus neutralization tests were conducted as follows. Equal volumes of Rauscher plasma virus (concentrated fivefold) and the undiluted aqueous phase after ether treatment were each mixed with either normal rabbit serum or equal volumes of rabbit immune serum prepared against intact Rauscher virus and were incubated at 37°C for 1½ hours. Newborn BALB/c mice were inoculated IC with 0.02 ml of each preparation. The leukemogenic activity of the nucleoids was not neutralized by specific antiserum that completely neutralized the intact virus (Table 2).

The effects of ether on the infectivity properties of the low-infectivity Rauscher virus propagated by tissue culture (JLS-V5) were also examined. The leukemogenic activity of the JLS-V5 virus has gradually decreased with prolonged subpassage (*4, 6, 7*). Since weanling BALB/c mice are not sensitive to intraperitoneal (IP) inoculation of many-passaged (> 150 transfers) JLS-V5 virus, concentrated 50-fold, infectivity assays were developed by using the IC route of inoculation into newborn BALB/c mice.

After the JLS-V5 virus had been extracted many times with cold ether, 0.02 ml of the undiluted aqueous phase was also infectious when inoculated IC into newborn BALB/c mice (Table 3). In some instances, infectivity of the JLS-V5 virus was apparently enhanced after treatment with ether. In one experiment, the mean time to palpation in mice inoculated with ether-treated virus was reduced to 30 days as compared with 134 days for intact virus (Exp. 2, Table 3). The enhancement of infectivity after treatment with ether was observed in another experiment (Exp. 3, Table 3), although, in this instance, the mean time to palpation was reduced by only 22 days. Since only one animal died in the intact groups the mean time to death could not be determined. In other experiments not reported in Table 3, some concentrated JLS-V5 preparations were not infectious in newborn mice. With these preparations, treatment with ether had no effect on enhancement or activation of viral infectivity.

For electron microscopy, the ether-treated aqueous phase of Rauscher



Figs. 1–4. Fig. 1. Thin section of intact Rauscher virus particles extracted from mouse plasma and fixed in glutaraldehyde and osmium. *N*, nucleoid; *E*, envelope. Fig. 2. Ether-extracted Rauscher virus. Thin section of pellet as in Fig. 1. Only nucleoids *N* are present; the envelope *E* has been removed. Fig. 3. Negative stain (PTA and OsO_4, see text) of intact Rauscher virus particles (same as for Fig. 1). The virion consists of a nucleoid *N* surrounded by an envelope *E*. Fig. 4. Ether-extracted Rauscher virus; PTA negative stain. Only nucleoids *N* can be seen; no trace of envelope *E* remains. See text for interpretation of density variations within nucleoids. The line marker represents 0.1 μ. Overall magnification for the four figures is × 130,000.

Table 2. Virus neutralization test employing both intact and ether-treated virus with immune serum prepared against whole virus. All mice inoculated intracranially, each with 0.02 ml of each virus-serum mixture. NRS, normal rabbit serum; RIS, Rauscher immune serum.

Inoculum	No. palpable/ No. inoculated*	No. dead/ No. inoculated*	Y_{50}†	
			Palpation	Death
Intact virus + NRS	10/10	10/10	25	41
Intact virus + RIS	0/10	0/10	0	0
Ether-extracted virus + NRS	9/10	9/10	46	60
Ether-extracted virus + RIS	7/10	6/10	63	59

* Number with palpable spleens or number dead over total number inoculated. †The average time in which 50 percent of the animals responded.

Table 3. Effects of treatment with ether on Rauscher virus cultivated in tissue culture (JLS-V5). All newborn BALB/c mice were inoculated intracranially with 0.02 ml of each inoculum.

Exp. No.	Inoculum (JLS-V5 virus)	Palpation		Death		Average wt. of spleen (mg)
		P/T*	Mean time (days)	D/T*	Mean time (days)	
1	Intact virus	10/12	32	10/12	51	>2000
	Ether-extracted virus	4/13	67	3/13	81	568
	Ether-extracted virus + ribonuclease	0/12		0/12		146
2	Intact virus	3/7	134	1/7		650
	Ether-extracted virus	12/12	30	10/12	36	>2000
3	Intact virus	5/15	92	1/15		400
	Ether-extracted virus	8/10	70	3/10	84	657

* Number palpable or number dead over total number inoculated.

mouse plasma virus was centrifuged in the SW 39 rotor for 2 hours at 38,000 rev/min in a Spinco model L centrifuge. The pellet was resuspended in cold PBS and spun an additional 5 hours at 38,000 rev/min in the SW 39 rotor. Both thin sections and negative stains were prepared for electron microscopy. The pellets were fixed in 2 percent glutaraldehyde in 0.01*M* phosphate-buffered saline, fixed again for 1 hour in 1 percent Dalton's chrome-osmium, dehydrated in ethanol (the 50 percent bath contained 2 percent uranyl acetate), embedded in epoxy resin, and sectioned with a diamond knife on an LKB ultrotome; the sections were stained with lead citrate (*8*). For negative staining the pellets were resuspended by vigorous pipetting in 2 percent aqueous phosphotungstic acid (PTA) that had been adjusted to *p*H 4.5 with 5*N* KOH. The film side of grids coated with Formvar and carbon was applied to the mixture of virus and PTA, and the meniscus formed on the grid upon withdrawal was drained onto filter paper. In some experiments, 1 percent aqueous OsO_4 was added to PTA in a ratio of 1:3 before mixing with virus. This rendered the envelope of intact virus permeable to PTA and resulted in better staining of nucleoids of the intact particles. Intact Rauscher plasma virus particles are illustrated in Fig. 1 (thin section) and Fig. 3 (negative staining). Nucleoids (*N*) of varying nonuniform density are surrounded by an envelope (*E*) which forms a tail-like protrusion that is shown well in negative stains. Ether-extracted particles are illustrated in Fig. 2 (thin section) and Fig. 4 (negative stain). The envelope is totally absent and only the nucleoids remain. In sections (Fig. 2), they have approximately the same varying nonuniform density as the nucleoids of intact virions (Fig. 1). Under these conditions no "unit-membrane" can be recognized on their surfaces. In negative stains (Fig. 4), a white peripheral line that is not as apparent in intact virions (Fig. 3) delineates each nucleoid. The significance of the density variations seemingly within the nucleoids is not clear, since they could be produced by particulate material either under or overlaying the nucleoids. However, since the nucleoid density also varies in sections, at least part of the variations seen in negative stains may be due to a true nucleoid substructure.

These nucleoids morphologically resemble those described by de Thé and O'Connor (*9*) and by O'Connor *et al.* (*10*), after treating Rauscher leukemia virus with a mixture of Tween 80 (polyoxyethylene sorbitan monooleate) and ether. Treatment of some myxoviruses, such as parainfluenza 2, with tween-ether results in a hemagglutinin (HA) titer that is higher than the HA titer of the virus prior to treatment with ether (*11*). The hemagglutinin, however, is noninfectious. Norrby (*12*) has described the disintegration of measles virus by the tween-ether treatment with accompanying release of subviral components, which show only complement-fixing and HA activity.

In these experiments, the primary structure of the intact Rauscher leukemia virion was altered by disassociating the outer membrane from the nucleoid by treatment with ether. Free nucleoids, released when the membrane ruptures, retained less than 1 percent of the infectivity shown by the intact virus from plasma. Once the viral envelope is removed, the nucleoprotein core is sensitive to degradation by ribonuclease.

Variation of the leukemogenic properties between the virus from plasma and the one propagated in tissue culture and their derived nucleoids can be attributed to the relative differences of the infectious properties of the respective virus population. The leukemogenicity of Rauscher mouse plasma virus is considerably greater than that derived from tissue culture. These differences in infectivity are even more pronounced when the infectious properties of the plasma virus and the JLS-V5 virus, which has low infectivity, are compared. The apparent enhancement of leukemogenic activity of nucleoids derived from JLS-V5 is evident only when it is compared with the activity of the low-infectious parent (tissue-culture propagated) virus. This enhancement may be more apparent than real since the original leukemogenicity of the tissue culture virus may merely be "unmasked" by exposure to ether; it has not been observed in plasma-derived nucleoids since the plasma virus is highly leukemogenic. Thus, the increase or decrease of leukemogenic activity associated with the nucleoids is relative to the infectivity of the parent virus.

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RNA and DNA Synthesis in Developing Eggs of the Milkweed Bug, Oncopeltus fasciatus (Dallas)

Abstract. *Ribosomal RNA synthesis in developing eggs of the milkweed bug,* Oncopeltus fasciatus *(Dallas), is turned on at gastrulation and is essentially turned off when organogenesis begins about 72 hours later. The pattern for DNA synthesis is similar although less pronounced.*

The general patterns of RNA and DNA synthesis during early development appear to be similar in amphibians, sea urchins, and fish (*1*). It is of interest to examine other organisms, in which embryological development is well documented, and which might be expected to yield useful information in this area. In earlier papers (*2*) we described changes in the concentrations of two groups of compounds, pteridines and nucleosides, which occur in the egg of the milkweed bug, *Oncopeltus fasciatus*. One group, at least, the nucleosides, might be expected to be involved in nucleic acid synthesis. In fact, we have demonstrated a correlation between nucleic acid synthesis and disappearance of the nucleosides inosine and guanosine in developing eggs. Now, in a more detailed examination of the synthesis of DNA and RNA, we have attempted to correlate their synthesis with embryological developments which are well documented in this organism (*3*). These studies reveal significant points of difference between the synthesis of nucleic acids in *O. fasciatus* and such synthesis in organisms that have been studied intensively heretofore.

Milkweed bugs were raised in plastic dish pans (about 35½ by 30½ by 15 cm) containing a supply of water and milkweed seeds (*4*). Gauze mats were placed in the containers to provide an area for egg-laying and to facilitate collection. Eggs were collected over 10-hour periods from 10 a.m. to 8 p.m. (*5*). They were removed from the gauze mats, divided into batches of 0.30 g (the quantity used in all experiments), and then allowed to develop at 21°C.

Since preliminary experiments (using dyes or radioactive glucose) showed that whole eggs were essentially impermeable, it was found necessary to remove part of the chorion before studies with isotopic tracers could be carried out. This was achieved by floating the eggs (0.3 g) on a 5.25 percent solution of sodium hypochlorite for 9 minutes (both concentration and time are crucial for successful experiments). They were then removed from the solution and thoroughly washed, in a small Buchner funnel (5 cm), with 1 liter of insect Ringer solution (0.15*M* sodium chloride, 0.004*M* potassium chloride, 0.002*M* calcium chloride). The partially dechorionated eggs were incubated at 25°C for 2 hours in 20 ml of Ringer solution without shaking, when they were again collected by filtration and placed in 10 ml of a standard incubation medium containing insect Ringer solution, 0.01*M* tris buffer, *p*H 7.5, and either 50 μc [^{3}H-5T]-uridine (23.0 c/mmole), or 50 μc[^{3}H-6T]-thymidine (14.5 c/mmole). After incubation for 2 hours at 25°C in the dark without shaking, the eggs were collected on a Buchner funnel and rapidly washed with several volumes of insect Ringer solution.

RNA was extracted from the eggs (0.3 g) by a modification of the phenol procedure of Kirby (*6*). The precipitated RNA was redissolved in 1.5 ml of acetate buffer and analyzed on a linear sucrose gradient (5 to 20 percent wt/vol in acetate buffer) with a Spinco centrifuge (SW 25 rotor for 17 hours at 25,000 rev/min and 4°C). Fractions (0.8 ml) were collected, diluted with water (0.6 ml), and analyzed for radioactivity (0.5-ml aliquot in 15 ml of Bray's solution, assayed on an Ansitron liquid scintillation counter) and optical density at 260 mμ (remainder of sample).

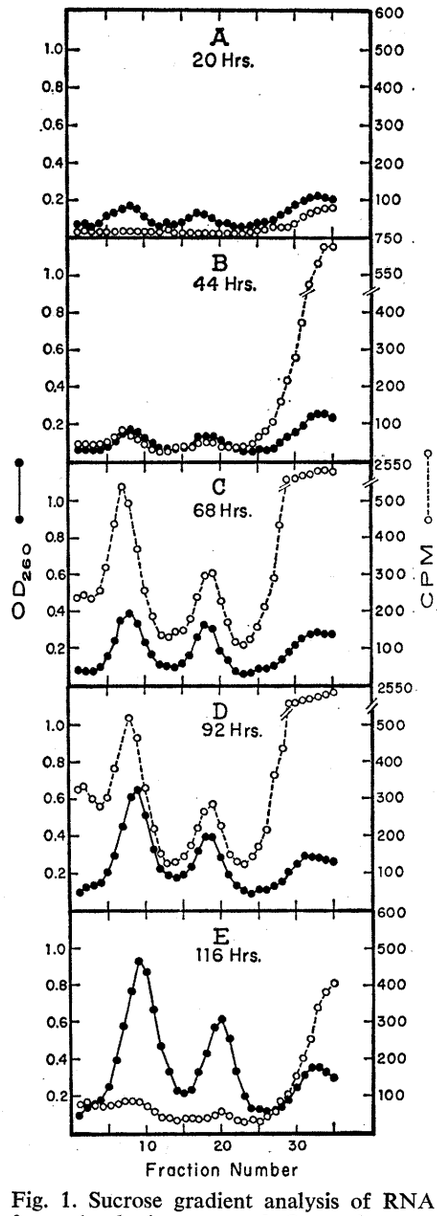

Fig. 1. Sucrose gradient analysis of RNA from developing eggs of *Oncopeltus*.

The specific activities of the two ribosomal RNA peaks were thus determined by using the equivalence, 41 μg RNA in 1 ml of fluid has an O.D.$_{260}$ of 1.0. Total tissue radioactivity was measured by using a 0.1-ml aliquot of the original homogenate, and radioactivity in ethanol-insoluble material was measured by summing all of the radioactivity measured from the sucrose gradients. Ribosomal RNA peaks were characterized by analysis on a model E analytical ultracentrifuge (*7*). They had S values of 24 and 16. The base ratios