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# A study on the isolation of leukemia virus of AKR mouse by fluorocarbon\*

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## **Abstract**

The authors have succeeded in isolating a biologically-active leukemia virus from leukemic tissues of AKR mice with a fluorocarbon. From the chemical analysis of the biologically-active virus fraction it has been clarified that the AKR leukemia virus is of the RNA type.

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## A STUDY ON THE ISOLATION OF LEUKEMIA VIRUS OF AKR MOUSE BY FLUOROCARBON

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Since Gross<sup>1</sup> first isolated leukemia virus from Ak mouse leukemia in 1951, several leukemogenic viruses have been discovered from murine leukemias<sup>2-5</sup>. Isolation of leukemia virus, however, by means of fluorocarbon finds no predecessor. We have been carrying out isologous, serial cell-free passage of AKR leukemia<sup>6</sup>. This communication deals with successful isolation of biologically-active virus by fluorocarbon from cell-free filtrate-induced leukemias in AKR mice and its biochemical properties.

### MATERIALS AND METHODS

AKR mice used in the experiment have been raised from litters sent to this University, in 1958, from Dr. A. Kirschbaum, Baylor University College of Medicine, Houston, Texas. They were fed Oriental laboratory chow and water *ad libitum*. Fluorocarbon used is trichlorotrifluoroethane (CCl<sub>2</sub>F-CClF<sub>2</sub>) manufactured by the Osaka Kinzoku, Osaka. The fluorocarbon, having a boiling point of 45.57°C and melting point of -35°C, is a colorless transparent liquid and is said to have a very low toxicity.

Mice with leukemia induced by the fourth cell-free passage served as donors. Leukemic liver, spleen, lymph nodes and thymus weighing 1 to 2 gm. were homogenized in 10 ml. of physiological saline solution or 10 ml. of McIlvaine's citrate buffer at pH 7.4. The homogenization was accomplished with a Waring blender run at 20,000 r. p. m. for 3 min. The homogenate was then centrifuged at 3,000 r. p. m. for 15 min. The supernate was removed and again centrifuged at 10,000 r. p. m. for 20 min. The resulting supernate was mixed with an equal volume of fluorocarbon and homogenized at 20,000 r. p. m. for 10 min. The homogenate was further centrifuged at 3,000 r. p. m. for 5 min., which resulted in the separation of three layers; the top thin fat layer, the middle aqueous layer containing virus and the bottom mixture layer of nonviral protein and fluorocarbon. The process was repeated one to several times until the supernatant aqueous layer became almost water-clear. The final supernate was inoculated into newborn AKR mice, less than 24 hours old, in the amount of 0.03

to 0.1 ml. either subcutaneously or intraperitoneally. The whole fractionation process was carried out at 4°C. For the measurement of ultraviolet absorption by the aqueous virus layer, a Beckman spectrophotometer, model QB-50 made by the Shimadzu Seisakusho, Kyoto was used.

## RESULTS OF EXPERIMENT

Spontaneous leukemias in 58 AKR mice were observed between 6 and 15 months of age with the peak incidence at 9 to 10 months (Fig. 1). Leukemias developing before the age of 6 months were considered induced by the inoculated

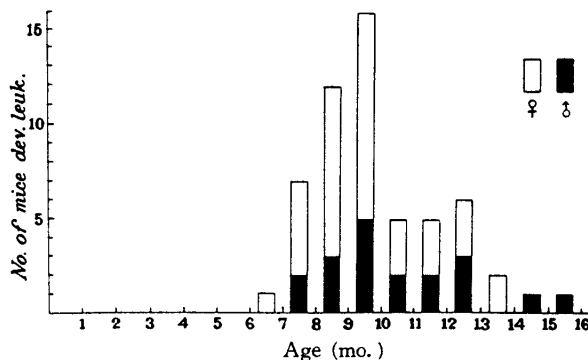


Fig. 1. Incidence of spontaneous AKR lymphatic leukemia.

virus. For each preparation of fluorocarbon extracts, different leukemic mice were used. A total of 23 newborn AKR mice comprising 4 litters were inoculated with partially fluorocarbon-purified AKR leukemia virus, and 8 (5 ♀ and 3 ♂) of them developed leukemia at 3 to 5 months of age. The susceptibility to acceleration of leukemia by virus varied among the 4 litters, and the incidence of leukemia ranged from 16.7 per cent to 66.7 per cent with an average of 34.8 per cent per litter. This litter difference might have actually represented an irregular potency of the leukemic extracts. A fluorocarbon extract of the leukemia virus was further prepared from leukemia thus induced. One of 5 AKR mice inoculated with the extract, when newborn, developed leukemia at the age of 2 months (Fig. 2 and Table 1). The incidence of leukemia

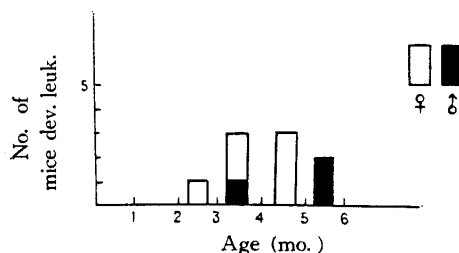


Fig. 2. Development of leukemia by the inoculation of AKR leukemia virus isolated by fluorocarbon



An AKR mouse of lymphatic leukemia induced by fluorocarbon-purified virus, showing the marked swelling of lymph nodes, spleen and liver.

Table 1. Leukemia incidence in isologous mice following inoculation of AKR leukemia virus isolated by fluorocarbon.

No. of passage	Date of birth	Date of inoculation	Leukemia incidence	Latent period (months)
5	14/II '62	14/II '62	2/4 (50 %)	3
5	18/II '62	18/II '62	2/3 (66.7%)	4~5
5	1/III '62	1/III '62	3/10 (30 %)	3~5
5	28/III '62	28/III '62	1/6 (16.7%)	4
6	21/V '62	22/V '62	1/5 (20 %)	2
Total			9/28 (32.1%)	

induced by fluorocarbon extracts was higher in female than in male mice, as in the case with filtrate-induced leukemia. All of the observed leukemias were of the lymphatic form of either thymic or extrathymic type.

Ultraviolet absorption of fluorocarbon extracts from AKR leukemic tissues was studied by a Beckman spectrophotometer. As a result, an absorption curve with the maximum absorption at  $260\text{ m}\mu$  and minimum at  $232\text{ m}\mu$  was obtained which is characteristic of nucleic acids (Fig. 3). The following optical density ratios resulted as  $O.D._{260}/O.D._{280} = 2.3$  and  $O.D._{260}/O.D._{232} = 1.2$ . A fluorocarbon extract with the maximum absorption of 1.65 was estimated for the amount of

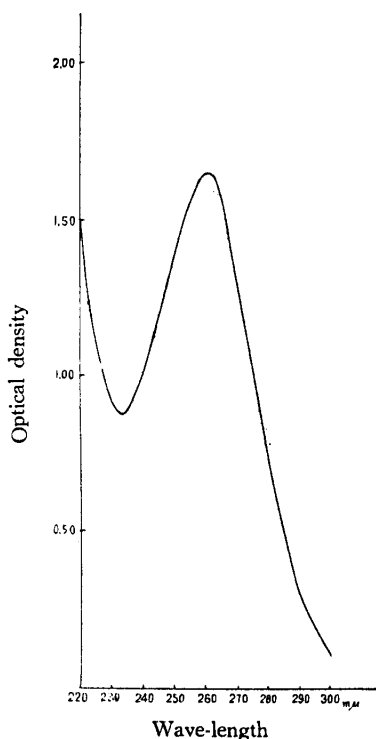


Fig. 3. Ultraviolet absorption spectrum of AKR mouse leukemia virus isolated by fluorocarbon

RNA by the orcinol color method and it was found to be 51  $\mu\text{g}/\text{ml}$ . On the other hand, DNA contained in the same extract was determined to be 0.05  $\mu\text{g}/\text{ml}$ . by the method of indole or diphenylamine reaction. It was shown that the leukemic tissue extract contained much more RNA than DNA. On the basis of this observation, it is considered that the AKR leukemia virus is composed of nucleic acid of the RNA type.

#### DISCUSSION

In 1956 GESSLER<sup>7</sup> employed fluorocarbon for the segregation of the Rous and vaccinia viruses but isolation of leukemia virus by fluorocarbon is not reported to date. The fluorocarbon technique appears to be superior to the filtration method usually employed for the preparation of cell-free leukemic extracts in that the former is less complicated, there is no loss of virus due to adsorption by filter candles, and the positive result can be higher than with filtration in certain cases.

It has been reported by BEARD<sup>8</sup> that the myeloblastosis virus contains RNA, and MOLONEY<sup>9</sup> reported that the Moloney leukemia virus contains only RNA. From our present observation that fluorocarbon extracts of AKR leukemic tissues contain a large amount of RNA in contrast to the negligible amount of DNA, we also feel that RNA is a main constituent of the nucleoid of the AKR leukemia virus. The fact that the virus is inactivated by ether<sup>10</sup> leads to an hypothesis that it also contains lipid, linking the RNA nucleoid and protein coat of the virus.

#### SUMMARY

The authors have succeeded in isolating a biologically-active leukemia virus from leukemic tissues of AKR mice with a fluorocarbon. From the chemical analysis of the biologically-active virus fraction it has been clarified that the AKR leukemia virus is of the RNA type.

#### ACKNOWLEDGEMENT

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