Tissue 1 are higher than those of Tissue 2. It is clear therefore that in some cases comparison of uptake of radioisotope by 2 whole tissues may give a misleading idea of their relative metabolic activities. As a corollary it follows that the more detailed the biochemical fractionation, the more meaningful are the data.

Summary. Some aspects of phosphorus metabolism of spleens of mice infected with Friend leukemia agent have been presented. A comparison has been made of concentrations of different phosphorus-containing cellular components as well as uptake of P^{32} into these components. Early in the infection, before significant spleen enlargement occurs, increase in uptake of P^{32} is restricted to microsomal and subparticulate lipid and nucleic acid. Later when spleen enlargement is marked, all phosphorus-containing fractions studied showed increased P^{32} uptake. X-irradiation, which caused marked reduction in spleen size, reduced P^{32} uptake into fractions of both control and infected spleens, with greater reduction in the infected ones.

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Stability Characteristics of Coe Virus.* (25575)

WILLIAM S. JORDAN, JR.
Depts. of Preventive Medicine and Medicine, School of Medicine, University of Virginia, Charlottesville

In preparation for studies of epidemiology of infections due to Coe virus, an agent isolated from the respiratory tract by Lennette and co-workers(1), it was important to investigate stability of this agent and to determine optimum conditions for handling and storing the virus. This paper presents evidence which indicates that Coe virus is relatively stable under varying conditions of pH and temperature.

Materials and methods. Tissue culture. Strain DMB cells derived from nasal mucosa were employed, using methods previously described(2). The nutrient fluid consisted of 40% pooled human serum and 60% Hank's balanced salt solution. Cells were washed 3 times with Hanks salt solution prior to use for virus growth. The maintenance fluid consisted of Scherer's MS 84.5%, chicken serum 8.0%, and tryptose phosphate broth 7.5% (3). Coe virus was supplied by Dr. E. H. Lennette after 6 passages in HeLa cells. Virus pools were prepared following 5 or 6 subsequent passages in DMB cells. *Infectivity Titrations.* Serial 1:3.2 ($10^{-0.5}$) dilutions were prepared in Hanks balanced salt solution, and 0.1 ml of each dilution was inoculated into each of 2 DMB cell culture tubes. Inoculated tubes were incubated in stationary racks at 37°C for 5 days. A culture was considered infected when 50% or more of cells had undergone specific cytopathic changes. Infectivity titer end-point expressed/0.1 ml inoculum was calculated as highest dilution of virus which infected one-half of tubes inoculated. *Alterations of pH.* Viral suspensions were adjusted to the desired pH with 0.5 N HCl or NaOH essentially as described by Ginsberg in a study of adenoviruses(4); in our study pH was measured with Beckman glass electrode meter. After adjustment to various hydrogen ion concentrations, the virus

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suspensions were allowed to stand at room temperature 30 minutes before readjustment to pH 7.3 - 7.5. The treated aliquots were then rapidly frozen and stored at −70°C until titrated simultaneously in DMB cells. Other methods of treatment and storage are described in relation to the results obtained.

Results. Localization of virus. To determine necessity for cell disruption when harvesting Coe virus infected cultures, the localization of virus at time of maximum cytopathic effect was investigated. Maximum CPE was obtained in 10 ml bottle in 48 hours. One-half of harvest was frozen and thawed 6 times, centrifuged at 2000 rpm for 10 minutes, and stored at −70°C. The supernatant fluid was removed from the other half after centrifugation of cells, and similarly stored. Cells were then washed 3 times in Hanks salt solution, and resuspended in an amount of maintenance fluid equal to volume of supernatant fluid removed. This suspension of cells was frozen and thawed 6 times, the debris centrifuged, and the fluid stored and titrated simultaneously with other aliquots. The titers were as follows: whole harvest after freezing and thawing, $10^{-6.0}$; supernatant fluid, $10^{-5.5}$; cells after freezing and thawing in new fluid, $10^{-5.5}$. Repetition of experiment gave the following results: titer of whole harvest, $10^{-6.0}$; supernatant fluid, $10^{-5.75}$; cells, $10^{-5.5}$; recombination of supernatant fluid and suspension of disrupted cells, $10^{-5.75}$. Since 25% to 50% of viral particles were still intracellular at time of maximum CPE, all subsequent harvests were subjected to freezing and thawing to disrupt the cells.

Stability after repeated freezing and thawing. After initial disruption cycle of 6 freezings and thawings, aliquots of Coe virus harvest were placed in sealed ampules and stored at −70°C. On successive days, the ampules were thawed at 37°C and refrozen in a dry ice-alcohol bath once each day, and returned to storage until completion of the tests. Repeated daily thawing of a virus suspension during 12 days of storage at −70°C resulted in no significant decrease in titer.

Stability on storage. Aliquots of virus suspensions were placed in sealed glass ampules, stored at 4°C or room temperature (22-26°C) for various periods of time, and then stored at −70°C until end of experimental period. Infectivity titrations were performed simultaneously on all 4°C samples; similarly, on all room temperature samples. Table I indicates that Coe virus retains its infectivity at these temperatures for 20 days.

TABLE I. Stability of Coe Virus at 4°C and Room Temperature (22°-26°C).

Days of storage	Infectivity titer (log 10) 4°C	Room temp.	Days of storage	Infectivity titer (log 10) 4°C	Room temp.
0	−6.0	−6.0	8	−5.5	nt
1	nt	−5.5	10	−5.25	−5.5
2	−5.5	nt	12	−5.25	nt
3	−5.5	−5.75	15	nt	−5.5
4	−5.5	−5.5	16	−5.25	nt
5	−5.5	−5.5	20	−5.5	−5.25

Stability at 37°C. Rate of inactivation of Coe virus when incubated at temperature used for propagation, *i.e.*, 37°C, was next determined. Undiluted culture fluid in sealed glass ampules was held at 37°C until assay. Although relatively stable when stored over a wide range in temperature, Coe virus deteriorated at 37°C. The geometric means of 2 experiments (Fig. 1) demonstrate that 90% of the virus was inactivated within 3 days, and that less than 1% of infectivity remained after 20 days.

Inactivation at 50°C. Rate of inactivation of Coe virus was determined by heating 1.0 ml volumes in a 50°C water bath for increasing periods of time. At each time interval a heated aliquot was immediately immersed in an alcohol-dry ice bath, and stored until infectivity titrations were done on all samples. Because of the persistence of infectious virus even after 30 minutes, the experiment was repeated 3 times, and the geometric means of infectivity end points calculated. Fig. II shows that more than 99.9% of virus was inactivated within 5 minutes. However, a small number of virus particles did survive for as long as 30 minutes. Virus infectivity could not be detected in the aliquot heated for 1 hour, nor was virus detected when this fluid was passaged undiluted and through one additional blind passage.

Inactivation by varying pH. Data relative

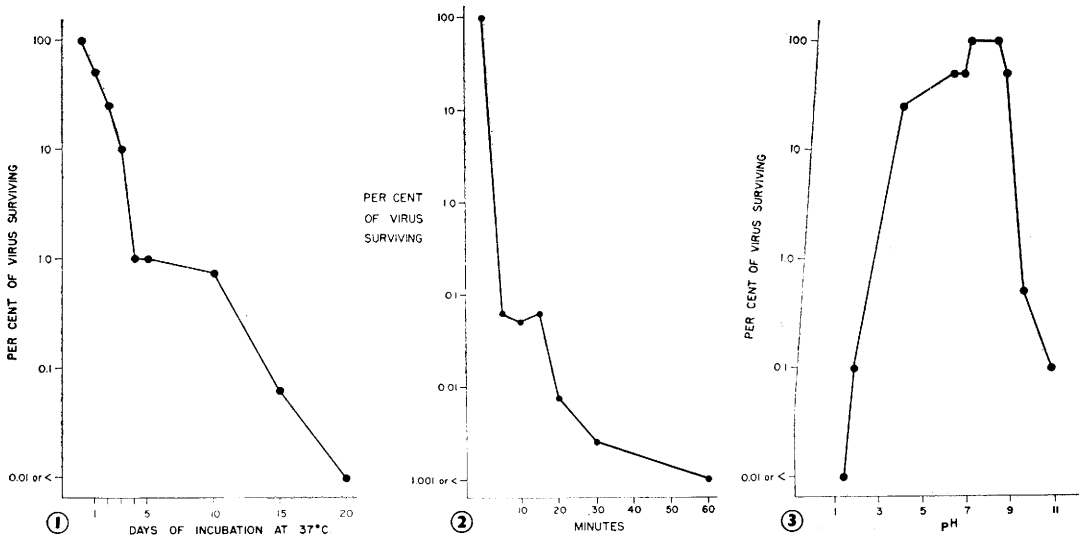

FIG. 1. Effect of continued incubation at 37°C on Coe virus in cell-culture maintenance mediums.

FIG. 2. Inactivation of Coe virus at 50°C.

FIG. 3. Inactivation of Coe virus by acid and alkaline pH changes.

to the stability of Coe virus when aliquots of a suspension were adjusted to varying pH values are plotted in Fig. 3. Coe virus was relatively stable at 22°-24°C between pH 6.0 and 9.0. Virus was inactivated outside of this range, but survival of virus even at pH's as low as 1.4 and as high as 10.8 emphasizes the stability of the infectious property.

Resistance to ether. Because of the importance accorded this characteristic in classifying viruses, the stability of Coe virus was tested by adding 0.4 ml of diethyl ether to 1.6 ml of virus suspension. The mixture was stirred at 4° for 15 hours, the ether removed following separation, and the virus titered. The titer of this suspension before treatment was $10^{-5.5}$; the titer after exposure to ether was $10^{-4.5}$. Thus, Coe virus was found to be ether resistant.

Discussion. Our data indicate that Coe virus is relatively resistant to inactivation by ether, by heat, and by pH changes. Pereira and Pereira(5) also found the agent to be ether resistant, and estimated size of Coe virus to be approximately 20 μ. Even though the virus does not grow in monkey kidney cells, these authors suggested that it be classed as an enterovirus. In terms of ether resistance and thermal stability, with deterioration of infectivity at 37°C, Coe virus does resemble a number of ECHO strains(6). It is equally true that the stability characteristics of Coe virus are similar to those of the adenoviruses (4), although Coe virus is more rapidly inactivated at 37°C.

Coe and adenoviruses grow in similar tissue culture cells—HeLa, KB, DMB, human amnion(1,5), but Coe virus dissociates much more readily from infected cells. When an adenovirus infected culture has undergone maximum cytologic alterations, only 2% to 6% of the virus detected in the cells is present in the fluid phase(7). Type 8 adenovirus is apparently an exception, for cell disruption is not necessary(8). Satisfactory titers of Coe virus can be obtained by harvesting the fluid phase alone, but the yield can be doubled by simultaneous disruption of the cells. The smaller Coe virus does not possess the common complement-fixing antigen shared by the adenoviruses; nor is Coe virus related to the myxoviruses or hemadsorption viruses(1). Since comparison of Coe virus with other animal viruses indicates that its stability to heat and pH alterations is of the same order as both the enteroviruses and adenoviruses(4,6), it is apparent that characteristics other than these must be used for classification.

The stability of Coe virus at 4°C, room temperature, and when maintained in the

frozen state, should facilitate attempts to isolate this agent from human sources, and to define further its role as a respiratory pathogen.

Summary. Coe virus dissociates from infected cells, but 25% to 50% of virus is still intracellular at time of maximum cytopathic effect. The virus was relatively stable when exposed to repeated freezing and thawing, and to storage at −70°C, 4°C, and room temperature. At 37°C, 90% of the virus was inactivated within 3 days, and less than 1% of particles were infectious after 20 days. Coe virus is rapidly inactivated at 50°C, but a few particles survive 30 minutes at this temperature. Coe virus is ether resistant and resistant to inactivation at pH 6 to 9.

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Development of Myeloid (Chloro-) Leukemia in Thymectomized C3H Mice Following Inoculation of Lymphatic Leukemia Virus.* (25576)

LUDWIK GROSS
(With technical assistance of Yolande Dreyfuss and Lorraine A. Moore)
Cancer Research Unit, Vet. Admin. Hospital, Bronx, N. Y. City

Results of recent preliminary experiments suggested(1) that thymectomy renders mice of an otherwise susceptible strain (C3H) resistant to leukemogenic action of a highly potent lymphatic leukemia virus (passage A). This report deals with a) further observations of injected, and thymectomized mice for delayed development of leukemia; b) an attempt to restore susceptibility by subsequent implantation of normal mouse thymus, and c) the unexpected development of myeloid (chloro-) leukemia in some of the thymectomized mice inoculated with the lymphatic leukemia virus, and in one additional non-thymectomized mouse inoculated with the same virus and implanted with normal mouse thymus.

Methods. Passage A strain of leukemic virus initially derived from spontaneous Ak leukemia(2) and then passed serially by cell-free inoculations through newborn mice(3,4) was used. Now in its 21st serial passage, this virus induces consistently lymphatic leukemia in 85 to 100% of suckling C3H mice (Bittner subline) inoculated at less than 10 days of age. The average latency is about 2½ to 3½ months. Adult mice are also susceptible; up to 65% develop leukemia following intraperitoneal inoculation of the virus, but the latency is prolonged, varying usually from 4 to 6 months. Leukemic filtrates (Selas 02) of 20% concentration were prepared(3,4) from pooled leukemic organs from several C3H donors with primary, passage A virus-induced leukemia. All mice were of the C3H, or foster-nursed C3H(f) lines, both of Bittner substrain, bred in our laboratory. Spontaneous lymphatic leukemia in untreated mice of our C3H and C3H(f) colonies does not exceed 0.5%(4). We have never seen myeloid leukemia developing spontaneously in these mice.

Results. Resistance of thymectomized mice to inoculation of virus. It appeared previously that thymectomy either a) following, or b) preceding inoculation of passage A virus ren-

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