THE EFFECT OF SUBLIMATION AND STORAGE ON AN EGG-ADAPTED STRAIN OF INFECTIOUS BRONCHITIS VIRUS

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THE EFFECT OF SUBLIMATION AND STORAGE ON AN EGG-ADAPTED STRAIN OF INFECTIOUS BRONCHITIS VIRUS

By

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Abstract

Samples of an egg-adapted strain of infectious bronchitis virus in the form of 2 ml portions of infected allantoic fluid were dehydrated by sublimation for 12, 24 and 48 hours, respectively, sealed in vacuo at a terminal pressure of 100 microns, and stored at 4° C, 20° C and 37° C for one, three and six months.

The samples were titrated in embryonating chicken eggs to determine the effect of sublimation and/or storage on viral activity.

The titer of the virus prior to drying was $10^{-7.2}$ infective doses per 0.1 ml. After 6 months storage at 4° C samples dehydrated for 12 hours had a titer of $10^{-6.5}$. Under the same conditions, the titer of samples dried 24 and 48 hours was $10^{-6.6}$ and $10^{-6.8}$, respectively.

Virus dried for 12 hours and stored at 20° C for 6 months had a titer of $10^{-5.5}$. Virus dried for 24 and 48 hours had titers of $10^{-4.8}$ and $10^{-5.7}$, respectively.

After storage at 37° C for 6 months virus dehydrated for 12 and 24 hours was completely inactivated. Samples dried for 48 hours had a titer of $10^{-1.2}$.

This study demonstrates that infectious bronchitis virus dried as long as 48 hours maintained a slightly higher level of infectivity during 6 months storage. From a practical viewpoint, this advantage is not great enough to warrant the additional time involved if large numbers of samples had to be

processed in a short period of time. If stored at 4° C, infectious bronchitis virus dried for 12 hours could be kept for 6 months without an appreciable loss of viral activity.

Richard Eugene Billings

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
REVIEW OF LITERATURE	2
MATERIALS AND METHODS	8
RESULTS	12
DISCUSSION	22
SUMMARY	25
BIBLIOGRAPHY	26

INTRODUCTION

This study was undertaken in an attempt to determine the effect of sublimation and storage on an egg-adapted strain of infectious bronchitis virus.

REVIEW OF LITERATURE

Schalk and Hawn (1931) in North Dakota were the first to describe infectious bronchitis in chickens. The most characteristic gross lesions in infected birds were found in the lungs and bronchi. The lungs were described as being in a state of acute congestion accompanied by a sero-mucoid exudate in the bronchi and bronchioles. Frequently, a mucoid exudate could be found in the nasal sinuses.

Berkefeld filtrates of exudates and tissue extracts were capable of inciting the disease in baby chicks.

Beach and Schalm (1936) showed that the disease could be produced by nasal, tracheal, and bronchial exudates passed through V, N, and W Berkefeld filters. Chickens recovered from the disease were resistant to further infection but were not immune to laryngo-tracheitis or infectious coryza.

They observed that the virus dried by Swift's method and stored in the refrigerator for 180 days was still capable of producing the disease. The virus remained infective for 80 days when stored in the refrigerator in 50 percent glycerol.

Beaudette and Hudson (1937) were the first to cultivate the virus in embryonating chicken eggs. Dwarfing of the embryo accompanied by enlargement of the liver was observed.

No lesions were produced on the chorioallantoic membrane which was thinner than normal and adhered closely to the shell membrane. They found that in early passages the virus was relatively non-lethal to the embryo but acquired lethality with continued passage.

Delaplane and Stuart (1941) confirmed the observations of Beaudette and Hudson on the acquired lethality of the virus for chicken embryos inoculated by the chorioallantoic membrane route.

Gross lesions consisted of white foci in the liver and congestion and enlargement of the kidneys. The embryos were dwarfed and occasionally hemorrhagic.

The virus survived storage in the fresh frozen state in the freezing compartment of an electric refrigerator for four and one-half months and at room temperature for five to seven days.

Cunningham and Stuart (1946) reported the effect of certain chemical agents on an egg-adapted strain of infectious bronchitis virus.

One part of the virus suspension was mixed with nine parts of the chemical agent, allowed to react for three minutes at room temperature, and injected into eggs via the allantoic sac route. The criterion for inactivation of the virus was survival of the embryo.

Phenol, 3 percent and 1 percent, liquor cresolis saponatus, 3 percent and 1 percent, tincture of metaphen, undiluted and 1 percent, potassium permanganate, 1-1,000 and 1-10,000, ethyl alcohol, 95 percent, 70 percent, 40 percent and 25 percent, Neoprontosil, 5 percent, and formalin, 1 percent produced complete inactivation of the virus.

Boric acid, 4 percent, and tincture of iodine, 0.01 percent were without effect.

Cunningham and Stuart (1947) studied the pH stability of an egg-adapted strain of infectious bronchitis virus.

For the first 60 days of the experiment the virus was more stabile in an acid medium than in an alkaline medium. From 60 to 170 days there was a shift to greater stability in an alkaline medium.

Virus in a phosphate buffer at a pH of 7.79 remained active for 170 days. Virus in allantoic fluid at a pH of 7.8 was active for 100 days. Undiluted virus at a pH of 8.20 remained active for 142 days. The criterion for viral stability was death of the embryos.

Cunningham and Stuart (1947) observed that egg-adapted infectious bronchitis virus stored at -25° C or -70° C had a higher virus activity than did samples stored at -10° C.

Freezing and thawing of infectious bronchitis infected allantoic fluid produced two types of precipitates, one soluble at room temperature and the other insoluble at room

temperature. The insoluble precipitate could be deposited by centrifugation without affecting the potency of the virus in the supernatant fluid.

No significant differences were noted when Difco nutrient broth, Difco tryptose phosphate broth, 0.85 percent saline, or M/10 phosphate buffer were used as diluents in comparative titrations.

An egg-adapted strain of infectious bronchitis virus was dried at 0.15 mm mercury pressure for 8 hours, sealed in vacuo and stored at 4° C. After 7 days the virus was reconstituted to volume with 1 ml of sterile distilled water and subsequent titration demonstrated a hundred-fold decrease in virus activity.

Delaplane (1947) reported that infectious bronchitis virus inoculated into embryonating chicken eggs via the allantoic sac route produced dwarfing of the embryo as early as the first passage.

Beaudette, Miller, Bivins, and Hudson (1948) dried egg-adapted infectious bronchitis virus from the frozen state over anhydrous phosphorus pentoxide in a vacuum desiccator. They found that samples stored over anhydrous phosphorus pentoxide in an evacuated desiccator in the refrigerator remained active up to 684 days.

Reagen, Hauser, Lillie, and Craige (1948), by means of electron microscopy, demonstrated the virus to be round with

a mean diameter of 90 mu. Filamentous projections were present on some of the particles.

Reagen, Brueckner, and Delaplane (1950), using improved techniques, reported the virus to be round with a mean diameter of 70 mu. Filamentous projections similar to those of Newcastle disease virus, were observed.

Preservation of Other Viruses

Olitsky (1939) reported that lyophilized brain tissue infected with avian encephalomyelitis virus was found active after 68 days.

Turner and Fleming (1939) observed that influenza virus, PR8 strain, in a 10 percent mouse lung suspension stored at -78° C for approximately 3 years was fatal to mice in essentially the same dilution as before freezing.

Under the same conditions similar results were obtained upon testing the virus of meningopneumonitis in a 10 percent mouse lung suspension.

The virus of lymphogranuloma inguinale in a mouse brain suspension remained pathogenic for mice after storage at -78° C for 10 months.

Wooley (1939) reported that the viruses of lymphocytic choriomeningitis and St. Louis encephalitis in brain tissue remained viable in storage 378 and 833 days, respectively, after being frozen, dried *in vacuo*, and stored at 5° C.

Bauer and Pickels (1940) found that yellow fever virus, in a medium rich in protein, desiccated at -18° C. and stored at refrigerator temperature was still virulent after 10 years.

Hoffstadt and Tripi (1946) observed that the viruses of the Levaditi and Cutter strains of vaccinia, herpes simplex, laryngotracheitis, and Rous sarcoma in human serum survived preservation by lyophilization for approximately 3 years with no alteration of characteristics.

The OA strain of Shope's fibroma under the same conditions did not survive preservation and storage for approximately 3 years.

After lyophilization and storage for approximately three years a human serum suspension of the virus of infectious myxomatosis of rabbits exhibited inconstant viability.

MATERIALS AND METHODS

The virus used was an egg-adapted strain, V11$\frac{4}{4}$D, of infectious bronchitis virus in the form of infected allantoic fluid.

Nine-day old embryos were used throughout the study. Inoculation was via the allantoic sac. The eggs were transilluminated for selection of an area of the chorioallantoic membrane free from large blood vessels about 3 mm below the air cell. A small hole was drilled through the shell without piercing the shell membrane, by means of a small drill attached to the chuck of an electric motor. Another hole was drilled through the shell over the top of the air cell. Tincture of metaphen was painted over the holes and allowed to dry. The shell membrane over the top of the air cell was punctured with a sterile teasing needle to allow equalization of pressure within the egg when the inoculum was injected into the allantoic sac and to prevent leakage of the inoculum from the site of injection. After injecting the inoculum, using a B-D Yale 1-cc capacity tuberculin syringe, fitted with a 27 gauge, 1/2-inch needle, the holes in the shell were sealed with melted paraffin and the eggs returned to the incubator. All incubation was at 99° F in an electric forced-draft incubator.

At the time of collection of the allantoic fluid the shell over the air cell was painted with tincture of metaphen and allowed to dry. This portion of the shell was cracked and removed with sterile forceps. Without removing the shell membrane, the allantoic fluid was harvested using a 5-cc syringe fitted with a 20 gauge, 1 1/2-inch needle.

Sixty-three eggs were inoculated using 0.1 cc of inoculum per egg. Within eighteen hours after inoculation the eggs were candled. Any deaths that occurred during this period were considered to be due to non-specific causes. After the initial candling, the eggs were candled every hour for eight hours and all dead embryos were refrigerated at 4° C. Embryos still living at the end of this period were also refrigerated at 4° C. for as long as 12 hours. The allantoic fluid from both living and dead embryos was collected, pooled, and dispensed in 20 ml portions into sterile 30 ml screw cap vials. The vials of infected allantoic fluid were stored at -30° C until used.

At the time of use 60 ml of the allantoic fluid was thawed quickly at room temperature and centrifuged to sediment the precipitate formed on thawing. Two ml portions were placed into sterile 10 ml pyrex ampoules using a sterile 2 ml volumetric pipette. The ampoules were placed in an ice bath until all the fluid was dispensed to minimize any decrease in activity that might be caused by exposure to room temperature. The allantoic fluid was shell frozen at -35° C and

attached to an Aminco freeze-dry apparatus.* Groups of samples were dried for periods of 12, 24 and 48 hours, respectively. At the end of the drying period the ampoules were sealed *in vacuo* using a cross-fire gas-oxygen torch.

After removal from the apparatus the ampoules were tested for leaks with a Tesla coil and labeled. Samples from the 12, 24 and 48 hour drying periods were stored at 4°, 20° and 37° C for comparative quantitative titrations at one, three, and six months. The samples stored at 20° C were placed in a laboratory bench cabinet where they would not be exposed to sunlight. Skinner and Bradish (1954) observed a decrease in infectivity titer of two to five log units when the viruses of influenza, strain B, Newcastle disease, fowl plague, vaccinia, and vesicular stomatitis were exposed to daylight for four hours.

To determine the effect of the drying process on viral activity, the infected allantoic fluid was titrated before sublimation. Immediately following drying, the contents of an ampoule of the dehydrated virus were reconstituted to volume with 2.0 ml of sterile distilled water for titration.

In all instances serial ten-fold dilutions, 10^{-1} through 10^{-8}, of the virus sample were made according to the procedure

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*American Instrument Company, Silver Springs, Maryland.

described by Cunningham (1952). The tubes were in an ice bath through the entire procedure. To each tube was added 4.5 ml of sterile nutrient broth. To the first tube 0.5 ml of the sample was added using a 2.0 ml serological pipette which was then discarded. With another pipette the contents of this tube were aspirated and expelled 20 times to insure adequate mixing of the broth and the virus. This constituted the 10^{-1} dilution of the virus. The same pipette was used to transfer 0.5 ml of this mixture to the second tube. This procedure was followed until all dilutions had been made using a separate pipette for each dilution.

Five eggs were inoculated via the allantoic sac using 0.1 cc of inoculum per egg from each virus dilution. The eggs were placed in the incubator and candled 18 hours later. Mortality occurring within this period was considered to be due to non-specific causes and was not included in the data for calculation of viral activity. After the initial candling, the eggs were candled daily for six days at which time they were discarded.

The tubes containing the broth-virus mixture were incubated at 99° F for six days. Lack of growth in the tubes was considered to be evidence of bacterial sterility.

RESULTS

The results of the titrations of the virus are shown in Tables I, II and III and Figures 1, 2 and 3.

The results of viral activity for each dilution are expressed as a fraction in which the denominator indicates the number of eggs inoculated, and the numerator, the embryo mortality due to viral activity. The 50 percent mortality and point, LD_{50}, was calculated according to the method of Reed and Muench (1938).

The effect of environmental influences was encountered when the dehydrated samples were reconstituted with sterile distilled water for the titration immediately following sublimation. While the difficulty is not directly related to the primary objective of the present study, it is given as an experience to be avoided by other investigators. Titration of the above samples showed a level of viral activity that was below a priori reasoning and at variance with the 100-fold decrease in activity of the same strain of virus as reported by Cunningham and Stuart (1947).

The distilled water used for reconstitution of the dehydrated virus, and the nutrient broth used as the diluent, had been stored in a refrigerator where two previously opened cans of ether were kept. It was considered that the

highly volatile ether had been absorbed by the distilled water and/or the nutrient broth and had reduced the viral activity. The results of the titration with ether-contaminated distilled water and nutrient broth are as follows:

Titration of Infectious Bronchitis Virus Using Ether-Contaminated Diluents

	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
12 hours	0/5	0/5	0/5	0/5	0/5
24 hours	0/5	0/5	0/5	0/5	0/5
48 hours	4/5	5/5	3/5	1/5	1/5

Using freshly prepared distilled water and nutrient broth that had not been in contact with ether, the virus was within the expected infectivity range. The results are shown in Tables I, II and III and Figures 1, 2 and 3.

These results show conclusively the virucidal properties of ether.

Due to the difficulties encountered with the ether contamination and the insufficient supply of nine-day old embryos, the titer of the virus samples immediately following sublimation could not be determined. Consequently, the titer of the frozen allantoic fluid was used as the basis to which the activity of the virus samples at the storage intervals could be compared.

TABLE I

EFFECT OF SUBLIMATION FOR 12 HOURS AND SUBSEQUENT STORAGE FOR 1, 3 AND 6 MONTHS AT 4° C, 20° C AND 37° C ON AN EGG-ADAPTED STRAIN OF INFECTIOUS BRONCHITIS VIRUS

	Virus Dilution									
	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	LD_{50}
	Infectious Bronchitis Virus Prior to Sublimation									
	-	5/5	5/5	5/5	5/5	5/5	5/5	3/5	0/5	$10^{-7.2}$
Time in Months					4° C					
1	5/5	5/5	5/5	5/5	5/5	3/5	3/5	0/5	-	$10^{-6.2}$
3	5/5	5/5	5/5	5/5	5/5	5/5	4/4	4/5	-	$10^{-7.4}$
6	5/5	5/5	5/5	5/5	5/5	5/5	3/5	2/5	-	$10^{-6.5}$
					20° C					
1	5/5	5/5	5/5	5/5	5/5	3/5	0/5	0/5	-	$10^{-5.2}$
3	5/5	5/5	5/5	4/4	5/5	4/4	0/5	0/5	-	$10^{-5.5}$
6	5/5	5/5	5/5	5/5	5/5	3/5	2/5	1/5	-	$10^{-5.5}$
					37° C					
1	5/5	5/5	5/5	4/5	1/5	1/5	0/5	0/5	-	$10^{-3.5}$
3	5/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	-	$10^{-1.6}$
6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	-	

Fig. 1. Effect of sublimation for 12 hours and subsequent storage for 1, 3 and 6 months at 4° C, 20° C and 37° C on an egg-adapted strain of infectious bronchitis virus.

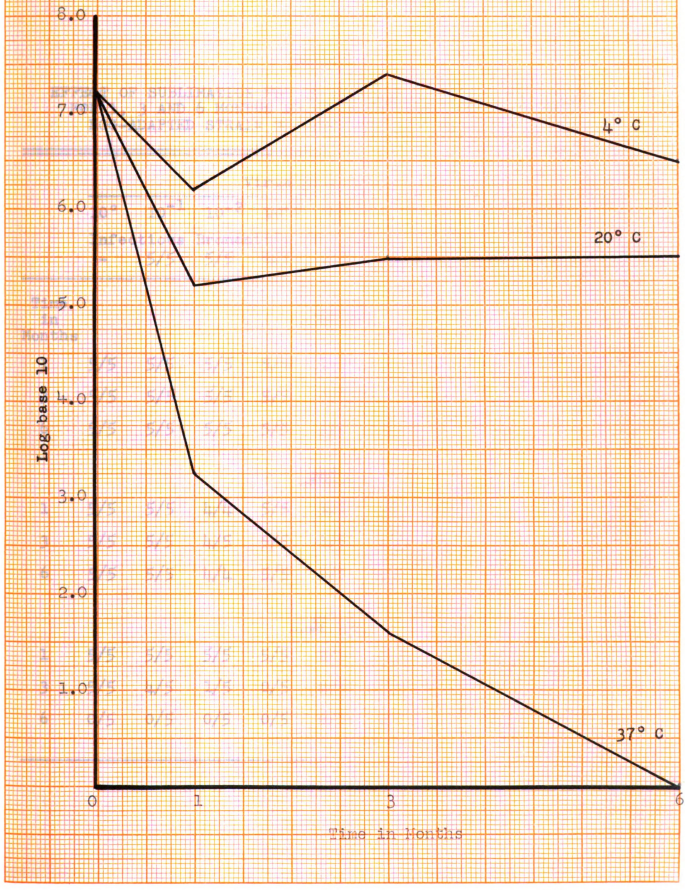


Fig. 1. Effect of [illegible] for [illegible] hours and subsequent storage for 1, 3 and 6 months at 4° C, 20° C and 37° C on an egg-adapted strain of infectious bronchitis virus.

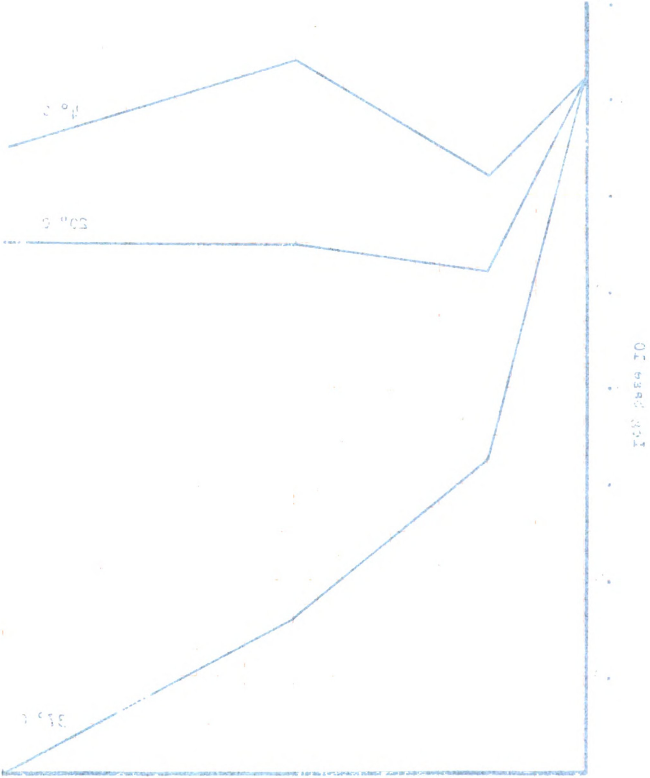
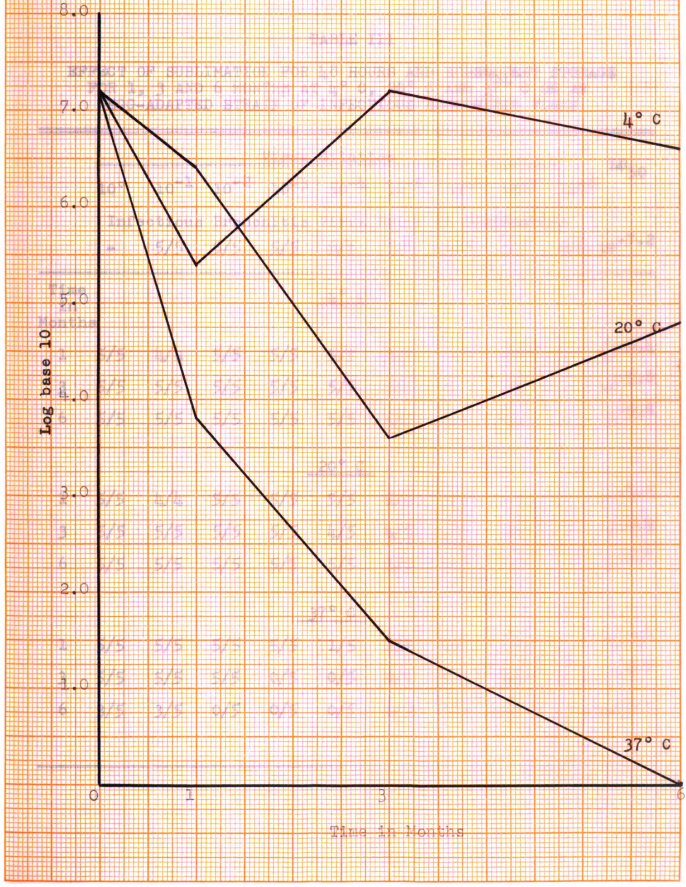


TABLE II

EFFECT OF SUBLIMATION FOR 24 HOURS AND SUBSEQUENT STORAGE FOR 1, 3 AND 6 MONTHS AT 4° C, 20° C AND 37° C. ON AN EGG-ADAPTED STRAIN OF INFECTIOUS BRONCHITIS VIRUS

	Virus Dilution									LD_{50}
	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
	Infectious Bronchitis Virus Prior to Sublimation									
	-	5/5	5/5	5/5	5/5	5/5	5/5	3/5	0/5	$10^{-7.2}$
Time in Months					4° C					
1	5/5	5/5	5/5	5/5	5/5	4/5	0/5	0/5	-	$10^{-5.4}$
3	5/5	5/5	5/5	5/5	5/5	5/5	4/5	3/5	-	$10^{-7.2}$
6	5/5	5/5	5/5	5/5	5/5	5/5	4/4	1/5	-	$10^{-6.6}$
					20° C					
1	5/5	5/5	4/4	5/5	5/5	5/5	4/5	0/5	-	$10^{-6.4}$
3	5/5	5/5	4/5	5/5	1/5	1/5	1/5	0/5	-	$10^{-3.6}$
6	5/5	5/5	4/4	5/5	5/5	2/5	0/5	0/5	-	$10^{-4.8}$
					37° C					
1	5/5	5/5	5/5	5/5	2/5	0/5	0/5	0/5	-	$10^{-3.8}$
3	5/5	4/5	1/5	0/5	0/5	0/5	0/5	0/5	-	$10^{-1.5}$
6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	-	

Fig. 2. Effect of sublimation for 24 hours and subsequent storage for 1, 3 and 6 months at 4° C, 20° C and 37° C on an egg-adapted strain of infectious bronchitis virus.



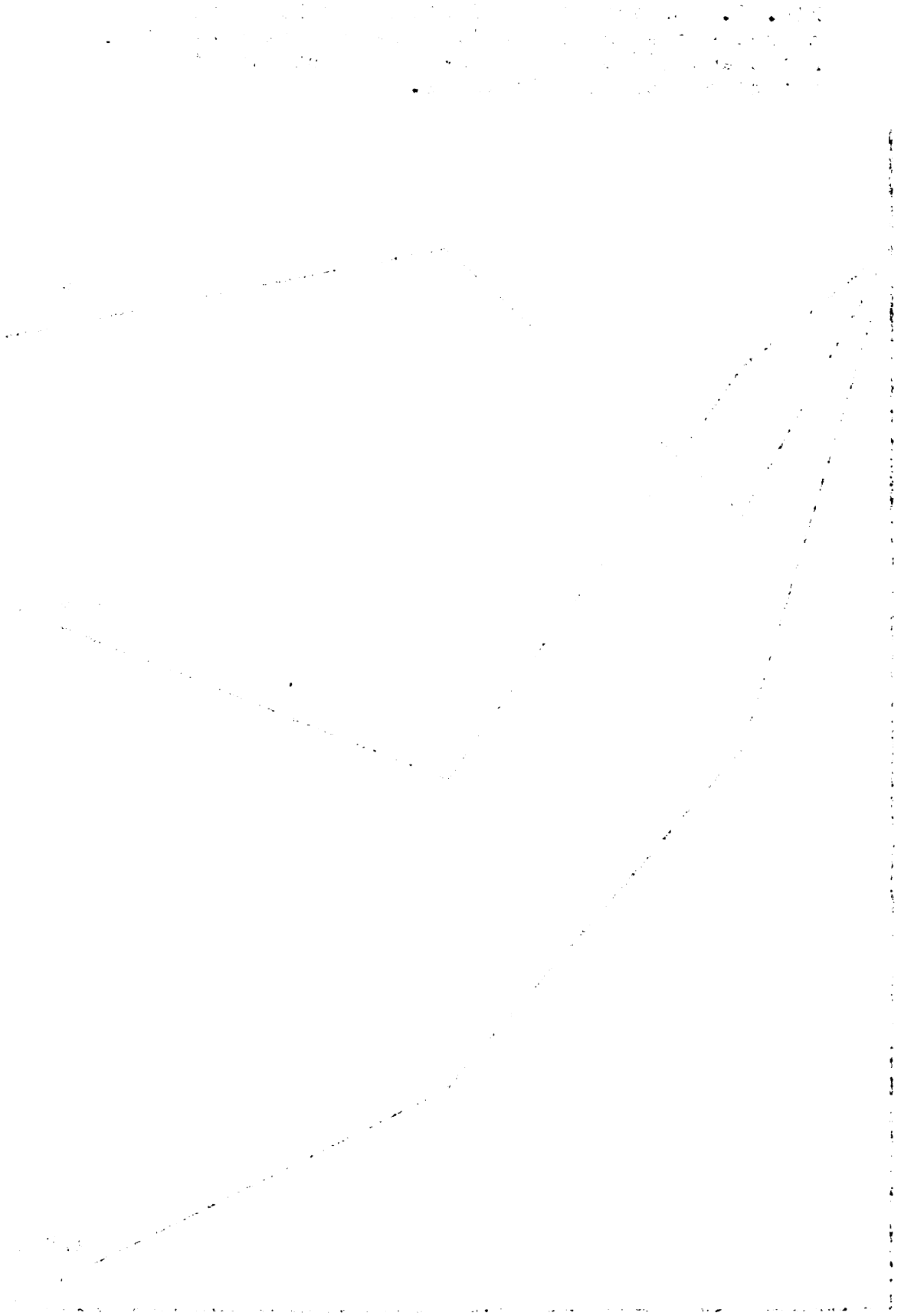
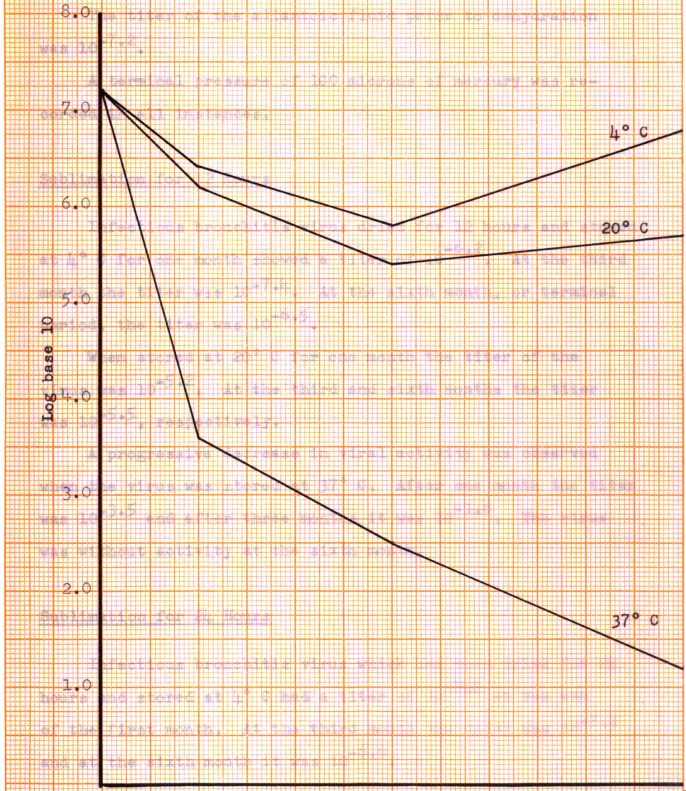


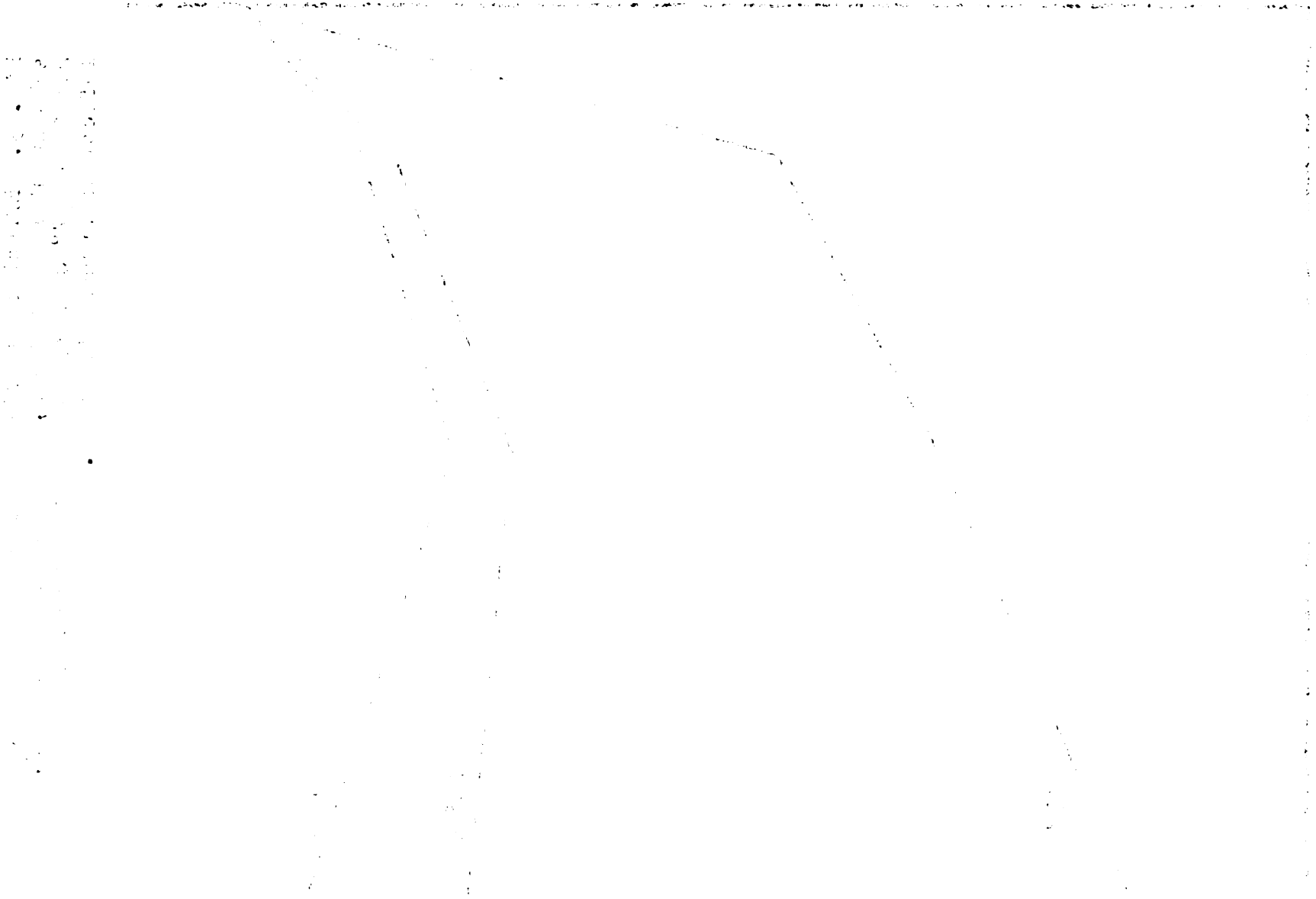
TABLE III

EFFECT OF SUBLIMATION FOR 48 HOURS AND SUBSEQUENT STORAGE FOR 1, 3 AND 6 MONTHS AT 4° C, 20° C AND 37° C ON AN EGG-ADAPTED STRAIN OF INFECTIOUS BRONCHITIS VIRUS

	Virus Dilution									LD_{50}
	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
	Infectious Bronchitis Virus Prior to Sublimation									
	-	5/5	5/5	5/5	5/5	5/5	5/5	3/5	0/5	$10^{-7.2}$
Time in Months					4° C					
1	5/5	4/4	5/5	5/5	5/5	4/5	4/5	0/5	-	$10^{-6.4}$
3	5/5	5/5	5/5	5/5	5/5	5/5	2/5	0/5	-	$10^{-5.8}$
6	5/5	5/5	5/5	5/5	5/5	5/5	4/5	2/5	-	$10^{-6.8}$
					20° C					
1	5/5	4/4	5/5	5/5	5/5	5/5	3/5	0/5	-	$10^{-6.2}$
3	5/5	5/5	5/5	5/5	4/5	4/5	0/5	0/5	-	$10^{-5.4}$
6	5/5	5/5	5/5	5/5	5/5	5/5	1/4	0/5	-	$10^{-5.7}$
					37° C					
1	5/5	5/5	5/5	5/5	1/5	1/5	0/5	0/5	-	$10^{-3.6}$
3	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	-	$10^{-2.5}$
6	3/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	-	$10^{-1.2}$

Fig. 3. Effect of sublimation for 48 hours and subsequent storage for 1, 3 and 6 months at 4° C, 20° C and 37° C on an egg-adapted strain of infectious bronchitis virus.





The titer of the allantoic fluid prior to dehydration was $10^{-7.2}$.

A terminal pressure of 100 microns of mercury was recorded in all instances.

Sublimation for 12 Hours

Infectious bronchitis virus dried for 12 hours and stored at 4° C for one month showed a titer of $10^{-6.2}$. At the third month the titer was $10^{-7.4}$. At the sixth month, or terminal period, the titer was $10^{-6.5}$.

When stored at 20° C for one month the titer of the virus was $10^{-5.2}$. At the third and sixth months the titer was $10^{-5.5}$, respectively.

A progressive decrease in viral activity was observed when the virus was stored at 37° C. After one month the titer was $10^{-3.5}$ and after three months it was $10^{-1.6}$. The virus was without activity at the sixth month.

Sublimation for 24 Hours

Infectious bronchitis virus which had been dried for 24 hours and stored at 4° C had a titer of $10^{-5.4}$ at the end of the first month. At the third month the titer was $10^{-7.2}$ and at the sixth month it was $10^{-6.6}$.

After storage at 20° C for one, three and six months, the titer of the virus was $10^{-6.4}$, $10^{-3.6}$ and $10^{-4.8}$, respectively.

Storage at 37° C resulted in a decrease in infectivity from $10^{-7.2}$ to $10^{-3.8}$ at the end of the first month. The third month the titer was $10^{-1.5}$. The virus was completely inactivated by the end of the sixth month.

Sublimation for 48 Hours

Virus which had been dried for 48 hours and stored at 4° C had a titer of $10^{-6.4}$ at the first sampling period. By the third month the titer had dropped to $10^{-5.8}$ and was $10^{-6.8}$ at the terminal period.

Virus samples stored at 20° C had titers of $10^{-6.2}$ at the first month, and $10^{-5.4}$ at the third month. After six months the titer was $10^{-5.7}$.

After storage at 37° C for one month the titer was $10^{-3.6}$. At the third and sixth months the titer was $10^{-2.5}$ and $10^{-1.2}$, respectively.

DISCUSSION

Three factors must be considered in evaluating the results of this study: the sublimation period, the storage period, and the storage temperature.

The storage temperature must, of necessity, be considered in relation to the length of the sublimation period. The amount of heat transferred to the virus particles is related to the moisture present in the dehydrated sample. The more moisture present the greater is the amount of heat transfer and, hence, the more rapid the rate of inactivation of the virus. At all temperatures the samples dried for 48 hours maintained a higher level of viral activity than did samples dried for either 12 or 24 hours. The difference is most striking in those samples stored at 37° C. due to the acceleration of the reaction between the virus and its environment. It was noted that in samples dried for 12 or 24 hours, a complete loss of infectivity occurred prior to, or during the sixth month of storage. Virus dried for 48 hours exhibited a marked decrease in activity but was not completely inactivated.

The duration of the storage periods played a role in retention of viral infectivity by determining the length of time the virus was exposed to environmental temperatures.

This would have become more obvious had the study been extended over a longer period of time. However, using the accelerated reaction at 37° C as an example, the loss of viral activity was related to the duration of the exposure to the environmental temperature.

Similarities were observed between all virus samples titrated after one month of storage at the various temperatures. There was a sharp initial decrease in viral infectivity presumably due to the effect of the dehydration process and the temperatures to which the samples were exposed. In samples stored at 20° C and 4° C this initial decrease was followed, in most cases, by a leveling off of viral activity. As has been stated before, storage at 37° C resulted in a progressive decrease in infectivity in all samples.

Technical difficulties, such as an error in measurement of the distilled water used for reconstitution of the virus, or improper mixing of the pooled allantoic fluid, may have contributed to the results obtained in the titrations of samples dried for 12, 24 and 48 hours and stored for three months at 4° C. No explanation can be given for the inability of the virus dried for 24 hours and stored at 20° C to maintain a level of activity consistent with that of the other virus samples subjected to the same conditions.

From a practical viewpoint, this study serves to point out that although the virus dried for 48 hours maintained a alightly higher viral activity than did samples dehydrated

for 12 and 24 hours, the difference was not sufficient to warrant the time involved if large volumes of virus were to be dried. Virus dried for 12 hours and stored at 4° C remained at a high level of infectivity for as long as six months.

SUMMARY

Infectious bronchitis virus in 2 ml portions was dehydrated for 12, 24 and 48 hours, sealed *in vacuo* at a terminal pressure of 100 microns, and stored at 4° C, 20° C, and 37° C. One, three and six months after sublimation the virus was titrated in embryonating chicken eggs. The results were expressed as the LD_{50}.

After six months storage at 4° C, virus samples dehydrated for 12, 24 and 48 hours showed titers of $10^{-6.5}$, $10^{-6.6}$ and $10^{-6.8}$, respectively.

After storage at 20° C for six months, virus samples dried for 12, 24 and 48 hours had titers of $10^{-5.5}$, $10^{-4.8}$ and $10^{-5.7}$, respectively.

Storage at 37° C for six months resulted in a complete loss of viral activity in virus samples dehydrated for 12 and 24 hours. Virus dried for 48 hours had a titer of $10^{-1.2}$.

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