

MAY 22 1951

DILUTION  
EFFECT

DOES THE MILK FACTOR DESTROY LANSING VIRUS OR  
MERELY NEUTRALIZE OR COMBINE WITH IT?

PURPOSE: To determine if all available virus is neutralized when a 10% blend of uncentrifuged Y-SK is mixed in a dilution of 1:10 with a pool of human milk which is known to neutralize Lansing virus. and the effect of diluting the milk which has combined with the virus.

VIROS: Y-SK Pool II of 11/24/50 10% blend

PROCEDURE: 0.1 ml of a 10% blend of Y-SK virus was added to 0.9 ml of milk and mixed. 0.1 ml of this mixture was removed and added to 0.9 ml of saline to determine if unneutralized virus was present. The same procedure was carried out in which autoclaved cow's milk was substituted for human milk. All mixtures carried out in an ice bath and inoculated immediately into mice.

MICE 18-20 gm Mayfield 20 per dilution

<u>CODE</u>	<u>SPECIMEN</u>
A	CGH HUMAN MILK POOL USED 9/7/50 0.9 ml. MILK + 0.1 ml. Y-SK VIRUS $10^{-2}$
B	CGH HUMAN MILK POOL USED 9/7/50 0.1 ml. of A + 0.9 ml. SALINE VIRUS $10^{-3}$
C	AUTOCLAVED COW'S MILK 0.9 ml. MILK + 0.1 ml. of Y-SK VIRUS $10^{-2}$
D	AUTOCLAVED COW'S MILK 0.1 ml. of C + 0.9 ml. of SALINE VIRUS $10^{-3}$
E	CONTROL SALINE + UNCENTRIFUGED YSK



SPECIMEN	VIROS NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	MORTALITY	
B (CONT.) 0.1 ml. of A + 0.9 ml. of YSK  (NO INCUBATION)		1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9/9	19/19	
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C AUTOCLAVED COW'S MILK + 10% UNCENTRIFUGED BLEND OF YSK  MILK 0.9 ml + 0.1 ml. of YSK  (NO INCUBATION)		1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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# EFFECT OF BOSTON MILK DIRECTORY - LATE HUMAN MILK OF 7/24/50 ON LANSING VIRUS

PURPOSE:

To determine if the anti-Lansing factor in Boston milk directory - late human milk of 7/24/50 destroys the virus or merely neutralizes it!

VIRUS:

Lansing virus: Pool VI of 2/15/51. 10% suspension in saline. Centrifuged at 2000 rpm for 10 minutes to remove flocculant ppt.

PROCEDURE:

0.9 cc of undiluted milk and 0.1 cc of Lansing virus were mixed so that the final concentration of milk was 90% and that of virus was  $10^{-2}$ . This mixture was incubated at room temperature for one hour and inoculated into 20 mice. Serial ten-fold dilutions of the milk-virus mixture were made so that dilution of milk & virus were: 9% and  $10^{-3}$ ; 0.9% and  $10^{-4}$ . These mixtures were inoculated without incubation.

As control on the effect of dilution of milk, the following mixtures of milk-virus were inoculated: 18% milk + virus 1:500 - final concentration 9% milk + virus  $10^{-3}$ ; 1.8% milk + virus 1:1,500 - final concentration 0.9% milk + virus  $10^{-4}$ .

Controls on virus were as follows: 0.9 cc saline + 0.1 cc virus 1:10 - virus final concentration of  $10^{-2}$ . This mixture was incubated at room temperature for one hour and inoculated into 20 mice. Serial ten-fold dilutions were made from this mixture of virus  $10^{-3}$ ,  $10^{-4}$  and inoculated without incubation.







MORTALITY

SPECIMEN	VIRUS NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		
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19/19

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SPECIMEN	VIRUS NO.	1	2	3	4	5	6	7	8	9	10
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MAR 10 1952

# THE EFFECT OF RAPID BLIND PASSAGE ON INCUBATION PERIOD OF LANSING VIRUS IN MICE.

## PURPOSE:

To develop a strain of Lansing virus which will have a uniform incubation period in mice. Because of the erratic behavior of this virus in which mice inoculated with dilution varying from  $10^{-1}$  to  $10^{-5}$  will become paralyzed from 2 to 5 days after inoculation, it was decided to attempt to alter the virus and bring about a uniform incubation period. It was felt that a given suspension of virus might contain a combination of "slow-growing" and "fast-growing" particles, and the combination of which might determine the rapidity with which mice are slain with paralysis. These could possibly be separated out by passage of the virus at limiting dilutions to pick up the "fast-growing" virus, followed by blind passage at 18 hour intervals.

## PROCEDURE:

The virus was passaged at dilution of  $10^{-3}$  and  $10^{-4}$  into groups of 10 mice. Any mouse which became paralyzed after 2 to 3 days was sacrificed and its cord + brain stem passaged again at  $10^{-3}$  and  $10^{-4}$ . After the seventh such passage the cord and stem of one mouse was passaged into a group of 10 mice as a  $10\%$  suspension. At 18 hours all mice were sacrificed, the cords and brain stems removed and again passaged at  $10^{-1}$ . This was repeated for 10 blind passages. On the 10<sup>th</sup> passage material & titration was carried out simultaneously with a titration on the original material from which the blind passage was initiated. Serial ten fold dilution ranging from  $10^{-1}$  to  $10^{-5}$  were made in saline of the 7<sup>th</sup> fast passage of fast growing virus and the 10<sup>th</sup> blind passage.

## VIRUS:

hearing virus

## MICE:

18-20 gram Mat-pulid albino  
20 per dilution.

MAR 10 1952

MORTALITY 1050

10-4.2

19/19

19/20

SPECIMEN	VIRUS NO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
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