TABLE II.
Nitrogen Loss by Precipitation of Virus Suspension with Methanol.

Virus CAF*	Mg N crude	Fold. conc.	Mg N after precipitation	% N reduction
Newcastle	.64	0	0.20	68.8
"	.62	0	0.35	43.8
"	.62	10	0.42	99.2
Influenza B	.54	0	0.21	62.0
" A	.47	0	0.14	70.3
" A	.47	10	1.12	76.2
Mumps	.56	100	2.91	94.9
"	.56	100	1.47	97.3
"	.56	100	0.35	99.3

* CAF — Chorio-allantoic fluid.

to pH 5 for one hour, and it was thereafter recovered with appropriate pH solutions. It has been reported that poliomyelitis virus can tolerate exposure to pH 1.6,[12] and on this basis it seems probable that the MEF virus was not destroyed when it failed to elute at the lower pH levels. Additional studies of the pH stability of MEF poliomyelitis virus indicate that while the virus was most stable at pH 6-7 it was able to tolerate pH 3 for 10 weeks.[13]

Summary. Five viruses (Eastern equine encephalomyelitis, MEF human poliomyelitis mumps, influenza B, and Newcastle disease) were utilized in studying the precipitating effect on them of methanol. Optimal methanol concentrations and optimal pH for elution were determined for each virus and are tabulated (Table I). All of the viruses which were studied demonstrated a relationship between the pH of the elution fluid and solubility: as the pH was raised there was a corresponding increase in solubility of the virus. The methanol precipitation technic results in considerable loss of nitrogen from the virus suspension, but this loss does not appear to be at the expense of the virus.

[12] Loring H., and Schwerdt, C. E., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 173.

[13] Pollard, M., and Connolly, J., Tex. Reports on Biol. and Med., Spring 1949.

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17168. Comparison of Newcastle Virus in Hamsters Exposed by Intracerebral Injection and Intranasal Instillation.

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Hamster-adapted Newcastle virus, California strain No. 11,914, has been continued through the 300th passage by serial intracerebral inoculation. Virus neutralization tests were conducted, using brain suspensions of the 245th and 300th intracerebral hamster passages with specific Newcastle virus immune and normal chicken sera, and with hamsters as test animals. Specific immune sera completely neutralized the hamster brain virus, whereas normal chicken serum had no effect. The virus of this 300th passage titred $10^{-4.77}$ intracerebrally in 4-week-old hamsters, in contrast to the virus of the 200th passage[1] which titred $10^{-4.25}$ in hamsters of the same age injected by the same route.

Newcastle virus infection in hamsters inoculated intranasally with 10% brain suspension of the 16th intracerebral hamster passage has been previously reported.[2] It was also reported that intranasal instillations

[1] Reagan, R. L., Lillie, M. G., Hauser, J. E., and Brueckner, A. L., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 293.

[2] Reagan, R. L., Lillie, M. G., Poelma, L. J., and Brueckner, A. L., *Am. J. Vet. Res.*, **8**, 427.

TABLE I.
Intranasal Passage of Hamster-Adapted Newcastle Virus.

Passage No.	No. inoculated	No. showing symptoms	No. days after inoculation symptoms occurred
1	6	5	3, 3, 4, 4, 5
2	5	5	2, 2, 3, 3, 4
3	8	6	4, 4, 4, 4, 5, 5
3a*	5	2	3, 3
4	6	6	4, 4, 6, 6, 6, 6
4a	6	2	4, 5
5	4	1	3
6	4	1	4
7	5	3	4, 4, 5
7a	6	2	4, 4
8	4	2	3, 4
9	5	0	
9a	8	8	3, 4, 4, 4, 4, 4, 4, 4

*"a"—Duplicate passages made from suspensions which were frozen and thawed.

TABLE II.
Titration in 4-Week-Old Hamsters of Hamster-Adapted Newcastle Virus.

Virus	Dilutions 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Titer[3]
Hamster brain 300th passage	—	4/4	4/4	3/4	2/4	$10^{-4.77}$
Hamster cord 300th passage	4/4	4/4	0/4	0/4		$10^{-2.5}$
Hamster brain from 4th intranasal passage	4/4	4/4	4/4	3/4	2/4	$10^{-4.77}$
Hamster brain from 7th intranasal passage	6/6	4/4	4/4	1/4		$10^{-3.66}$

Numerator of fraction denotes number of deaths.
Denominator of fraction denotes number of hamsters inoculated.

of lung or brain suspension from the affected animals failed to produce symptoms of central nervous system involvement in hamsters of the primary passage. Repeated trials with intranasal instillation of 10-20% infected brain suspension after the 16th intracerebral passage and up to the 245th intracerebral passage failed to produce typical symptoms of Newcastle virus infection, except in the first exposure. When a 20% brain suspension of the 245th intracerebral subculture was passed intranasally in young hamsters, symptoms involving the central nervous system, similar to those observed after intracerebral injection of the hamster-adapted Newcastle virus, were observed. These intranasal instillations were continued through 9 uninterrupted passages. Virus neutralization tests were conducted, using brain suspension of this 9th passage with specific Newcastle virus immune and normal chicken sera, with hamsters as test animals. Specific immune serum completely neutralized the hamster brain virus while normal chicken serum had no effect. Symptoms of central nervous system involvement appeared between the 2nd and 6th days following intranasal exposure. Table I gives the results of these 9 passages. Table II gives results of titrations in 4-week-old hamsters of the hamster-adapted Newcastle virus from various sources.

Discussion. Similar symptoms were shown by hamsters infected by the intracerebral route and by intranasal instillation, despite the fact that the incubation period was much shorter by the first method. Virus of the higher numbered intracerebral passages, and

[3] Reed, L. J., and Muench, H., *Am. J. Hyg.*, **27**, 493.

including the 300th, produced marked nervous reactions within 24 hours after intracerebral injection. This was true when dilution was carried as high as 1 to 1000. Hamsters exposed by intranasal instillation of 20% brain suspension in amounts as small as 0.06 cc became infected. In exposure by intranasal instillation the incubation period was regularly increased and ranged from 2 to 6 days. Brain suspension from hamsters infected intranasally in the 4th passage titred $10^{-4.77}$ by intracerebral injection, whereas the same form of material from the 7th passage titred $10^{-3.66}$ by the same route. These titrations were made with 0.03 cc of the various brain suspension dilutions. Spinal cord of hamsters of the 300th intracerebral passage, injected in 10% suspension intracerebrally, produced typical nervous symptoms. This suspension of virus titred $10^{-2.5}$, as shown in Table II.

Summary. Infected hamster brains from the 300th intracerebral passage and from the 4th intranasal passage titred $10^{-4.77}$, whereas infected brains from the 7th intranasal passage titred $10^{-3.66}$. Positive Newcastle chicken serum neutralized the virus from the 245th and 300th hamster passage, whereas normal chicken serum had no effect.

Nasal instillation trials were conducted from the 16th and 300th hamster passage but were not successful (except for the 1st passage) until after the 245th passage.

The incubation period of hamsters infected by intranasal instillation is longer than by intracerebral injection. Hamster-adapted Newcastle virus was carried through 9 passages intranasally, and brain material from this 9th nasal passage was neutralized by positive Newcastle chicken serum. Hamsters infected intranasally and intracerebrally showed symptoms of irritability followed by involuntary motor reactions and paralysis.

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17169. The Stability and the Stabilization of Testicular Hyaluronidase.

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Over a period of years, numerous preparations of hyaluronidase have been stored in these Laboratories as bulk powder at room temperature without any appreciable loss in activity. It has been observed, however, that decreases in potency occur when the enzyme is stored under other conditions. We have therefore conducted an investigation of the factors which influence the stability of testicular hyaluronidase. This involved the development of an accelerated aging test and the use of stabilizing agents. The stability of hyaluronidase in bulk, in aqueous solution and in sterile vials has been studied at 10°C, at room temperature and under conditions of accelerated aging. The influence of various bacteriostatic agents on the lability of the enzyme is reported.

Methods. The turbidimetric method of assay employed in this investigation has recently been described in detail.[1] This procedure permits the use of hyaluronates of varying degrees of purity as substrates and gives consistent results because of the stabilization of the protein indicator and the prompt termination of the reaction by heat. The activity of the enzyme is expressed in turbidity reducing units (T.R.U.) which are defined as that amount of enzyme which will hydrolyze hyaluronic acid until it gives a turbidity corresponding to half the amount of substrate employed. Using this method of assay the units have numerical values lower than those obtained by other procedures: *e.g.* 100 TRU

1 Tolksdorf, S., McCready, M. H., McCullagh, D. Roy, and Schwenk, E., *J. Lab. Clin. Med.*, 1949, **34**, 74.