may be recalled that Ramon[10] has shown that the addition of lanolin to diphtheric toxin promotes antitoxin production; there are no data in Ramon's papers as to the duration of antitoxin-formation after the injection of toxin combined with lanolin.

Conclusions. When guinea pigs receive an injection of horse serum combined with a lanolin-like substance and killed tubercle bacilli suspended in oil, sensitization to horse serum is similar to that seen in guinea pigs injected with living tubercle bacilli and horse serum insofar as the reactions to the intracutaneous injection of horse serum lasts longer than 48 hours and may be necrotic. The duration of intense sensitization is remarkably long. Precipitin-titers are higher in guinea pigs immunized with the aid of adjuvants. Under the conditions of these experiments, the use of the lanolin-like substance seems to be essential.

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Concentration of Dilute Solutions of Virus of Mouse Encephalomyelitis by Pervaporation and Ultracentrifugation.*

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The finding of the virus of poliomyelitis in sewage emphasizes the importance of methods for its detection in dilute, aqueous solutions.[1, 2] A satisfactory method should be able to concentrate the virus from dilute, non-infectious solutions into small infectious volumes, free from bacteria and chemical and bacterial toxins. To attain this end the experiments to be described represent an attempt to exploit the principles of pervaporation and ultracentrifugation.

Mouse encephalomyelitis virus (Theiler's mouse poliomyelitis virus) was chosen because its properties with respect to filtration, thermal inactivation, and stability to ether, and its capacity of producing an infectious myelitis in mice indicate a close similarity to

10 Ramon, G., Lemetayer, E., and Richou, R., *Rev. d'Immunol.*, 1937, **3**, 202.

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† National Research Council Fellow in the Medical Sciences, 1941-42.

1 Paul, J. R., Trask, J. D., and Gard, S., *J. Exp. Med.*, 1940, **71**, 765.

2 Gard, S., *J. Exp. Med.*, 1940, **71**, 779; Trask, J. D., and Paul, J. R., *J. Exp. Med.*, 1942, **75**, 1.

human poliomyelitis virus.[3, 4] Moreover Olitsky and Schlesinger[5] have recently shown that in mice there are no essential histological differences between the neural lesions produced by Theiler's virus and those caused by the Lansing strain of poliomyelitis.

Methods. The virus used in these studies was the FA strain (which was kindly supplied to us by Dr. Max Theiler of the International Health Division of the Rockefeller Foundation). A 10% pooled brain extract, kept at −64°C in a solid CO_2 refrigerator, served as the source of the virus. For each experiment an aliquot of this extract was thawed, and a 4000 cc dilute, non-infectious solution of the virus (10^7-fold dilution of brain) made with distilled water. In all cases the volume of inoculum used was 0.03 cc; it was injected intracerebrally into Swiss mice (28-32 days old).‡ The mice were observed for 20 days, and the time noted for the onset of encephalitic or myelitic symptoms. In the calculation of the average incubation time the survivors were considered to have remained well only 20 days.

Control titrations of each lot of virus were made to account for any loss in viral activity because of the time necessary for the concentration procedures. These control solutions were kept at 8 to 10°C until the concentrates were prepared. Both controls and concentrates were then injected within the same hour into groups of mice.

Criteria for the detection of the virus consisted of (a) the development (after a variable incubation period of at least several days) of weakness, ataxia, and, if death did not ensue, of paralysis of extremities and neck; and (b) the failure of brains of animals killed while sick to exhibit any bacterial growth when cultured on blood agar plates. Occasionally intracerebral passage of brain suspensions from sick animals was made, and histological sections of the brains and cords of sick mice were examined for lesions.

Concentration by Pervaporation. The passage of water through cellophane tubing placed before a fan (pervaporation) has been used for the concentration of protein from serum.[6] In the present experiments 4 cellophane tubes, 4.2 cm in diameter and 100 cm in

[3] Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49.

[4] Olitsky, P. K., *J. Exp. Med.*, 1940, **72**, 113.

[5] Olitsky, P. K., and Schlesinger, R. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 79.

‡ All mice used were obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine.

[6] Hartman, F. W., *J. Am. Med. Assn.*, 1940, **115**, 1989.

length, were filled with 4000 cc samples of the 10^7-fold dilutions of the virus-infected brains. These were placed before 2 electric fans either in an air-conditioned room maintained at 23°C and 45% humidity, or in the laboratory at 18-23°C and about 30% humidity. Because of the rapid evaporation, the temperatures of the virus solutions were about 8 to 9° below that of the room temperature.

A typical experiment presented in Table II, indicates that the virus may be concentrated without inactivation by this method. Etherization (8%) appears to stabilize the virus during the time necessary for the concentration to take place, and the ether does not interfere with the passage of water through the membrane. In six experiments it has been possible to concentrate 4000 cc samples of inactive solutions into small active volumes (40 to 80 cc) in from 2 to 3 days.

Concentration by Ultracentrifugation. By means of the air-driven ultracentrifuge, it is possible to sediment the virus from extracts of infected brains (Table I). Gard and Pedersen[7] have utilized this technic in their isolation of the FA virus. The procedure adopted in the present work is illustrated in the following experiment.

October 13, 1941: 40 cc of a 10% brain suspension in 0.05 M sodium phosphate buffer at pH 8.0§ were centrifuged in an angle centrifuge at 3000 rpm for 15 minutes. The opalescent supernatant fluid was then spun in a Beams ultracentrifuge at 45,000 rpm (142,000 times gravity) for 60 minutes. The 10° angle rotor of Masket[8] was used. After the run the water-clear supernatant fluid was collected, and the surface of the pellets and the sides of the tubes were washed 3 times with distilled water. The pellets were then extracted with 40 cc of pH 8.0 buffer, and the aggregated material was subsequently removed in the laboratory centrifuge. The data in Table I indicate that this pellet extract was equal in infectivity to the original 10% brain extract. Thus practically all of the virus must have been sedimented in the ultracentrifuge. The residual infectivity found in the 40 cc of the supernatant fluid reveals that only about 1% of the original activity failed to be sedimented.

Inasmuch as the above experiments show that the FA virus in brain extracts is large enough to be sedimented, ultracentrifugation was employed as a step in the method for concentrating dilute solu-

[7] Gard, S., and Pedersen, K. O., *Science*, 1941, **94**, 493.

§ A buffer at pH 8.0 was used in view of the fact that Theiler and Gard[3] found the FA virus to be most stable at this hydrogen ion concentration.

[8] Masket, V., *Rev. Sci. Inst.*, 1941, **12**, 429.

TABLE I.
Ultracentrifugation of Mouse Encephalomyelitis Virus.

	Dilution of test sol.	Morbidity ratio*	Avg incubation time (days)†
Original extract (40 cc)	10^0	6/6	2.3
	10^1	15/15	4.8
	10^2	8/8	6.5
	10^3	8/8	7.3
	10^4	7/8	11.0
Pellet " "	10^1	7/7	4.6
	10^3	13/13	7.5
Supernatant fluid "	10^0	8/8	5.5
	10^2	9/13	14.1

*Denominator indicates number of mice inoculated intracerebrally; numerator indicates number of mice which developed symptoms compatible with encephalomyelitis.

†Incubation period to the onset of symptoms.

tions of the virus. After concentration by pervaporation of 4000 cc samples of 10^7-fold dilutions of infected brains to from 40 to 80 cc, the resulting solutions were spun at 36,000 rpm for 100 minutes. No visible pellets were present in the lusteroid centrifuge tubes. The supernatant fluid was poured off; and the walls of the tubes were washed thoroughly (rubber-tipped glass rod) with a total volume of 1.0 cc or less of 0.05 M sodium phosphate buffer at pH 8.0. If the pervaporation had been allowed to take place in the presence of ether, the latter was removed *in vacuo* before ultracentrifugation.

The results of 4 experiments, one of which is cited in Table II, indicate that the FA virus even in dilute solutions may be sedimented to yield an active, although invisible, lining on the bottom surface of the ultracentrifuge tubes. The active material in 4000 cc samples of 10^7 brain dilutions has been concentrated into fractions less than 1 cc in volume.

TABLE II.
Concentration of Mouse Encephalitis Virus by Pervaporation and Ultracentrifugation.

December 6, 1941. 4000 cc of a solution representing a 10^7 fold dilution of infected brains were concentrated by pervaporation to 50 cc. The pervaporation concentrate was spun at 36,000 R.P.M. (92,000 times gravity) for 100 minutes, and the pellet extracted with 0.4 cc of 0.05 M phosphate buffer, pH 8.0.

	Morbidity ratio	Avg incubation time
Control 10^3 fold dilution of brain	7/7	8.1
" 10^5 " " " "	8/8	11.3
" 10^7 " " " "	0/7	
Pervaporation concentrate	7/8	9.9
Ultracentrifugal supernatant fluid	2/9	19.2
" pellet extract	8/9	8.5

Summary. Mouse brains infected with the FA strain of Theiler's mouse encephalomyelitis virus have been diluted ten million times so that the solutions were no longer infective. By means of pervaporation it has been possible to concentrate 4000 cc samples of such inactive solutions into volumes of 40 to 80 cc. The virus present in the pervaporated solutions has been further concentrated by sedimentation in the ultracentrifuge into active fractions less than 1 cc in volume.

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Identification of Amines in Anaerobic Kidney Extracts.

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In recent studies on the formation of pressor amines by the kidney, Holz and Heise[1, 2] found that under anaerobic conditions, renal extracts could transform l-tyrosine and l-dihydroxyphenylalanine (l-dopa) into their corresponding amines. Bing and Zucker[3] reported that the decarboxylating enzymes in the kidney are specific for certain amino acids and that species differences exist. It was also found that the perfusion of kidneys with l-dopa, under conditions of oxygen lack, would result in the formation of a pressor substance, presumably hydroxytyramine.[4] Victor, Steiner and Weeks[5] prepared an anaerobic extract of kidneys that had marked pressor activity. Schroeder and Adams[6] found that tyrosinase would reduce the blood pressure of hypertensive dogs and rats, and would also abolish the pressor activity of Victor's extract, suggesting that the pressor activity of extract was due to amines. In view of this work it is of interest to determine the presence of amines in the extract of Victor, Steiner and Weeks, and to identify the amines if possible.

Methods. Extracts of ground hog kidneys were prepared ac-

1 Holz, P., *Klin. Woch.*, 1937, **16**, 1561.

2 Holz, P., and Heise, R., *Arch. Exp. Path. und Pharm.*, 1939, **191**, 87.

3 Bing, R. J., and Zucker, M. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 343.

4 Bing, R. J., *Am. J. Physiol.*, 1941, **132**, 497.

5 Victor, J., Steiner, A., and Weeks, D. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 767.

6 Schroeder, H. A., and Adams, M. H., *J. Exp. Med.*, 1941, **73**, 531.