tuberculin employed exerted a marked toxicity on the monocytes of all preparations including explants from normal animals. This confirms the observations of Shwartzman.[10]

Discussion. It is a reasonable assumption that if the *in vitro* cytotoxic effect of tuberculin on the tissues of sensitive animals is the outcome of the interaction of a sessile antibody with specific antigen, specific desensitization should accomplish saturation of antibody and consequent abolition of this cytotoxic sensitivity.

The fact that the desensitization treatment used in the present experiments did not completely abolish *in vitro* cytotoxic sensitivity does not necessarily militate against the theory that this sensitivity is dependent upon antibody. One possibility is that desensitization of the animals was not complete. It would be of interest to determine whether more complete desensitization of the animals can be accomplished and also whether desensitization of tissues could be accomplished *in vitro* by exposure to a series of increasing concentrations of tuberculin.

It does not seem likely that a blocking antibody was responsible for the results obtained in the present experiments in view of the speed with which a refractory state developed in the tissues of the animals given desensitization treatment. Above all, the specificity of the effect of desensitization makes it quite certain that antibody is responsible for the *in vitro* tuberculin sensitivity of tissue explants of tuberculin-sensitive animals.

Summary. Experiments are presented which show that the *in vitro* effects of tuberculin in suppressing the initial migration of leucocytes from splenic explants of tuberculous guinea pigs is markedly reduced by specific desensitization.

This finding indicates that the cytotoxic effect of tuberculin on tissue explants of tuberculin-sensitive animals is the result of the interaction of a sessile antibody with specific antigen.

[10] Shwartzman, G., *Arch. Path.*, 1928, **6**, 773, 790.

16501

Purification of Mouse Poliomyelitis Virus by Methanol Precipitation at Low Temperatures.*

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(Introduced by M. B. Visscher.)

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Purification of viruses, which is essentially the separation of viral protein from non-viral protein, has been the object of a large number of investigations. In an extensive review Beard[1] has critically examined the present status of the purification of animal viruses which principally involve ultracentrifugal procedures. Cox, Van der Scheer, Aiston, and Bohnel[2] were the first to report the purification and concentration of influenza and other viruses by using organic solvents to precipitate the proteins from solutions. Recently Gollan[3] has adapted the procedures of Cox *et al.*[2] to the purification of a mouse strain of MM poliomyelitis virus from mouse brain.

This investigation concerns the purification of Theiler's GDVII strain of mouse encephalomyelitis virus by methanol precipitation, and the comparison of methanol precipitates from infected mouse brain with those obtained from

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[1] Beard, J. W., *J. Immunol.*, 1948, **58**, 49.

[2] Cox, H. R., Van der Scheer, J., Aiston, S., and Bohnel, E., *J. Immunol.*, 1947, **56**, 149.

normal uninfected mouse brain.

Materials and methods. The procedures used in this investigation were similar to those developed by Cox *et al.*[2] in the purification of influenza viruses and modified by Gollan[3] in the purification of MM poliomyelitis virus. Briefly, the procedures included freezing and thawing, removal of some of the tissue lipid with ether, and the precipitation of virus protein with methanol at low temperatures. In this investigation Theiler's GDVII strain of spontaneous mouse encephalomyelitis virus was used.

Mice were infected with Theiler's virus by intracerebral inoculation. At the height of paralysis the mice were killed and their brains were recovered aseptically. The brains were weighed, ground in a mortar, and the resultant emulsion was diluted to a 20% suspension in distilled water. The tissue suspension was then subjected to additional grinding in a Potter Elvehjem homogenizer, after which it was frozen over night at –20°C. After thawing at room temperature, the suspension was centrifuged for 1 hour at 5,000 R.P.M. at 4°C. The supernatant fluid was withdrawn, frozen, thawed, and centrifuged at the same rate and temperature. The supernate was again withdrawn, dispensed in 20 ml aliquots, and stored at –20°C until used. The material obtained at this point will be referred to as crude virus suspension.

To purify the crude virus, some lipoidal material was first removed by ether extraction. Twelve ml of ethyl ether was added to each 20 ml of crude virus, and the mixture was shaken and then centrifuged at 5,000 R.P.M. for 30 minutes at 4°C. The lipid layer was removed and the aqueous virus suspension was subjected to a vacuum to remove the dissolved ether. The suspension was then frozen at –20°C, thawed, and again centrifuged for 30 minutes at 4°C. The supernatant fluid was diluted to 200 ml with 0.1 M phosphate buffer, pH 7, and cooled to –1°C. Absolute methyl alcohol, cooled to –40°C, was added drop by drop by means of a capillary pipette and with constant stirring while the suspension was kept at –1°C (to avoid heat of dilution) until the suspension contained the desired concentration of alcohol. The resultant turbid suspension was held at –4°C for 1 to 4 hours and then centrifuged for 1 hour at 5,000 R.P.M. and at 4°C. The precipitates were washed with phosphate buffers of low pH values in attempts to remove more of the non-viral protein and subsequently were placed in 0.1 M phosphate buffer at pH 7. This was frozen and thawed before the precipitate went into solution. The final suspensions appeared water-clear.

The infectivity of the crude and purified virus suspensions was tested by preparing serial 10-fold dilutions and by inoculating 0.03 ml of each dilution into each of 3 Swiss albino mice intracranially. The infective titers were determined by the 50% end-point method of Reed and Muench.[4] Total nitrogen determinations were made by the micro-kjeldahl method of both crude and purified virus suspensions. In order to obtain comparative infectivity and nitrogen values, all calculations were made on the basis of equal volumes of the crude and purified fractions.

Normal uninfected mouse brains were subjected to the same purification procedure, in which total nitrogen determinations and activity tests were also made.

The factors that might possibly be influential in affecting the activity and the relative purity of the final purified product were thought to be concentration of methanol, the pH of the suspension from which the methanol precipitation was made, and the pH and molarity of the buffers used to wash the methanol precipitates. Variations that undoubtedly would occur as a result of temperature variations were not investigated. Methanol was used in concentrations of 25% and 30%. Suspensions were precipitated at pH 7 and pH 6. The precipitated proteins were washed with phosphate buffers of pH 4.6, 5.1, and 5.6 with molar concentrations of .01 M and .02 M.

30% methanol, precipitation at pH 7. Purification, in terms of the percentage of total

[3] Gollan, F., PROC. SOC. EXP. BIOL. AND MED., 1948, in press.

[4] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

TABLE I.
Purification of Theiler's Virus by Methanol Precipitation.

Exp. No.	Methanol conc.	pH ppt'n	pH wash	Mg N per ml crude	Mg N per ml purified	% removal N	Titer (LD_{50}) crude	Titer (LD_{50}) purified	MLD per mg N
A1	30%	7	—*	1.52	.0386	97.5	$10^{-8.5}$	$10^{-7.5}$	1.6×10^8
A2	"	"	4.6	1.52	.33	78.0	$10^{8.5}$	$10^{-8.5}$	1.9×10^8
F15	"	"	5.1	2.42	.026	98.9	$10^{8.5}$	10^{-6}	7.7×10^6
F16	"	"	"	2.42	.027	98.9	$10^{8.5}$	10^{-7}	7.4×10^7
D8	"	"	5.6	0.49	.0046	99.0	$10^{6.7}$	$10^{7.2}$	6.8×10^8
B4	25%	"	5.1	1.60	.195	87.8	$10^{7.2}$	10^{7}	1.0×10^7
C5	"	"	"	2.51	.018	99.5	$10^{5.7}$	10^{6}	1.7×10^7
D9	"	"	"	0.49	.0098	97.1	$10^{6.7}$	$10^{6.2}$	3.2×10^7
H21	"	"	"	1.38	.0256	98.2	$10^{6.5}$	$10^{6.2}$	1.2×10^7
C6	"	"	5.6	2.51	.112	95.3	$10^{5.7}$	$10^{6.2}$	2.8×10^6
J1	30%	6	5.1	1.26	.123	91.2	10^{6}	10^{6}	
Normal brain	"	7	"	1.56	.018	98.6			
"	"	6	"	1.56	.085	94.6			

* Precipitate was not washed.

nitrogen removed, varied from 97.5% to 99%. This variation occurred irrespective of whether the pH of the buffers used to wash the precipitates was 5.1 or 5.6. The activities of the purified samples, based on infectivities of equivalent volumes of crude and purified virus suspensions, varied from 0 to 100-fold. When crude suspension was only diluted to 40 ml and when this methanol precipitate was washed with phosphate buffer at pH 4.6, only 78.2% of the total nitrogen was removed but all of the virus was recovered. In 2 parallel purifications it was observed that remarkably close checks were obtained in the nitrogen determinations. The results are shown in Table I.

25% methanol, precipitation at pH 7. Again the percentage of nitrogen removed was subject to variation, ranging from 87.8% to 99.5% when buffers at pH 5.1 and 5.6 were used to wash the precipitate. The activities in this case, however, consistently remained undiminished, and it appeared that approximately 100% of the virus was always recovered.

30% methanol, precipitation at pH 6. On the basis of one determination slightly less nitrogen appeared to have been removed, although the methanol precipitate appeared to be considerably heavier. There was approximately 100% recovery of the virus activity, as shown in Table I.

Normal brain precipitated with 30% methanol at pH 6 and pH 7. It was interesting to observe that when normal uninfected mouse brain was subjected to the same purification procedures, the amount of nitrogen recovered in the purified product was not significantly different from some of the nitrogen values obtained in purified infected brain. Precipitation at pH 7 yielded slightly purer products. The results are presented in Table I. As would be expected, normal brain did not exhibit virus activity.

Yields. The activities of each determination appeared to vary widely in some cases. The inaccuracy of the animal titrations may be responsible in part for this variation, although it was noticed that when the methanol precipitates were held at –4°C for 4 hours,

increased yields were obtained.

Conclusions. Although at first glance the data may appear rather inconsistent, closer inspection reveals that the results are consistent when consideration is given to the experimental error involved in some of the methods. The variation in the nitrogen determinations of the crude virus suspensions were thought to be due in turn to expected variation in the amount of protein removed in the freezing and thawing steps. Estimations of the degree of purity have been presented in terms of the amount of nitrogen eliminated from the crude sample. Since purified normal brain gave nitrogen values which bore a close similarity to those obtained with purified infected tissue, it can be seen that the so-called purified fractions still contain a large amount of normal brain protein. There is no doubt that the non-viral protein greatly overbalances the viral proteins in the purified fraction. However, a relatively large amount of protein is removed during the procedure, as compared with the amount removed in the ultracentrifugation methods of purification cited by Beard.[1] The activities of crude and purified virus fractions, with a few exceptions, remain fairly constant, indicating that most of the active virus is recovered. Although the titrations may not be accurate enough to determine the exact yield, they indicate that in many of the determinations 100% of the virus appeared to be recovered. The number of infectious units of virus per milligram of nitrogen depends, in part, on the original titer of the virus, and therefore is a relative value. When the methanol precipitate was held at −4°C for 4 hours the yield of virus was greater. Apparently no significant difference resulted from the use of 25% or 30% methanol. Also, the use of buffers at pH 5.1 or pH 5.6 to wash the precipitates did not seem to appreciably alter the results. One step that did result in the reduction of nitrogen was the immediate freezing of the final precipitate at pH 7 before it went into solution. Upon thawing, insoluble protein was removed by centrifugation.

16502 P

Secretion of Fructose and Citric Acid in Transplants of Rat Seminal Vesicle and Coagulating Gland.*

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Earlier investigations[1,2] established fructose and citric acid as secretory products of certain accessory glands of reproduction in several mammalian species; in rats the first is found in the coagulating gland and dorsal prostate, and the latter in seminal vesicle proper and ventral prostate. Further it was demonstrated that the secretion of fructose and citric acid depends upon and is regulated by, the male sex hormone; following castration there was gradual disappearance of both substances though at varying rates, and after treatment with testosterone their level was restored.[3] In the present study more evidence has been accumulated which emphasizes the effect of testosterone upon the accessory reproductive organs as regards their ability to produce fructose and citric acid. Using subcutaneous

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1 Mann, T., *Biochem. J.*, 1946, **40**, 481.

2 Humphrey, G. F., and Mann, T., *Nature*, London, 1948, **161**, 352.

3 Mann, T., and Parsons, U., *Nature*, London, 1947, **160**, 294.