

tissue. In Ringer's solution, at a protein concentration of 1 to 1.5 mg per cc, infectivity does not diminish within 7 days at 5°C and the sedimentation pattern is unchanged for periods of several weeks. In 0.9% NaCl or Ringer-buffer salt solution (NH₄OH-NH₄Cl, 0.05 M), however the protein loses much infectivity in 96 hours. In the latter solution the boundary may remain intact for 3 weeks when infectivity has diminished 6 decimal dilutions or more.

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Immunization with Non-Infectious Formalin Derivative of Purified Equine Encephalomyelitis Virus Protein.*

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Previous ultracentrifugal studies¹ of crude equine encephalomyelitis chick vaccines² have failed to yield definite evidence of the character of the immunizing principle. Information concerning its probable nature has been sought in the present work by study of the purified virus protein treated with formaldehyde.

Purified protein (Eastern strain),³ dissolved in Ringer's solution, pH 8-9, 2.0 mg per cc, was treated with various concentrations of CH₂O. Before exposure to CH₂O, one m.i.u.⁴ of protein was 10^{-13.5} g. When the concentration of CH₂O was less than 0.01 M, inactivation was not always complete in 2 weeks, and tests for immunizing capacity were not made. With other concentrations of CH₂O used, inactivation was complete in the time shown in Table I, as judged by the failure of 10^{-5.5} g of protein to infect mice.

The effect of CH₂O on the protein molecules was somewhat similar

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¹ Taylor, A. R., Sharp, D. G., Finkelstein, H., and Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 462.

² Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 490.

³ Taylor, A. R., Sharp, D. G., Finkelstein, H., Beard, D., and Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 648.

⁴ Cox, H. R., and Olitsky, P. K., *J. Exp. Med.*, 1936, **63**, 745.

FORMALIN PURIFIED EQUINE ENCEPHALOMYELITIS VIRUS 651

TABLE I.
Immunization of Guinea Pigs with the Non-Infectious Formaldehyde-treated
Equine Encephalomyelitis Virus Protein.

CH ₂ O molar	Inactivation time, days	pH	Total protein* per guinea pig, mg	Test for immunity†	
				Vaccinated	Control
.01	7	7.0	.125	3/3‡	0/5
			.250	6/6	"
.02	4	6.5	.125	4/4	"
			.250	5/6	"
.04	3	6.3	.125	3/4	"
			.250	4/6	"

*Given subcutaneously in 2 equal doses in 0.1 cc at interval of 1 week.

†500 to 1,000 mouse infectious units of virus given intracerebrally.

‡Numerator = survivals; denominator = No. of test guinea pigs.

to its action on tobacco mosaic virus protein.⁵ With CH₂O 0.01 M or less, the protein remained in solution but lost slightly in homogeneity, showing a slightly diffuse boundary with a sedimentation pattern similar to that of the untreated protein³ persisting with little change for more than 3 weeks. In 0.02 M and higher concentrations of CH₂O, the protein rapidly became quite inhomogeneous and increasingly insoluble, losing the definite boundary in 2-4 days. In no instance was there evidence of molecular disruption to small soluble protein fragments of uniform size, nor was there evidence of the component $S_{20}^{\circ} = \text{ca } 60 \times 10^{-13} \text{ cm sec}^{-1} \text{ dynes}^{-1}$ previously described.⁶

The non-infectious, soluble though slightly inhomogeneous protein derivative with 0.01 M CH₂O has immunized all guinea pigs receiving it, as shown in the typical experiment in Table I. The more inhomogeneous fragments resulting from 0.02 M and higher concentrations of CH₂O definitely immunize less well.

The results show that the immunizing material derived with CH₂O from the pure protein consists of an inhomogeneous group of degradation products nearly the size of the intact protein or somewhat smaller. With time, as the CH₂O reacts with the protein, there is a progressive increase in inhomogeneity and insolubility of the products when the pH is allowed to drop from the initial value of 8-9 to pH 7.0 to 6.3, as is shown in Table I. It is probable that the immunizing material derived with CH₂O from the virus in crude vaccines is of the same nature, and this probability has been empha-

⁵ Wyckoff, R. W. G., Biscoe, J., and Stanley, W. M., *J. Biol. Chem.*, 1937, **117**, 57.

⁶ Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 89.

sized by the isolation of such a material with a definite, though slightly diffuse boundary from crude vaccines less than 48 hours old (infectivity diminished at least 5 decimal dilutions). Older vaccines fractionated at pH below 7.0, however, yield materials that show only suggestions of a boundary or completely diffuse shadows in the sedimentation pattern where boundaries corresponding to this approximate particle size should appear.

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Water-Soluble Antihemorrhagic Compounds.

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The interest in vitamin K analogues suitable for parenteral medication is evident from the current literature. Usually patients with hypoprothrombinemia due to obstructive jaundice receive orally vitamin K and bile or bile salts. Frequently these latter substances are badly tolerated and it seems that they are not needed, if the antihemorrhagic material is administered parenterally. In cases of severe hepatic insufficiency vitamin K orally is apparently not beneficial, but intravenous injections of a vitamin K analogue, such as e.g. 2-methyl-1,4-naphthoquinone, may be effective in raising the prothrombin content of the blood, as reported by Rhoads and Fliegelman.¹

The first synthetic antihemorrhagic substance to be employed parenterally in the clinic was phthiocol.^{2, 3} Relatively large doses (50-100 mg) are required, because the potency of this compound is small.⁴ Flynn and Warner⁵ determined that phthiocol had but 1/500 the activity of methyl-naphthoquinone, and we had reported pre-

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¹ Rhoads, J. E., and Fliegelman, M. T., *J. Am. Med. Assn.*, 1940, **114**, 400.

² Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R., *J. Am. Med. Assn.*, 1939, **113**, 380.

³ Butt, H. R., Snell, A. M., and Osterberg, A. E., *Proc. Staff Meeting Mayo Clin.*, 1939, **14**, 497.

⁴ Ansbacher, S., and Fernholz, E., *J. Am. Chem. Soc.*, 1939, **61**, 1924.

⁵ Flynn, J. E., and Warner, E. D., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 190.