and an excessive number of eosinophils in the bronchial walls, a finding not uncommon in control rats. The conclusion is that chronic toxicity of penicillin in rats is not demonstrable with the doses used.

6. *Percutaneous Absorption.* Penicillin was applied to the freshly clipped abdominal skin of rabbits by gentle rubbing on two occasions. For the first trial, 1000 units of penicillin were dissolved in 5 cc of monoethylether of diethylene glycol (carbitol) and applied over 15 minutes; for the second, 5000 units were dissolved in 2.5 cc of the same vehicle and applied over 1 hour. In neither case was there any measurable penicillin in the blood at the end of the period of application. A mixture of 4000 units of penicillin in 0.05 gm of zephiran was administered sublingually to a man. No penicillin appeared in the urine during the next 45 minutes.

Summary. In spite of the lethal effect which penicillin exerts on bacteria, power to alter or poison normal physiological processes significantly has not been demonstrated. It has no demonstrable effects on contraction of the isolated aorta or pregnant uterus, or on the vomiting reflex of pigeons; it produces no serious meningeal irritation; and, in small doses, it produces no chronic tissue changes in rats. Although the penicillin used inhibited the liberation of oxygen from hydrogen peroxide by catalysis, the inhibition persisted after inactivation of the penicillin and, therefore, may be due to some other substance in the impure products. Monoethylether of diethylene glycol did not promote its percutaneous absorption.

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Collodion Particle Adsorption of Equine Encephalomyelitis Virus.

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The flocculation of talcum powder or of organisms such as *Proteus vulgaris* or *Eberthella typhi* in *Escherichia coli* filtrate upon the addition or anti-coli serum was reported as early as 1898 by Nicolle.[1] That protein is adsorbed upon various particles has been shown by changes in physical properties such as isoelectric point or cataphoretic mobility.[2-4] The agglutination of collodion particles as a result of the reaction of the adsorbed protein with specific antiserum was observed by Jones[5] and by Freund.[6]

Collodion particle suspensions have been used in many studies involving their adsorption of antigen.[7, 8] In most cases, protein solutions containing relatively large amounts of antigen have been employed for adsorption upon the collodion particles. The adsorption of virus antigens from tissue suspensions, where the specific antigen is in very small amount, upon cells of *Serratia marcescens* with the demonstration of agglutination by virus antisera has been reported by Roberts and Jones[9] and Weil.[10] Later, Pearson[11] was unable to show agglutination with specific antisera and collodion particles or cells of *S. marcescens* which had adsorbed material from

1 Nicolle, C., *Ann Inst. Pasteur.*, 1898, **12**, 161.

2 Coulter, C. B., *J. Gen. Physiol.*, 1921, **3**, 309, 513.

3 Northrup, J. H., and DeKruif, P. H., *J. Gen. Physiol.*, 1922, **4**, 655.

4 Loeb, J., *J. Gen. Physiol.*, 1923, **5**, 395.

5 Jones, F. S., *J. Exp. Med.*, 1927, **46**, 303.

6 Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 65.

7 Zozaya, J., *J. Exp. Med.*, 1932, **55**, 325.

8 Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 365.

9 Roberts, E. C., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 75; 1942, **49**, 52.

10 Weil, A. J., *J. Immunol.*, 1942, **45**, 187.

11 Pearson, H. E., *J. Immunol.*, 1944, **49**, 117.

TABLE I.
Agglutination of W.E.E. "sensitized" collodion particles.

Series	Source of virus for sensitization of collodion particles	Antiserum used*	Agglutination: Serum dilutions†					
			1:2	1:4	1:8	1:16	1:32	Saline
1	Allantoic fluid	W.E.E.	....	+	+	+	—	—
	"	Normal	+	+	—	—	—	—
2	"	W.E.E.	—	—	—	—	—	—
	"	Normal	—	—	—	—	—	—
3	"	W.E.E.	+	+	—	—	—	—
	"	Normal	+	—	—	—	—	—
4	"	W.E.E.	+	—	+	—	—	—
	"	Normal	—	+	—	—	—	—
5	Chick embryo	W.E.E.	—	—	—	—	—	—
	"	Normal	—	—	—	—	—	—
6	Mouse brain	W.E.E.	—	—	—	—	—	—
	"	Normal	—	—	—	—	—	—

* The sera are designated according to the nature of the mouse brain used to immunize the rabbits.

† Agglutination is recorded as positive whether partial or strong. Complete agglutination was not observed.

influenza, poliomyelitis, St. Louis encephalitis or eastern equine encephalomyelitis virus suspensions. We have attempted to adsorb the virus of western equine encephalomyelitis (W.E.E.) upon collodion particles and to cause their agglutination by specific hyperimmune rabbit sera.

Methods. Collodion particle suspensions were prepared according to Cannon and Marshall's[8] modification of Loeb's[12] method. Three sources of virus were used: allantoic fluid of infected 11-day chick embryos; clear supernate from suspensions of 10% infected chick embryo in 0.8% saline; and clear supernate from 1% infected mouse brain suspension in 0.8% saline containing 1% rabbit serum. These tissues were harvested under conditions in which the virus was close to maximal titer. No attempt was made to control pH during adsorption; buffers were avoided since they may cause the precipitation of collodion particle suspensions.[8] The collodion particles were added to the virus suspensions in amounts varying from one to two volumes per volume of virus suspension, and of a turbidity which when diluted twice would be comparable to tube 3 or 4 of McFarland's standards.[13] After standing in the ice-box overnight, or sometimes 36 hours, the collodion particles were washed 3 times in 0.8% saline. They were then diluted to a turbidity equal to tube 5 of McFarland's standards and 0.5 ml introduced into 0.5 ml of each of a series of double dilutions of anti-W.E.E. or of control antiserum.

Hyperimmune sera were prepared by repeatedly injecting rabbits with mouse brain suspensions of a mouse-adapted strain of W.E.E.* and control antisera were prepared by similarly inoculating rabbits with suspensions of normal mouse brain. We used the allantoic fluid from W.E.E. infected chick embryos because the low protein[14] and high virus content[15] of this tissue might be expected to reduce non-specific reactions to a minimum.

Attempts to demonstrate agglutination with virus antigen-antibody systems. Collodion particles which had been treated with virus-containing allantoic fluid gave agglutination with about the same dilutions both of W.E.E. and of control antisera (see Table I).

Infected chick embryo suspensions and mouse brain suspensions were then used as virus sources. 11-day-old chick embryos moribund with W.E.E. were triturated in 0.8 % saline to give a 10% suspension, and the clear supernate obtained after centrifuging for one hour at 4000 rpm in an angle head, was used. The mouse brain suspension was pre-

[12] Loeb, J., *J. Gen. Physiol.*, 1922, **5**, 109.

[13] McFarland, J., *J.A.M.A.*, 1907, **49**, 1176.

* Obtained originally from Dr. Schlessinger at the Rockefeller Institute.

[14] Needham, J., *Chemical Embryology.* The Macmillan Co., New York, 1931.

[15] Bang, F. B., *J. Exp. Med.*, 1943, **77**, 337.

TABLE II.
Amount of virus adsorbed by collodion particles.

Dilution of materials	W.E.E.C.E.S.* adsorbed with collodion particles	W.E.E.C.E.S. control
10^{-4}	2/2†	2/2
10^{-5}	2/2	2/2
10^{-6}	0/2	1/2
10^{-7}	0/2	0/2

*Western equine encephalomyelitis chick embryo suspension.

†Numerator = number of 10-day chick embryos infected.

Denominator = number of chick embryos inoculated.

pared as is the complement fixing antigen of Casals and Palacios.[16] The brains of mice moribund with W.E.E. were triturated to give a 10% suspension in 0.8% saline containing one percent rabbit serum; after freezing and thawing 5 times and centrifuging as with the chick embryo suspension, the clear supernate was employed.

After adsorption from the chick embryo suspension or the mouse brain suspension, the collodion particles gave no agglutination with either the W.E.E. hyperimmune serum or with the control antisera (see Table I).

Tests upon antisera. The hyperimmune sera were tested for neutralizing antibody by introducing virus dilutions into undiluted serum, and about 10^4 mouse protective units per ml were observed.

Complement fixation tests with the Casals and Palacios[16] mouse brain antigen and the antisera inactivated for half an hour at 65° C to remove non-specific antibody to brain protein showed that these W.E.E. antisera fixed complement at dilutions of the order of 1:40; the normal mouse brain antisera gave incomplete fixation at dilutions of 1:8 or less.

Amount of virus adsorbed by collodion particles. Since the agglutination reactions were essentially negative although the sera contained antibody to W.E.E., we wondered whether sufficient virus had been adsorbed on the collodion particles to give a positive reaction. Titration of virus suspensions before and after adsorption with collodion particles indicated that the virus was only slightly adsorbed.

[16] Casals, J., and Palacios, R., *Science*, 1941, **93**, 162.

TABLE III.
Effect of decreasing percentage of ovalbumin in 2% protein solution during sensitization of collodion particles. Overnight exposure.

% of protein as ovalbumin	Agglutination: anti-ovalbumin serum dilutions 1:20	1:40	1:80	1:160	1:320	Saline
100	+	+	+	±	—	—
66	+	+	±	—	—	—
40	+	+	+	—	—	—
20	+	±	±	—	—	—
6	—	—	—	—	—	—
2.5	—	—	—	—	—	—

\+ complete agglutination
± partial agglutination
— no agglutination

The following experiment is typical: Fifteen ml of supernate of a 25% W.E.E. chick embryo suspension in 0.8% saline were added to 7½ ml of collodion particle suspension of a turbidity which when diluted 4 times matched tube 3 of McFarland's standards. This mixture was allowed to stand overnight in the ice box and a portion was then removed and centrifuged 20 minutes at 4000 rpm. The supernatant fluid was treated about half an hour with 1:20,000 Zephiran (a mixture of high molecular alkyl-dimethyl-benzyl-ammonium chlorides)[17] to kill the bacteria introduced with the collodion particles and tenfold dilutions were made in tryptose broth for titration with 10-day chick eggs. A control portion similarly treated but not adsorbed was also titrated. The results of these titrations are recorded in Table II.

In such determinations, the decrease of virus in the suspension treated with collodion particles was little more than that observed in the control suspension. Therefore, the washed collodion particle suspensions when diluted to two or three times the original volume for the agglutination test would contain less than 10 chick embryo infective units per ml. Merrill[18] has accumulated data indicating that for a virus of this size about 10^{11} virus particles per ml would be necessary for a visible immunological reaction. The sensitivity of collodion particle agglutination is not likely to surmount such a differential.

[17] The Personnel of U. S. Naval Laboratory Research Unit No. 1, *Science*, 1942, **96**, 543.

[18] Merrill, M. H., *J. Immunol.*, 1936, **30**, 169.

TABLE IV.
Effect of decreasing percentage of ovalbumin in 2% protein solution during sensitization of collodion particles. One-half hour exposure.

% of protein as ovalbumin	Agglutination with anti-ovalbumin serum dilutions									
	1:2	1:4	1:8	1:16	1:20	1:40	1:80	1:160	1:320	Saline
100	....	....	....	....	+	+	+	+	—	—
75	....	....	+	+	±	±	—	—	—	—
50	....	±	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—	—	—

\+ complete agglutination
± partial agglutination
— no agglutination
.... not examined

Effect of the presence of other proteins in agglutination by adsorption. The effect of the large amount of non-specific tissue protein in suspensions of virus-containing tissue was approached artificially. In 2 experiments, when collodion particles were sensitized in mixtures of ovalbumin and sheep serum, the agglutination titer of an anti-ovalbumin rabbit serum decreased with the amount of ovalbumin in the protein mixture. In the first experiment the collodion particles remained in the protein solution overnight, while in the second the exposure was for half an hour. Results of these experiments are given in Tables III and IV.

There is 0.5% protein by micro-Kjeldahl analysis in the supernate from a 10% W.E.E. mouse brain suspension prepared according to Casals and Palacios[16] method for complement-fixing antigen. 0.02 ml of a 10^{-6} dilution of this suspension was infectious for young mice. Assuming 50 virus particles per mouse infectious dose—special methods are necessary to show one virus-particle infectious[19, 20]—and a mass of 3.7×10^{-15} g per particle, then each ml of suspension would contain about 10^{-6} g of virus. The virus content would then be 0.0001% and the portion of protein as virus would be 0.02%. The decrease in agglutinating titer of an antiserum with the percentage decrease of specific protein in a mixture of proteins indicates that the low percentage of virus in respect to tissue might be responsible for negative results.

Conclusions. Only a small amount of the virus of western equine encephalomyelitis is adsorbed upon collodion particles from the clear supernates of virus-containing tissue suspensions during an overnight period in the icebox. This amount is not sufficient to give a positive agglutination reaction with specific antiserum. Experiments with collodion particles sensitized with protein mixtures indicate that it will probably be necessary to concentrate and partially purify the W.E.E. virus in order to obtain collodion particles adequately sensitized for agglutination with virus antisera.

19 Parker, R. F., and Rivers, T. M., *J. Exp. Med.*, 1936, **64**, 439.

20 Parker, R. F., *J. Exp. Med.*, 1938, **67**, 725.

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Vaccination of Mice Against Typhus.

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Ever since the discovery of its susceptibility by Nicolle *et al.*[1] the guinea pig has been the animal most widely used in typhus studies. Its reaction to established murine strains,