TABLE I. Response of Cotton Rats and Swiss Mice to Rabies Virus. All showed symptoms.*

Min-max incubation period† (in days)	
Cotton rats	Swiss mice
7-8	9-10
7-8	7-8
9-11	7-8
7-8	7-8
12-14	10-11
8-11	7-8
5-6	6-7
5-10	8-11
—	14

* Except cotton rats by intranasal inoculation.
† Incubation period—time between exposure to virus and onset of symptoms.

dead rats or mice were also examined for Negri bodies.

Results and discussion. Table I shows the response of cotton rats and Swiss mice injected with rabies virus by different routes of exposure. All rats and mice exposed, except the cotton rat group exposed by intranasal instillation, died from the disease or were sacrificed when symptoms appeared.

The intranasal group of rats was held 30 days without showing symptoms. They were then sacrificed and touch preparations of their brains examined. No Negri bodies were seen.

No cotton rats or Swiss mice showed symptoms of furious rabies as found in Syrian hamsters infected with this strain. The incubation period in rats and mice was between 5 and 14 days by these various routes of exposure. The cotton rats were as susceptible as the Swiss mice to this V 308 strain of rabies virus. This strain of mice was the one recommended by Webster as being especially susceptible to neurotropic viruses(5).

Summary. A strain of rabies isolated from a dog brain and passaged intracerebrally 2 times in Swiss albino mice and one time in cotton rats has been successfully transmitted to the same rodents by the following routes of exposure: intracerebral, intraperitoneal, intradermal, intramuscular, intracardial, intralingual, oral and rectal. The cotton rats and Swiss mice were equally responsive to this strain by the above routes of exposure. Mice were susceptible to intranasal instillation but cotton rats exposed by this method remained symptom free during a 30-day observation period and there was no evidence of Negri bodies when their brains were examined at the end of that time. This strain of virus, which had produced furious rabies in Syrian hamsters in a previous experiment, produced the dumb form of rabies in the cotton rat and Swiss albino mice.

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An Adsorption Technique for Partial Purification of Japanese Encephalitis Virus in Chick-Embryo Tissue Suspensions. (19188)

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Crude suspensions of chick-embryo tissues infected with the virus of Japanese encephalitis contain a miscellany of tissue materials differing widely in particle size, composition, and physical properties. As a preliminary step toward the isolation of the virus it would be desirable to utilize some simple method to remove most of the extraneous material, leaving a partially purified and considerably more uniform suspension of unimpaired infectivity.

A certain amount of the extraneous material can be removed by differential centrifugation.

The coarse particles which are much larger than the virus can be separated readily in this way and in some instances the tissue material of smaller size can be separated by sedimentation of the virus particle itself. The latter technic can be used with chick-embryo suspensions infected with Eastern, Western or Venezuelan equine encephalomyelitis(1) but has not proved effective in separating Japanese encephalitis virus. The centrifugal separation of Japanese encephalitis virus from infected mouse-brain tissues has also failed to be practicable(2).

In this laboratory experiments have been conducted to explore the possibility of adsorption as a means of removing the smaller tissue constituents of a crude chick-embryo suspension. The adsorbent which has been used is Attaclay SF,* a naturally active, highly sorptive clay which is available in large quantities as a dry finely-divided powder. The clay is an essentially inert, neutral material with an average particle diameter of 400 to 600 mμ. The exceedingly small particle size of this material suggested that it would be a poor adsorbent for the virus particles and would tend to reject them in favor of the smaller constituents of the suspension.

Methods. The virus used in these experiments was the Nakayama strain of Japanese encephalitis. Tissue suspensions were prepared from normal or infected chick embryos homogenized in a Waring Blendor with sufficient phosphate-buffered physiological saline (pH 7.8) to make 20% suspensions. Coarse tissue fragments were removed by centrifugation at 2000 rpm for 10 minutes.

Samples of the supernatant fluid from this centrifugation were treated with various volumes of dry, sterile (autoclaved) Attaclay SF. The volumes used were measured in graduated glass cylinders with tapping to pack the clay particles reproducibly. In each instance the amount of clay was specified as ml of clay per 100 ml of fluid. The fluid and clay were placed in a stoppered centrifuge tube of such size that the volume of the contents was about half the total capacity of the tube. Tube and contents were shaken vigorously by hand for 5 minutes. During the shaking process the temperature of the fluid ranged from 10 to 15°C and within this range did not appear to be a critical factor. After this shaking the tubes were cooled to 2 to 4°C and centrifuged for 15 minutes at 3200 rpm. The clarified supernatant fluid was decanted and if necessary, centrifuged again to remove the last traces of the adsorbent. Before and after treatment with the clay the suspensions were sampled for infectivity titrations and for total nitrogen assay by the micro-Kjeldahl method. Infectivity was determined by the intracerebral inoculation of 0.03 ml doses of suspension using groups of 5 Bagg strain, Swiss albino mice for each serial 10-fold dilution. The infective titer was expressed as —log LD_{50}.

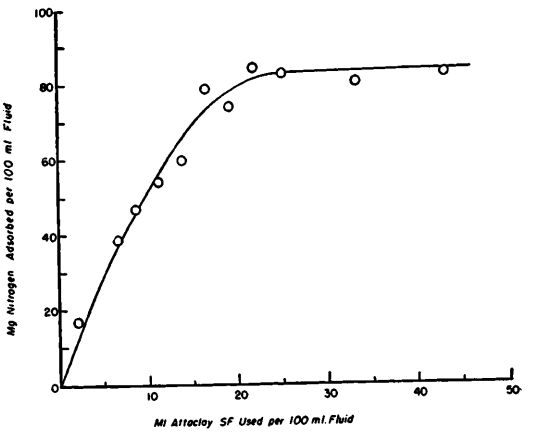

FIG. 1. Adsorption of nitrogenous material from 20% normal chick embryo suspension using varying amounts of Attaclay SF.

Results. The relationship between the quantity of adsorbent used and the amount of nitrogenous material removed from 20% suspensions of normal chick embryo tissue is illustrated in Fig. 1. Twenty ml of clay per 100 ml of fluid appeared to be the optimal amount of adsorbent. This amount removed almost all of the nitrogenous material which could be adsorbed and larger quantities of adsorbent were not appreciably more effective. It was noted that the pink color in the crude suspensions due to hemoglobin was usually removed by as little as 10 ml of clay per 100 ml of fluid.

The effect of the clay treatment upon the

* Supplied by courtesy of Attapulgus Clay Co., Philadelphia, Penna.

TABLE I. Infective Titer and Total Nitrogen Content of Infected 20% Chick-Embryo Tissue Suspensions Before and After Adsorption with Attaclay SF.*

Trial No.	Infective titer ($-\log LD_{50}$) Before	After	Total N (mg/ml) Before	After	% N reduction
1	7.7	6.9	.93	.20	79
2	6.8	6.6	1.01	.24	76
3	8	7.2	1.11	.29	74
4	6.5	6.6	1.36	.45	67
5	5.5	5.2	1.07	.27	75
6	6.2	6.6	1.39	.40	71
7	6.6	7	1.13	.29	74

* 20 ml of dry packed clay used for each 100 ml of tissue suspension.

infectivity and the nitrogen content of the suspensions is summarized in Table I. The data show that it is possible by this method to remove about 75% of the nitrogen content of a crude virus suspension without serious loss of infectivity. It should be pointed out that the figure expressing the infective titer of a virus preparation is inherently of a low order of accuracy ($\pm$ 0.3 log unit). The apparent losses of infectivity in Trials 1 and 3 do not appear significant when considered in context with the apparent gains in Trials 6 and 7. Some of the infective particles are undoubtedly lost in the adsorption process and in the subsequent centrifugation. However, the loss is usually not sufficient to result in a significant decrease in infective titer.

The simplicity and convenience of the technic described suggest that it may have a useful application as a preliminary step in virus purification and in the preparation of vaccines and diagnostic antigens.

Summary. Japanese encephalitis virus in 20% suspensions of infected chick-embryo tissues may be partially purified by adsorption of inert material on particles of Attaclay SF, a naturally-occurring sorptive clay of exceedingly small pore diameter. Removal of about 75% of the nitrogen content of a crude virus suspension may be accomplished without a prohibitive loss of infectivity.

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Excretion of Amino Acids in Cystinuria. (19189)

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The precise nature of the metabolic disturbance responsible for cystinuria remains a matter of conjecture. At one time the disease was considered by many to be linked exclusively to aberrations in the metabolism of the sulfur containing amino acids. Numerous reports have appeared, however, indicating that amino acids other than cystine may be excreted in abnormal amounts by the cystinuric. References to the early literature on the subject may be found in Garrod(1). More recently, Yeh, Frankl, Dunn, Parker, Hughes and Gyorgy(2) employed microbiological assay to analyze the urine of an 8-year-old cystinuric girl and observed a consistent and unusually high level of lysine and arginine excretion. Since then Dent(3) and Dent and Rose(4) have employed paper chromatography to investigate the amino acid content of a number of cystinuric urines. Although the paper chromatographic method is not quantitative, Dent and Rose were led to