from the air. The only data we have found in the literature on this subject are in a paper by Andrewes and co-workers,[3] in which a very brief statement was made to the effect that by the use of bactericidal mists, presumably NaOCl, they found that a few viruses, including influenza, were susceptible to the mist action as judged by their reduced infectivity for mice. No mention was made of the method of testing. Since the most direct and convincing method of determining the antiviral effect of propylene glycol vapor would be protection aaginst air-borne infection, experiments were undertaken toward that end. That spontaneous experimental infection with influenza virus from infected to normal animals does occur by the aerial route has been shown by Andrewes and Glover[4] in experiments with ferrets. Eaton's[5] observation that normal mice may contract influenza from close contact with infected mice provides suggestive though not conclusive evidence for droplet infection.

Our experiments consisted in exposing 5 to 10 gram mice in a 60 liter glass-walled chamber to mouse-adapted influenza virus[6] (the PR8 strain of Francis[7]) in the form of a fine mist produced with a Graeser atomizer.[8] The virus, consisting of dilutions of finely ground infected mouse lungs suspended in broth containing 20 per cent. normal horse serum, was sprayed into the chamber in quantities of 0.2 to 1 cc. The mice were exposed to the virus mist for periods of time ranging from 5 minutes to 1 hour. Exposure of several hundred mice to sprays of 10^{-2} dilution of the virus resulted regularly in extensive consolidation of the lungs and death within 4 to 10 days. Less numerous tests with higher dilutions of virus have shown that pulmonary lesions are produced constantly with amounts as small as 10^{-4} but not all the animals succumb to the infection at this dilution. Still higher dilutions produced pulmonary lesions occasionally, but no deaths.

Experiments on the protective action of propylene glycol vapor were carried out as follows: Mice were placed in a chamber into which propylene glycol vapor was introduced in concentrations of 1 gram of propylene glycol to two to three million cc of air.[9] Then 0.2 to 1 cc of a 10^{-2} dilution of the virus was sprayed into the chamber and the mice exposed for periods of 15 minutes to 1 hour. All these animals remained well, whereas the control mice similarly exposed to the same suspension of influenza virus alone died within 4 to 10 days of influenza and showed extensive consolidation of the lungs from which the virus was recovered. In other experiments the test mice were killed after 6 to 8 days to determine whether they had been completely protected against infection. A protocol of one such test is shown in Table 1. In this particular experiment the mice were shielded from the spray during the introduction of the virus. They were kept in the chambers for 30 minutes. The fact that mice in the propylene glycol atmosphere were exposed in many instances directly to the influenza virus spray, yet failed to contract infection suggests that the interaction between vapor and virus droplets is exceedingly rapid and may approach the rate at which the glycol vapor kills bacteria suspended in air.[10]

TABLE 1

PROTECTIVE ACTION OF PROPYLENE GLYCOL VAPOR

	Amount of virus sprayed into chambers	Result
32 mice in chamber containing glycol vapor 1 : 2,000,000	0.39 cc 10^{-2} dilution	All remained well; killed 8th day; lungs normal*
35 mice in control chamber	0.37 cc 10^{-2} dilution	All died 6–10 days with extensive consolidation of lungs

* One mouse showed a small area of consolidation about one mm in diameter in the left upper lobe.

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PROPERTIES OF THE ISOLATED MACROMOLECULAR COMPONENT OF NORMAL CHICK EMBRYO TISSUE[1]

STUDIES on the ultracentrifugal isolation of the equine encephalomyelitis virus[2] revealed the presence

[3] C. H. Andrewes, *Lancet*, 2: 770, 1940.

[4] C. H. Andrewes and R. E. Glover, *Brit. Jour. Exp. Path.*, 22: 91, 1941.

[5] M. D. Eaton, *Jour. Bact.*, 39: 229, 1940.

[6] We are indebted to Dr. Thomas Francis, Jr., and Dr. Frank Horsfall, Jr., for supplying us with this strain of virus. Their methods for preparation of the virus suspensions have been used in these experiments.

[7] T. Francis, Jr., SCIENCE, 80: 457, 1934.

[8] J. B. Graeser and A. H. Rowe, *Jour. Allergy*, 6: 415, 1935.

[9] The methods employed will be described in detail in a subsequent publication.

[10] When these experiments had been largely completed, the senior author received a manuscript by Drs. Werner Henle and Joseph Zellat, in which they reported the protection of mice against air-borne influenza virus by means of propylene glycol aerosol. These authors adapted the technique previously described by us (SCIENCE, 93: 213, 1941) to their particular experiments with the virus.

[1] This work was aided by the Dorothy Beard Research Fund and by a grant from Lederle Laboratories, Inc., Pearl River, N. Y.

in diseased chick embryo tissue of two heavy components, one with a sedimentation constant of $S_{20°}$ = ca 250×10^{-13} cm sec^{-1} dynes^{-1} and the second with a sedimentation constant of $S_{20°}$ = ca 70×10^{-13} cm sec^{-1} dynes^{-1}. The former is present only in diseased tissue, while a component similar to the latter is found also in normal chick embryo tissue.[3] In the earlier experiments both components obtained from diseased tissue appeared equally infectious. Subsequent experiments have indicated that the heavier material with $S_{20°} = 253 \times 10^{-13}$ cm sec^{-1} dynes^{-1} represents the equine encephalomyelitis virus.[4] However, in order to eliminate the possibility of some relation between the virus complex and the lighter component of diseased tissue, systematic studies have been made of the properties and the behavior of the latter.

Since infectivity of the lighter material from diseased embryos could have been due to contamination[2] with small quantities of the virus complex, a better separation was sought by changes in the isolation technique. This has been accomplished by the extraction with distilled water of diseased embryo tissue for 42 to 96 hours without adjustment of pH. The extracts, filtered through celite, were then subjected to two or three cycles of alternate low- and high-speed centrifugation at 17,000 g and 67,000 g for 15 and 30 minutes, respectively. Distilled water was the solvent throughout, the pH remaining between 7.2 to 7.4. The significant changes were: (1) prolonged extraction (42 to 96 hours as compared with two hours previously used); (2) pH (7.2 to 7.4 instead of 8.5 to 9.0) and (3) filtration with celite.

The procedure yields a product entirely different with respect to infectivity from that previously reported. While infectivity is practically always associated with the material, the infectious unit has been $10^{-8.5}$ grams or greater, a difference of the order of 100,000 or more in comparison with the unit of the virus complex, which is consistently 10^{-13} to 10^{-14} grams.[2] These findings substantiate the suspicion of previous imperfect separation of the two components. Studies on the nature and properties of the lighter component indicate that it is in no way related to the virus complex.

Solutions containing 1 mg or more of the lighter component from diseased or normal tissue per cc are opalescent and, in the higher concentrations, exhibit a distinct yellow cast. The material is precipitated by the usual protein coagulants and gives positive xanthoproteic, Millon and biuret tests, but a negative glyoxylic acid test. The Molisch test is negative, while that for pentose with Bial's reagent and the acrolein test for fat are strongly positive. Heat coagulation occurs at 73 to 76° C. The yield has varied from 1 to 4 mg per gram of embryo tissue. The ultraviolet absorption spectrum showed a maximum at 2600 Å.

Analysis of 440 mg of the substance, dialyzed against distilled water and dried from the frozen state and further dried to constant weight over P_2O_5, revealed an elementary constitution of C, 55.2; H, 8.3; N, 9.5; P, 2.3; and S, 0.22 per cent. Extraction successively with acetone, alcohol-ether (1–1) and benzene revealed a fat content of 34.6 per cent. of which phospholipid constituted 67.0; cholesterol, 18.4; and fatty acid 20 per cent. The total carbohydrate of the whole complex was 7.0 per cent. The partial specific volume by pyknometer measurement was 0.79, corresponding to a specific gravity of 1.27.

Fractionation of 181 mg of the lipoid-free fraction for nucleic acid yielded 28.5 mg of a white amorphous product giving a positive Bial's test but negative Feulgen and diphenylamine tests. The biuret test was negative. On hydrolysis crystalline adenine picrate was isolated, and the murexide test for guanine was positive. Tests for cytocine or uracil or both were positive. The test for thymine was entirely negative. This ribonucleic acid represented 10.5 per cent. of the whole complex, and the ultraviolet absorption curve was indistinguishable from that obtained with yeast nucleic acid.

Ultracentrifugal sedimentation diagrams of the undamaged material reveal sharp boundaries indicative of high homogeneity, comparable in this respect to the papilloma virus protein[5] and the equine encephalomyelitis virus complex.[6] The sedimentation constant at pH 7.0 in water is $S_{20°} = 73 \times 10^{-13}$ cm sec^{-1} dynes^{-1} and in borate buffer solution at the same pH $S_{20°} = 78.7 \times 10^{-13}$ cm sec^{-1} dynes^{-1}. Molecular stability studied in 0.005 M buffer solution by means of the analytical ultracentrifuge was at a maximum between pH 7.0 and 8.2, precipitation occurring at pH 4.8, degradation above pH 8.3 to smaller relatively homogeneous fragments. The complex is extremely salt labile, breaking down in three or four days even in 0.005 M salt solutions.

Comparison of the properties of the lighter com-

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

[3] D. G. Sharp, A. R. Taylor, H. Finkelstein and J. W. Beard, *Proc. Soc. Exp. Biol. and Med.*, 42: 459, 1939. It should be emphasized that this component is wholly different from the normal chick embryo component of Claude (*Proc. Soc. Exp. Biol. and Med.*, 39: 398, 1938) reported (K. G. Stern and F. Duran-Reynals, SCIENCE, 89: 609, 1939) to have a sedimentation constant of $S_{20°}$ = ca 532×10^{-13} cm sec^{-1} dynes^{-1}.

[4] D. G. Sharp, A. R. Taylor, D. Beard, H. Finkelstein and J. W. Beard, SCIENCE, 92: 359, 1940.

[5] J. W. Beard, W. R. Bryan and R. W. G. Wyckoff, *Jour. Infect. Dis.*, 65: 43, 1939.

[6] A. R. Taylor, D. G. Sharp and J. W. Beard, *Jour. Infect. Dis.*, 67: 59, 1940.

ponent with those of the virus complex[4,6] reveals pronounced differences. Further, in the degradation of the virus complex by any means yet studied, no material with $S_{20°}$ = ca 70×10^{-13} cm sec^{-1} $dynes^{-1}$ has been seen. It appears reasonable to conclude that the lighter component in diseased embryo tissue is identical with that of normal chick embryo tissue and as such is not specifically concerned with the disease process associated with the virus.

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CRYSTALLINE CATALASE FROM BEEF ERYTHROCYTES

As early as 1911 Wolff and de Stoecklin[1] obtained rather highly purified catalase solutions from erythrocytes (species not stated). In 1923 Tsuchihashi[2] described a method for the purification of the horse erythrocyte catalase.

In our laboratory Tria[3] made considerable progress in purifying the catalase of beef blood, and recently A. L. Dounce[4] obtained this catalase in crystalline condition. But Dounce's method is time-consuming and the yield is very small.

We have prepared crystalline catalase from washed and laked cow erythrocytes by the following steps:

1. Adsorption on aluminum hydroxide[5] at pH 5.7, repeated washing of the adsorption complex with dilute acetate buffer pH 5.5, and elution with M/10 phosphate buffer at pH 8.0.

2. Precipitation at pH 5.7 with 30 gm ammonium sulfate for every 100 cc of enzyme solution. This precipitation was then repeated, using one tenth of the initial volume.

3. Dialysis, followed by precipitation at pH 5.5 by enough alcohol to make 40 per cent. concentration.

4. Solution in 1.25 per cent. NaCl and adsorption at pH 5.5 on $Al/OH/_3$ followed by elution with phosphate buffer pH 8.0 (no preliminary washing).

5. Precipitation of the eluted catalase with 30 gm of solid ammonium sulfate per 100 cc, at pH 5.7. The precipitate is mixed with a minute amount of water, centrifuged and allowed to stand in the ice-chest. Crystals form. The yield is increased by adding saturated ammonium sulfate slowly.

The catalase obtained by this method had a "Kat.f" of 43,000 and the crystals had the shape of small needles. After a second recrystallization from ammonium sulfate solution the crystals were mostly plates, while the "Kat.f." was 48,000. The iron content was 0.12 per cent. The visible absorption spectrum is identical with that for liver catalase.

Unlike crystalline beef liver catalase, erythrocyte catalase gives no blue color upon treatment with acetone and hydrochloric acid, but a reddish-brown color. The reddish-brown material has been identified as hemin.

These preliminary findings indicate that beef erythrocyte catalase differs from beef liver catalase in possessing a greater activity and in lacking prosthetic groups which furnish the "blue substance" or biliverin.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A GRINDER FOR HOMOGENIZING BACTERIAL CLUMPS OR INFECTED TISSUES

THE problem of preparing homogeneous suspensions of acid-fast bacteria, infected tissues or small amounts of various substances in the laboratory has long since resulted in the production of various grinders to facilitate the process. Recently, the need arose for a considerable supply of sterile grinding devices for homogenization of leprous nodules to recover the bacilli. It was found that remarkably complete homogenization and suspension of subcutaneous nodules, after they were cut down to small pieces by means of scissors, could be brought about in Pyrex grinders of the type illustrated in Fig. 1.

The important features in the construction of such grinders are: (*a*) selection of pairs of tubes so that one fits closely within the other, (*b*) careful boring of the holes in the rubber stoppers which join the inner grinding tube to the glass-shaft, and (*c*) grinding of the paired tubes with emery powder in water to produce roughened surfaces which rotate against each other without letting the small bits of tissue lodge where they may escape grinding. Due to the type of joint used, the inner grinding tube never rotates perfectly on its axis, but has a wabble which causes it to scour the walls of the outer tube. The rotating shaft

[1] J. Wolff and E. de Stoecklin, *Compt. Rend. Acad. Sci.*, 152: 729, 1911.
[2] M. Tsuchihashi, *Biochem. Zeitschr.*, 140: 63, 1923.
[3] E. Tria, unpublished.
[4] A. L. Dounce, unpublished.
[5] G. Tracey and W. H. Welker, *Jour. Biol. Chem.*, 22: 55, 1915.
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