maximum degree of growth within 24 hours of incubation. At the end of this time, and also following a more prolonged period of incubation, the contents of the culture tubes revealed a white, turbid suspension of organisms. In the presence of increasing concentrations of sodium sulfathiazole, the more resistant strains produced a yellow color, which first appeared at the end of 24 hours of incubation. If the cultures remained at incubator or room temperature for another 24 to 48 hours, the color became more intense. The color was so pronounced with some strains that a deep brown-orange pigment was observed. This pigment appeared only in the presence of the higher concentrations of the sulfonamide, and uniformly, shortly after maximum growth had been attained. Strains, whose growth was completely inhibited by 200 mgs per 100 cc of sodium sulfathiazole, showed pigment production occurring in the presence of 40 to 100 mgs concentrations of the drug. There was little or no inhibition of bacterial growth in the tubes showing the presence of the pigment. The pigment appeared also in the presence of sulfanilamide, sodium sulfapyridine and sodium sulfadiazine. It is of interest that strains 7 and 14, which were studied by Landy and his group, did not produce a demonstrable pigment. These strains are not highly sulfonamide-resistant.

The nature of this pigment is being investigated at the present time. The pigment is not of the usual type produced by many strains of staphylococci, since it is not soluble in fat solvents. Evidence at hand would indicate that the pigment is derived from PAB. It would appear that under the experimental conditions described, some strains of staphylococci reproduce readily in the presence of the sulfonamides because of the synthesis of significant amounts of PAB. After maximum growth has been attained, the PAB is changed from a colorless state to a yellow-brown pigment.

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INTRA-OCULAR VIRUS INFECTIONS

IN investigation of the virus of epizootic fox encephalitis[1,2] we have found that the method of intra-ocular injection is a most useful procedure. This technique appears to be of great potential value in the study of other virus diseases. Observations of infections resulting from intra-ocular injections have been reported by a number of investigators in the virus field, principally in work on the pathogenesis of infections of the nervous system. It is felt, however, that the possibilities of this procedure have not been fully utilized in the approach to the more common problem of demonstrating the presence of viable virus.

Fox encephalitis can be experimentally transmitted to dogs, wolves and foxes. Evidence of infection has, in the past, consisted in death of the animal with characteristic symptoms and the demonstration of inclusion bodies in vascular endothelium in the brain and elsewhere. Studies of fox encephalitis have been hampered by a low or variable mortality of inoculated animals. This ranged from 10 to 80 per cent., depending upon the virulence of the strain of virus used and the natural resistance of the animals injected. Accurate titration of the amount of virus in tissue suspensions and the titration of antisera have been wholly impracticable, since the large number of foxes or dogs required was prohibitive. Employment of the simple technique of intra-ocular injection has changed this picture completely. Intra-ocular inoculation results in 100 per cent. infection of the eyes of foxes; the infection is easily observed grossly and is readily confirmed by demonstration of the inclusion bodies in smear preparations. Previously a 1 to 10 per cent. tissue suspension has been used[3] as an inoculum, but it has now been found that each of the several stock tissue suspensions tested was fully infective by intra-ocular inoculation in a dilution of 1 part in 100,000.

About 0.2 ml. of virus is injected into the anterior chamber after aspiration of a slightly greater volume of aqueous humor. Infection from the fox encephalitis virus is usually apparent grossly by the third day, and in all cases there is a diffuse opacity of the cornea on the fifth day. A significant amount of purulent conjunctival exudate is not seen unless bacterial infection has occurred.

On the fifth day the eye is removed either under anesthesia or after the animal has been killed. Aqueous humor is aspirated with a Pasteur pipette for bacterial culture. Inclusion bodies are demonstrated in the endothelial cells that line the internal surface of the cornea by the technique routinely employed in making smears of the epithelium of the urinary bladder in the diagnosis of canine distemper.[4] Using a modified Shorr's stain,[5] we have frequently found more than half of the cells in a smear to contain large inclusion bodies.

Neutralization tests are carried out by injecting mixtures of serum and virus dilutions into the anterior

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[4] R. G. Green and C. A. Evans, *Cornell Vet.*, 32: 190–193, 1942.
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chamber of the eye. The results are clear-cut and unequivocal, and may be verified by examination of smears. Exploration of the range of susceptible species of animals has been greatly facilitated by intra-ocular injection of virus. The raccoon, previously considered resistant to fox encephalitis, has been easily infected by this method. These results indicate that some viruses may be isolated and transmitted by intra-ocular inoculation of animals that are resistant to inoculation by other routes.

The eye contains many types of cells and is susceptible to a great variety of viruses. In a survey of the effects of intra-ocular injection, it has been found that infection is visible grossly in the eyes of rabbits when the animals otherwise appear well in equine encephalomyelitis, ornithosis[6] and several other virus diseases.

Failure to cause *visible* infection of animals that are susceptible (capable of supporting the multiplication of virus) is an obstacle to work with many virus diseases other than fox encephalitis; in some of these, as in fox encephalitis, intra-ocular injection should prove of great value. It appears that this may be a successful method for the initial demonstration of some viruses.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PREPARATION OF METHIONINE AND TRYPTOPHANE-FREE CASEIN HYDROLYSATES[1]

TOENNIES and Kolb[2] have shown that methionine is selectively oxidized by hydrogen peroxide in the presence of perchloric acid. More recently, Toennies[3] has found that the methionine in casein is rendered biologically inactive by peroxide oxidation of the whole protein dispersed in formic acid. The concurrent observation made in this laboratory that methionine is also selectively oxidized by hydrogen peroxide in the presence of 30 per cent. sulfuric acid has been found to afford the simple and inexpensive procedure for the preparation of a methionine and tryptophane-free casein hydrolysate to be described.

One kilogram crude casein was hydrolyzed under reflux for 20 to 23 hours with a mixture of 500 ml concentrated sulfuric acid and 1 liter of water. After cooling to 80° C., 200 ml of 30 per cent hydrogen peroxide (technical) was added and the mixture allowed to stand 24 hours at room temperature. Now, 2 liters of water and 4 liters of 16 per cent. calcium oxide suspension were added. The slightly alkaline mixture was thoroughly stirred and resulted in the evolution of ammonia. After standing overnight, it was filtered through a norite-precoated filter and the resulting calcium sulfate cake resuspended in 2 liters of hot tap water. This mixture was stirred mechanically for 30 minutes, filtered and the combined filtrate and washings concentrated *in vacuo* at 50–60 ° to approximately 2 liters. The resulting ammonia-free concentrate was made neutral to litmus with 50 per cent. sulfuric acid, cooled under the tap and filtered.

The protein equivalence ($N \times 6.25$) of the preparation was estimated from micro-Kjeldahl analysis of a suitable aliquot. Approximately 650 grams of methionine and tryptophane deficient product were obtained. The methionine content of the final product by the method of Kolb and Toennies[4] varied from 0.12–0.21 per cent. of the protein. No tryptophane could be detected.[5] Histidine, arginine, threonine and serine determinations indicated that these amino acids had not suffered any destruction by the treatment.

For use in a methionine deficient rat diet[6] the solution was supplemented by 1.5 per cent. l-tryptophane and 1 per cent. l-cystine. Bioassay in rats showed that the weight loss incurred by feeding the methionine deficient product as the protein moiety (14.7 per cent.) of the diet was regained and normal growth resumed on supplementation of the diet by 3 per cent. d-l methionine.

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