

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<sup>6</sup> 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<sup>1</sup>

TOENNIES and Kolb<sup>2</sup> have shown that methionine is selectively oxidized by hydrogen peroxide in the presence of perchloric acid. More recently, Toennies<sup>3</sup> 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 prepa-

<sup>1</sup> This investigation was aided by grants from the Rockefeller Foundation, Merck and Company and E. R. Squibb and Sons.

<sup>2</sup> G. Toennies and J. J. Kolb, *Jour. Biol. Chem.*, 140: 131, 1941.

<sup>3</sup> G. Toennies, *Jour. Biol. Chem.*, 145: 667, 1942.

ration 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<sup>4</sup> varied from 0.12–0.21 per cent. of the protein. No tryptophane could be detected.<sup>5</sup> 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<sup>6</sup> 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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<sup>4</sup> J. M. Stickney and F. R. Heilman, *Proceedings of the Staff Meetings of the Mayo Clinic*, 17(24): 369–375, 1942.

<sup>5</sup> J. J. Kolb and G. Toennies, *Ind. and Eng. Chem. Analyt. Ed.*, 12: 723, 1940.

<sup>6</sup> A. A. Albanese and J. E. Frankston, *Jour. Biol. Chem.*, 144: 563, 1942.

<sup>6</sup> A. A. Albanese and W. Buschke, *SCIENCE*, 95: 584, 1942.

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