# Effect of DL-Ethionine on Poliomyelitis Virus Growth in Tissue Culture.* (18782)

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The role of certain amino acids in the growth and multiplication of viruses *in vivo* is becoming more and more evident. Sprunt (1) found that mice fed a low protein diet showed an increased resistance to infection with swine influenza virus with a reversal of this effect when methionine was incorporated in the diet suggesting that methionine is essential for viral multiplication. Jones, *et al.*(2) noted that low protein or low tryptophane diets delayed symptoms of Lansing type poliomyelitis infection in mice during the first two weeks and Rasmussen *et al.*(3) also report a lengthened incubation period with tryptophane deficiency in mice and that the deficiency is aggravated by 6-methyl tryptophane, an analogue of this amino acid. Rafelson, Pearson and Winzler(4) have shown that large amounts of certain amino acids may interfere with the phosphorus metabolism of mouse brain tissue supporting the growth of Theiler's virus in tissue culture. The inhibitory effects of one of these, lysine, could be partially overcome by the addition of methionine. The importance of methionine in the biosynthesis of influenza virus has been demonstrated by Ackermann(5) who studied the effects in tissue culture. By incorporating structural analogues of methionine into the tissue culture medium he found that virus propagation was inhibited. Furthermore, this inhibitory effect could be reversed or blocked by the addition of methionine to the fluids. He concluded that the inhibitors do not act by destroying the virus, the host tissue, or by preventing infection of the cells, but by interfering with the synthesis of the virus.

The present study was undertaken to determine the effect of dl-ethionine upon another virus, the Lansing strain of poliomyelitis, cultivated in tissue culture.

*Experimental.* A line of mouse adapted Lansing strain of poliomyelitis virus which has been maintained in this laboratory was used to initiate infection of the tissue. The mouse infectivity of the original pooled virus suspension was $10^{-4}$. Virus was grown *in vitro* using the tissue culture technic described by Enders, Weller and Robbins(6). Brain tissue from human embryos of 3 to 4 months was minced and distributed under perforated cellophane in 25 ml flasks. Each flask then received 3 ml of Hank's solution plus Simms ultrafiltrate, to which had been added penicillin (90 u/ml) and streptomycin (0.1 mg/ml).

The flasks were tightly stoppered and incubated at 37°C. When the pH decreased to approximately 6.7 as indicated by a small amount of phenol red in the fluids, they were removed and fresh fluid at pH 7.2 was added, usually at intervals of 2-4 days. 0.1 ml of virus was added at the first fluid change and the titer of virus in subsequent fluid changes was determined by mouse infectivity tests in groups of 8 mice per dilution. The virus for subsequent experiments was always from a previous tissue culture, and the titer of the fluid at the start of each experiment was

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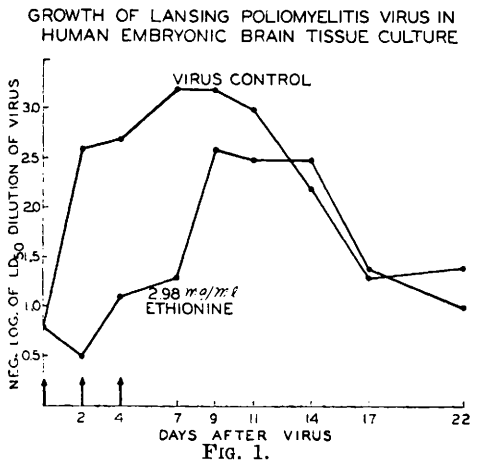


FIG. 1.

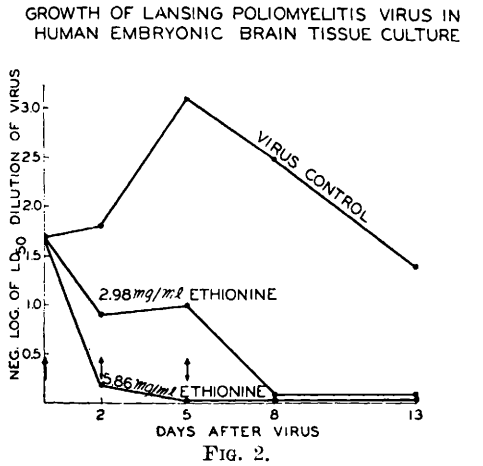


FIG. 2.

recorded as 1/30 (0.1 in 3 ml) of the amount of virus added.

*Results.* The peak of the virus titer, $10^{-3}$ to $10^{-3.3}$ was always obtained between the 4th and 7th days after addition of virus to the culture, but significant quantities were present between the 2nd and 11th days. It was observed that the best growth curves were obtained with tissue of embryos most recently viable.

Fig. 1 shows a typical growth curve and also the effect of ethionine on virus propagation. When 2.98 mg/ml were added to some of the flasks concurrently with the virus and again 2 and 4 days later at the 1st and 2nd fluid changes, a marked suppression of viral growth was detected. The tissue, however, was still capable of supporting multiplication of the virus as evidenced by a distinct increase in virus titer when ethionine was withheld from subsequent fluid changes.

Fig. 2 shows another experiment in which two different quantities of ethionine were employed. When the original concentration was doubled there was almost complete inhibition of virus.

In order to determine the effect of ethionine on the virus itself 3 ml of Hank's solution plus 0.1 ml of tissue culture virus having an infective titer of $10^{-3.2}$ were incubated at 37°C with and without ethionine (6 mg/ml). These conditions exactly duplicated the tissue culture technic except for the absence of living tissue. In addition, 2 ml of the same preparation of virus was allowed to stand at 37°C in the presence and absence of ethionine. The various mixtures were titrated in mice after 18 and 44 hours. The results presented in Table I show that ethionine has no destructive action on the virus itself under these conditions.

If the action of ethionine is specifically directed against its analogue, methionine, the addition of the latter to cultures containing ethionine should result in reversal of the inhibition. To test this hypothesis the experiment portrayed in Fig. 3 was conducted. In agreement with the results of previous tests, the growth curve of the virus in the control flasks was much higher than in the flask containing ethionine. The flasks containing ethionine together with methionine showed an intermediate growth curve, by no means as high as in the controls, but considerably higher than when ethionine alone was used. This demonstrates that under these conditions methionine limits the inhibitory effect of ethionine; further experiments using other proportions of the two compounds are being conducted in order to determine the optimal quantitative relationship. In an effort to

TABLE I. Effect of DL-ethionine on Mouse Infectivity Titer of Lansing Poliomyelitis Virus Incubated at 37°C.

| | 18 hr | 44 hr |
|---|---|---|
| Hank's sol'n 3 ml + virus .1 ml | $10^{-1.1}$ | $10^{-.1}$ |
| Hank's + virus + ethionine 6 mg/ml | $10^{-1.1}$ | $10^{-.6}$ |
| Virus 2 ml | $10^{-2.4}$ | $10^{-2.1}$ |
| Virus 2 ml + ethionine 6 mg/ml | $10^{-2.2}$ | $10^{-2}$ |

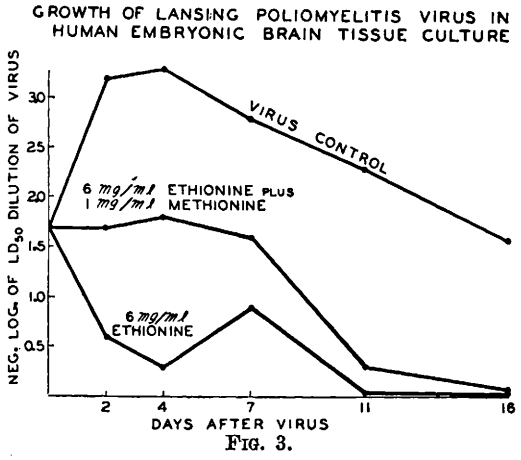


FIG. 3.

demonstrate true reversal of ethionine effect, methionine was added after several fluid changes to flasks already showing ethionine inhibition. No observable increase in virus growth took place but the significance of this experiment is rendered doubtful by the fact that cultures were in a state of deterioration at the time.

Attempts were made to substitute betaine and homocysteine for the methionine in the above experiment but the viral growth curve closely paralleled that with ethionine alone indicating that embryonic brain tissue is not able to synthesize methionine under these conditions from metabolites which in other biological systems are its immediate precursors (7).

*Summary.* 1. DL-ethionine inhibits the growth of Lansing strain of poliomyelitis virus in tissue cultures of human embryonic brain. 2. When the inhibitor is withheld from subsequent fluid changes virus growth is resumed indicating that the tissue itself is not irreversibly affected. 3. The effect of ethionine was shown not to be on the virus itself under conditions identical in all respects except for the absence of living tissue. 4. The inhibitory effect of ethionine was reduced by the addition of methionine to the cultures.

*Conclusions.* The Lansing strain of poliomyelitis virus requires L-methionine for its growth and multiplication in cultures of human embryonic brain tissue. Its growth can be materially inhibited with DL-ethionine, a structural analogue of the essential amino acid. The inhibitor acts by interfering with the biosynthesis of the poliomyelitis virus just as it suppresses the growth of influenza virus. This technic affords a procedure for studying the growth requirements of poliomyelitis virus and for testing materials which may influence its growth.

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## A Non-Particulate, Dinitrocresol-Resistant, Glycolytic, Phosphorylating Mechanism Present in Malignant and Certain Normal Tissues. (18783)

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The present experiments deal with the respective abilities of cell-free fractions from normal and tumor tissues to derive energy for phosphorylation from oxidation of succinate or from glycolytic breakdown of hexose diphosphate. Cell-free, particulate systems from a number of normal tissues can apparently utilize up to 50 or 60% of the energy derived from oxidation of α-ketoglutarate, glutamate, or succinate for synthesis of phosphate compounds(1,2), which are in turn available for other cellular syntheses. These oxidative, phosphorylating, particulate systems have been shown by Loomis and Lipmann(3) and by Cross *et al.*(4) to be highly sensitive to the

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