CHANGES IN MICROSOMAL PROTEIN SYNTHESIS CAUSED BY IN VIVO VIRUS INFECTION

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An understanding of the mechanism of the pathogenic effect of a virus infection must come, fundamentally, from a knowledge of its effect on host cell metabolism. The question of the synthetic capability of the host, specifically protein synthesis, could be basic to the problem of retention of cellular integrity. The role of cellular ribonucleic acid (RNA) in protein synthesis has been well established (Hoagland and Zamecnik, 1959; Harris, 1961). Although exogenously added viral RNA has been shown to stimulate protein synthesis by microsomal preparations (Nirenberg and Matthaei, 1961; Ofengand and Haselkorn, 1962), there has been no report of protein synthesis by microsomal preparations derived from animals infected with an RNA virus. Consequently, it was decided to study protein synthesis by microsomes obtained from brain cells of mice infected with the virus of Venezuelan equine encephalomyelitis (VEE). VEE is an RNA virus[1] which, in the mouse, manifests its virulence through central nervous system (CNS) involvement. Although the virus appears very rapidly in many tissues and more slowly in the brain, it rises to higher levels and remains elevated in the brain for longer periods of time[2]. Because of its chemical nature, the possibility exists that this virus might act through interference with the normal role of cellular RNA.

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[1] Miesse, M. L., this laboratory, unpublished data.

[2] Tasker, J. B., this laboratory, unpublished data.

Material and Methods

Bagg Strain, male, white mice, 25-30 gms, obtained from the animal colony at the Walter Reed Army Institute of Research were employed in this study. Mice to be infected were inoculated intraperitoneally with 0.2 or 0.3 ml of a 10^{-7} dilution of egg slurry containing 400-1700 $MIPLD_{50}$'s[3] of Trinidad Strain VEE. This virus is virulent for mice in that an infecting dose is a lethal dose. Fifty mice were sacrificed by cervical fracture each day for six days following inoculation with VEE Virus. Control animals (not inoculated) were sacrificed on the day following the last day of sacrifice of the infected animals.

The mice for each day's sacrifice were divided into two groups; from one group of forty-four mice the brains were removed and immediately placed in ice-cold Medium A (Keller and Zamecnik, 1956) for separation of the microsomes and fractionation of the pH 5 soluble enzymes. The brains of the second group of six mice were then removed, pooled, and stored at -50°C to be titered for infectious virus at the completion of the sacrifice schedule.

Microsomes and soluble pH 5 enzymes were prepared as previously reported (Keller and Zamecnik, 1956). Infectivity titration for infectious virus were performed on a sample of each day's microsomes and soluble pH 5 enzymes as well as the pool of six brains removed for this purpose. The method employed for infectivity titration was that previously described for this laboratory. (Berge, et al, 1961). Virus levels were quantitated as lethal doses according to standard methods (Reed and Muench, 1938).

The final incubation mixture contained pyruvate kinase, 0.05 mg; phosphoenol-pyruvate, 25 μM; ATP, 1.25 μM; GTP, 0.3 μM; $MgCl_2$, 5 μM; L-leucine, 0.13 μM containing approximately 50,000 cpm of uniformly labeled L-leucine-C-14; pH 5 enzymes, 3.0 mg enzyme protein; microsomes, 4.4 mg microsome protein; in a final volume of 1.0-1.3 ml. The mixtures were incubated for 15 minutes at 37°C in air

[3] Mouse intraperitoneal lethal dose.

Trichloracetic acid insoluble protein was isolated as previously described (Seikevitz, 1952). The final protein product was dissolved in 1.0 ml of 0.25N NaOH and an 0.1 ml aliquot was pipetted onto flat nickel planchets for assay of the radioactivity. All counting was done in an internal gas flow counter to an error of 5% or less. Inasmuch as all planchets had the same amount of NaOH and approximately the same amount of protein, no correction for self-absorption was made.

Enzyme and microsome protein analyses were carried out by the micro-Kjeldahl method (Kabat and Mayer, 1948).

Results and Discussion

It was found that there was a marked increase in the protein synthetic activity of microsomes obtained from the brains of the mice infected with Trinidad strain VEE when incubated with the pH 5 enzymes from the same tissue. This is evident from Figure 1 which summarizes the uptake of L-leucine-C-14 as

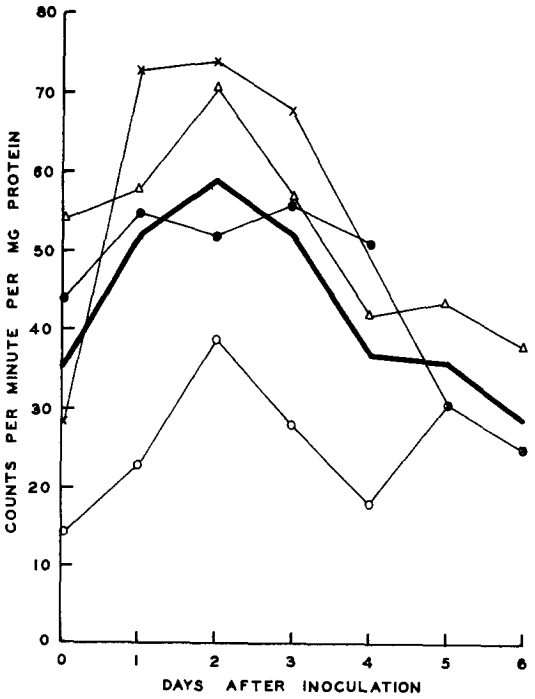

Figure 1. Changes in protein synthesis by microsomal preparations obtained from brains of VEE virus infected mice. See text for conditions. Each light-lined curve represents a replicate experiment. Heavy line is the averages. Zero (0) time is the control value.

a function of time after challenge. The maximum activity appears to occur about day 2 post-inoculation. The increase in synthetic ability occurs during the time that the virus titer is increasing in the brain. However, the subsequent drop in synthetic ability occurs while the titer is either still rising or remaining at a high level. Figure 2 is illustrative of this point. From

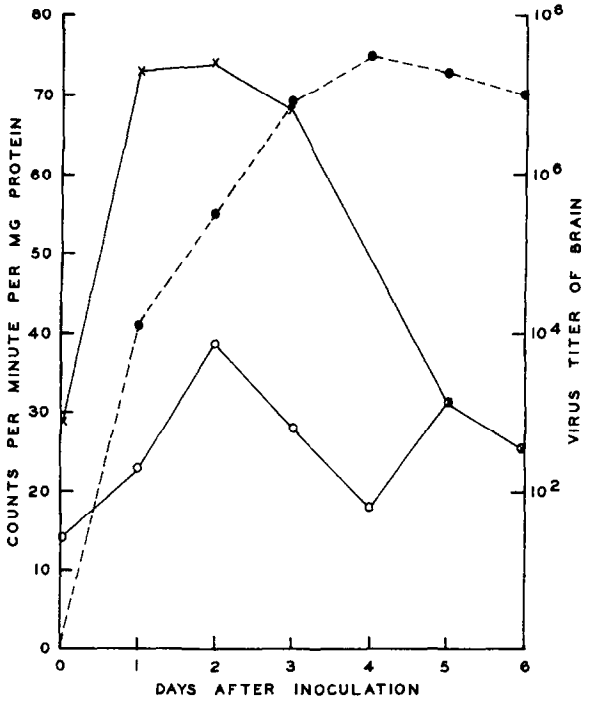

Figure 2. Relation of virus titer of the brain to the change in protein synthesis by microsomal preparation. For details see text. The solid lines are the protein synthesis rates and correspond to the high and low curve of Figure 1. The dashed line is the virus titer and is the mean of six experiments.

Figure 2, it can be seen that the brains of the infected animals contain significant quantities of virus. The virus titer of the microsomal and soluble enzyme fraction has also been measured and found to be about 10^7 $MIPLD_{50}$'s and about 10^5 $MIPLD_{50}$'s respectively.

From these results, it is apparent that in the presence of a virulent virus, there is an immediate stimulation of the protein synthesizing system in mouse brains. The data illustrated in Figure 1 cover a rather broad

range of uptake of the L-leucine-C-14. However, each light-lined curve representing an individual experiment follows the same pattern as the average curve (heavy line). In addition, two additional experiments, not reported in these data are in accord with the reported findings.

It is interesting to assess the meaning of these findings in the pathogenesis of this viral disease. It has been shown in this laboratory that not until day 4 following inoculation with Trinidad VEE virus do mice begin to show CNS involvement (Gleiser, et al, 1962). At about day 3-4 the mouse begins to lose its temperature regulating ability. Finally, it is not until this same time that any histological evidence of brain tissue damage can be observed (Gleiser, et al, 1962). It is apparent then, that overt signs of illness due to VEE do not occur until the rate of protein synthesis has begun to decline and the integrity of the brain tissue is lost. Although the initial stimulation of protein synthesis by the microsomes from the infected brains, as reported above, is apparently sufficient to meet the needs of the tissue, it is not unreasonable to attribute the loss of cellular integrity to the decreased ability of the cells to make host protein.

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