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PLAQUE FORMATION OF ARBOVIRUSES UNDER AN OVERLAY OF TISSUE CULTURE MEDIUM AND LIQUID PARAFFIN

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THE plaque technique provides a sensitive and accurate method for the quantitative estimation of virus infectivity in cell cultures. This technique was first introduced by Dulbecco in 1952 for animal viruses. Since then it is widely employed in the field of virology with several modifications though the principle of the original technique essentially remains the same. Most viruses require solid overlay medium to produce plaques and agar is commonly used in such studies. However, substances such as agarose, methylcellulose[2], carboxymethyl cellulose[3], startch[1], Tragacanth gum[4] or any other substance which inhibits the production of secondary plaques can also be used for the formation of plaques. A viscous substance is usually added to overlay medium which solidifies or remains viscous. Vaccinia is the only virus which produces clear plaques even under liquid tissue culture medium[5,6]. In the present study formation of plaques of two arboviruses under an overlay consisting of a combination of liquid tissue culture medium and liquid paraffin was successfully carried out.

Vero cell culture was employed and the tests were conducted in linbroplates. Vero cell monolayers were grown in wells of linbroplates employing MEM (Glasgow) with Earle's base and 5 per cent goat serum (G.S.).

Commercially available liquid paraffin (LPFN) obtained from Agenta Chemicals, Bombay, India and MEM double strength with 10% G.S. were used in the overlay. 0·1 per cent Amido black[3] was employed for staining the cells. Tissue culture stocks of two arboviruses: Sindbis (Group A) and Chandipura (Vesicular Stomatitis group) were studied.

The method followed for conducting the experiments was as follows:

(*a*) The medium was removed from the wells of linbroplate with confluent monolayers of Vero cell culture and dilutions of virus were inoculated in 0·1 ml quantities to each well and adsorbed for one hour at 35° C with intermittent rocking of the plates.

(*b*) After adsorption MEM double strength with 10% G.S. was added in 0·5 ml quantities followed by one ml quantities of LPFN into each well. MEM double strength was used instead of MEM as the cell sheet was degenerating faster when the latter medium was employed. The LPFN formed a separate layer on top of the liquid tissue culture medium. While growing Vero cell monolayers and after virus inoculation, the plates were incubated at 35° C in carbon dioxide incubator. Results were noted by staining with 0·1 per cent Amido black on appropriate post-inoculation days as determined by earlier experiments. Sufficient precaution was taken to avoid unnecessary handling and movement of the test plates during incubation to avoid development of secondary plaques.

The size and titre of the plaques of the two viruses (Sindbis and Chandipura) are presented in Table 1. Figures 1 and 2 show plaques of these two viruses.

TABLE I

Virus	Overlay	Day of appearance of plaque	Plaque titre $\log_{10}$ PFU/ML.	Plaque diameter
Sindbis (AR 339 Gr. A,	Liquid paraffin	3	7·3	Micro-plaques
Chandipura (653514) (Vesicular stomatitis group)	do.	1	8·3	2 mm

FIG. 1. Plaques of Sindbis Virus.

Plaques were produced in our experiments by employing a combination of liquid tissue culture medium and LPFN in overlay. However, when overlaying was done with only liquid tissue culture medium, cytopathogenic effect was produced. LPFN alone in the absence of liquid tissue culture medium failed to produce plaques. The mechanism responsible for the formation of plaques under this overlay is the pressure exerted by the layer

of liquid paraffin which prevents the rapid spread of infective foci that results in the formation of plaques.

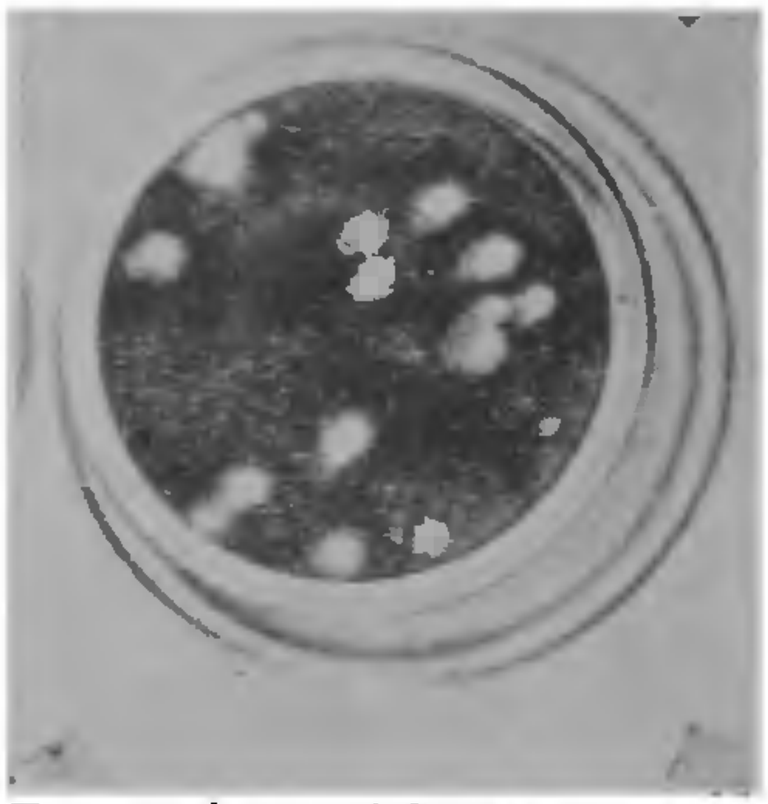

FIG. 2. Plaques of Chandipura Virus.

The advantage of plaquing under this overlay is that it permits the virus to form plaques in liquid tissue culture medium itself and the effect of LPFN is only indirect on the plaques. The easy availability of LPFN makes it an useful adjunct to plaque study.

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PHYSIOLOGICAL STUDIES ON THE EFFECTS OF NUTRITIONAL IMBALANCE ON THE CENTRAL NERVOUS SYSTEM: EFFECTS OF THIAMINE DEFICIENCY ON THE REGIONAL PROTEIN METABOLISM IN THE BRAIN OF CHICKEN, *GALLUS DOMESTICUS*

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ABSTRACT

The levels of RNA, protein and the activity levels of Aspartate amino-transferase (AAT) and Alanine aminotransferase (AlAT) in the cerebrum, cerebellum and medulla oblongata of thiamine-deficient chicken increased indicating an augmentation in the activity of the protein synthetic machinery in B_1-hypovitaminous birds.

THE proteins in the brain of vertebrates are known to exist in a dynamic state[1]. The synthesis and catabolism of proteins in the mammalian brain have been extensively studied[2–4]. It has been demonstrated that the levels of protein, total nitrogen and DNA remain practically unchanged, even in states of severe protein deficiency or starvation[6–8].

However, information about the regional distribution and metabolism of protein in the vertebrate brain during thiamine deficiency is lacking. Since marked changes in the enzymes connected to carbohydrate metabolism occur in animals with thiamine deficiency[5,9], it might be expected that the brain would also show alterations connected to protein metabolism during thiamine deficiency. Little work has been done to test this possibility. Hence, the present study was undertaken.

MATERIAL AND METHODS

Two days old, male white leghorn chicken, *Gallus domesticus* (10–12 g) were reared in the laboratory at $36 \pm 2°$ C. The controls were fed on standard chicken feed. The experimental animals were fed on polished rice to induce thiamine deficiency[5]. Water was available *od libitum* to both the groups.

The normal and experimental birds were decapitated after 28 days. The brain was dissected and kept in Ringer at 0° C. The different regions of the brain (cerebrum, cerebellum and medulla oblongata) were separated with sterilized instruments, weighed in ice-cold Ringer and immediately used for analysis.

Proteins from the samples were precipitated by trichloro acetic acid (BDH) and estimated by the micro-biuret method[10].

RNA was extracted by the method of Schmidt-Thanhausser-Schneider[11] and estimated by orcinol colour reaction following the colorimetric procedure described by Glick[12].

The Aspartate aminotransferase (AAT, E.C. 2·6·1·1) and alanine amino transferase (AlAT, E.C. 2·6·1·2) activities were determined following the colorimetric procedure of Reitman and Frankel as described by Bergmeyer[13].