interest to analyse the other related genera, *Amphipnous* and *Symbranchus* to know about the possible derivation of the presently determined karyotype.

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## STUDIES ON INTERFERON INDUCTION BY FIVE STRAINS OF WEST NILE VIRUS IN BRAINS OF SUCKLING MICE

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IN a previous study[1], it was reported that thirty-seven arboviruses, belonging to different antigenic groups could be divided into three groups—high, moderate and low inducers—depending on their ability to induce interferon in brains of suckling mice. However, only one strain (prototype and/or topotype) of each virus was used in that study. Different strains of a virus[2-3] including some arboviruses[4-7], are known to differ in their ability to induce interferon. In the present study, interferon inducing abilities of five strains of WN virus isolated from bat, mosquito and human sources have been determined. Further, an attempt has been made to correlate this property with other biological properties described earlier[8, 9].

Details regarding the strains employed in the study are listed in Table I. Virus pools were prepared in brains of Swiss albino suckling mice and stored after lyophilization at $-20°$ C. Viral infectivity titrations were done in 3–4 week old Swiss albino mice by intracerebral inoculation. Preparation of interferon was similar to the method described earlier[10]. Approximately 2·6–3 dex $LD_{50}$ of each virus strain was inoculated intracerebrally into groups of 3-day old mice. The actual dose inoculated, determined by back titration, is given in Table II. Brains of mice inoculated with different strains were harvested simultaneously at the end of 72 hr, and processed for infectious virus, interferon and complement fixing (CF) antigen as described earlier[1]. CF test was done using hyperimmune ascitic fluid having a homologous titre of 1 : 128. The assay of interferon was done in L–M cell line using vesicular stomatitis virus plaque reduction method. Standard mouse interferon (G–002–904–511) from NIH and "mock" interferon from normal mouse brains served as controls. One unit of interferon in our system was equal to approximately three international units.

The results are presented in Table II. Bat (68856) and mosquito (G 2266, G 22886) isolates induced higher amounts of interferon as compared to human (672698, P 4230) isolates. No relationship could be observed between infectious virus, CF antigen titres and titres of interferon (Table II). Comparing the infectious viral titres of five strains, all seem to be equally pathogenic[8] in suckling mice. Correlation between titres of viruses and amount of interferon induced is not apparent, probably due to varying

TABLE I

*West Nile virus strains employed for interferon induction*

| WN virus strain | Passage level (infant mice) | Source of isolation | Year | Locality |
|---|---|---|---|---|
| 68856 | M 47 | *Rousettus leschenaulti* | 1968 | Horabail (Karnataka) |
| G 22886 | M 7 | *Culex "vishnui"* | 1958 | Sathuperi (Tamil Nadu) |
| G 2266 | M 10 | *Culex "vishnui"* | 1955 | Sathuperi (Tamil Nadu) |
| 672698 | M 35 | Human | 1967 | Kaisodi (Karnataka) |
| P 4230 | M 8 | Human* | 1956 | Poona (Maharashtra) |

* Laboratory infection.

TABLE II

*Infectious virus, CF antigen and interferon titre in brains of West Nile virus infected suckling mice*

| WN virus strains | Dose inoculated Dex $LD_{50}$/ 0·03 ml | Virus titre Dex $LD_{50}$/ 0·03 ml | Titre of M.Br. CF antigen | Interferon* titre IU/ml |
|---|---|---|---|---|
| 68856 | 2·6 | 7·5 | 1 : 256 | 13887 |
| G 2266 | 3·0 | 6·2 | 1 : 128 | 11678 |
| G 2266 | 2·7 | 6·6 | 1 : 128 | 10339 |
| 672698 | 2·8 | 6·5 | ⩾1 : 256 | 6730 |
| P 4230 | 2·7 | 7·0 | 1 : 128 | 6694 |

* Geometric mean of three separate assays.

sensitivity of different strains to interferon induced during multiplication[11]. Only one correlation that could be derived in the study was between interferon inducing ability and size of plaques on Vero cell line[8]. Human strains (672698, P 4230) which produced large size plaques (1·5 to 2·9 mm in diameter) were poor inducers of interferon. Whereas bat and mosquito strains which produce small size plaques (0·3 to 0·9 mm) were found to be efficient inducers of interferon This observation lends support to the suggestion by Umrigar and Pavri[8] that bat strain (68856), might be a good inducer of interferon in mice. The correlation between interferon inducing ability and plaque size in Vero cell line, a heterologous system, is difficult to assess. Moreover, Vero cell line has been shown to be incapable of production of interferon[12]. Period of isolation and passage level also did not seem to influence variation observed in interferon titres. Cole and Wisseman[13] have observed that low, medium and high mouse brain passaged lines of dengue type I human isolate did not differ significantly in rate of virus multiplication or amount of interferon induced in suckling mice brains.

Finter[14] has reported that brains of adult mice inoculated with an unnamed strain of WN virus are a rich source of mouse brain interferon (Geometric mean titre 9,500 units/ml). Earlier, Vainio *et al.*[10] and Haahr[15] have studied interferon induction by E 101 strain of WN virus. All the studies mentioned above have employed adult mouse brain as source of interferon and have not included international reference preparation of interferon in their assay. Hence, it is difficult to compare the present results with those reported earlier.

The present study has revealed that out of five strains, three isolated from non-human sources induced higher amount of interferon as compared to human isolates. Further, there seems to be no clear relationship between biological properties of five strains of WN virus and quantitative ability to induce interferon in suckling mouse brains. The observation that human strains of WN virus are less efficient in iinduction of interferon in brains of suckling mice needs further study.

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## CHELATING AGENT EDTA DECREASES THE TOXICITY OF COPPER TO FISH

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COPPER toxicity is well investigated in the field of toxicology because the pollutant has adverse effect on water quality, fish production and aquatic ecosystem[1–4]. Furthermore, even trace concentrations of copper (one tenth to the twentieth of the accepted standards for drinking water) can be lethal for fish in soft water[5].