

TABLE I  
Different metabolic constituents of wild and pyridoxineless mutants of *A. nidulans*

Constituents*	Wild strain (Y)	Mutant ( $\gamma_1$ :pyro <sub>8</sub> )
Mycelial weight in mg/50 ml culture	370	182
Glycogen	13.2	12.0
Total protein	8.5	7.0
RNA	3.1	2.6
DNA	1.0	0.8
RNA : DNA	3.10	3.30
Total lipids	6.55	8.98
Sterols (estimated as cholesterol)	0.36	0.25
Triglycerides	1.70	2.70
Phospholipids	0.75	0.62
Fatty acids	2.30	1.90
Unidentified lipids	1.50	3.50

\* Weight expressed in mg per 100 mg dry mycelia; values are given as an average of six determinations.

thus to study the exact role of pyridoxine in lipid metabolism.

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#### A NOTE ON THE ADAPTATION OF LENTOGENIC STRAIN (CDF66) OF RANIKHET DISEASE VIRUS IN CELL CULTURE

VELOGENIC and mesogenic strains of Ranikhet Disease Virus (RDV) adapt at a faster rate in cell cultures. The adaptation of lentogenic strains is difficult and cumbersome<sup>1</sup>. The lentogenic strain CDF66, originally isolated from the respiratory tract of a pig<sup>2</sup> has been successfully adapted to grow in chicken embryos. This strain of virus, a potential immunogenic strain, without any pathogenic effect, needed cell culture adaptation for future extensive work, since no attempt seems to have been made for its cell culture adaptation. The virus was given serial passages in chicken embryo-fibroblast cell culture (CEF) and the evidence of adaptation and multiplication of the virus was determined through several parameters of study, viz., haemagglutination (HA), haemagglutination-inhibition (HI), Cytopathogenic effect (CPE) in monolayer cells, haemadsorption (HAD) with chicken red blood cells, plaque formation in agar overlay, neutralization of the virus by specific hyperimmune serum and 50% embryo-infectivity dose (EID<sub>50</sub>).

The allantoic fluid, having a HA titre of 512 was used as the initial inoculum for CEF cells infection. The virus was given three blind passages in the CEF cells and the HA of the culture fluid was assayed every time and is presented in Table I. The evidence of cytopathogenic effect was absent till 4th passage. In the 5th serial passage slight evidence of CPE was noticed which became well marked after 8th passage (Table I). In the 10th passage the CPE was evinced by spider web appearance, of the monolayer due to

detachment of the cell sheet at places. There was marked shrinkage and syncytia formation of the cells. The appearance of aggregates of degenerated and giant cells were obvious with the evidence of degeneration of the nuclear material.

TABLE I  
*Haemagglutination and cytopathogenic score of the virus in each passage*

Passage number	Reciprocal of HA titre		Observable CPE
	Inoculum	Harvest	
I.	512 allantoic fluid	..	..
II.	..	..	..
III.	..	16	..
IV.	16	64	..
V.	64	64	+
VI.	64	128	++
VII.	128	128	++
VIII.	128	256	+++
IX.	256	256	+++
X.	256	256	+++

The HI titre of the 10th passage virus with ND hyperimmune serum was 128, indicating the specificity of the virus for the ND antibody.

Further evidence of adaptation and propagation of the virus was obtained by HAD test. The CEF cells at 10th passage had  $1 \times 10^5$  HAD ID<sub>50</sub>/ml. In agar overlay technique, the virus could not produce plaque, which had been a difficult process<sup>3,4</sup>. The evidence of propagation was further confirmed, by virus neutralization test, from the TCF of the 10th passage. Through constant serum-varying virus method the TCID<sub>50</sub> was found to be  $1 \times 10^6$ . The TCID<sub>50</sub> neutralization index of serum per ml was calculated to be  $\log 1 \times 10^4$ . The egg embryo infectivity EID<sub>50</sub> at 10th passage level of the virus was found to be  $1 \times 10^6$ .

The evidence presented has obviously pointed out the adaptation of the virus in the CEF cells. The strain in the beginning of the passages had little tendency to adapt; but after 5th passage, the evidences were well marked and at 10th passage level, the virus had every evidence of being adapted in CEF cells. The HAD test indicated no plaque formation in the agar overlay. This does not point out to a negative finding rather lentogenic strains under ordinary overlay techniques do not produce plaques unless the technique is modified,

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#### THE EFFICACY OF CELLULOSE ACETATE ELECTROPHORESIS IN FRACTIONATION OF CEREBROSPINAL FLUID PROTEINS

THE electrophoretic fractionation of proteins often helps in the diagnosis and management of certain human disorders involving liver, kidney and central nervous system (CNS). Different media such as paper, agarose, starchgel etc. have been used for separating the various protein fractions. This paper deals with the use of cellulose acetate (CA) for separation of proteins in cerebrospinal fluid (CSF) of patients with various CNS disorders.

##### *Material and Methods*

The material for this study was obtained from patients admitted in the clinical units of this Centre. Samples of CSF were collected in such of those cases where it was indicated for diagnostic formulations.

The CSF proteins were first concentrated by dialysis using the Amicon Concentrator—CS 15 (M/s Amicon Products, USA). The CSF proteins were fractionated using the Beckman Microzone electrophoresis apparatus\* (Beckman Instruments, USA)<sup>1</sup>. Quantitation was done by using the Gelman Densitometer with integrated recorder\* (M/s. Gelman Instruments, USA).

##### *Observations and discussion*

The protein fractions in CSF samples from patients of different neuropsychiatric disorders were matched against control samples. Qualitatively, the normal serum samples show distinct separation of Albumin, Alpha<sub>1</sub>, Alpha<sub>2</sub>, Beta and Gamma globulins, while normal CSF shows besides these a pre-Albumin band (see Fig. 1). CSFs with protein content of 20–40 mg% showed 85–90% of Albumin and the remaining was globulin. Such CSFs also showed increased Beta globulin and decreased gamma globulin as compared to serum<sup>2</sup>. The normal values found in 50 control CSF samples are indicated in Table I.

By using the CA electrophoretic technique, samples of CSF obtained from patients with different neuropsychiatric disorders have been analysed and the results obtained are indicated in Table II.