detachment of the cell sheet at places. There was marked shrinkage and syncytiata 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**

<table>
<thead>
<tr>
<th>Passage number</th>
<th>Reciprocal of HA titre</th>
<th>Observable CPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculum</td>
<td>Harvest</td>
</tr>
<tr>
<td>I.</td>
<td>512 allantoic fluid</td>
<td>..</td>
</tr>
<tr>
<td>II.</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>III.</td>
<td>..</td>
<td>16</td>
</tr>
<tr>
<td>IV.</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>V.</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>VI.</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>VII.</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>VIII.</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>IX.</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>X.</td>
<td>256</td>
<td>256</td>
</tr>
</tbody>
</table>

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 $1 \times 10^8$ HAD ID$_{50}$/ml. In agar overlay technique, the virus could not produce plaque, which had been a difficult process$^3$. 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$_{50}$ was found to be $1 \times 10^6$. The TCID$_{50}$ neutralization index of serum per ml was calculated to be $1 \times 10^4$. The egg embryo infectivity ELD$_{50}$ at 10th passage level of the virus was found to be $1 \times 10^8$.

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.

*Division of Microbiology, Central Drug Research Institute, Lucknow 226001, January 31, 1980.


**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, agargel, 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). 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 Albunin, Alphaa, Alphabeta, and Globulin, while normal CSF shows besides those a pre-Albunin band (see Fig. 1). CSFs with protein content of 20–40 mg% showed 85–96% of Albunin and the remaining was globulin. Such CSFs also showed increased Beta globulin and decreased Gamma globulin as compared to serum$. 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.
From Table II, it could be seen that pre-albumin is virtually absent in cases of meningitis. Increase in gamma globulin is observed in CSF of patients with encephalitis, with a concomitant increase in beta-globulin and decrease in albumin. Increase in gamma-globulin is also seen in cases with GPI. In cases of Guillain-Barre (GB) syndrome, the elevation of proteins is largely due to albumin followed by a relative increase in the beta-globulin.

Compared to the conventional electrophoretic methods using paper, agar or starch gel techniques, the CA method used in the present studies was found to yield information of considerable significance in certain conditions. For instance, in TB Meningitis, the pre-albumin band is absent during the acute phase and with suitable treatment and recovery this band reappears. Compared to patients with Neurosyphilis (GPI), where a relative increase in globulin is noted, cases with TB Meningitis show elevation of both albumin and globulin due to the breakdown of the blood-brain barrier. The studies on variation in the protein fraction before, during and after therapy are being studied longitudinally.

The results obtained during the present study on CSF from diseased states indicate the potential usefulness of this technique. The new items of information that could be got by this technique are: the variation in the pre-albumin band in CNS disorders where the blood-brain barrier is broken down as in TB Meningitis, the variation in A/G ratio as a result of local inflammatory reactions such as in GPI, relative differences in the albumin and globulin fractions in GB syndrome; and differential diagnosis of brain tumors as compared to cerebral vascular episodes, the former having

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**Table I**

<table>
<thead>
<tr>
<th>Protein fractions in 50 cases</th>
<th>CSF (%)</th>
<th>Serum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-albumin</td>
<td>3.5 ± 1.0</td>
<td>...</td>
</tr>
<tr>
<td>Albumin</td>
<td>55.5 ± 7.5</td>
<td>60.0 ± 5.0</td>
</tr>
<tr>
<td>Alpha-1-globulin</td>
<td>4.6 ± 1.0</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>Alpha-2-globulin</td>
<td>7.0 ± 1.5</td>
<td>6.0 ± 1.5</td>
</tr>
<tr>
<td>Beta globulin</td>
<td>16.0 ± 3.5</td>
<td>10.0 ± 2.0</td>
</tr>
<tr>
<td>Gamma globulin</td>
<td>13.4 ± 3.0</td>
<td>20.0 ± 2.5</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>CSF Protein fractions in certain CNS disorders</th>
<th>No. of cases</th>
<th>Total Protein (mg%) (Mean)</th>
<th>CSF Electrophoresis</th>
<th>Pre-albumin %</th>
<th>Albumin %</th>
<th>Alpha₂ G %</th>
<th>Alpha₄ G %</th>
<th>Beta G %</th>
<th>Gamma G %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Subjects</td>
<td>10</td>
<td>32.0</td>
<td>3.5</td>
<td>55.5</td>
<td>4.6</td>
<td>7.0</td>
<td>16.0</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td>4</td>
<td>48.2</td>
<td>3.6</td>
<td>56.0</td>
<td>6.6</td>
<td>8.6</td>
<td>15.2</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>GB Syndrome</td>
<td>5</td>
<td>78.0</td>
<td>3.0</td>
<td>56.3</td>
<td>4.4</td>
<td>5.0</td>
<td>20.6</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td>GPI</td>
<td>4</td>
<td>64.2</td>
<td>4.2</td>
<td>47.5</td>
<td>4.0</td>
<td>5.0</td>
<td>11.0</td>
<td>28.3</td>
<td></td>
</tr>
<tr>
<td>Encephalitis</td>
<td>5</td>
<td>78.2</td>
<td>4.0</td>
<td>44.0</td>
<td>4.4</td>
<td>7.0</td>
<td>20.4</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td>TBM</td>
<td>14</td>
<td>112.5</td>
<td>1.6</td>
<td>56.5</td>
<td>6.8</td>
<td>7.0</td>
<td>14.6</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>Pyogenic meningitis</td>
<td>8</td>
<td>182.0</td>
<td>0</td>
<td>56.2</td>
<td>5.2</td>
<td>7.0</td>
<td>16.2</td>
<td>15.6</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Showing the separation of the various protein fractions (in the middle) and the densitometric recording (top curves) along with the integrated recording (at the bottom).
elevation of all fractions as compared to the latter. 
Apart from these, the present technique gives quan-
titative data in less than an hour, a thing which cannot 
be done by using conventional techniques.

Thus the advantage of cellulose acetate electro-
phoresis of CSF proteins lies in the fact that valuable 
information could be got for purposes of diagnosis 
and management with speed and accuracy. In some of 
the CNS disorders seen in relatively large numbers in 
tropical countries and where early diagnostic for-
mulations would help in therapeutic management, the 
present technique is found to provide valuable infor-
mation.

Dept. of Neurochemistry, M. N. SUBHASH, 
National Inst. of Mental B. S. SRIDHARA RAMA RAO, 
Health and Neuro Sciences, Bangalore, July 9, 1979.

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* Gifts of WHO-SEARO.


A PRELIMINARY OBSERVATION ON THE 
ACTION OF METOCLOPRAMIDE ON 
SKELETAL MUSCLE OF FROG 
AND GUINEAIG ILEUM

METOCLOPRAMIDE is one of the recently introduced 
antiemetic. It promotes oesophageal and gastric 
peristalsis, increases the tone of the cardiac sphincter 
and tends to speed gastric emptying. Extrapyramidal 
reaction is one of the few side effects produced by this 
drug. Its therapeutic actions are antagonised by 
anticholinergic drugs like atropine. It has been 
reported, that the drugs inducing extrapyramidal 
reactions like chlorpromazine, chloroquine, quinine, morphine, atropine, some of the antihista-
mes, other phenothiazines, ergot alkaloids, and 
metallic tremoregents like copper, mercury, lead and 
manganese (unpublished data), when tested on rectus 
abdominis muscle of frog, exhibit a curarimimetic as 
well as a cholinomimetic effect. On superfused intestine, 
produce a contraction and also potentiate acetylcholine 
(Ach)5,6. Since, metoclopramide is also a tremo-
regent13, the present study was undertaken to test 
whether it produces similar response on the biological 
preparations mentioned above.

The rectus abdominis muscle of frog was super-
fused as reported earlier4. After recording sub-
maximal response of the tissue to Ach, metoclopramide 
was added in 1, 10, 100 ng and 1, 10 and 100 mcg 
concentrations, followed by Ach. The guineapig 
ileum was superfused as reported from our labo-
atory6,8. Metoclopramide was used in the same 
concentrations as mentioned above. The response 
of the tissue was recorded on a slow moving smoked 
drum. Atropine sulphate was used as an antagonist.

On skeletal muscle, the metoclopramide in 1, 10 
and 100 ng concentrations produced an unpredictable 
effect on the action of Ach, i.e., sometimes an 
immediate potentiation of Ach, a curarimimetic effect 
and a delayed potentiation of Ach. Whereas in 1, 10 
and 100 mg concentrations it produced a predictable 
and graded potentiation of Ach (Fig. 1); at 100 mcg 
concentrations the tissue responded with a maximum 
contraction, and during recovery Ach was potentiated. 
The guineapig ileum responded with a contraction to 
all concentrations of metoclopramide in a graded 
fashion. Ach was also potentiated (Fig. 2). Atropine 
which is reported to exhibit a dual action on intestinal 
smooth muscle6, produced a contraction of guineapig 
ileum (Fig. 2) but blocked the contraction induced 
by metoclopramide partially and with increasing 
concentrations completely.

Fig. 1. Showing effect of metoclopramide on 
rectus abdominis muscle of frog, all contractions are 
induced by 0-1 mcg of Ach, 1 and 10 indicate the 
addition of 1 and 10 mcg of metoclopramide followed 
by Ach.

Metoclopramide has been reported to potentiate 
Ach on human intestine4. It potentiates Ach on 
skeletal muscle of frog and guineapig ileum and produces 
contraction of the latter which is antagonised by atro-
pine (Figs. 1 and 2). The therapeutic action of meto-
clopramide is antagonised by anticholinergic drugs9. 
Its mechanism of antiemetic effect is due to the 
increasing gastric peristalsis and frequent gastro 
emptying. Our results indicate that it has produced 
similar effect on both skeletal muscle as well as on 
intestinal smooth muscle like other tremoregents 
reported from our laboratory. Though it is said to 
induce extrapyramidal reactions by antagonizing