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 1×10^5 HAD ID_{50}/ml. In agar overlay technique, the virus could not produce plaque, which had been a difficult process[3,4]. 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 1×10^6. The $TCID_{50}$ neutralization index of serum per ml was calculated to be log 1×10^4. The egg embryo infectivity EID_{50} at 10th passage level of the virus was found to be 1×10^5.

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, 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)[1]. 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, $Alpha_1$, $Alpha_2$, 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[2]. 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.

TABLE I

Protein fractions in 50 cases

	CSF (%)	Serum (%)
Pre-albumin	3·5±1·0	..
Albumin	55·5±7·5	60·0±5·0
Alpha-1-globulin	4·6±1·0	4·0±1·0
Alpha-2-globulin	7·0±1·5	6·0±1·5
Beta globulin	16·0±3·5	10·0±2·0
Gamma globulin	13·4±3·0	20·0±2·5

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 starchgel techniques, the CA method used in the present studies was found to yield information of considerable significance in certain conditions. For instance, in TB Meningits, the pre-albumin band is absent during the acute phase and with suitable treatment and recovery this band reappears. Compared to patients with Neuro-syphilis (GPI), where a relative increase in globulin is noted, cases with TB Meningitis show elevation of both albumin and gobulin 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.

TABLE II

CSF Protein fractions in certain CNS disorders

Disease	No. of cases	Total Protein (mg%) (Mean)	CSF Electrophoresis (Mean Values)					
			Pre-albumin %	Albumin %	$Alpha_1$ G %	$Alpha_2$ G %	Beta G %	Gamma G %
Control Subjects	10	32·0	3·5	55·5	4·6	7·0	16·0	13·4
Epiepsy	4	48·2	3·6	56·0	6·6	8·6	15·2	10·0
GB Syndroue	5	78·0	3·0	56·3	4·4	5·0	20·6	10·7
GPI	4	64·2	4·2	47·5	4·0	5·0	11·0	28·3
Encephalitis	5	78·2	4·0	44·0	4·4	7·0	20·4	20·2
TBM	14	112·5	1·6	56·5	6·8	7·0	14·6	13·5
Pyogenic meningitis	8	182·0	0	56·2	5·2	7·0	16·2	15·6

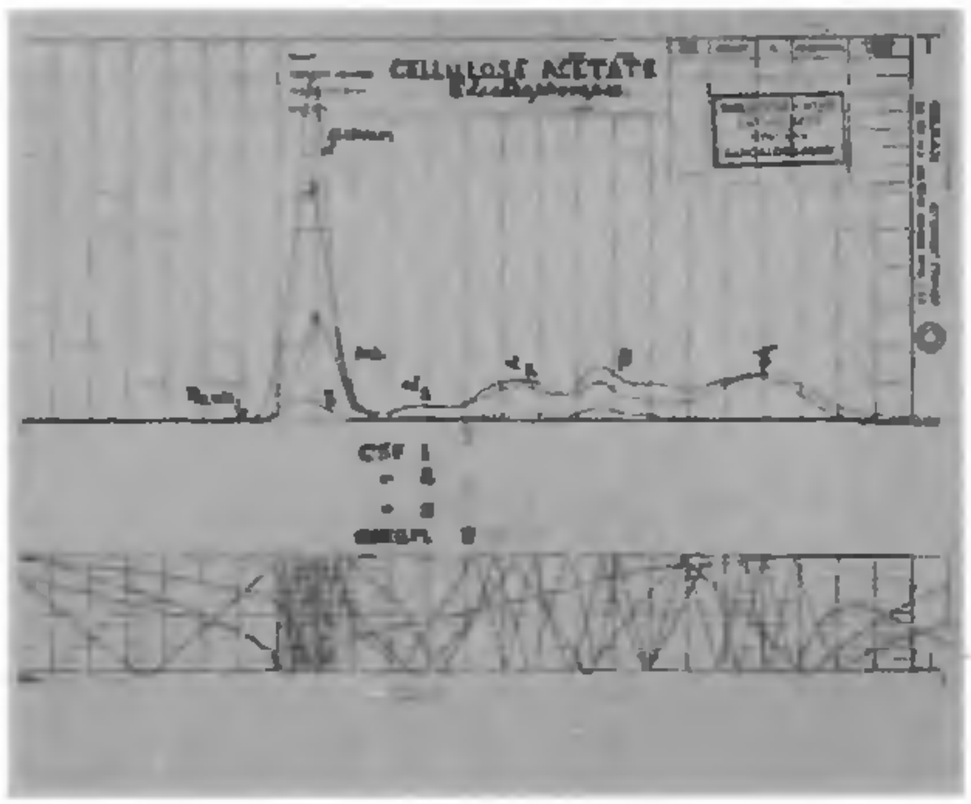

FIG. 1. Showing the separation of the various protein fractions (in the middle) and the densitometric recording (top curves) along with the integrated recording (at the bottom).

The results obtained during the present study on CSF from diseased states indicate the potential useful ness 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 inflam-matory reactions such as in GPI, relative differences in the albumin and globulin fractions in GB syndrome; and differential diagnosis of brain tumours as compared to cerebral vascular episodes, the former having

elevation of all fractions as compared to the latter. Apart from these, the present technique gives quantitative data in less than an hour, a thing which cannot be done by using conventional techniques.

Thus the advantage of cellulose acetate electrophoresis 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 formulations would help in therapeutic management, the present technique is found to provide valuable information.

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A PRELIMINARY OBSERVATION ON THE ACTION OF METOCLOPRAMIDE ON SKELETAL MUSCLE OF FROG AND GUINEAPIG 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[15]. It has been reported, that the drugs inducing extrapyramidal reactions like chlorpromazine[1], chloroquine[2,3], quinine, morphine[9], atropine[5,6], some of the antihistamines[6], other phenothiazines, ergot alkaloids[9] and metallic tremorogens 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)[6,10]. Since, metoclopramide is also a tremorogen[13], the present study was undertaken to test whether it produces similar reponse on the biological preparations mentioned above.

The rectus abdominis muscle of frog was superfused as reported earlier[4]. After recording submaximal 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 laboratory[6,8]. 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 mcg 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 muscle[5], produced a contraction of guineapig ileum (Fig. 2) but blocked the contraction induced by metoclopramide partially and with increasing concentrations completely.

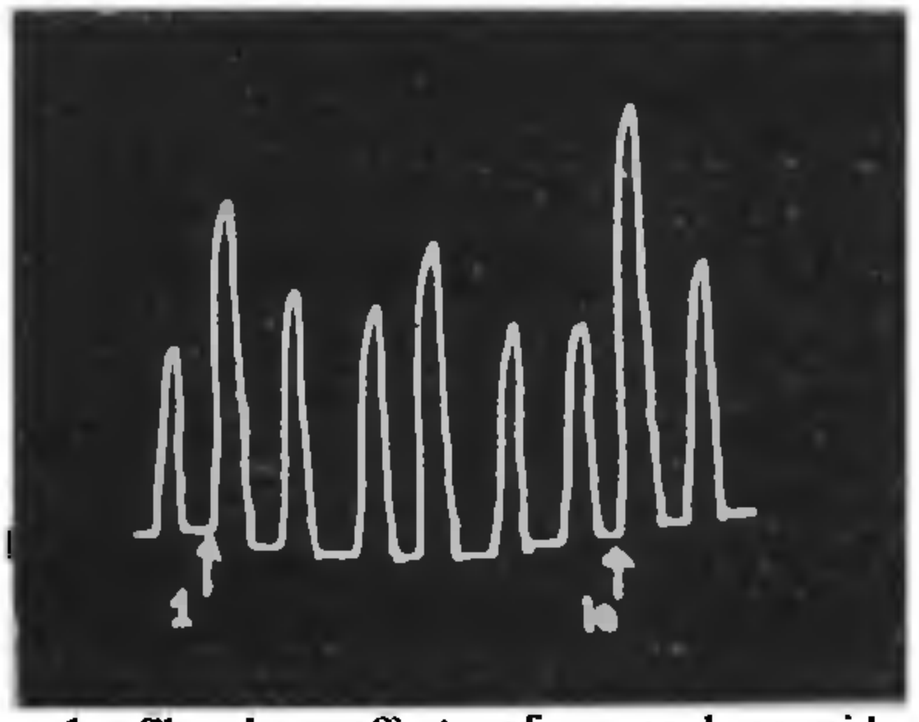

FIG. 1. Showing effect of metoclopramide on rectus abdominis muscle of frog, all contractions are induced by 0·1 mcg of Ach, 1 and 10 indicate the addition of 1 and 10 mcg of metoclopramide followed by Ach.

Metoclopramide has been reported to potentiate Ach on human intestine[14]. It potentiates Ach on skeletal muscle of frog and guineapig ileum and produces contraction of the latter which is antagonised by atropine (Figs. 1 and 2). The therapeutic action of metoclopramide is antagonised by anticholinergic drugs[15]. Its mechanism of antiemetic effect is due to the increasing gastric peristalsis and frequent gastric emptying. Our results indicate that it has produced similar effect on both skeletal muscle as well as on intestinal smooth muscle like other tremorogens reported from our laboratory. Though it is said to induce extrapyramidal reactions by antagonising