## Logarithmic-Normal Distribution of Cerebrospinal Fluid Folate Concentrations

Folate deficiency is known to be deleterious to the foetal nervous system, resulting in malformations[1]. Caused by malnutrition, malabsorption, toxic or drug-induced metabolic interference, e.g. nutritional deficiency, anticonvulsant drugs, alcohol, psychopharmaca, etc., this deficiency has been implicated in several disorders of mature neural tissue, e.g. neuropathies[2], deterioration and dementia in epileptics[3], psychosis in epileptics[4] and cerebellar dysfunction associated with anticonvulsant therapy[5-7]. Mostly, microbiological assays of serum folate with *Lactobacillus casei* have been used as the earliest and most sensitive index of subclinical deficiency. Unlike vitamin $B_{12}$, which is selectively excluded from the cerebrospinal fluid (CSF), folates are reported to be selectively concentrated[6,7]. This fact has been assumed to be related to the folate requirements of the central nervous system[8], and CSF folate assays are suggested to reveal folate deficiency in the central nervous system with better discrimination and accuracy[9]. Using this criterion, it has been reported that epileptics have significantly lower values[7].

Since, however, little seems to be known of the range of CSF folate values, a normal symmetric distribution has usually been presumed, and calculations of differences between rather small and selected groups founded upon this assumption; the following findings are reported.

*Materials and methods.* Specimens of CSF were obtained by lumbar puncture from a random sample of 416 neurological patients, and assayed in triplicate microbiologically using *Lactobacillus casei*[10]. Serum folate values in our laboratory for healthy controls ($N = 104$) were; range 2.3–14.8, median 5.0, interquartile range 2.1, arithmetic mean 5.45 ng/ml, respectively. In addition to conventional analyses of the CSF specimens (including protein electrophoresis), their vitamin $B_{12}$ concentrations were measured. Serum folate and vitamin $B_{12}$ values were also determined. For comparison with previously published data, arithmetic means and S.D. were calculated from the ungrouped values.

*Results.* The range of individual CSF folate values for 416 patients was 1.3–80.0 ng/ml, median 11.6 ng/ml, and arithmetic mean 14.25 ng/ml and S.D. $\pm$ 10.01 ng/ml. The distribution diagram of grouped values shown in Figure 1 clearly suggests a logarithmic-normal distribution. This hypothesis was tested by plotting on probability paper (Figure 2), the log-values for class limits (log ng/ml CSF folate) on the abscissa, against the corresponding cumulative distribution frequency values on the ordinata (normal cumulative distribution percentual scale). The evident nice linear function thus confirmed the idea that log CSF folate follows normal distribution. By graphic probit analysis[11], the log mean and log S.D. for this material were found to be $1.065 \pm 0.323$. From the log-normally fitted equation the anti-log values for percentiles were obtained by the same procedure. These were: 1.0 = 2.1, 2.0 = 2.5, 5.0 = 3.4, 10.0 = 4.5, 25.0 = 7.0, 50.0 = 11.6, 75.0 = 19.2, 90.0 = 30.1, 95.0 = 39.4, 97.0 = 47.0, 99.0 = 65.4, ng/ml CSF folate respectively (1st and both last percentile values extrapolated). Thus, median coincided with geometric mean = 11.6 ng/ml, S.D. = $\pm$ 10.7 ng/ml, interquartile range being 12.2 ng/ml.

None of the subjects had hematological signs of either folate or vitamin $B_{12}$ deficiency; there was no positive correlation between paired serum and CSF folate values in the individuals, which constituted a representative sample of patients with various disorders in a large neurological department. Cases with low or high CSF values had no specific general metabolic disorders, neurological feature, nor CSF abnormality in common[12].

*Conclusions.* Our findings show a marked trend towards higher folate concentrations in CSF compared with serum

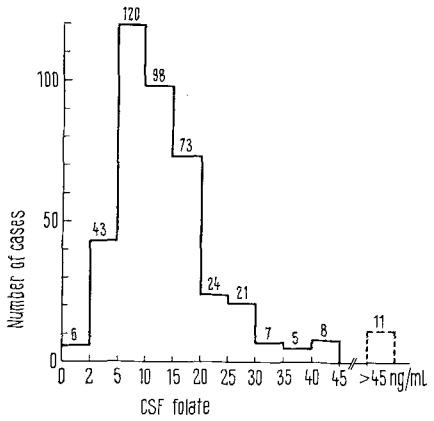


Fig. 1. Logarithmic-normal distribution of CSF folate.

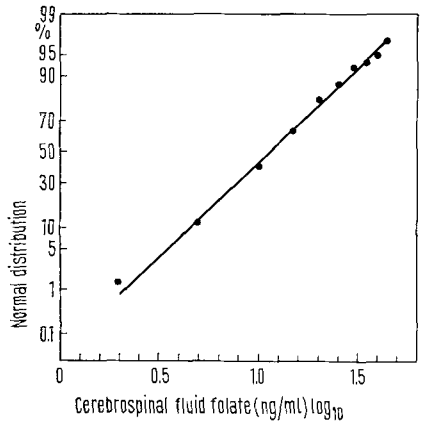


Fig. 2. Logarithmic-normal distribution of CSF folate concentrations.


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values, the implications of which are subject to further studies. The considerable range, and especially the pronounced logarithmic-normal distribution, are to our knowledge new and important observations, which indicate obvious sources of error in the interpretation of previously published data. Thus they emphasize that when the results of CSF folate assays are subjected to statistical analysis (*t*-test, analysis of variance, correlation analysis), the logarithm of the folate concentration should be used. This appears especially urgent in view of the serious practical consequences previous reports may have caused, i.e. ambiguity and confusion leading to inadequate anticonvulsant treatment of epileptics[13].

*Zusammenfassung.* Die Folatkonzentration der Zerebrospinalflüssigkeit wurde bei 416 neurologischen Patienten mikrobiologisch bestimmt. Es wurde festgestellt, dass die Werte einer logarithmisch-normalen Verteilungskurve folgen.

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## Reversible Inhibition of Embryonic Mitosis by Phytohemagglutinin from *Phaseolus vulgaris*

Phytohemagglutinin (PHA), an extract from *Phaseolus vulgaris*, is well known not only as a hemagglutinating agent[1] but also as a stimulant of mitosis[2]. PHA M and PHA P have the property of transforming lymphocytes and this transformation is the precursor of mitosis[3]. Joachim[3] also detected stimulation of mitosis of cells other than lymphocytes, such as tissue cells, neoplastic cells etc. in presence of PHA. A very spectacular case is the mitotic outburst induced by PHA in soil amoebae[4]. A number of reports have also appeared in the literature on the molecular basis of the nature of action of PHA but little can be said as yet save the very general statement that PHA stimulates RNA synthesis. Pogo et al.[5] and Joachim[3] reviewed some of these investigations.

In view of the widely discussed phenomenon of mitotic stimulation by PHA, especially in soil amoeba[4], we wish to report the *inhibitory* effect of this substance on cleavage of embryonic cells, i.e. a system of rapid mitosis. The present report describes the results obtained with embryos of *Limnaea* sp. The eggs were collected from the underside of aquatic leaves in the pond and in vessels kept in the laboratory where these molluscs can be reared by feeding with lettuce. Bacto-phytohemagglutinin M and P (Difco laboratories) were both used by dissolving in the medium, i.e. distilled water in which these molluscs can develop normally.

The most surprising feature of these treatments is that both PHAP and PHAM *reversibly* block development, i.e. embryonic cleavage or mitosis in all stages of morula or blastula. The Table shows the concentrations at which such arrestation takes place. It should be noted that Bacto-phytohemagglutinin M and P as supplied by Difco laboratories has been standardized on the basis of the biological activity. A vial for 5 ml may contain different quantities of the dry material because the product is standardized by the manufacturers on the basis of its mitotic stimulatory activity. Taking the standard concentration (i.e. one vialful of dry material dissolved in 5 ml) as *n*, we have used *n*, *n*/2, *n*/10, *n*/20, *n*/100, *n*/200 concentrations.

As is evident from the Table, a higher concentration of PHAM (than of PHAP) is required in order to arrest cleavage. This is in agreement with the findings that PHAP is stronger than PHAM[3,4]. However the point to be emphasized is that arrestation by neither of the agents was permanent; on being washed and then left in water at various intervals of time, the treated eggs began to undergo cleavage. This has been tested with PHAP on uncleaved eggs, morula, blastula and gastrula and with PHAM at uncleaved, 2-cell and 4-cell stages. The most significant finding is that even after being left for 24 or 48 h in PHAP, the arrested blastula etc. on being put to water began to develop and were followed up to post-trochophore stages, save when they disintegrated altogether on being put back to water. It is most unlikely that PHA stimulates RNA synthesis in the arrested eggs because as we have found[6] the incorporation of $P^{32}$ in 2-cell stage is significantly higher than in the uncleaved stage and increases continuously up to late trochophore stage. Thus, although at present nothing can be said about the mechanism of this inhibitory action, the facts reported indicate a new property of PHA[7].

Effective concentration of PHA

| Concentration | Action PHAP | PHAM |
|---|---|---|
| *n* | | + |
| *n*/2 | | + |
| *n*/10 | + | + |
| *n*/20 | + | – |
| *n*/100 | – | – |
| *n*/200 | – | – |

Signifies inhibition of mitosis.

*Zusammenfassung.* Phytohämagglutinin hemmt die Furchungsteilung, im Gegensatz zu differenzierten Geweben, bei denen es als Mitoseaktivator wirkt. Wenn die Eier ausgewaschen werden, läuft die Entwicklung normal weiter.

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