

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 epileptics¹³.

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 agent¹ but also as a stimulant of mitosis². PHA M and PHA P have the property of transforming lymphocytes and this transformation is the precursor of mitosis³. JOACHIM³ 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 amoebae⁴. 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 JOACHIM³ reviewed some of these investigations.

In view of the widely discussed phenomenon of mitotic stimulation by PHA, especially in soil amoeba⁴, 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 its 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 found⁶ the incorporation of P³² 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 PHA⁷.

Effective concentration of PHA

Concentration	Action PHAP	PHAM
<i>n</i>		+
<i>n/2</i>		+
<i>n/10</i>	+	+
<i>n/20</i>	+	—
<i>n/100</i>	—	—
<i>n/200</i>	—	—

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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⁷ We are grateful to Difco Laboratories for generous gift of PHAP and PHAM.