of great importance in understanding the processes leading to mitosis. Considerable doubt has, however, been cast on the interpretation of Barnett's results, by the fact that the spin label used has since turned out to be a mixture of three compounds². Consequently, an independent study has been undertaken to examine the effects of the mitogen phytohaemagglutinin (PHA), using several different spin labels that probe different regions of the membrane.

The cells examined were human tonsil lymphocytes, separated (>90% purity) by teasing through a mesh and suspended in Hank's medium. ESR measurements were made using a Varian E-9, X-band spectrometer, equipped with a variable temperature accessory and a dual cavity operating in the H_{014} mode. Quantitation of the spectra was aided by the use of a Nicolet 1020A signal averager. Spin labels were purchased from the Syva Corporation, Palo Alto, California. These were the androstane label

and the stearic acid labels

where $m = 1$, $n = 14$; $m = 5$, $n = 10$ and $m = 12$, $n = 3$. All these labels are known to incorporate into the lipid regions of membranes. Two different methods were used to label the cells, namely deposition of the label on to the walls of a flask by evaporation of an alcoholic solution, and addition of $1 \mu\text{l}$ of a 10^{-1} M solution of label in ethanol to 1 ml of cell suspension. In both cases, cells were incubated with the label for 15 min at 37°C . They were then washed three times with medium, to remove free label and resuspended. A third method was also tried for label I ($m = 12$; $n = 3$), this being dissolved in an aqueous solution of sodium carbonate before addition to the cell suspension; however the method of introduction of label seemed to have no influence on the results obtained. Introduction of a spin label into the cells also had no detectable effect on their integrity. The PHA used

was Wellcome purified reagent. The optimum mitogenic dose for this material was found to be $1 \mu\text{g ml}^{-1}$ and this is the concentration used, except in one experiment where a higher dose ($10 \mu\text{g ml}^{-1}$) was used.

The androstane label and the stearic acid label I ($m = 1$; $n = 14$) both gave weakly immobilised, three-line spectra when added to the tonsil lymphocytes. The ratio of the heights of the three lines, which gives a measure of the mobility of the label³, was found to be unaffected by incubation with PHA for times up to 90 min. The label I ($m = 5$; $n = 10$) gave a complex spectrum showing both weakly and strongly immobilised components, possibly a result of some localisation of the polar nitroxide group in a hydrophilic region. Although difficult to analyse, this showed no detectable change with PHA. The label I ($m = 12$; $n = 3$) gave a strongly immobilised 'powder' spectrum when incorporated in the cells. From the separation of the extrema, an order parameter, S , was calculated⁴. This was determined with an accuracy of $\pm 1\%$ by means of the Nicolet 1020A. No change in the value of S could be detected, up to 90 min after the addition of PHA at 37°C . Use of a higher concentration of PHA also showed no effect on the value of S . When spectra were recorded at $21 \pm 1^\circ\text{C}$, the values of S were 0.678 ± 0.004 in the absence of PHA and 0.674 ± 0.007 in the presence of PHA. When measurements were made at 35°C , the fluidity of the membrane was increased and the value of S correspondingly reduced to 0.57 ± 0.01 .

In one experiment, cells were suspended in medium containing serum, in order to promote growth. As previously reported⁵, however, it was found that after introduction of spin label the observed spectrum was due primarily to label complexed with serum albumin.

Investigations into the fluidity of erythrocyte membranes in myotonic muscular dystrophy⁶ suggest that the spectral amplitude ratio A/B is more sensitive to change than the order parameter, S .

Consequently the present spectra were also analysed in terms of this parameter, but in no case could any change as a result of PHA be detected.

The present results indicate that PHA has no demonstrable effect on the fluidity of human tonsil lymphocyte membranes and it is unlikely that other human lymphocytes will behave differently. An alternative interpretation of the findings of Barnett *et al.*¹ must therefore be found. Since individual labels, in the mixture they used, would give differing values for the order parameter, the changes following mitogenic stimulation are consistent with a change in the relative proportions of the three labels present in the membranes. The cause of such a change could be as important as the apparent change in fluidity to which it was ascribed and this is now being investigated.

I thank Drs M. Moore and M. R. Potter of the Immunology Section for help and advice. The work was supported by the MRC.

N. J. F. DODD

Paterson Laboratories,
Christie Hospital and Holt Radium
Institute,
Manchester M20 9BX, UK

- ¹ Barnett, R. E., Scott, R. E., Furcht, L. T., and Kersey, J. H., *Nature*, **249**, 465-466 (1974).
- ² Barnett, R. E., Scott, R. E., Furcht, L. T., and Kersey, J. H., *Nature*, **255**, 262 (1975).
- ³ Smith, I. C. P., in *Biological Applications of ESR*, (edit. by Swartz, H. M., Bolton, J. R., and Borg, D. C.), 483-539 (Wiley-Interscience, New York, 1972).
- ⁴ Seelig, J., *J. Am. chem. Soc.*, **92**, 3881-3887 (1970).
- ⁵ Landsberger, F. R., Paxton, J., and Lenard, J., *Biochim. biophys. Acta*, **266**, 1-6 (1971).
- ⁶ Butterfield, D. A., Chesnut, D. B., Roses, A. D., and Appel, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 909-913 (1974).

Chandler Wobble and viscosity in the Earth's core

AN addition is necessary to the references given by Dr Rochester¹. The (I_1 , m_1) he is using are those of the reference² given as (1950a) on p.257 of *The Earth*, fourth edition.

BERTHA JEFFREYS

160, Huntingdon Road,
Cambridge CB3 0LB, UK

- ¹ Rochester, M. G., *Nature*, **255**, 655 (1975).
- ² Jeffreys, H., *Mon. Not. R. astr. Soc.*, **110**, 460-466 (1950); *Collected Papers*, 3, 547-553 (Gordon and Breach, London, 1974).

Erratum

In the original contribution by Rochester¹ the following text corrections should be made: para 1, line 12, $(\dot{I}_1^2 + \dot{m}_1^2)/\omega^2$. and para 2, line 17, $\gamma = (\dot{I}_1^2 + \dot{m}_1^2)^{1/2}/\omega$.

- ¹ Rochester, M. G., *Nature*, **255**, 655 (1975).

Matters arising

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