Immunology, 1970, **18**, 393.

Response of Human Foetal Thymocytes to Phytohaemagglutinin (PHA)

H. E. M. Kay, Jean Doe and A. Hockley

Foetal Tissue Bank, Royal Marsden Hospital, London, S.W.3

(*Received* 14*th July* 1969)

Summary. Human foetal thymocytes first respond to phytohaemagglutinin at about 14 weeks gestation. This corresponds to the completion of thymic differentiation and is perhaps the earliest, but still indirect, evidence of immune competence in the foetus.

INTRODUCTION

It would be valuable to know the earliest age in the human foetus when immune function or capacity can be demonstrated. By comparison with animals where experiments have been performed (Silverstein and Kraner, 1965; Cotes, Hobbs and Bangham, 1966) it might be expected that the development of immune competence would be extended over a long period of time during foetal and post-natal life but that the earliest demonstrable activity might coincide with the formation and differentiation of the lymphoid organs.

This supposition would be strengthened if immune activity of the lymphoid cells could be shown *in vitro*. Van Furth (1964) demonstrated γ-globulin synthesis by spleen cells from foetuses of 21 or more weeks but the emergence of blood lymphocytes and the development of the thymus are prior events which happen about the 12th to the 14th weeks. However, since the lymphocyte may not be functional when first detectable, or may be confused with other non-immune small round cells, a test of their properties is necessary. The response to phytohaemagglutinin (and similar substances) is to some extent correlated with immune status as judged clinically and by other tests, although responsive cells form only one component of the system of cellular immunity.

METHODS

Normal human foetuses of different ages from 9 to 28 weeks gestation (6–24 cm crown–rump length) were dissected soon after the operation of hysterotomy and the thymic tissues placed in TC 199 at 4° for up to 48 hours. The thymus was chopped finely with scissors and filtered through nylon gauze to make a cell suspension.

All cultures were set up with a final cell concentration of 10^6 cells/ml in a mixture of TC 199 (four parts) and AB serum (one part). Phytohaemagglutinin (Wellcome Research Laboratories, Beckenham) was used at a standard concentration (1 volume to 100 volumes of medium). Whenever possible four cultures of 5 ml each were grown in stationary bijou screw-capped bottles, two with PHA and two controls. From small thymuses smaller volumes were cultured (0·5 and 1·0 ml) in 1-ml disposable syringes sealed with the needle bent.

After 3 days incubation at 37°, tritiated thymidine was added to all cultures, 5 μCi/ml of culture medium and incubation continued for a further 4 hours.

The material was prepared for scintillation counting by precipitation with trichloroacetic acid and solution in 25 per cent tetrabutylammonium hydroxide. After addition of a scintillation compound, dimethyl POPOP, the samples were counted in a Packard Tricarb Scintillation counter. Results were corrected for quenching.

Parallel studies of cell morphology showed typical 'blast transformation' of a variable proportion of the thymic cells which in autoradiographs showed heavy labelling. The concentration of PHA, the cell concentration, timing and other details of culture are those which have produced the most consistent results in other laboratories (e.g. Knight, Bradley, Oppenheim and Ling, 1968; Weber, 1968) as well as our own. A single experiment with concentrations of PHA of five times and of one-fifth of the standard showed decreased uptake at both levels of about 40 per cent.

Results were expressed as corrected counts per millilitre of cultured cells and this was compared with controls in two ways, either as excess counts by merely subtracting the control values or by a factor derived from the expression:

$$\frac{\text{Mean PHA counts—Mean control counts}}{\text{Mean control counts}}$$

This takes into account the variable amount of cell growth which occurs in control cultures. There was good agreement between most duplicates but a few discrepant results due to lack of growth in one PHA bottle occurred and these results were excluded.

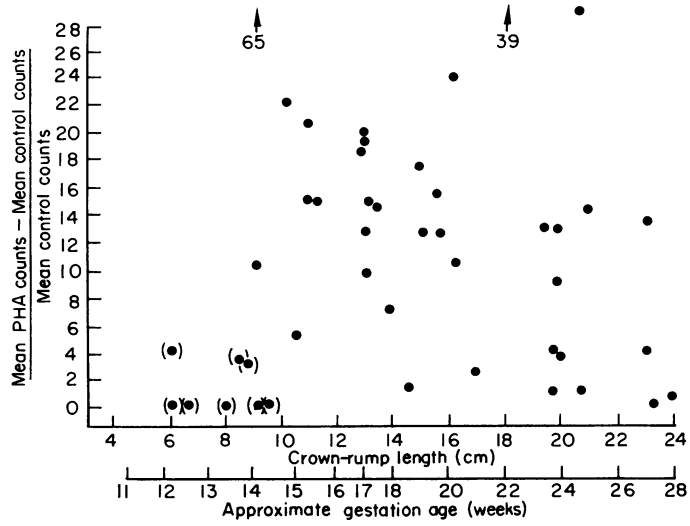

FIG. 1. Thymidine uptake by foetal thymocytes. ●, Duplicated results; (●), single estimates.

RESULTS AND DISCUSSION

Thymidine uptake values are shown in Fig. 1 expressed in relation to crown–rump length with the approximate foetal age given below. Three points stand out: the lack of PHA-responsiveness (with one unduplicated exception) below 9·0 cm crown–rump length (about 13–14 weeks), the variable but sometimes very high results corresponding to

a foetal age of 15–20 weeks and, less certainly, a slight tailing off in the foetuses of 22–28 weeks.

The genesis of PHA-responsiveness takes place in the phase of thymic development where cortex and medulla become clearly demarcated and when the earliest Hassall's corpuscles appear. At this stage the medulla sometimes seems to occupy the major portion of the tissue (see Fig. 2) and the very high transformability of thymic cells in some foetuses of this period corresponds with evidence that PHA-responsive cells are located in the medulla rather than the cortex (Weber, 1966).

This is also the phase of development when the blood lymphocyte count increases rapidly (Playfair, Wolfendale and Kay, 1963). These cells also are responsive to PHA at 14–16 weeks as can be demonstrated both morphologically and by uptake studies. Unfortunately, however, owing to difficulties of cell separation, small quantities and the presence of many cells which proliferate in the absence of PHA in the blood of young foetuses, it has not been possible to determine the timing of this development with any accuracy.

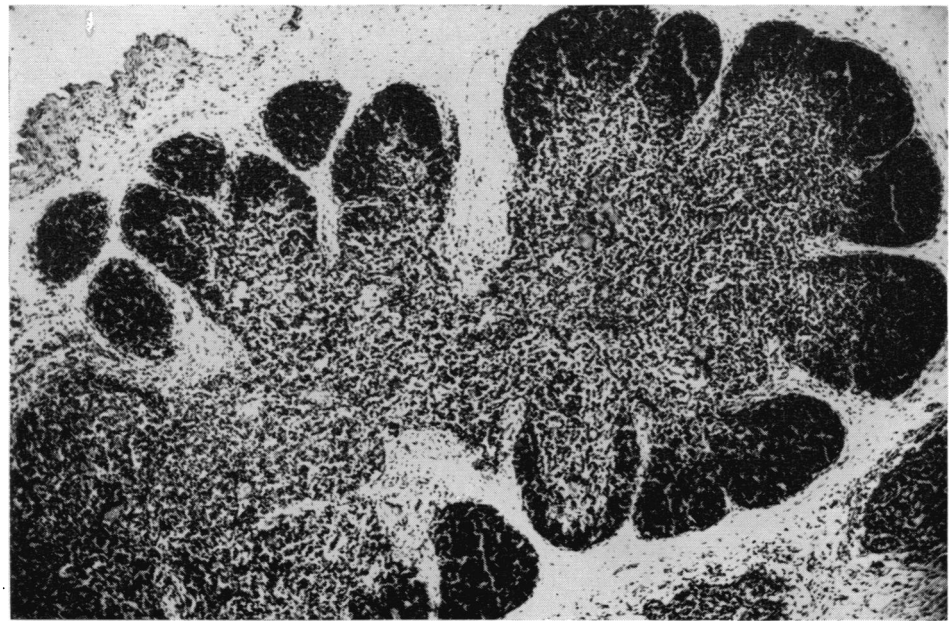

FIG. 2. Foetal thymus at 16 weeks. Crown–rump length 11·7 cm. ×60.

Thereafter there appears to be a variable decline in the proportion of PHA-responsive cells to the levels found post-natally, although these are also extremely variable (McIntyre and Segel, 1966; Lischner and Punnett, 1966; Winkelstein and Craddock, 1967; Claman and Brunstetter, 1968). Part of this decline may be attributable to the higher proportion of cortex in the thymus but the variability from one foetus to another and from one post-natal thymus to another probably has its origin in other immunological circumstances.

ACKNOWLEDGMENTS

The Foetal Tissue Bank at the Royal Marsden Hospital is supported by a grant from the Medical Research Council. The collaboration of Dr M. J. G. Allan, Dr B. Markowski, Mrs D. M. Boyd and others is gratefully acknowledged.

REFERENCES

CLAMAN, H. N. and BRUNSTETTER, G. H. (1968). 'The response of cultured human thymus cells to phytohemagglutinin.' *J. Immunol.*, **100**, 1127.

COTES, P. M., HOBBS, K. R. and BANGHAM, D. R. (1966). 'Development of the immune response in the foetal and newborn rhesus monkey.' *Immunology*, **11**, 185.

KNIGHT, S., BRADLEY, J., OPPENHEIM, J. J. and LING, N. R. (1968). 'The *in vitro* transformation of thymocytes and lymphocytes from humans, rabbits and guinea-pigs and from thymomas.' *Clin. exp. Immunol.*, **3**, 323.

LISCHNER, H. W. and PUNNETT, H. H. (1966). 'Phytohaemagglutinin and thymic lymphocytes.' *Lancet*, **ii**, 640.

MCINTYRE, O. R. and SEGEL, W. D. (1966). 'PHA and thymic lymphocytes.' *Lancet*, **i**, 1265.

PLAYFAIR, J. H. L., WOLFENDALE, M. R. and KAY, H. E. M. (1963). 'The leucocytes of peripheral blood in the human foetus.' *Brit. J. Haemat.*, **9**, 336.

SILVERSTEIN, A. M. and KRANER, K. L. (1965). 'Studies on the ontogenesis of the immune response.' *Molecular and Cellular Basis of Antibody Forms* (Ed. by J. Sterzl *et al.*), p. 341. Academic Press, New York.

VAN FURTH, R. (1964). *The Formation of Immunoglobulins by Human Tissues* in vitro. Luctor et Emergo, Leiden.

WEBER, W. T. (1966). 'Difference between medullary and cortical thymic lymphocytes of the pig in their response to phytohaemagglutinin.' *J. cell Physiol.*, **68**, 117.

WEBER, W. T. (1968). 'Comparative responses to phytohaemagglutinin of appendiceal thymic and splenic lymphocytes of rabbits.' *Immunology*, **15**, 173.

WINKELSTEIN, A. and CRADDOCK, C. G. (1967). 'Comparative response of normal human thymus and lymph node cells to phytohemagglutinin in culture.' *Blood*, **29**, 594.