The Development of Immunity in Fetal Life and Childhood

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Among the weapons man and other vertebrates have evolved to combat infection are specific antibodies and specific cell-mediated immunity. Of equal importance, and closely associated with antibody-antigen interaction, is complement, the heat labile multimolecular complex of 11 components which is best known for its part in the lysis of antibody-sensitised cells. Perhaps more important than all, and older phylogenetically, is phagocytosis. Although essentially non-specific, phagocytosis is enhanced by complement fixation and itself contributes to the afferent phase of immunisation. Most of this review refers to the development of these four factors and their interaction, but mention will also be made of lysozyme and interferon. Much more is known about the development of antibody formation than the other components mentioned. It is, however, believed that a high degree of competence exists at birth in all these functions. Great interest has centred on fetal and neonatal immunological development, since basic mechanisms are laid down at this stage, and massive antigen contact first occurs in the neonatal period.

The development of lymphoid tissue is essential to the development of antigen responsive, specific, immunological competence. It is convenient to consider the development of general lymphoid tissue separately from that of special lymphoid tissue, such as the thymus.

Recognisable nodules of lymphoid tissue have been found in the connective tissue of the neck of the 25 to 28 mm (8 week) human fetus (Gilmor, 1941). Although some lymphocytes are present in the fetal circulation at five weeks, they are few, and it is not until the third month (48 mm) that large numbers of lymphocytes have appeared in lymph vessels and bone marrow and definite lymph nodes are found. The blood lymphocyte count is about 1,000 at 12 weeks and increases to 5,000 to 10,000 cells per mm^3 at 24 weeks (Playfair et al., 1963). Although the splenic anlage is present at five weeks, lymphopoiesis in the spleen is delayed until the third month (Valdes-Dapena, 1957). In normal circumstances, in the absence of intra-uterine infection, lymph nodes are devoid of primary follicles, and lymphoid tissue contains few plasma cells before birth. The histogenesis of lymphatic tissue is uncertain, but both general lymphatic tissue and thymic lymphocytes probably arise from blood stem cells in the areas vasculosa and yolk sac (Moore and Owen,

1967). The occurrence of antigen-sensitive cells in other tissues, notably the liver, has been observed in animals (Tyan *et al.*, 1967) and probably occurs in the placenta in man (Good and Zak, 1956).

Thymic tissue is functionally different from other lymphoid tissues in that immunoglobulin synthesis is minimal in the thymus and the rate of thymic lymphopoiesis is independent of antigen stimulation.

The thymus is derived from the third and fourth pharyngeal pouches and the epithelial component is found at 11 to 12 mm (sixth week) (Arey, 1946). By the middle of the third month (35 mm) the thymic cortex is mainly composed of small lymphocytes (thymocytes) and the medulla contains epithelial cells and Hassal's corpuscles (Pinkel, 1968).

The role of the thymus in the development of immunity has been studied partly in animal experiments and partly in the immunity deficiency syndromes of man. Briefly, the facts may be summarised as follows (Miller, 1966).

Thymectomy in neonatal mice is accompanied by—

1. Lymphopenia (Metcalf, 1960).
2. Failure of allograft-rejection (Miller, 1962).
3. Poor humoral response to some antigens (Miller *et al.*, 1963).
4. Atrophy of paracortical tissue in lymphoid glands (Parrott *et al.*, 1966).

In the mature animal, thymectomy causes gradual failure of response to new antigens. Restoration of immunological competence following neonatal thymectomy in the mouse can be achieved either by grafting splenic tissue or grafting a large amount of thymic tissue. However, restoration may also follow grafting thymic tissue in diffusion chambers, indicating that a thymic hormone may be present (Osoba and Miller, 1964). Where diffusion chambers are not used, a large proportion of the lymphocytes subsequently found in grafted thymic tissues proved to be immigrants from lymphoid tissues, especially the bone marrow, of recipient rather than donor origin (Harris, 1964), implying the large-scale passage of cells into the thymus.

The experimental consequences of thymectomy and reconstitution suggest that the thymus has an instructional role in the development of immunological competence. The consequences of neonatal thymectomy vary from species to species and depend on the level of maturation of immunological function at birth. Man is considerably more mature at birth than the mouse (Good and Papermaster, 1964) and the rare instances (Wilmers and Russell, 1963; Karatlis *et al.*, 1964) of therapeutic thymectomy in man in early infancy have occurred after the neonatal period, so that precise comparisons are lacking.

Another organ of specialised lymphoid tissue is found in the chicken—the bursa of Fabricius. This organ is adjacent to the cloaca and its removal after

hatching results in a marked diminution in the synthesis of humoral antibody. Although no definite equivalent has been found for the bursa in mammals, comparisons have been made between the thymectomised neonatal mouse and the bursectomised chicken with certain types of immunity deficiency syndrome in man. Di George's syndrome, congenital absence of the thymus and parathyroids, resembles the former, and severe congenital hypogammaglobulinaemia, in which plasma cells are absent, the latter. In severe examples of the combined immunity deficiency syndrome, however, in which both cell-mediated and humoral immunity are deficient, there is dysplasia of both thymus and the other lymphoid tissues. It is likely that more primitive stem cells are deficient (*Lancet*, 1969) because reconstitution can be achieved only by grafting compatible bone marrow (for review see Fudenburg *et al.*, 1971). The immunological deficiency diseases are among those disorders that have been described as 'experiments of nature' (McQuarrie, 1944) and although phylogenetic analogies should not be exaggerated the concept of complementary immunological systems in which 'thymus dependent' lymphocytes mediate cellular immunity and 'bursa dependent' lymphocytes contribute to antibody synthesis has become established.

Humoral antibodies are synthesised by plasma cells, which are believed to differentiate from antigen-sensitive lymphocytes. They are found exclusively in the immunoglobulins of which five classes are now recognised (Fig. 1).

Class	Adult serum concentration mg/100 ml	Molecular weight	Distribution	Complement fixation	Opsonification	Placental passage	Reaginic activity
IgG	1158 ± 305[a]	150 000	Blood Body fluids	+	+	+	−
IgA	200 ± 61[a]	Serum form 170 000	Blood Body fluids	−		−	
		Secretory form 400 000	Some mucous and exocrine secretions	(+) see text			
IgM	9 ± 27[a]	900 000	Blood Body fluids	+	+	−	−
IgD	0·3 ± 40[b]	150 000	Blood Body fluids	−	−	−	−
IgE	250 ng/ml[c]	200 000	Blood Cell membranes Some mucous and exocrine secretions	−	−	−	+

[a] From Stiehm and Fudenburg, 1966; [b] From Rowe and Fahey, 1965; [c] From Johansson, 1968.

Fig. 1. Some properties of the classes of immunoglobulin.

The linkage of one pair of either κ or λ light chains and one pair of heavy chains by disulphide bonds occurs in all classes of immunoglobulins and the antibody combining site is contributed to by both types of chain. The heavy chain determines other properties, however, such as complement fixation and the passage from mother to child through the placenta of IgG. A number of genetically determined variants occurs in both heavy and light chains (for review see Pink *et al.*, 1971).

It has been shown by trace labelling of the proteins synthesised in cultures of organs from non-infected fetuses that IgG and IgM can be formed from the 20th week of gestation (Van Furth *et al.*, 1965); more recently it has been claimed that synthesis of IgG may begin at the 12th week and IgM from the 11th week (Gitlin and Biasucci, 1969). During normal gestation, however, the amounts of IgG and IgM synthesised are probably low.

During the latter part of intra-uterine life the human fetus reserves a large endowment of maternal IgG and the resulting concentration of IgG in the fetal serum is logarithmically related to gestational age (Papadatos *et al.*, 1969). The evidence for the maternal gift of immunoglobulins can be summarised as follows—

1. Allotypic differences between mother and child for IgG are hard to detect in the newborn (Grubb and Laurell, 1956; Linnet-Jepsen *et al.*, 1958).
2. Maternal IgG antibodies against red cells, bacteria and viruses are detected in cord sera (Wiener and Berlin, 1947; Vahlquist, 1958; Hitzig, 1959; Freda, 1962; de Muralt, 1962) (Fig. 2).
3. Labelled IgG has been found in cord serum after the injection of labelled IgG into the mother (Gitlin *et al.*, 1964).

Antitoxins	Diphtheria
	Tetanus
	Streptococcal erythrogenic toxin
	Streptolysin O
Virus neutralising antibodies	Measles
	Poliomyelitis
	Vaccinia
	Herpes
	Japanese B encephalitis
Complement fixing antibodies	Mumps
	Influenza
	Toxoplasma
Other antibodies	Anti Rh

Fig. 2. Antibodies transmitted to fetus.

Cord blood IgM and IgE levels are unrelated to the maternal concentrations, suggesting that fetal synthesis of these classes of immunoglobulin also

occurs, for they are not transmitted across the placenta. IgA and IgD synthesis appears almost always to start after birth. Thereafter, the synthesis of all classes of immunoglobulin gradually increases, but the IgG concentration passes through a trough because of the catabolism of the large maternal endowment of IgG of the fetus (Fig. 3).
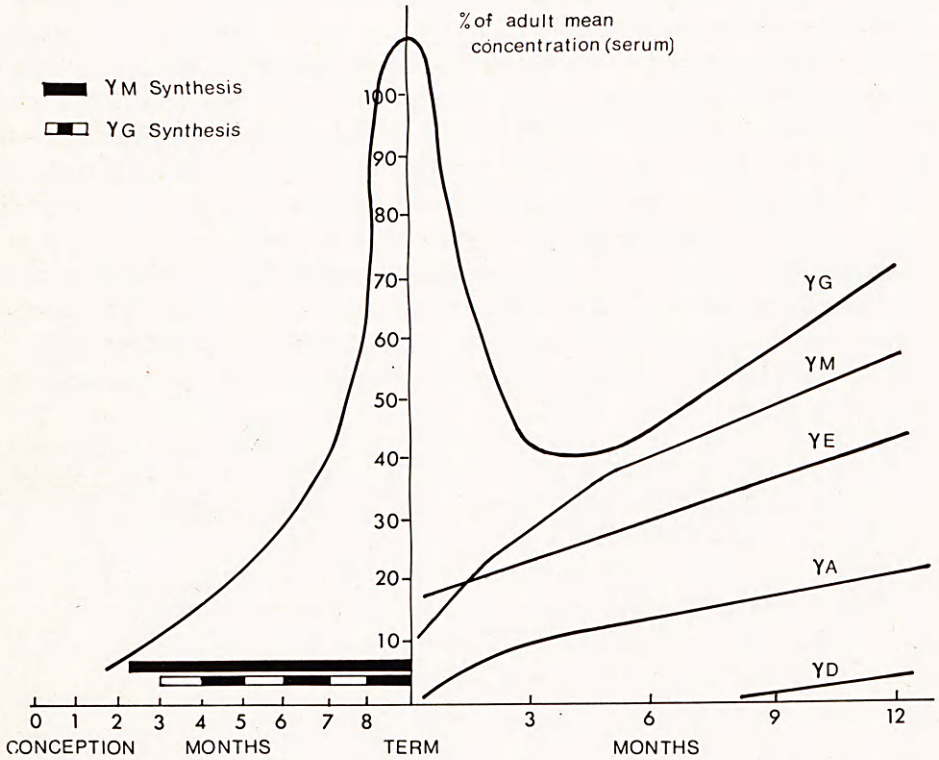

Fig. 3. Development of immunoglobulins: serum concentrations during early human development (*from* Rowe *et al.*, 1968; Stiehm and Fudenburg, 1966; Johansson, 1968; Papadatos *et al.*, 1969; Gitlin and Biasucci, 1969).

The placenta is not a perfect barrier to infections of the fetus and the embryopathies associated with rubella, herpes simplex, toxoplasma, cytomegalovirus, and syphilis are well known. In a proportion of such cases, but certainly not all, fetal infections are accompanied by synthesis of antibodies of predominantly IgM and also, probably, IgA immunoglobulin class (Alford *et al.*, 1967; McCracken and Shinefield, 1965; Remington and Miller, 1966; Sieber *et al.*, 1966; Scotti and Logan, 1968; Hanshaw *et al.*, 1968). Because of the maternal endowment of IgG, fetal synthesis of IgG is difficult to demonstrate. In rubella embryopathy the cord serum IgM may be increased

in 20 per cent of cases (McCracken *et al.*, 1969) and is more likely to be raised in severe cases (Hardy, 1971). A cord serum level of 20 mg/100 ml has been suggested at which intra-uterine infection should be suspected (Alford *et al.*, 1969). Doubt has recently been expressed, however, about the validity of this level and it has now been suggested that a cord level of 30 mg/100 ml or raised levels in the first few months are better indicators of infection. Increased cord levels of IgM may also follow maternal respiratory tract infection (Hardy, 1971). Raised levels of IgA in the cord serum are particularly likely to be found in more severe intra-uterine infections (Mellits, 1971).

The fact that IgM antibodies do not always appear during intra-uterine infections raises the question: Can all fetuses respond to all antigens, or can some fetuses respond to certain antigens and not others? Among outstanding work in this field is that of Smith's group (Smith *et al.*, 1964), who showed that both full-term and short-term gestation babies would respond within 7 to 14 days with synthesis of IgM antibodies to salmonella H antigens but that there was no response to O antigens until considerably later. The type of immunoglobulin synthesised changed from IgM to IgG after 30 to 40 days. Other workers have shown that salmonella H antibodies were produced in the IgM class if immunisation was given at birth. IgG antibodies are formed later (Fink *et al.*, 1962). IgM anti-I cold agglutinins (Adinolfi, 1965) and IgM antibodies to light chain determinants (Epstein, 1965) and to trypsinised human cells (Mellbye, 1966) are frequently found in cord blood, further evidence for the synthesis of antibodies by the fetus *in utero*, although the mechanism of sensitisation to these antigens is difficult to identify.

Human IgG contains sub-populations with different heavy chain primary structures, and these, known as subclasses, are not all transmitted equally across the placenta. Subclasses IgG 1, 3 and 4 of human IgG appear to be much more effectively transmitted to the fetus than IgG 2—an unexplained phenomenon—which may result in certain maternal antibodies being unavailable to the fetus (Hay *et al.*, 1971). Antibodies passed from mother to fetus *in utero* may inhibit the response to prophylactic immunisation in the very young (Perkins *et al.*, 1959; Butler *et al.*, 1962), although there is some evidence that a small amount of antibody may enhance immunisation (Levi *et al.*, 1969), perhaps by assisting the uptake of antigens by phagocytes in the afferent phase of immunisation.

IgA immunoglobulin appears both in the serum and in secretions; the concentrations achieved in each type of fluid are independent. The appearance of the secretory form (Tomasi and Bienenstock, 1968) which is a dimer formed of two IgA molecules linked by secretory piece, in the saliva of the newborn, may precede the appearance of IgA in the serum (Haworth and

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Dilling, 1966). Secretory IgA may appear several days before serum IgA and in increased amount when the mucosal surfaces have been infected (South *et al.*, 1967). Prophylactic immunisation may be achieved through the mucosa of the upper air passages, and with influenza vaccine this is reported to be more effective than parenteral injection, especially in young recipients (Fulk *et al.*, 1970). The recent discovery that urinary tract infections are accompanied, in up to 77 per cent of cases, by larger than normal amounts of secretory IgA in urine, confirms the suspected importance of the secretory IgA system in areas other than the respiratory tract. The levels were age related (Uehling and Stiehm, 1971). Secretory piece has been detected in the urine of premature infants (Remington and Schafer, 1968).

Recent studies have produced striking evidence of the local, IgA-mediated immunological role of the tonsils and the adenoids. When intra-nasal immunisation with polio virus was followed by tonsillectomy and adenoidectomy in a group of young children the titre of anti-polio antibodies of IgA class in the pharyngeal secretion diminished (Ogra, 1971). The effect was more marked in boys than in girls.

Unlike IgG antibodies, IgA antibodies in maternal serum are not available to the fetus. Secretory IgA antibodies are, however, present in colostrum and breast milk and, although not absorbed from the gastro-intestinal tract of the newborn, probably contribute to the control of its immunological flora. Polio vaccine does not so easily infect the gastro-intestinal tract of the breast-fed babies if their mothers have polio antibodies (Sabin *et al.*, 1963). It has recently been shown that serious neonatal infections are less frequent in babies whose milk intake in the first few days of life is predominantly from the breast than in those who are artificially fed (Weinberg and Wessner, 1971).

The development of humoral immunity after infancy is largely a matter of maturation conditioned by experience. As progressively more antigens are encountered, primary responses are replaced by quicker secondary responses.

Factors that may influence individual variations in antibody responses are not yet well understood. The influence of sex chromosomes on immuno-globulin concentration is, however, striking, for the levels of IgM and IgG are lower in XY and XO karyotypes (Stoop *et al.*, 1969; Butterworth *et al.*, 1967; Wood *et al.*, 1970) than in XX karyotypes. IgM levels have been found to be higher in females with XXX karyotype than in normal females (Rhodes *et al.*, 1969). The levels of *E. coli* haemagglutinins and rubella antibodies after immunisation have been found to be lower in male than female children aged 6 to 14 months (Michaels and Rogers, 1971).

The recent demonstrations of immunity deficiency syndromes in patients with abnormalities of components of complement C′3 (Alper *et al.*, 1970)

and C′5 (Miller and Nilson, 1970) have been among the discoveries that have added to our knowledge of the importance of complement, which comprises a considerable amount of the globulin in the serum and has been shown to have lytic and opsonic properties *in vitro*. Using methods similar to those used for detecting IgG and IgM synthesis, C′3 (β_{IC}-β_{IA}) have been found in fetal organ cultures as early as 14 weeks of gestation (Adinolfi *et al.*, 1968). C′4 (β_{IE}) was detected at 18 weeks. β_{IC}–β_{IA} has also been found in cultures from 29 day fetuses (Gitlin and Biasucci, 1969). Several organs participate in complement synthesis but the liver probably predominates. At birth, the level of haemolytic complement in cord serum is at about 50 per cent of that in adult life and this corresponds to the concentrations of β_{IC}-β_{IA} (C′3) and β_{IE} (C′4) (Adinolfi, 1970) which are also less than those of the adult.

At this point it is appropriate to discuss the ontogenesis of phagocytosis. Phagocytic cells are probably present in the fetus at the time of development of vascular organs (Bierman, 1961). The phagocytic capacity of leucocytes in term and low birth weight infants is similar when opsonins are provided from adult sera. However, the phagocytosis by leucocytes from low birth weight infants when their cells are tested with their own serum as the source of opsonin, proves to be much less efficient (Forman and Stiehm, 1969) (Fig. 4). The testing systems used involved *staphylococcus aureus* and paracolon
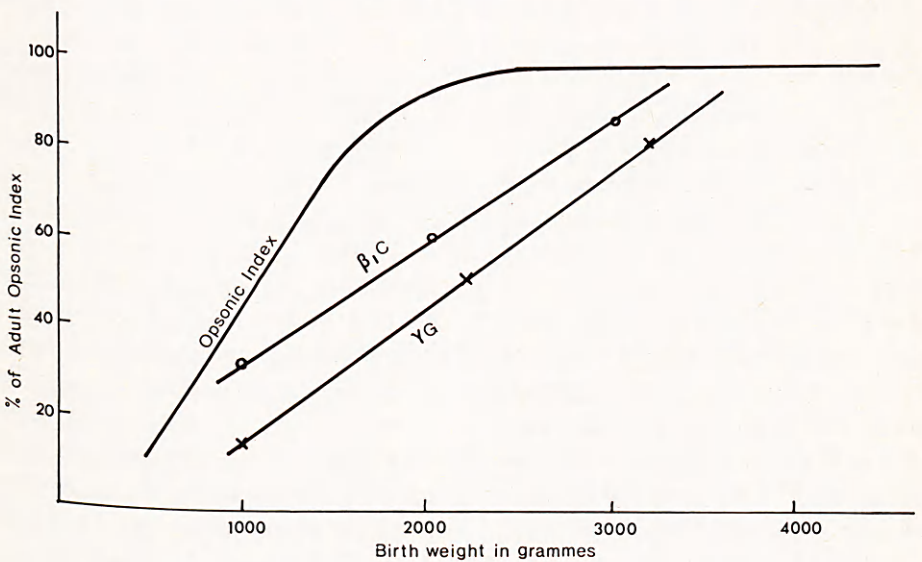
Fig. 4. Ontogenesis of phagocytosis and opsonification: the interaction of β_{IC} and γG immunoglobulin in phagocytosis in relation to birth weight (*after* Forman and Stiehm, 1969).

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bacillus and the opsonic index was closely related to the IgG and complement level. The intra-cellular killing of phagocytosed staphylococci by leucocytes from the newborn is as effective as that by leucocytes from adults (Park *et al.*, 1970). These authors found, moreover, that oxygen consumption and nitro-blue tetrazolium reduction, which are accompaniments of phagocytosis, were greater in resting leucocytes from both normal and pre-term newborn, than in those from adults. During *in vitro* phagocytosis, both these activities increased still further, but in premature infants with pre-existing bacterial infections, nitroblue tetrazolium reduction was less than in term babies (Cocchi *et al.*, 1971). The significance of these observations is not yet known. In chronic granulomatous disease, in which intra-cellular bacterial killing is abnormally reduced, these metabolic activities are also much diminished.

Little is known of the effectiveness of phagocytosis in the processing of antigen in the afferent phase of immunisation in the fetus and newborn, but if such processing were inadequate, the capacity to synthesis in antibodies in the very young might be impaired. In newborn rats quantitative and qualitative differences consistent with this possibility are reported (Nossal, 1967).

Before leaving the humoral phase, mention should be made of another non-specific component. Lysozyme, which was discovered by Sir Alexander Fleming and affects the mucopeptide layer of the bacterial cell wall, is present in large amounts in the cord serum and in fetal tissue, independently of maternal levels (Fig. 5), suggesting that it is synthesised in the fetus (Glynn

Molecular weight	14,500
Structure	Single polypeptide chain
Mean concentration	
Maternal	9·5 μg/ml
Newborn	13·6 μg/ml
Colostrum	230 μg/ml

Fig. 5. Lysozyme: properties and concentration
(from Glynn *et al.*, 1970).

et al., 1970). The fetal serum concentration may be 5 to 8 μg/ml at 16 weeks. It is also present in breast milk and, together with secretory IgA antibody and complement, has a bacteriolytic action (Glynn, 1968) which may be important in the gastro-intestinal tract.

It has recently been shown that the leucocytes of the human fetus, low birth weight and term babies can synthesise interferon, an inhibitor of virus replication in amounts similar to those in adults when allowance is made for the number of leucocytes in the cultures (Ray, 1970; Cantell *et al.*, 1968).

The capacities to develop delayed hypersensitivity and to reject homografts are accepted clinical measures of the efficiency of cell-mediated immunity,

which is especially involved in resistance to virus and fungal infections. Cell-mediated immunity depends on the stimulation of antigen-sensitive lymphocytes which may then undergo blast-cell transformation, develop cytotoxic properties and synthesise lymphokines which can be detected in *in vitro* cultures (Dumonde *et al.*, 1969). The lymphokines are a group of proteins with molecular weights in the range 25,000 to 80,000. They include macrophage inhibition factor and are concerned in the mobilisation of the components of delayed hypersensitivity. In contrast, the synthesis of immuno-globulin by these cells is minimal and their function is distinct from, but complementary to, those which have become committed to differentiation to immunoglobulin synthesising plasma cells. Transformation can be induced both by non-specific mitogenic agents such as phytohaemagglutinins and by specific antigens (for review see Ling, 1968). Quantitative *in vitro* procedures have been set up to measure cell-mediated immune competence in this way. Blast cell transformation can be induced in thymic lymphocytes from very young fetuses (14 weeks) by phytohaemagglutinin, foreign antigens and the antigenic array of leukaemic cells (Pegrum, 1971; Kay *et al.*, 1970). Many tissues are dividing in the very young fetus and so quantitative characterisation of the behaviour of peripheral lymphocytes is difficult. At birth, the reaction to transforming agents is probably at a lower level than in older children and adults (Ayoub and Kasakura, 1971). The capacity to manifest delayed hypersensitivity *in vivo*, however, depends on the capacity of lymphocytes to transform and on the production of the lymphokines. After BCG immuni-sation at birth, just over 80 per cent of infants in a very large trial (Gaisford, 1965) showed delayed hypersensitivity in the form of Mantoux conversion at four weeks. The conversion figure rose to 90 per cent at six weeks. The smallest infant in the study, a premature baby weighing 1·8 kg developed a positive tuberculin test at eight weeks. An experiment to sensitise premature babies to the hapten dinitrochlorobenzene was successful in a small proportion of low birth weight babies, the weight of the smallest who reacted being 1·1 kg (Uhr *et al.*, 1960).

While there is, therefore, much evidence that delayed hypersensitivity reactions can be mounted in the newborn period, developmental studies of lymphokine production are urgently needed.

Maternal infection may sensitise fetal lymphocytes, for it is found that lymphocytes in babies whose mothers have had coliform infection during pregnancy show a response to coliform antigen challenge in culture that is greater than would be expected in an infant who had had no previous experi-ence of this antigen (Brody *et al.*, 1968). It has recently been found that lymphocytes from the cord blood of infants whose mothers show delayed

hypersensitivity to PPD show a greater degree of reactivity to PPD in culture than those from infants whose mothers are tuberculin negative (Field and Caspary, 1971). How sensitivity is transmitted to the child is uncertain, for actual transfer of cells from mother to fetus appears to be an uncommon event in man (Turner *et al.*, 1966). The possibility exists that the transplacental passage of lymphokines or similar substances may be responsible for the transfer of information to the fetus but there is as yet no firm information on this point. In mixed leucocyte cultures, if leucocyte inhibition is used as a test of lymphocyte stimulation, the effect is less marked when the reacting cells are from mother and cord than when other more grossly allogenic pairs are used (Field and Caspary, 1971). This suggests information has passed from mother to fetus, but in this example it is of a kind such as to depress immunological responsiveness.

The evidence now available indicates that the newborn is well equipped to mount primary humoral and cell-mediated response to antigen challenge. After the fetal and neonatal period, increasing immunological experience renders the infant more and more capable of developing secondary responses more rapid and powerful than his primary responses. As the young child meets parasites for the first time, whether or not infection becomes established depends on how quickly the well-equipped but inexperienced defences can be mobilised. For most mildly pathogenic micro-organisms the response to a primary infection is adequate to ensure survival and primes an immunological memory that ensures a brisk secondary response if the parasite is encountered again. A rapid secondary immune response can be ensured by prophylactic immunisation against the more important and dangerous micro-organisms. Although very young infants will respond with high titres of the appropriate antibody to the prophylactic antigens, maternal endowment of IgG may depress the response in the first 2 to 3 months. Immunisation schedules, which are beyond the scope of this review, take account of this, and also of the need to arrange schedules that are socially convenient and likely to be accepted in the community at large.

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