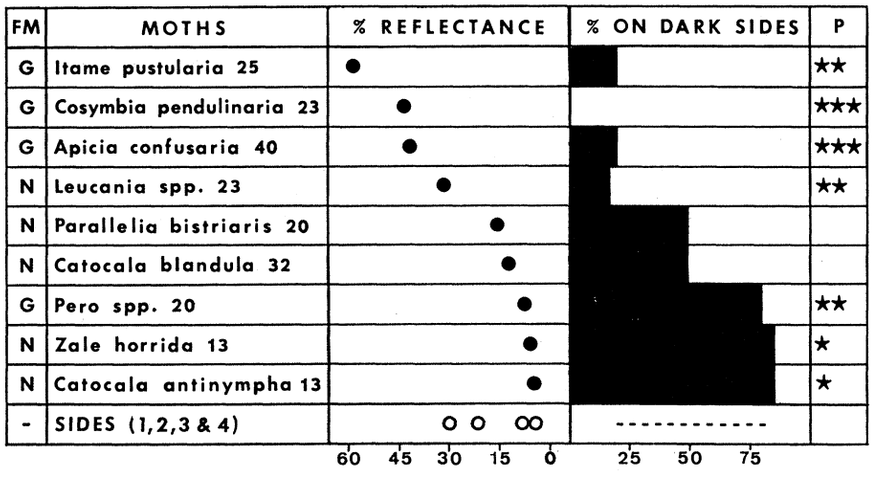

Fig. 1. Reflectances and background selections of nine common moths, with reflectances of the sides of the experimental box. The number of individuals tested is given after each species name. FM, Family; G, Geometridae, N, Noctuidae. Significant deviations from chance selections of the light and dark sides are indicated by stars for probabilities (P) of less than 0.05 (one), 0.01 (two), and 0.001 (three).

circle 2.85 cm in diameter within this montage. The reflectance of the construction paper was 7.33 percent.

The reflectances and background selections of the lightest and darkest species, of which 12 or more specimens were collected, are shown in Fig. 1. In addition, two species of intermediate reflectances are included. (*Pero spp.* are considered dark moths, as the basal two-thirds of the forewings were darker than side 3 of the box.)

In chi-square analyses, the moths of each species were divided into two groups: those selecting the two lighter sides and those selecting the two darker sides of the box. This procedure was used because of the small numbers of some species and the relatively close reflectance values of the sides being combined. All of the light and dark species exhibited significant deviations from a chance distribution on the lighter and darker sides of the box (Fig. 1). In addition, the summed distribution of the light moths differed from that of the dark moths, and both of these distributions differed from that of the intermediate moths (all $P < .001$).

These results indicate that some species of moths are able to select backgrounds which tend to match the reflectance of their forewings. However, Kettlewell's earlier results with *Biston betularia* (*2*) indicated that distinct phenotypes of one species might also select appropriate backgrounds. A similar finding was obtained during the present study with two forms of *Catocala ultronia*. The most common moth in the study area was the form *lucinda*, characterized by relatively pale median areas on each forewing. A number of the melanic form *nigrescens*, characterized by nearly uniform brown-black forewings, were also collected. The percent reflectance value of *lucinda* was between the values for sides 2 and 3 of the experimental box, and the value of *nigrescens* was less than that for side 4. Of 85 *lucinda* tested, 57 selected sides 2 and 3, and 22 selected side 4; of 12 *nigrescens* tested, 2 selected sides 2 and 3, and 10 selected side 4. The difference between these distributions is significant ($P<.001$). Similar differences in background selections by different forms of a single species have been suggested from field observations of *Oenosandra boisduvalii* and *Ectropis consonaria* (*4*). These results suggest that background matching in some species may be a phenotype or individual, rather than a species, attribute.

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11 October 1966

Immunologic Maturation in utero: Kinetics of the Primary Antibody Response in the Fetal Lamb

Abstract. *The kinetics of the primary antibody response to bacteriophage ϕX174 have been studied in the fetal lamb* in utero *after permanent indwelling catheterization of the fetal blood vessels. The initial antibody response by the developing fetus to this form of antigenic stimulus is comparable to that found in adult animals and shows none of the characteristics of the immature immunologic response that have generally been ascribed to fetal and neonatal animals.*

The manner in which developing animals achieve immunologic competence and the timing of this development have attracted much interest for theoretical as well as practical reasons. Whereas it was believed that immunologic maturation occurs only at or after birth among mammalian species (true of many small laboratory rodents), recent evidence (*1*) indicates that a variety of immunologic functions may be manifested by the fetuses of many species (human, rhesus-monkey, ovine, bovine, and others). Moreover, the fetus becomes immunologically competent to respond to each antigen at a particular gestational age. Competence for some antigens appears extremely early in gestation, for other antigens later, and for some antigens only after birth. Thus, one cannot speak of an overall state of immunologic competence or incompetence in the developing animal.

At the time of the young animal's transition from immunologic nonreactivity to ability to respond to a given antigenic stimulus, it has generally been supposed that the initial attempts at response are somewhat hesitant and weak (*2*). The term "immunologic immaturity" generally connotes such a transition period of initial feeble response by the young animal. We have reported (*3*) that when the fetal lamb develops the capacity (shortly after midgestation) to reject orthotopic skin homografts, there is apparently no hesitancy or immaturity on the part of the fetus in its first re-

sponse to homografted tissue. Before about the 85th day of gestation (full term is 150 days), the fetus cannot respond to the graft antigens, but after this age the fetus rejects skin homografts as rapidly and as competently as the adult animal does. We now describe studies of the early response of the fetal lamb *in utero* to another form of immunologic stimulus involving a separate mechanism of immune response—the formation of circulating antibodies to bacteriophage ϕX174. Our data indicate that despite the apparent previous lack of any immunologic experience by the developing fetus, the kinetics of antigen elimination and of the subsequent appearance of circulating antibody are typical of those observed in mature animals. This typical "immune elimination" constitutes normal catabolism of the antigen, its subsequent rapid clearance resulting from combination with the earliest antibody, and finally the appearance of free circulating antibody.

Our study of the kinetics of the primary antibody response by the fetus *in utero* has been made possible by the development of a technique permitting permanent indwelling catheterization of the fetal blood vessels. At the time of surgery a catheter was inserted into the jugular vein or carotid artery of the fetal neck (*4*); at this time the fetus was injected intravenously at another site with a predetermined dose of bacteriophage ϕX174. The fetus was then replaced into the uterus, and the catheter was led, in turn, through the fetal membranes, the uterine wall, the maternal abdomen, and the skin, emerging on the back of the ewe. Serial samples of heparinized fetal blood could then be removed through the catheter for analysis of elimination of bacteriophage antigen during the early stages, and for the estimation later of the increasing titers of circulating antibody in response to the antigenic stimulus. Random-bred sheep of known date of conception were used throughout. For convenience in the surgical procedure, fetal lambs were used during the last third of gestation (100 to 135 days), when the fetus weighs some 1500 to 2000 g. The preparation and purification of bacteriophage ϕX174 has been described (*5*). The bacteriophage-neutralizing antibody titer (k) of each serum was determined by the standard titration for phage antibody (*6*) before and after incubation with 0.1*M* 2-mercaptoethanol at 37°C for 30 minutes to differentiate γM from γG antibodies.

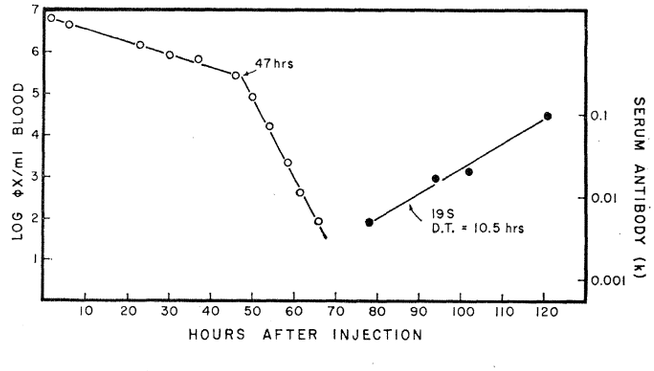
The data of Uhr and co-workers (*5, 7*) indicate that the primary antibody response of the normal adult to bacteriophage ϕX174 includes the following principal elements: (i) a period of relatively slow catabolism of injected antigen lasting about 1 to 2 days; (ii) the more rapid immune elimination of antigen from the blood associated with the initial formation of antibody; (iii) the early appearance of circulating 19*S* γM antibody, with doubling time from the initial concentration of some 6 to 10 hours; (iv) the cessation of γM antibody formation and its rapid disappearance from the blood, with little or no formation of 7*S* γG antibody in response to the injection of relatively little antigen; and (v) the continued production of γM antibody and, somewhat later, initiation of γG antibody formation in response to higher initial doses of antigen.

The primary antibody response of the fetal lamb *in utero* is similar in all major respects to those responses reported earlier, and apparently there are no differences ascribable to immaturity of the immune response. (Figs. 1–3). The initial formation of antibody and immune elimination of circulating antigen started within 47 hours after injection (Fig. 1). Disappearance of circulating antigen from the blood was accompanied by the appearance of circulating antibody, the concentration of which increased with a doubling time of about 10.5 hours.

The immune elimination of smaller quantities of bacteriophage antigen, followed by the transient formation of circulating γM antibody, is illustrated in Fig. 2. Immune elimination started 44 hours after injection of antigen, and formation of γM antibody reached a peak titer at 150 hours and then essentially vanished from the circulation by the end of the 8th day. During the course of this experiment, only very low levels of γG antibody ($k = 0.01$) were observed.

The typical primary antibody response sequence of first γM and then γG antibody is illustrated in Fig. 3. An inadequate number of samples was taken during the first phase of the response to permit construction of the immune-elimination curve in this instance, although all antigen had disappeared within 50 hours after immunization. As early as 54 hours after injection γM antibody was evident, increasing with a doubling time of about 9.5 hours; the titer reached 10^3 times the minimum detectable value during the 4th day, and continued to increase thereafter. At the same time, γG antibody was first detected during the 4th day after immunization, and the titer slowly increased during the 11 days of the experiment.

Apparently the kinetics of the primary antibody response in the fetal



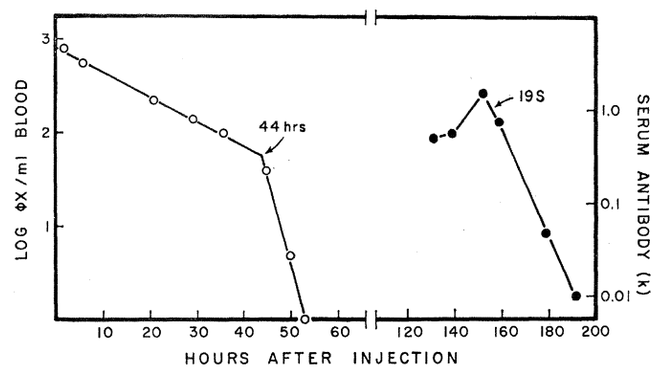


Fig. 1 (left). Immune elimination of 1×10^{10} plaque-forming units of bacteriophage ϕX174 from the blood of a fetal lamb at 103 days gestation. The early 19*S* γM antibody titer increased, with a doubling time of 10.5 hours. At 14 days, the blood of this fetus had 55 times as much antibody per milliliter as on the 5th day. Fig. 2 (right). Immune elimination of 5×10^8 plaque-forming units of bacteriophage ϕX174 from the blood of a fetal lamb at 116 days gestation. The γM antibody titer reached a peak on the 5th day and then rapidly disappeared.

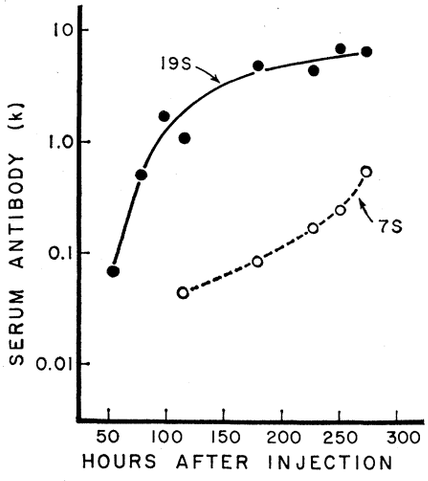


Fig. 3. The sequence of appearance of 19*S* γM and 7*S* γG antibodies in the circulation of a fetal lamb at 131 days gestation, after injection with 3×10^9 plaque-forming units of bacteriophage φX174.

lamb *in utero* are qualitatively similar to those reported for premature human newborns and older children (*8, 9*), for newborn and adult guinea pigs (*5, 7*), and for adult rabbits (*10*). These features include the initiation of immune elimination of antigen during the 2nd day after immunization, the formation of 19*S* γM antibodies with a rapid initial doubling time unaccompanied by appreciable γG antibody formation when small doses of antigen are used, and subsequent formation of γG antibody in response to larger doses of antigen. Even in its quantitative aspects, moreover, the fetal response appears to be consistent with that observed in more mature individuals, with allowance made for the reported difference in the degree of response (*8*) to different batches of bacteriophage φX174 antigen. Thus the initial γM antibody titers in the serums of immunized fetuses increased, the doubling time being about 10 hours, whereas within 1 week of immunization the amount of antibody detectable in the blood was 10^3 to 10^4 times the minimum detectable amount, with continued increase in antibody levels thereafter. A correction of the foregoing values for the rapid growth of the fetus added to a correction for the large volume of extracorporeal blood in the placenta would result in higher estimates of the amount of serum antibodies formed at any given time after immunization. We were, unfortunately, unable to compare the fetal lambs' response with that of adult sheep, owing to the presence of a phage-neutralizing factor, presumably antibody, in the adult serums. The similarity of response in the three different species mentioned above suggests, however, that the aforesaid features of the response are probably typical of the mammalian response to this antigen.

Our data imply that, once the developing fetus is able to respond in any specific manner to antigenic stimulus with the relatively strong immunogens thus far examined, it manifests no significant immaturity in any of the attributes of this response. In this respect, these data are in agreement with those cited above on the earliest manifestation of specific homograft rejection by the fetal lamb, and they agree also with other data (*11*) that the earliest antibody response by the fetal rhesus monkey *in utero* involves the same proportion of its total population of lymphoid cells as is employed by the mature animal (*11*).

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21 September 1966

Discrimination Learning and Inhibition

Abstract. *Pigeons learned to discriminate between a white vertical line on a dark background (S+) and a monochromatic circle of light (S−) either with or without responses to S− (errors). Gradients of inhibition, which were centered around S−, and which had greater than zero slopes, were obtained only from those subjects who learned to discriminate with errors. The results indicate that the occurrence of errors is a necessary condition for S− to function as an inhibitory stimulus. This finding is consistent with other performance differences in subjects who have learned to discriminate with and without errors.*

During the past decade our knowledge of how a discrimination is acquired has been considerably enhanced by new empirical findings and theoretical analyses. The peak shift and behavioral contrast have both been established as reliable characteristics of discrimination performance. The peak shift, first studied by Hanson (*1*), derives its name from the finding that, after subjects are successively trained to discriminate between a stimulus correlated with reinforcement (S+) and a stimulus not correlated with reinforcement (S−), the peak of a generalization gradient does not occur at S+ but is instead shifted away from S−. Behavioral contrast, a phenomenon studied by Reynolds (*2*), was originally called "induction" by Pavlov (*3*) and was later referred to as "contrast" by Skinner (*4*). The term refers to an increase in the strength of the response to S+ that accompanies a decrease in the strength of the response to S− during discrimination training. Behavioral contrast derives its name from the fact that the rates of responding to S+ and S− diverge. According to classical generalization theory, these rates should converge (*5*).

Recent experiments on "errorless" discrimination learning suggest a possible relationship between the peak shift and contrast (*6*). Errorless discrimination learning is achieved by starting discrimination training immediately after the response to S+ has been conditioned with a large S+-S− difference that is progressively reduced to its final smaller value. These experiments show that a subject can be trained to discriminate without the occurrence of responses to S− (errors),