SERUM IgM AS AN INDICATOR OF INTRAUTERINE INFECTION IN COLOMBIAN NEWBORNS[1]

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Highly elevated levels of serum IgM (over 50 mg per cent) and detectable levels of serum IgA (over 1 mg per cent) were correlated with overt infection in less than 1 per cent of 2,029 Colombian newborns. Toxoplasmosis and cytomegalovirus infection were the most frequent specific infections encountered. Apparently noninfectious antigenic stimulation in utero, as evidenced by moderately elevated serum IgM (20-50 mg per cent), is quite common in this population.

Introduction

Infections in the perinatal period are a significant cause of morbidity and mortality in newborns throughout the world. The clinical manifestations of perinatal infections are dramatic. They include fetal growth retardation, embryopathy, central nervous system involvement, perceptual and cognitive disabilities, and immune dysfunction (*1, 2*). Estimates of the incidence of intrauterine infection vary from 0.2 to 4 per cent of all newborns, depending upon the study population (*3, 4*). Determination of this incidence is complicated by the fact that many children infected prenatally are asymptomatic at birth (*5, 6*). Sequellae of infections may appear only months or years later, when the association with a perinatal event is difficult to establish (*7*).

The diagnosis of "silent" intrauterine infections is a prerequisite for the proper treatment and follow-up of affected children, but this diagnosis is complicated by lack of a reliable indicator of infection in the absence of symptoms. To help deal with this problem, several investigators have demonstrated the usefulness of measuring immunoglobulin M (IgM) in cord or newborn serum (*8-10*). Concentrations of IgM in normal newborns tend to fall in the range 0-20 mg per cent, while infected neonates usually have IgM levels exceeding 20 mg per cent (*11*). Unfortunately, some infected fetuses do not produce elevated amounts of IgM (*12*); and, conversely, some children born with IgM concentrations greater than 20 mg per cent never show any signs of disease (*13*). Hence the clinical evaluation of both groups requires follow-up for at least several months to detect the delayed appearance or continued absence of symptoms.

A growing list of infectious agents have been associated with intrauterine infections. These include the so-called TORCH agents (*Toxoplasma gondii*, rubella virus, cytomegalovirus, herpesvirus), *Treponema pallidum*, and others (*3*). Differentiation between these disease entities is often impossible on purely clinical grounds, necessitating identification of the causative agent by cultural or serologic means (*14*). The latter, employing immunofluorescent tests for specific IgM antibodies to

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each of the common pathogens (*15*), has proved the most feasible identification method.

A few previous studies suggest that antigenic stimulation and in utero infection are more common among economically disadvantaged populations in both industrialized (*16*) and developing societies (*17,18*). Mata et al. (*19*) have reported that up to 15 per cent of the newborns in a rural Guatemalan village had elevated levels of serum IgM at birth, although many of these children were not obviously infected (*19*). Similar data for Colombian populations are not available in the literature.

The present study was designed to accomplish three objectives: (1) to evaluate the usefulness of measuring serum IgM as an indicator of intrauterine infection in Colombian newborns; (2) to determine the incidence of infection in a poor urban population; and (3) to identify some of the specific microbial pathogens responsible for intrauterine infections in this population.

Patients and Methods

A total of 2,029 infants born in the *Hospital Universitario del Valle* in Cali, Colombia, between November 1976 and April 1978 were screened for elevated serum immunoglobulin M (IgM). Informed consent was obtained from all mothers of participating newborns. A small blood sample was taken by heel puncture with a lancet during the first 24 hours of life. The blood sample was allowed to clot at room temperature, and the serum was separated by centrifugation and tested within a few hours.

The IgM level in each newborn's serum was determined by two techniques, radial immunodiffusion (RID) and counterimmunoelectrophoresis (CIE). The RID test is commonly used in clinical immunology laboratories to quantify immunoglobulins and other serum proteins (*20*). The test was performed by incorporating commercial monospecific anti-human IgM antiserum (Hyland-Trave-

nol) into 1 per cent Noble agar at a concentration of 1:60 on a microscope slide. Holes were then cut into the agar and the infants' sera were used to fill the wells. Forty-eight hours later, the diameter of the precipitin ring around each well was measured using an ocular micrometer. A set of four standard dilutions of human IgM were tested with each batch of infant sera, and the results from the standards were used to construct a graph on semi-logarithmic paper from which the unknown serum IgM concentrations were calculated. The results were expressed as mg of IgM per 100 ml of serum (mg per cent). The minimum detectable level of IgM using this procedure was found to be about 1.0 mg per cent.

The CIE test was designed as a rapid, semi-quantitative assay to detect elevated levels of IgM within 2 hours rather than 2 days. The test was performed using a Gelman deluxe electrophoresis chamber and employing a 1 per cent Agarose overlay on a tray of microscope slides. Pairs of adjacent wells were cut into the agar. A 1:10 dilution of commercial monospecific anti-human IgM antiserum (Hyland-Travenol) was placed in each well on the anode side, and the infant's serum was placed in the corresponding well on the cathode side. The electrophoresis was carried out using high-resolution buffer (Tris-barbital – sodium barbital; pH 8.8; $\mu = 0.05$) at a constant voltage of 250 v and an effective current flow of approximately 2-3 milliamperes per tray of six slides. Six infant sera were tested simultaneously on each slide, making a total of 36 per tray.

After a total electrophoresis time of 90 minutes, the slides were examined for the presence of a precipitin band between pairs of wells. (Preliminary tests showed that a positive test was only obtained under these conditions when the serum IgM concentration was greater than 25-30 mg per cent. Therefore, a precipitin band between the wells indicated an elevated level of IgM in the newborn's serum.) Following this initial examination, all the slides were incubated at room temperature

for an additional 24 hours to detect the delayed appearance of a precipitate.

Infants who were identified as having elevated serum IgM based upon the CIE test were given a complete physical examination within 24 hours of birth. This examination included X rays of the long bones, chest, and cranium; a fundoscopic examination for retinopathy, a complete blood count, and palpation for organomegaly. The gestational age of the infant was determined using the physical and neurological criteria of Dubowitz (*21*). In addition, a venous blood sample was taken for further immunologic studies. Seventy-five healthy newborns with normal IgM levels (less than 20 mg per cent) were selected at random throughout the study to provide control values.

The immunologic tests included quantitation of serum IgG, IgA, IgD, and C3 complement using a RID test similar to that described for IgM and employing monospecific commercial antisera (Hyland-Travenol). The test for IgA was modified to detect the very low levels of IgA expected in newborn sera. The minimum detectable level for this test as performed was 1.0 mg per cent. The lymphocyte blastogenesis assay was performed in vitro on peripheral blood lymphocytes from these infants, using a whole-blood culture technique adapted from the procedure described by Han and Pauly (*22*).

Each patient's cells were cultured in the presence or absence of two plant lectins— phytohemagglutinin (PHA) and pokeweed mitogen (PWM)—that stimulate blast transformation and mitosis in either thymus-derived (T) lymphocytes alone (PHA) or both T and B (bone marrow – derived) lymphocytes (PWM). The amount of blastogenic activity was measured by incorporating tritiated thymidine (^{3}H-TdR; specific activity 6.7 mCi/mM; New England Nuclear) into the cell cultures after they had been kept 4 days at 37°C and counting the radioactivity in a liquid scintillation counter after an additional 24 hours. The results were expressed as a lymphocyte blastogenesis index, which is the

ratio of the mean counts per minute (cpm) of ^{3}H-TdR in the stimulated culture to the mean cpm in the unstimulated (control) culture. Duplicate cultures were tested in all cases. All counts were corrected for background and quenching and were adjusted to represent the activity of 10^6 lymphocytes.

In addition to these tests of general immunologic competence, serologic tests for specific IgM antibodies to some of the common pathogens that cause intrauterine infection were performed. The indirect immunofluorescence test was used to detect specific IgM antibodies against *Toxoplasma gondii*, herpesvirus (types 1 and 2), and cytomegalovirus. Commercial kits (containing an antigen preparation fixed to a microscope slide) and a fluorescein-conjugated anti-human IgM antiserum were used for each test (Microbiological Research Corporation). Each infant serum was tested over a series of two-fold dilutions and an end-point was determined. Each test included a negative control serum as well as one strong and one weak positive control serum. The minimum dilutions accepted as positive were 1:100 for cytomegalovirus; 1:100 for herpesviruses 1 and 2; and 1:250 for *Toxoplasma gondii*.

Newborns who were obviously ill upon examination were retained in the newborn unit for treatment and observation. Infants with no detectable disease were sent home with their mothers. Both symptomatic and asymptomatic children were subsequently visited in their homes at 2, 3, and 6 months of age and were examined for the delayed appearance of disease.

Results

Of the 2,029 sera tested, a total of 315 (16 per cent) were found to contain IgM concentrations of 20 mg per cent or greater. Table 1 shows the newborns' mean serum IgM concentrations—grouping the newborns arbitrarily into those with less than 20 mg per cent IgM, between 20 and 50 mg per cent, and over 50 mg per cent. It is obvious from these

Table 1. Serum IgM concentrations in newborns tested at the *Hospital Universitario del Valle.*

Grouping (by observed IgM concentration)	No.of children	Observed IgM concentrations(mg%)	
		(range)	(mean ± SEM[a])
< 20 mg%	1,714	0-19.5	6.00 ± 0.15
20-50 mg%	286	20.0-50.0	26.60 ± 0.41
> 50 mg%	29	65-520	222.67 ± 35.64
All sera	2,029	0-520	12.02 ± 0.78

[a]SEM = Standard error of the mean.

averages that most of the children in the 20-50 mg per cent group had IgM levels near 20 mg per cent, while most of those in the over 50 mg per cent group had IgM levels much greater than 50 mg per cent. Overall, the mean serum IgM concentration for the 2,029 children was about 12 mg per cent.

Nearly 39 per cent of the children had IgM concentrations below the minimum detectable level (1.0 mg per cent). On the other hand, nearly 1.5 per cent of the newborn sera contained over 50 mg per cent IgM. The highest IgM concentration measured was 520 mg per cent.

Figure 1 graphs the distribution of serum IgM levels in the newborns studied (light bars) together with the relative ability of the CIE test to detect elevated IgM concentrations (dark bars). Table 2 compares the results obtained with the RID and CIE tests for serum IgM. Both tests were performed on all the sera tested. The CIE results are presented as the presence (positive result) or the absence (negative result) of a precipitin band under the test conditions described. At the two extremes of IgM concentration (under 20 mg per cent and over 50 mg per cent) there was complete agreement between the two tests. All children with markedly elevated (over 50 mg per cent) IgM at birth yielded positive CIE results, as did 35 of the 39 with over 40 mg per cent IgM. On the other hand, only 23 of 85 (27 per cent) of the newborns with IgM concentrations in the range of 21-40 mg per cent yielded positive CIE results.

Of the 58 newborns identified by the CIE test as having elevated serum IgM levels, a total of 14 (24 per cent) were found to have signs and symptoms of overt disease upon exhaustive physical examination. The most

Figure 1. Distribution of serum IgM concentrations (light bars) and positive counterimmunoelectrophoresis for IgM (dark bars) in sera from Colombian newborns.
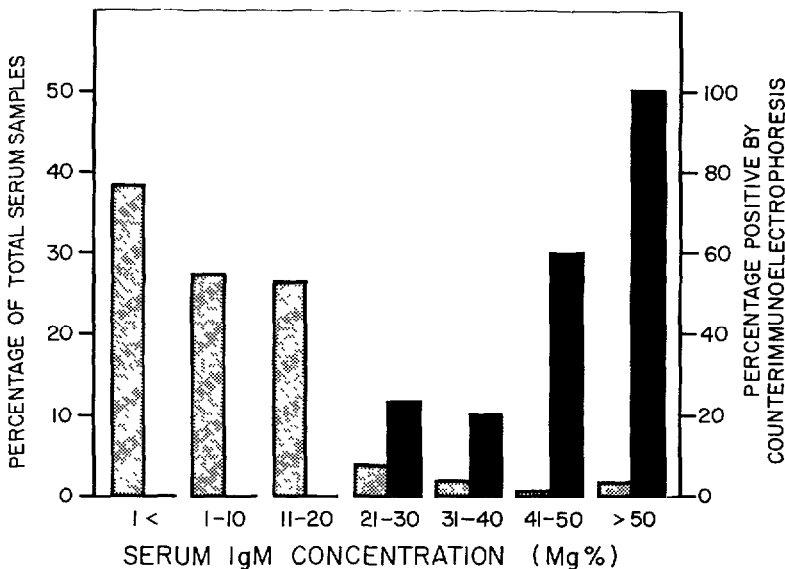
Table 2. Comparison of results from radial immuno-diffusion (RID) and counterimmunoelectrophoresis (CIE) tests used to detect elevated serum IgM in newborns at the *Hospital Universitario del Valle.*

IgM detected by radial immuno-diffusion (in mg%)	CIE results for sera with different IgM values		No. of negative sera	Total No. of sera
	Sera positive			
	No.	%		
0-20 mg%	0	0%	1,882	1,882
21-30 mg%	16	23%	55	71
31-40 mg%	7	19%	30	37
41-50 mg%	6	60%	4	10
> 50 mg%	29	100%	0	29
Total	58	2.8%	1,971	2,029

common physical findings included hepato-magaly (10 of 14), splenomegaly (7 of 14), icterus (6 of 14), retinopathy (7 of 14), and calcifications of the cranium and/or long bones (3 of 14). The other 44 children with elevated IgM levels were completely asymptomatic.

Table 3 compares immunologic data for the symptomatic and asymptomatic newborns with elevated IgM (as detected by the CIE test) with the group of 75 newborns showing normal serum IgM levels at birth. In addition to the striking differences in mean serum IgM concentrations, symptomatic neonates had significantly more IgA in their sera at birth. Lymphocyte blastogenesis (upon stimulation with phytohemagglutinin and pokeweed mitogen) was significantly decreased in the symptomatic infants, although the average number of circulating lymphocytes observed did not differ markedly from that found in the normal newborns.

Newborns with overt diseases weighed significantly less at birth than either the control cohort or the asymptomatic cohort, although there was no significant difference in their mean gestational ages. Concentrations of serum IgG and the third component of complement were about the same in all groups. Except for a significant elevation in serum IgM, the asymptomatic neonates appeared similar to the normal newborns with respect to the other immunoglobulins and in vitro lymphocyte function.

Serologic tests for specific IgM antibodies to *Toxoplasma gondii,* herpesvirus types 1 and 2, and cytomegalovirus were performed on 14 symptomatic and 23 asymptomatic newborns

Table 3. Comparison of selected immunologic parameters in newborns with normal and elevated serum IgM concentrations.

Variable	Normal serum IgM (mean ± standard error of the mean; n = 75)	Elevated serum IgM (> 20 mg%); mean ± standard error of the mean	
		Asymptomatic (n = 35)	Symptomatic (n = 14)
Serum IgM (mg%)	8.9 ± 3.2	40.2 ± 4.3[a]	202 ± 51[a]
Serum IgA (mg%)	1.4 ± 0.3	0.9 ± 0.9	13.1 ± 6.6[a]
Serum IgG (mg%)	1,095 ± 85	1,083 ± 74	1,061 ± 184
Serum C3 (mg%)	95 ± 7	100 ± 9	83 ± 7
Lymphocyte blastogenesis[b] with phytohemagglutinin	23.2 ± 4.3	17.5 ± 6.4	5.7 ± 2.1[a]
Lymphocyte blastogenesis[b] with pokeweed mitogen	16.8 ± 4.8	17.6 ± 6.1	8.9 ± 4.4[a]
Total No. of lymphocytes per mm^3	4,468 ± 314	4,818 ± 460	4,875 ± 533
Body weight (gm)	3,380 ± 102	3,231 ± 93	2,814 ± 229[a]

[a]Significantly different from control values at p < .05.

[b]Lymphocyte blastogenesis index = $\dfrac{\text{cpm in stimulated culture}}{\text{cpm in unstimulated culture}}$

with elevated IgM, as well as on 20 control infants. No IgM antibodies to these disease agents were found in any of the control infants. Two of the asymptomatic neonates were found to have significant IgM titers to cytomegalovirus, as did four of the 14 newborns with overt disease. In addition, three symptomatic infants had IgM antibodies specific for *T. gondii*, and one was found to yield a significant titer of anti-herpesvirus type 2 antibodies of the IgM class. In all, a total of eight (57 per cent) of the 14 symptomatic neonates showed serologic evidence of infection caused by one of the disease agents tested. Only two of 23 (9 per cent) of the asymptomatic newborns harbored antibodies that indicated previous exposure to these pathogens.

Several children were lost in the course of the follow-up visits at 2, 3, and 6 months postpartum. Three of the symptomatic newborns died during this period, and three others became unavailable due to relocation of their families. The eight remaining infants with overt disease at birth became asymptomatic during the follow-up period. Six appeared normal upon physical examination at 2 months of age.

Eight of the asymptomatic infants could not be located after leaving the hospital. However, none of the remaining asymptomatic children, including the two with significant IgM antibody titers to cytomegalovirus, developed disease signs or symptoms during their first 6 months of life that could be related to their intrauterine environment.

Discussion

Antigenic stimulation of the human fetus during the second or third trimester usually results in active production of antibody, predominantly of the IgM class (*12*). This is especially true when the antigen takes the form of an infectious agent.

Several previous studies have documented the usefulness of elevated serum IgM at birth as an indicator of intrauterine infection (*8-10*). However, the utility of this measurement depends upon the definition of "normal" IgM levels in the population at hand, since normal IgM concentrations vary somewhat from one population to another (*16, 17*).

Particular interest has focused upon the hypothesis that intrauterine infections are more common among populations characterized by low income, crowding, malnutrition, and increased exposure to infection (*23*). Our results do not support this hypothesis. If one accepts as evidence of intrauterine infection the presence of signs or symptoms within the first 6 months of life and/or the presence of specific IgM antibodies to a suspected pathogen in the newborn's serum, then the incidence of intrauterine infection in our study population was less than one per cent, a figure comparable to that obtained in industrialized societies. The mothers of these infants came predominantly from a poor urban area of Cali.

Socioeconomic data on families similar to the ones we studied have been published elsewhere (*24*). It must be noted, however, that our study group may not be completely representative of a poor urban population. Many mothers from this segment of society do not come to the hospital to deliver their babies, and no records are available on early abortions that may have resulted from infection in utero. Thus, our estimate of the incidence of intrauterine infection in the total population may be somewhat low.

In contrast to the relatively low incidence of overt disease (less than 1 per cent), we observed a rather high incidence (over 15 per cent) of infants born with elevated serum IgM levels (at or above 20 mg per cent). This could not be accounted for by contamination of samples with maternal blood, since all serum samples were obtained directly from the infant by heel puncture. Mata et al. (*19*) have reported a similar incidence of elevated IgM (15 per cent) in a rural Guatemalan population. As in the present study, Mata found no evidence of infection in many of the newborns with IgM concentrations above 20 mg per cent. Similarly, Alford et al. (*16*) have

reported finding elevated serum IgM more frequently among infants born to women from low socioeconomic strata (16).

The absence of detectable disease among the large number of infants with elevated serum IgM levels in our sample suggests that 20 mg per cent may not be a good discriminatory value for certain disadvantaged populations. Our data clearly distinguish between infected newborns, all of whom had IgM levels greater (usually much greater) than 50 mg per cent, and asymptomatic newborns with IgM concentrations between 20 and 50 mg per cent. The clinical significance of a "moderately" elevated IgM level (i.e., 20-50 mg per cent) is still uncertain. The data suggest, however, that noninfectious types of antigenic stimulation of the fetus may be more common in populations like that studied.

A similar conclusion has been reached by investigators studying a group of 523 Hawaiian newborns in whom the mean serum IgM concentration at birth was found to be 17 mg per cent. These authors concluded that an IgM level greater than 20 mg per cent was not necessarily a good indication of intrauterine infection in asymptomatic neonates (25). However, there seems a real need to clarify the nature of the in utero stimulation that results in significantly greater IgM production among study populations such as these.

The overall mean IgM concentration in our newborns' sera (12.0 ± 0.8 mg per cent) was very similar to the value obtained in a previous study of 101 Colombian newborns (13.9 ± 1.0 mg per cent) (26). This latter group also contained several infants with IgM levels greater than 20 mg per cent, none of whom had overt signs of disease.

Of the other immunologic parameters tested, serum IgA appeared to be associated most closely with clinical disease in our newborns. In fact, detectable IgA (over 1 mg per cent) was found primarily in the sera of diseased newborns. Nevertheless, not all infected newborns had elevated IgA levels.

Other authors have reported finding elevated IgA levels among infants with intra-uterine infection (27). Cederqvist et al. (28) have reported a predominance of IgA in cord sera from infected newborns, and Mason et al. (29) have found a strong correlation between detectable levels of serum IgA (1 mg per cent or more) and infection with cytomegalovirus. Our results suggest that the measurement of newborn serum IgA, in addition to IgM, would permit clinicians to identify infected neonates with increased certainty.

Cytomegalovirus appears to be a common cause of intrauterine infection in the Cali area, followed by *Toxoplasma gondii* and herpesvirus. The true relative incidence of these specific infections cannot be ascertained from the present study because of the small sample involved and the fact that specific serologic tests for other potential pathogens (e.g., rubella virus, *T. pallidum*, etc.) were not performed. As in previous studies, no specific etiologic agent could be identified in a significant proportion (50 per cent) of the symptomatic newborns.

Our results thus confirm the findings of other authors, who have reported that toxoplasmosis and cytomegalovirus infection are frequent causes of fetal morbidity (8, 14). Mata et al. (23) have reported strikingly similar results from a study of seroconversion among 61 Guatemalan women in a rural village. During gestation, 6 per cent of the women developed antibodies to cytomegalovirus, 5 per cent developed antibodies to herpesvirus, and nearly 2 per cent developed antibodies to *T. gondii*.

One difficulty in using the standard IgM radial diffusion (RID) test for identification of potentially infected newborns is the delay (24-48 hours) in obtaining results. This is an especially serious problem in developing societies where hospitalization for delivery is usually quite brief (24 hours or less). The counterimmunoelectrophoresis (CIE) test was found to provide a rapid and useful screening method for detecting elevated serum IgM in newborns. The results were available within 2 hours of receipt of the serum sample, and the test detected nearly all of the newborns with

serum IgM concentrations greater than 40 mg per cent—including every child with overt disease and/or positive serology for one of the specific pathogens investigated. In general, the CIE procedure has become increasingly useful in diagnosing specific infections, and our data support its adoption as a routine screening procedure for elevated IgM under conditions where rapid identification of infants with elevated IgM is required.

Perhaps one of the most interesting results of this study was the demonstration that symptomatic newborns had impaired in vitro cell-mediated immunity. Despite mild lymphocytosis, the blastogenic response of peripheral blood lymphocytes from newborns with overt disease was significantly depressed (see Table 3). The immunosuppressive effects of infection with *Toxoplasma gondii (30),* cytomegalovirus *(31),* and rubella virus *(32)* have been documented previously in humans and experimental animals. It appears that both humoral and cell-mediated components of the immune response may be influenced. Experimental evidence suggests that a variety of mechanisms may be responsible, including direct viral infection of lymphoreticular cells *(31),* impaired thymic development and function *(33),* antigenic competition, and alteration of lymphocyte subpopulations *(34).* The latter could result in either the loss of a responder population or active suppression of in vitro blastogenesis by increased suppressor lymphocyte activity. Our results are more suggestive of such a change in lymphocyte function, since the number of lymphocytes was not decreased.

Although the correlation between in vivo and in vitro cell-mediated immune function is not absolute, the demonstration of impaired lymphocyte blastogenesis implies that intrauterinely infected newborns may not mount a normal immune response to their primary infection and may be at risk of developing secondary infections as well. Hence the clinical relevance of immunologic sequellae among children infected in utero is a question that deserves further study.

ACKNOWLEDGMENTS

SUMMARY

A study of 2,029 Colombian newborns from a poor urban population revealed that less than 1 per cent suffered from overt intrauterine infection that showed itself through signs or symptoms within the first 6 months of life or through the presence of specific IgM antibodies to suspected pathogens. Counterimmunoelectrophoresis provided an effective method for quickly detecting elevated serum IgM concentrations in newborn sera, thereby permitting rapid identification of potentially infected infants.

Sixteen per cent of the newborns studied had serum IgM levels elevated above the commonly accepted criterion of 20 mg per cent. Symptomatic newborns all had greatly elevated serum IgM levels (over 50 mg per cent) and detectable serum IgA levels (over 1 mg per cent). In contrast, asymptomatic newborns had moderately elevated serum IgM levels (20-50 mg per cent) and no detectable IgA levels (under 1 mg per cent). The mean IgM level for all newborns was about 12 mg per cent.

Of the three microbial pathogens for which specific IgM serologic tests were performed, cyto-

megalovirus (in 4 of 14 symptomatic infants) and *Toxoplasma gondii* (in 3 of 14) were the most prevalent.

Symptomatic newborns exhibited impaired cell-mediated immune function, as evidenced by a significant reduction in mitogen-stimulated lymphocyte blastogenesis in vitro. Our results suggest that antigenic stimulation in utero, but not necessarily infection, may be more common among economically disadvantaged populations than it is among other populations.

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RABIES IN THE UNITED STATES

A total of 5,150 laboratory-confirmed rabies cases were reported in the United States and its territories in 1979. This total was 67 per cent higher than the average annual total for the preceding five years and was 1,852 cases over the total reported for 1978. Forty-eight states and Puerto Rico reported infected animals in 1979; only the District of Columbia, Idaho, Guam, Hawaii, and the Virgin Islands reported no cases. Seven kinds of animals accounted for 98 per cent of the reported cases. These were skunks (59 per cent), bats (15 per cent), raccoons (10 per cent), cattle (4 per cent), dogs (4 per cent), cats (3 per cent), and foxes (3 per cent). Wild animal species accounted for 87.6 per cent of the total reported cases, domestic animals accounted for 12.3 per cent, and humans accounted for 0.1 per cent.

The general trend was one of across-the-board increases vis-a-vis 1978. For example, there were five human cases (versus four in 1978), 636 domestic animal cases (versus 469 in 1978), and 4,509 wild animal cases (versus 2,285 in 1978). However, cases among skunks exhibited the sharpest reported leap, increasing from 1,657 to 3,031 cases—a rise of 67 per cent over 1978.

Source: Morbidity and Mortality, vol. 29, no. 37, 1980.