

The Journal of
ALLERGY
and
CLINICAL IMMUNOLOGY

VOLUME 48

NUMBER 2

Development of IgE in newborn human infants

**Donald D. Stevenson, M.D., H. Alice Orgel, M.D.,
Robert N. Hamburger, M.D., and Robert T. Reid, M.D.**

With the technical assistance of

Drusilla E. Richardson, R.N.

La Jolla, Calif.

Thirty-four maternal-neonatal pairs were skin tested with anti-IgE, and 18 of 34 babies were skin tested again with anti-IgE until they converted from negative to positive skin test reactions. Quantitation of serum IgE by a primary binding blocking technique was accomplished in 13 maternal-neonatal pairs and in some infants' sera during follow-up. Although human newborn skin will passively accept adult atopic serum and respond with a positive reaction to anti-IgE injections, there appears to be a delay (mean 21.7 days) before the baby's skin responds to anti-IgE with a positive wheal and flare reaction. The time of conversion from a negative to a positive skin test response was quite variable and bore no relationship to cord or follow-up serum IgE levels nor to atopic hereditary background. However, breast-fed babies developed positive skin reactions to anti-IgE later than the group fed with cow's milk.

In 1968, Ishizaka and Ishizaka,¹ reported injecting highly specific anti-IgE into the skin of monkeys and human volunteers. Injected anti-IgE, in concentrations of 10^{-4} μ g of antibody N per milliliter (1:80,000), produced a wheal and flare reaction in all human subjects regardless of an atopic history. Anti-IgG, anti-IgA, anti-IgM, and anti-IgD in concentrations of 0.2 to 0.5 μ g of antibody N per milliliter (1:100) failed to produce a wheal and flare response when injected into the skin of the same human volunteers and monkeys. Newcomb and Ishizaka,² using guinea pig anti-IgE (1:2,000 highest concentration), skin tested atopic and nonatopic children who were over the age of

From the Division of Allergy-Immunology, Scripps Clinic and Research Foundation, and the Pediatric Immunology and Allergy Division, School of Medicine, University of California, San Diego.

Supported by National Institutes of Health Grant PHS AI 10126.

Received for publication Dec. 17, 1970.

Reprint requests to: D. D. Stevenson, Division of Allergy and Immunology, Scripps Clinic and Research Foundation, 476 Prospect St., La Jolla, Calif. 92037.

5 years. IgE was present in the skin of all tested individuals but was quantitatively greater in amount in the atopic group. In 12 of 12 children with a variety of immunologic deficiency diseases, skin IgE could be demonstrated, but the skin test responses were smaller than in the normal and atopic groups. In another study, 11 of 16 patients with ataxia telangiectasia had negative skin test reactions to anti-IgE skin test.³

There is some information concerning IgE serum levels in neonates and children. Johansson⁴ has shown that 37 cord sera contained a mean IgE level of 36.3 ng. per milliliter with a range of 12.9 to 102 ng. per milliliter, as contrasted with the baby's atopic mother's mean IgE serum level of 286 ng. per milliliter. There was no correlation between individual maternal and cord IgE levels. Groups of babies and children from 1½ months to 5 years old were found to have mean IgE levels which progressively increased from 60.6 ng. per milliliter in the younger group to 155 ng. per milliliter in the 3- to 5-year-old group. These data are consistent with neonatal synthesis of IgE. Yet, the general impression has been that newborn infants are not reaginic. This concept is based in part on the work of Sherman and associates⁵ who studied infants born to atopic women. They found that even though the mother had positive immediate wheal and flare skin test reactions to various allergens, the same allergens elicited a negative response in the newborn infants. They concluded that the babies were not synthesizing reagins and that maternal reagins were not crossing the intact placental barrier. Further studies by Kuhns⁶ have shown that the human newborn skin is capable of accepting reagins in passive transfer and in responding with a wheal and flare reaction when the appropriate antigen was introduced to the transfer site in the newborn's skin.

The purpose of this study was to combine the assessment of serum IgE levels in mothers and their newborn infants with the development of positive wheal and flare reactions to anti-IgE in the skin of the babies.

MATERIALS AND METHODS

Preparation of anti-IgE

Myeloma IgE serum was obtained from the New Hampshire myeloma patient (P. S.)⁷ through the courtesy of Dr. Joseph Feldman. The serum was passed through a DEAE column (pH 7.0 to 0.025M phosphate), and peak II, containing mostly IgE with possible trace amounts of IgG, transferin, IgA, and IgD, was injected into rabbits and a goat using Freund's complete adjuvant. Antiserum was harvested, and antibodies against IgG, Fab determinants, and transferin were absorbed out with human IgG, IgG F(ab)₂, and transferin. This did not absorb out antibodies against idiotypic IgE antigens. On Ouchterlony plates, goat and rabbit anti-IgE united in a single line with myeloma E serum (P. S.), and no precipitin lines were observed against whole normal human serum. The goat serum was shown to be sterile and pyrogen free, conforming to Public Health Service regulation 42-73. It was diluted 1:10 in sterile phosphate-buffered saline with 0.05 per cent phenol preservative and stored in rubber-stopped glass vials at 4° C. On the days of the testing, 1:10 goat anti-IgE serum was diluted to 1:1,000, 1:10,000, and 1:100,000. The most concentrated dilution used for reversed cutaneous anaphylaxis was 1:1,000, and the maximum injected volume of 0.02 ml. contained less than 10 µg of protein.

Procedure for quantitation of serum IgE

A primary binding blocking test was employed in which 0.5 ml. of a standard dilution of a monospecific goat anti-IgE preparation, known to bind 50 per cent of 5 ng. of myeloma

IgE protein in 1:100 normal goat serum, was incubated with varying amounts of myeloma IgE. On the following day, 5 ng. of I¹²⁵ myeloma IgE (P. S.) in 0.5 ml. of 1:100 normal rabbit serum was added to the incubation medium. Anti-IgE antibodies not bound to unlabeled IgE were then available to bind the radioactively labeled IgE. After a second overnight incubation, the I¹²⁵ IgE bound to goat anti-IgE was separated from the unbound I¹²⁵ IgE by precipitating the complexes with 0.5 ml. of rabbit antigoat IgG. The precipitate was collected by centrifugation, washed once with cold saline, and recentrifuged. The amount of I¹²⁵ IgE protein in the pellet was calculated by counting the radioactivity in the bottom of the tube. A standard blocking curve was then constructed by plotting the per cent of the I¹²⁵ IgE precipitated versus the amount of unlabeled myeloma IgE protein added to the tubes. The amount of IgE in an unknown sample could be quantitated in terms of myeloma IgE by adding the unknown sample in place of the known amount of myeloma IgE and interpolating from the standard blocking curve.^{8,9} When increasing concentrations of IgE (P. S.) were inserted into this blocking system, the dose response curve started at 50 per cent precipitation of I¹²⁵ and blocked to 0 per cent. Insertion of atopic and nonatopic reference sera into the system produced curves which descended to 20 per cent precipitation of I¹²⁵ but would not block further, even with increased additions of atopic and nonatopic IgE. The myeloma and reference sera curves were divergent at the extremes but fused in the 20 to 40 per cent precipitation region which represented the steepest part of the slope in the dose-response curve. Sera containing very high or very low IgE levels were difficult to quantitate accurately. However, the range of IgE levels recorded in this paper could be accurately and reproducibly measured in this system. The same nonatopic and atopic reference sera were used routinely throughout the study. Dr. Gerald J. Gleich kindly also assayed these reference sera for us. He uses a double-antibody immunoassay, with specific antibody to one IgE myeloma protein (ND) in the first stage of the assay and another IgE myeloma protein (P. S.) as standard.¹⁰ Our values of 42, 125, and 1,096 ng. per milliliter were lower than his respective values of 117, 209, and 5,996 ng. per milliliter. The primary binding blocking method which we employed was reproducible (less than 5 per cent difference) from one assay to the next.

Assay for cord serum IgA and IgM

A standard radial immunodiffusion technique was used for the measurement of cord serum IgA and IgM. Low level IgA (Lot No. 7092H002B1) and IgM plates (Lot No. 7082H001A1) were obtained from Hyland Laboratories.

Study procedure

After gaining written permission from 34 mothers of newborn babies at University Hospital of San Diego County, the following was carried out: Mothers were interviewed by one of us (D. S. or A. O.) to elicit any family history of atopic disease and the mother's intent with respect to breast- or bottle-feeding her infant. All skin tests were performed by the same experienced allergy nurse (D. R.). The mothers were skin tested with codeine phosphate, 1 mg. per milliliter (injecting 0.02 ml.), to demonstrate their capacity to release histamine from dermal cells, and with 0.02 ml. of goat anti-IgE at dilutions of 1:100,000 to 1:1,000 as well as with normal goat serum, diluted 1:1,000, as a control. Sera were obtained for measurement of maternal IgE. Their newborn babies were skin tested in a similar manner at ages one through 3 days, using the skin over the backs and testing with 0.02 mg. codeine phosphate in 0.02 ml., 1:1,000 normal goat serum control, and 1:1,000 dilution of goat anti-IgE. Tracings of the wheal and flare reactions were recorded, and the wheal area was measured in planimeter units. Three to 5 ml. of cord serum, collected at delivery, constituted a requirement for admission to the study group. There was no intentional selection of maternal-neonatal pairs, but permission seemed to be less easily obtained from Mexican-American mothers, small volume or no collection of cord serum eliminated other pairs, and patients with black skin could not be included because of difficulties in accurately measuring the wheal and flare reactions.

Of the original 34 pairs, 18 mothers cooperated by returning with their infants for

TABLE I. Anti-IgE skin test results in 34 pairs of mothers and their newborn infants

Maternal neonatal pair	Family atopic history		Maternal*			Neonatal		
			3 Dilutions of anti-IgE/codeine†			Age tested (days)	1:1,000 anti-IgE /codeine†	Normal goat serum‡
	Pat.	Mat.	1:100,000	1:10,000	1:1000			
1, Ga.	0	0	0.28	0.57	0.89	2	Neg.	Neg.
2, Pa.	+	+	0.27	0.42	0.50	1	Neg.	Neg.
3, Ro.	0	+	0.33	0.60	0.86	1	Neg.	Neg.
4, We.	0	+	0.17	0.61	0.72	1	Neg.	Neg.
5, Ne.	0	0	0.38	0.66	—	1	0.20 (?)	0.20
6, Gr.	0	+	0.31	0.50	—	2	Neg.	Neg.
7, McH.	0	+	0.12	0.87	1.00	1	Neg.	Neg.
8, Mor.	0	0	0.47	0.83	1.28	2	Neg.	Neg.
9, Bo.	0	0	0.24	0.63	—	1	0.50 (?)	0.75
10, Ca.	?	0	0.63	—	—	2	1.00 (+)	Neg.
11, Pit.	+	+	0.23	0.62	0.93	½	Neg.	Neg.
12, Si.	+	+	0.36	0.54	1.00	1	Neg.	Neg.
13, Br.	?	+	0.19	0.47	—	3	Neg.	Neg.
14, Pi.	+	+	0.41	0.48	0.52	2	Neg.	Neg.
15, St.	0	0	0.87	1.34	—	1	Neg.	Neg.
16, La.	+	+	0.40	.60	—	1	Neg.	Neg.
17, Cl.	0	0	0.25	1.20	1.0	2	1.0 (+)	Neg.
18, Kr.	0	+	0.55	—	—	2	Neg.	Neg.
19, Mar.	+	+	0.40	0.43	—	2	0.2 (±)	Neg.
20, Jo.	?	+	0.67	0.35	0.84	1	Neg.	Neg.
21, Lau.	0	+	1.00	1.00	—	2	0.33 (±)	Neg.
22, Fr.	0	0	0.44	0.56	1.00	3	Neg.	Neg.
23, Es.	+	0	0.43	0.78	—	3	0.66 (+)	Neg.
24, Go.	0	0	0.66	2.0	—	3	0.75 (+)	Neg.
25, Mo.	0	+	0.11	0.78	—	3	Neg.	Neg.
26, Wi.	0	+	0.24	—	—	3	Neg.	Neg.
27, Al.	0	?	0.5	1.00	—	2	0.75 (+)	Neg.
28, Fl.	?	+	0.17	0.29	0.55	2	Neg.	Neg.
29, Ma.	0	0	0.50	0.80	0.90	1	Neg.	Neg.
30, L.	0	0	0.63	0.54	0.63	1	Neg.	Neg.
31, M.	0	0	0.32	0.50	0.58	1	0.20 (±)	Neg.
32, Va.	0	0	0.43	0.73	—	2	Neg.	Neg.
33, Sil.	0	0	0.23	0.32	0.54	1	Neg.	Neg.
34, Rod.	?	0	0.36	—	1.10	2	Neg.	Neg.

*Control tests with normal goat serum were negative in all mothers.

†Wheal produced by anti-IgE in planimeter units divided by the units of the wheal produced by 0.02 mg. codeine phosphate.

‡Wheal in planimeter units.

follow-up skin testing. Eight of these babies underwent femoral venipuncture between one and 35 days after birth. The 18 returning mothers were requested about the milk feedings of their infants, and their responses were checked with their original intentions and their baby's neonatal charts. Four neonates who had negative skin tests to goat anti-IgE received adult atopic and/or nonatopic serum by passive transfer, and after 48 hours anti-IgE was injected into the transfer sites. One maternal serum was found to have a high titer of reagins against grass pollens, and this was compared to her baby's cord serum by P-K testing.

RESULTS

When codeine phosphate, 0.02 mg. in 0.02 ml., was injected intradermally, a wheal and flare response was elicited in all subjects. Maternal wheals varied in size from 6 to 46 planimeter units (mean 19.7), and those of their newborn infants varied from 2 to 10 planimeter units (mean 3.9). The capacity to identify skin-fixed IgE with anti-IgE after combination and release of histamine is clearly influenced by this difference in histamine release. Therefore, the ratio

TABLE II. Follow-up skin testing with anti-IgE in 18 babies

Baby	Family history		Feeding		Cord serum IgE (ng./ml.)	Days After Birth									
	Pat.	Mat.	Breast	Cow's milk		0	5	10	15	20	25	30	35	40	45
1, Ga.	0	0		+	—	0	0		+						
2, Pa.	+	+		+	—	0	+		+						
3, Ne.	0	0	+		20.4	±		0	19.6*	0		+			
4, Gr.	0	±		+	—	0			0			+			
5, McH.	0	+	+		18.0	0		0		0	18.4*	+	17.6*		
6, Mor.	0	0		+	—	0			0		+				
7, Ca.	?	0		+	60.5	+			+	112.3*					
8, Pit.	+	+	+		25.9	0		±		0	+		+	23.8*	
9, Si.	+	+		+	21.6	0				0	+		+		
10, Br.	?	+	+		28.1	0		0	108.0*				+		
11, Pi.	+	+	+		116.6	0	0		0		0		+		
12, St.	0	0		+	—	0			0		±				
13, La.	+	+	+		30.2	0		0			0	19.7*	±	+	
14, Cl.	0	0		+	—	+								+	
15, Kr.	0	+		+	90.7	0		+							
16, Mas.	+	+		+	17.6	±		0			+				
17, Jo.	?	+		+	—	0	0					0	+		
18, Lau.†	0	+		+	19.2	±		0		0	+		0		0

Results of Anti-IgE skin tests: 0 = negative; ± positive but not reproducible; + significantly positive.

*IgE serum levels, nanograms per milliliter.

†Day 57, +; Day 71, 0; Day 120, 0.

of the area of the wheal produced by anti-IgE divided by the wheal area produced by codeine phosphate injection is used for comparison of results. Experience using this approach has shown that ratios less than 0.4, although usually significant, are not consistently reproducible and are recorded as ± wheal and flare reactions. A ratio greater than 0.4 is recorded as positive (+), and these reactions have been found to be highly reproducible.

In Table I, all mothers except Wi. exceeded a wheal ratio of 0.4 during testing with anti-IgE dilutions of 1:100,000 through 1:1,000. Mother Wi. had an unusually large codeine wheal of 46 planimeter units, and even a large wheal response to 1:100,000 anti-IgE was converted into a deceptively low ratio by our formula. Further testing at higher anti-IgE concentrations was felt to be improper. In most of the mothers, a progressive increase in the ratio occurred when they were tested with higher concentrations of anti-IgE. Two of 34 newborn babies (Ne. and Bo.) had positive skin test reactions to normal goat serum. Three of 34 had ± reactions to anti-IgE, but 5 of 34 were positive to anti-IgE during the first 3 days of life. These 5 babies with early skin-fixed IgE were the products of parents who were predominantly nonatopic. Babies from 5 families in which both parents were atopic were negative for early skin-fixed IgE, as were the other 21 newborn infants.

Table II records the follow-up data obtained from 18 babies. The conversion from a negative anti-IgE skin test to a clearly positive test (> 0.4 ratio) occurred between Day 1 and Day 39, with a mean conversion time for 16 of 18 babies of 21.7 days. Baby 12 (St. was ± at Day 25 but unfortunately was lost to follow-up, and an expected positive response could not be obtained

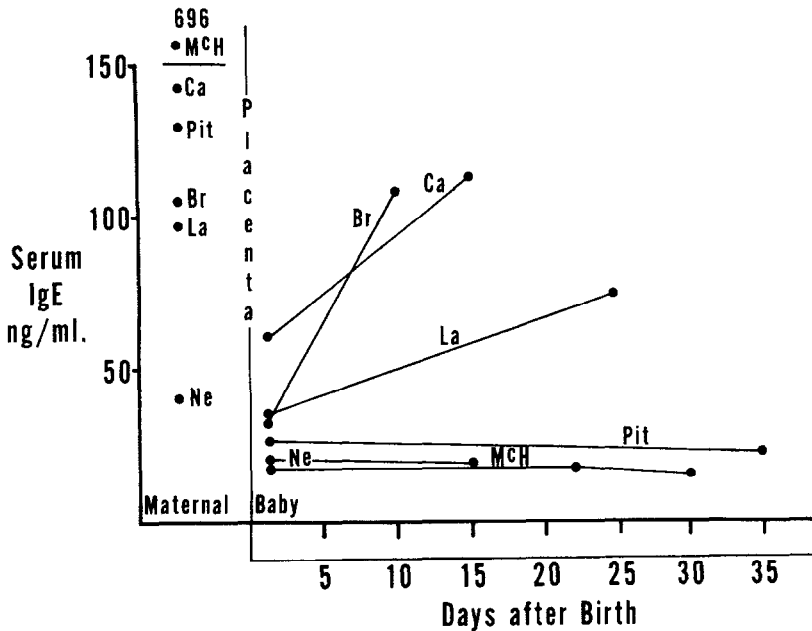


FIG. 1. Serum IgE levels in 6 infants: Compared with the day anti-IgE skin test first became positive. **Ca**, Day 2; **Br**, Day 33; **La**, Day 37; **Pit**, Day 23; **Ne**, Day 29; **MCH**, Day 30.

at a later date. Baby 18 (Lau.) was tested on multiple occasions, and the skin testing results were confusing. This baby received Sedadrops (phenobarbital, pentobarbital, and homatropine) from Day 14 through Day 120 with each feeding. At 4 months of age the serum IgE level of baby Lau. was 18 ng. per milliliter, contrasted with 19.2 ng. per milliliter at birth, and her skin test response to anti-IgE was negative. Because there is no experience to evaluate these variables in neonates, even though the codeine phosphate-induced wheal and flare reaction increased by 120 days, it seemed prudent not to include babies St. and Lau. in the data used to calculate the means.

Of particular interest is the relationship between follow-up serum IgE levels and the conversion to positive anti-IgE skin tests depicted in Table II and in Fig. 1. The 3 babies (Ca., Br., and La.) whose follow-up serum IgE levels increased above their cord serum levels were either positive to anti-IgE at birth (Ca.) or converted to positive at 32 days and 39 days after birth. Three other babies maintained low serum IgE levels and converted the anti-IgE skin tests to positive during a time when serum IgE titers were low and stable.

As shown in Table II, if one contrasts the 6 babies who were fed maternal breast milk with 10 who were fed cow's milk, the mean conversion rates for development of positive anti-IgE were Day 29.1 for the breast-fed group and 17.2 for the cow's milk-fed group. Using the Wilcoxon two-sample test, the difference of the means is statistically significant ($p < 0.01$). In contrast, if one analyzes the conversion time in those babies who came from unilateral

TABLE III. Maternal and cord serum IgE, IgA, and IgM levels

Maternal-neonatal pair	Maternal IgE (ng./ml.)	Neonatal (cord serum)		
		IgE (ng./ml.)	IgA (mg. %, normal 0-6 mg. %)	IgM (mg. %, normal 5-30 mg. %)
1, Ca.	155.5	60.5	< 6	7
2, Br.	114.5	28.1	—	5.4
3, La.	103.7	30.2	—	6.1
4, McH.	696.6	18.0	< 6	12.0
5, Pit.	142.6	25.9	—	9.5
6, Ne.	41.0	20.4	—	8.0
7, We.	77.8	34.6	—	4.6
8, Ro.	125.3	25.9	—	11.0
9, Pi.	168.5	116.6	—	21.0
10, Mar.	291.6	17.6	< 6	—
11, Si.	90.7	21.6	—	7.0
12, Kr.	101.5	90.7	—	9.5
13, Lau.	183.6	19.2	< 6	6.1
Mean	177.2	39.1	< 6	8.9

or bilateral atopic backgrounds as opposed to that of those babies from non-atopic families, the means are 24.9 and 16.1 days, respectively. The difference in means is not statistically significant ($p > 0.05$).

In Table III serum IgE levels are shown in 13 paired maternal and neonatal sera. The mean level of IgE in maternal sera was 177.2 ng. per milliliter and in cord sera it was 39.1 ng. per milliliter, or 22 per cent of the mean maternal IgE serum level. Concomitant cord IgA and IgM levels are normal for neonates, do not approach known maternal serum levels, and provide evidence against the hypothesis of maternal transfusion of immunoglobulins into the newborn circulation during delivery.

Of further interest was the fortunate observation that one mother (Pi.) had spring hay fever and was highly reaginic (Table IV), with a P-K titer against perennial rye grass (*Lolium perenne*) of 1:640. Despite this hereditary background and a high titer of cord serum IgE, her baby's undiluted cord serum, when injected into the skin of the same recipient, failed to respond to injections of house dust, slender wild oat, and rye grass which had elicited large reactions in undiluted maternal serum P-K sites.

As shown in Table V, one maternal-neonatal pair (Ro.) showed a fivefold difference between cord and maternal serum IgE levels. Furthermore, the cord serum IgE level and Day 1 serum IgE levels were identical, indicating that no dilution of a hypothetical transfused bolus of maternal IgE occurred during the baby's first day of life. In the other pair (We.), the baby's serum IgE level dropped to $\frac{1}{2}$ the cord level in the first day, but further data are needed to show that this represented dilution of maternal IgE.

Table VI shows that newborn skin is capable of accepting adult IgE and responding to anti-IgE with a positive wheal and flare response. This was difficult to demonstrate because 2 of 4 babies (Pa. and Ro.) converted their own skin responses to anti-IgE or normal goat serum during the 48 hour fixation of atopic serum in the transfer sites. However, even in one of these babies (Ro.) there was a marked difference in the wheal size after anti-IgE

TABLE IV. Passive transfer of maternal and cord serum into sites on a P-K recipient: Comparison of reagin activity and serum IgE levels

Sites	Mother Pi. (serum IgE 168.5 ng./ml.)	Baby Pi. (cord serum) (serum IgE 116.6 ng./ml.)
	<i>w/v*</i>	<i>Wheal†</i>
Undiluted serum		
House dust	1:100	6
<i>Avena fatua</i>	1:1,000	16
<i>Avena barbata</i>	1:1,000	22
<i>Cynodon dactylon</i>	1:1,000	10
<i>Lolium multiflor.</i>	1:1,000	28
<i>Lolium perenne</i>	1:1,000	23
<i>Bromus ridigus</i>	1:1,000	16
<i>Poa annua</i>	1:1,000	25
<i>Holcus halepensis</i>	1:1,000	4
<i>Olea europaea</i>	1:1,000	10
<i>Quercus dumosa</i>	1:1,000	6
Saline		0
P-K titer		
<i>Lolium perenne</i> , 1:1,000 <i>w/v*</i>	<i>P-K serum dilutions</i>	<i>Wheal†</i>
testing antigen	1:10	23
	1:20	21
	1:40	20
	1:80	13
	1:160	12
	1:320	12
	1:640	3

*0.02 ml. injected intradermally into each P-K site.

†Area of wheal measured in planimeter units.

was injected into the P-K site as contrasted to the normal skin sites. In the other two babies (Ga. and We.), the appropriate controls remained negative and atopic serum was shown to fix to their skin and respond to anti-IgE injections with significant wheal and flare reactions. In Baby Ga., nonatopic serum, containing a measured level of IgE (47 ng. per milliliter), did not respond to anti-IgE after being transferred to the baby's skin, even though the nonatopic adult who provided the serum consistently responds to 1:1,000 anti-IgE with wheal areas of 15 planimeter units (wheal diameter 14 mm.).

DISCUSSION

It has been shown by several workers^{5, 6} that reagins do not cross the placental barrier. Therefore, one might assume that maternal immunoglobulin E does not cross the placenta and should not appear in cord serum. However, Johansson⁴ has demonstrated and, using a different myeloma IgE primary binding method, we have confirmed his observations that IgE can be consistently detected in cord sera. Our mean cord IgE level of 39.3 ng. per milliliter is similar to his mean level of 36.3 ng. per milliliter. Furthermore, the difference in serum IgE levels between individual maternal-neonatal pairs⁴ (Table III) eliminates the possibility of a passive equilibrium between the maternal-fetal circulation, such as appears to exist for IgG.¹¹ These IgE detection methods, which rely upon a single myeloma IgE standard, are

TABLE V. Comparison of cord and 1-day-old neonatal serum to maternal serum and anti-IgE skin test results

Maternal-neonatal pair	Maternal			Neonatal		
	Serum IgE (ng./ml.)	Anti-IgE skin test		Serum IgE (ng./ml.)		Skin test with Day 1 Anti-IgE 1:1,000*
		Anti-IgE dilutions	Anti-IgE* /codeine	Cord	Day 1	
1, We.	77.8	1:100,000	0.17 ($\pm$)	34.6	17.6	Neg.
		1:10,000	0.61 (+)			
		1:1,000	0.72 (+)			
2, Ro.	125.3	1:100,000	0.33 ($\pm$)	25.9	25.9	Neg.
		1:10,000	0.60 (+)			
		1:1,000	0.86 (+)			

*Planimeter units for the area of anti-IgE wheal reaction, divided by codeine wheal area.

TABLE VI. Passive transfer of adult serum containing IgE into newborn skin

Baby	Age tested (Days)*	First skin tests			48 Hours later		
		Normal goat serum	Anti-IgE 1:1,000	Codeine 0.02 mg. (wheal in plan. units)	Passive sites: 1:1,000 anti-IgE† /codeine	Baby's normal skin	
						1:1,000 anti-IgE† /codeine	Normal goat serum
1, Ga.	2	Neg.	Neg.	3	(A) 1.66 (+) (NA) 0 (0)	Neg.	Neg.
2, Pa.	1	Neg.	Neg.	3	(A) 1.00 (+) (NA) 1.32 (+)	2.0 (+)	Neg.
3, Ro.	1	Neg.	Neg.	5	(A) 3.0 (+) (NA) —	0.80 (+)	0.20
4, We.	1	Neg.	Neg.	11	(A) 0.5 (+) (NA) —	Neg.	Neg.

*On the day baby initially tested, skin on the back was injected with 0.1 ml. of serum from a known atopic (A) and a nonatopic (NA) human subject; serum IgE level (A) = 1,700 ng. per milliliter and (NA) = 47 ng. per milliliter.

†Planimeter units of anti-IgE wheal divided by codeine wheal.

measuring class-specific IgE in terms of the myeloma IgE. Because Johansson used anti-IgE absorbed with IgE Fab, the antisera he used should have detected IgE Fc only and not detected idiotype antigens on the IgE Fab piece. In our system we did not have sufficient amounts of myeloma IgE (P.S.) to absorb with IgE Fab. Among groups interested in measuring IgE, standardization of methods and results is incomplete. Therefore, it would not be surprising if future measurements of newborn serum IgE levels produced slightly or even considerably different absolute values. Although we cannot exclude the possibility that we are measuring some idiotype IgE antigens in our system, the majority of the evidence would suggest that this is a minor or even negligible component of our stated IgE values. The fusion of reference serum and myeloma serum dose-response curves in the 30 per cent I¹²⁵ precipitation region and the use of this area for measuring unknown samples suggest that we detected major class-specific IgE antigens. In addition, the fact that our mean cord serum IgE level is close to Johansson's recorded mean level speaks against idiotype antigen measurement in our

system. Dr. Gleich's independent assay of our reference sera detected higher IgE levels. Since his double-myeloma assay system virtually excludes the possibility of measuring idiotypic antigens our lower values would suggest that few idiotypic antigens were measured in our system. Finally, serial IgE measurements in the sera of some babies showed a change in IgE concentration (Fig. 1). Since the babies' idiotypic antigens, which might cross-react with P. S. myeloma IgE idiotypic antigens, should remain constant, one would expect any measured change in IgE concentration to represent an increasing quantity of class-specific IgE.

The failure to demonstrate any reaginic activity in the cord serum of Baby Pi. (Table IV), even though it was present in high titer in her mother's serum and the observation that their respective serum IgE levels were similar (116.6 ng. per milliliter versus 168 ng. per milliliter) support the earlier observations that reagins do not cross the placental barrier. Since demonstrable reagins have not been shown to cross the intact placenta but IgE is found in cord serum, one must assume that either the biologic activity of reagin is lost when it crosses the placenta or that IgE is synthesized by the fetus. The second possibility seems more likely and is further supported by a rise in serum IgE titers in some infants during their first few weeks of life (Fig. 1). Even a stable serum titer of IgE in other infants may not reflect the true dynamics of newborn immunoglobulin E synthesis, since there may be rapid diffusion from the vascular space. Waldmann¹² has shown a short 2 day half-life of serum IgE in adults. In a recent abstract, Levin and associates¹³ described a highly sensitive modified bacteriophage antibody detection system, which allows quantitation of as little as 0.2 to 2.0 ng. of antibody per milliliter. Using this method they detected antibodies in sera from newborn infants which were directed against penicillin hapten groups. Further studies, using heat inactivation and immunoabsorbents, have shown that many of these antibodies are in the IgE class. They conclude that these IgE antibodies are actively produced by the fetus in response to a known antigen, in this case, penicillin.

If IgE synthesis begins in fetal life, does newborn IgE possess the same biologic capacity to fix to dermal cells which is characteristic of adult IgE? It seems curious that IgE could be available in babies' cord or follow-up sera but could not be consistently and coincidentally demonstrated in their skin with the use of the anti-IgE skin test technique (Table II). Ishizaka and associates¹⁴ have published data which show that anti-IgE, when reacted with leukocyte-fixed IgE, releases histamine. The mechanism of this reaction is such that sera with higher concentrations of IgE did not necessarily sensitize leukocytes for a more vigorous release of histamine with the addition of a comparative quantity of anti-IgE antibody. Furthermore, they found that nonatopic IgE from 5 individuals participated poorly in the described chain of events and required larger concentrations of anti-IgE in order to release minimal amounts of histamine. This was particularly interesting in view of the fact that the leukocytes from some atopic patients released histamine by anti-IgE reactions even though their serum IgE concentrations were lower than those of 2 normal individuals whose leukocytes did not release a significant amount of histamine

after exposure to the same amount of anti-IgE. The authors suggested that the atopic state may be expressed in the capacity to efficiently fix IgE to histamine-containing cells or to release larger amounts of histamine after the bridging of IgE molecules by anti-IgE antibodies. These observations may be relevant to our discussion, since the maturation of an efficient IgE cell-fixing and histamine-releasing system in newborn babies may be a more critical factor than specific serum levels of IgE. The majority of our data support this explanation. Codeine produces very small wheal and flare responses in newborn skin when compared to adult skin. Very few babies developed positive responses to anti-IgE in the first few days of life. Although considerable scatter occurred (range 2 to 39 days), most newborn infants did not react to anti-IgE with positive wheal and flare skin responses until about 3 weeks of age (mean 21.7 days). This suggests that the day of birth is an arbitrarily imposed point upon a maturation process which has been operative for an indefinable time period in utero. In Fig. 1, the serum levels of IgE do not parallel such a proposed maturation process. Although available IgE must be a necessary component, the function of newborn dermal cells may be the more critical factor in allowing detection of cell fixed IgE by a tertiary immunologic detection system.

On the other hand, it is difficult to exclude the possibility that neonates are synthesizing two or more populations of IgE immunoglobulins with the predominant early population less able to fix to dermal cells or to participate in the anaphylactic process. This theory might explain why newborn skin could accept adult atopic serum passively (Table VI) and respond with large wheal and flare reactions to injections of anti-IgE in the passive sites. However, an alternative explanation, such as supersaturation of newborn dermal cells with IgE (1,700 ng. per milliliter) might also be operative.

Could antigenic exposure influence this synthetic process and selectively stimulate synthesis of cell fixing IgE? It has been known for some time that ingested protein in the newborn infant is absorbed in part as immunogenic macromolecules.¹⁵ A large body of work^{16, 17} has demonstrated precipitating or primary binding antibodies against cow's milk antigens in a high percentage of infants and adults. Furthermore, suspected cow's milk hypersensitivity reactions in newborn infants have suggested to some physicians¹⁸ that antibody formed against cow's milk antigens may participate in anaphylactic or hypersensitivity reactions. Therefore, we separated the breast milk-fed babies and analyzed these two groups for time of conversion to a positive anti-IgE skin test response. As recorded in the results section, the cow's milk-fed group converted earlier than did the breast-fed group, and the difference in the means was statistically significant. If one further analyzes these two groups for the relationship between type of feeding and heredity, one finds that the breast-fed group was populated with babies from atopic parents (5 of 6), whereas the cow's milk-fed group contained 5 of 10 babies from atopic backgrounds. Atopy appeared to be a factor only in the type of milk selected for feeding. Specifically, we did not observe a preponderance of positive anti-IgE skin test responses during the early part of neonatal life in babies who came from atopic families. Early maturation of functional dermal cells did not appear to be related to an

atopic family background in this study. The observations on milk feeding are interesting but incomplete, since our protocol did not include skin testing the babies with cow's milk antigens to show that reagins against the presumed immunogens appeared at or near the time of conversion from negative to positive anti-IgE skin test reactions. Clearly this is an important point, both from the standpoint of identifying and understanding the development of biologically functional IgE, as well as in the practical advice one might give for feeding newborn infants.

Final conclusions about the early synthesis of IgE and its biologic activity cannot be made from the available data. In human subjects it appears that IgE is synthesized by the time of birth. Sometime during the first month of extrauterine life neonatal IgE fixes to dermal cells and, after union with anti-IgE, can participate in histamine release in a manner indistinguishable from that which is known to occur with adult IgE.

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