Factors Influencing the Immunization of Rh-negative Mothers

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According to Nevanlinna and Vainio (1962) the probability that an Rh-negative mother who has had two Rh-positive children will be immunized is 4.5%. In the third and later births the probability of immunization decreases rapidly. The reason why most mothers do not become immunized is intriguing. It may depend on individual differences in immunological reactivity, and perhaps the response of some mothers to the antigenic stimulus of the foetus is exceptionally strong. However, this factor is unlikely to be the main one, as a transfusion sensitizes the majority of mothers (Nevanlinna, 1953). When small amounts, 0.2–0.6 ml. of foetal red cells were injected (group O, Rh-positive), 4 out of 15 women became immunized (Zipursky, Pollock, Chown, and Israels, 1965).

Levine (1943) observed that ABO compatibility* between the mother and her husband favours, and incompatibility inhibits, the development of maternal Rh-sensitization. His observation has been confirmed by many authors. The probability of sensitization is even lower if mother and foetus are incompatible than if mother and husband are incompatible (Knox, Murray, and Walker, 1960; Murray, Knox, and Walker, 1965). In complete agreement with these findings, ABO compatible Rh-positive red cells injected into Rh-negative volunteers immunized the volunteers much more readily than ABO incompatible ones (Stern, Davidsohn, and Masaitis, 1956).

Most of the studies on the subject of maternal Rh-sensitization stress the association between ABO incompatibility and lack of Rh-sensitization. The recent discovery that Rh-sensitization could be prevented by injections of anti-D γ -globulin was based on an analogy with the reduction in sensitization associated with ABO incompatibility (Clarke, 1966).

However, even if ABO incompatibility completely prevented maternal Rh-sensitization (which it does not), the essential problem remains unsolved. Supposing that the probability of Rh-sensitization is 4.5% and that the frequency of ABO compatible matings is 65%, then more than 90% of mothers do not become sensitized in spite of ABO compatibility. So, other ways in which Rh-sensitization may be affected should be looked for. According to Race and Sanger (1962), both Brambell and Mitchison have suggested that the mothers who were not immunized had, while foetuses, become tolerant of the Rh-positive antigens of their own mothers. However, this does not seem to be the true explanation (Race and Sanger, 1962). Another tolerance factor which might interfere with immunization is the tolerance of mothers to the non-self qualities of the foetus, such as the male antigen. Similar non-self antigens are likely to be present more often in dizygotic twins. The presence or absence of the male antigen affects the results of skin grafting (Eichwald-Silmser phenomenon) and may play a role in determining whether there are more boys or girls within a given sibship (Renkonen, 1964a, b). A certain degree of tolerance has been postulated in mothers with twin foetuses (Renkonen, 1966).

The critical time for immunization of mothers seems to be at the end of pregnancy, especially during parturition (Boorman, Dodd, and Mollison, 1945; Clarke, 1966; Knox, Murray, and Walker, 1961). Whatever the role of transplacental haemorrhage and abnormal pregnancies (Clarke, 1966), the entry of foetal cells into the mother is necessary for sensitization. The entry of foetal cells might be influenced, and favoured, by compatibility between mother and foetus.

The sex ratio (M/F) seems to be very high among the sensitizing firstborn children in families where the second child is affected (Renkonen and Seppälä, 1962). However, because of the small size of the series presented, further data were required.

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* No maternal isoagglutinins against the red cells of her husband (or foetus).

TABLE I

SIBS BORN BEFORE THE FIRST ERYTHROBLASTOTIC CHILD

Birth Orders of Healthy Sibs	Birth Orders of Affected Children*			Total
	II	III	IV and over	
I				
Singles	211	119	155	485
Boys	134	68	82	284
Girls	77	51	73	201
M/F	1.74	1.33	1.12	1.48
Pairs of twins	6	2	—	8
	(2mm, 4ff)	(mm, mf)		
Total of singles and pairs of twins	217	121	155	493
II				
Singles		121	154	275
Boys		64	77	141
Girls		57	77	134
M/F		1.12	1.00	1.05
Pairs of twins		—	1	1
			(mf)	
Total of singles and pairs of twins		121	155	276
III and over				
Singles			349	349
Boys			163	163
Girls			184	184
Sex Unknown			2	2
M/F			0.89	0.89
Pairs of twins			5	5
			(2mm, 3mf)	
Total of singles and pairs of twins			354	354
Total				
Singles	211	240	658	1109
Boys	134	132	322	588
Girls	77	108	334	519
Sex Unknown	—	—	2	2
M/F	1.74	1.22	0.96	1.13
Pairs of twins	6	2	6	14
Total of singles and pairs of twins	217	242	664	1123
Sibs born immediately before affected				
Singles	211	121	152	484
Boys	134	64	75	273
Girls	77	57	77	211
M/F	1.74	1.12	0.98	1.29
Pairs of twins	6	—	3	9
	(2mm, 4ff)		(2mm, mf)	
Total of singles and pairs of twins	217	121	155	493

* m = male; f = female.

Present Investigation

The present investigation covers the children born in the University Central Hospital, Helsinki, during the years 1963–65 and parts of 1962 and 1966. Only those erythroblastotic children were included whose precedent sibs were reported healthy, or were not suspected of suffering from erythroblastosis. No attention was paid to the legitimacy of the children. Table I describes the healthy sibs.

The high sex ratios (M/F) 134/77 and 108/75 among the sensitizing firstborn children observed in this series and in the earlier ones (Renkonen and Seppälä, 1962)

indicate that there are ($p < 0.001$) more boys than expected among these firstborn children as compared to normal firstborn children in Finland (M/F 1.062).

It is noteworthy that there were six pairs of twins among the 217 births of sensitizing firstborn children (Table II). The early series (Renkonen and Seppälä, 1962) also contained six pairs of twins* out of 189 births. Thus the frequency of twin births seems to be very high 12/406, i.e. 2.96% among these firstborn children. The general frequency of twin births in Finland (1961–63) was $991/90 \cdot 748 = 1.09\%$ among first-born children. Statistically the difference is highly significant ($p < 0.01$).

TABLE II

FIRST ERYTHROBLASTOTIC CHILDREN AND THEIR BIRTH ORDERS

	Birth Orders			Total
	II	III	IV and over	
Singles	212	117	153	482
Boys	109	52	80	241
Girls	102	65	73	240
Sex unknown	1	—	—	1
M/F	1.07 ± 0.14	0.80 ± 0.18	1.09 ± 0.16	1.00 ± 0.09
Pairs of twins	5	4	2	11
(One* or both affected)	(3mm, mf, ff)	(mf, 3ff)	(mf, ff)	
Total of singles and pairs of twins	217	121	155	493

* Both twins were usually affected. One pair of male and one of female twins born at the second birth each consisted of one healthy and one affected child.

Discussion

The high frequency of boys and twins among the sensitizing children is puzzling. It could be due to the strong immunological response of mothers to double the amount of antigen (twins), or to doubly different antigens (male, Rh-positive). The uterine trauma and transplacental bleeding, likely to be more severe at the birth of twins, might also increase the likelihood of sensitization by twins. Perhaps, the high frequencies of boys and twins indicates that these mothers are tolerant of the non-self antigens carried by their foetuses.

The high incidences both of boys and twins seems to be limited to firstborn children who are immediately followed by an affected child. When the birth order of the first affected child is three or higher, the sex ratio of both the firstborn children and of the last healthy sibs are normal (Table I).

* The 9 families with twins in those series were not reported in the paper of Renkonen and Seppälä (1962). There were 6 pairs of twins among the firstborn children and 4 pairs among the affected (second) children. (In one family both the sensitizing and affected children were twins.)

The risk of having an affected child is known to decrease rapidly with increasing birth order (Nevanlinna and Vainio, 1962). The amount of foetal cells entering the maternal circulation therefore should also decrease. There could be several reasons for this, among them an increase in the mother's reactivity against her foetuses. The M/F rate is known to diminish somewhat with increasing birth order and to be correlated with the number of boys born earlier (Renkonen, 1964a, b).

The sex ratio 1.00 ± 0.09 among the (first) erythroblastotic children was low in our series, but the frequency of twins 2.2% (Table II) was as high (2.1%) as in the series of Renkonen and Seppälä. The frequency of all twins (13/527) among the affected children born second or third was almost significantly ($p \sim 0.05$) higher than the expected frequency (7.58/527) in Finland (second births, 1.37%; third 1.67%).

Twins are generally considered to be vulnerable to immunological factors. If their mothers are more tolerant than normal, which for various reasons has been thought to be the case (Renkonen, 1966), then the high frequency of twins among the affected children would be understandable.

The mean birthweight of 152* sensitizing girls (firstborn children immediately preceding the affected child) was high, 3.344 ± 0.04 kg., compared to that of all firstborn girls born in the same hospital, 3.254 kg. (Timonen, Uotila, Kuusisto, Vara, and Lokki, 1966). The mean birthweight of the 241* sensitizing boys was 3.340 ± 0.04 kg. and of the controls 3.386 kg. If the high birthweight of the sensitizing girls could be verified, it might mean that the gestation time of those children is longer (tolerance?, oedema?). In considering the normal mean birthweight of the boys, one should not forget that this group might contain individuals (in the low weight groups) who would have died had not the mother been exceptionally tolerant (high sex ratio). The only other paper dealing with the

weight of the sensitizing children (Knox *et al.*, 1961) does not confirm the present findings. The mean weight of 42 ABO incompatible (?) sensitizers was 3117 g. (6 lb. 14 oz.), that of 43 controls 3288 g. (7 lb. 4 oz.).

Summary

Among the firstborn children that were followed by an erythroblastotic child there were more boys and twins than expected. The ratio M/F was 1.74 in this series (expected 1.062). The rate of twin births was 2.96% (expected 1.09%).

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* The series of Renkonen and Seppälä is included. The weight of one sensitizing boy is not known.