CHANGES IN LYMPHOCYTE SUB-POPULATIONS IN THE BLOOD OF HEALTHY AND SICK NEWBORN INFANTS[23]

(Lymphocyte sub-populations in neonates)

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SUMMARY

Normal values for the percentages of lymphocytes carrying surface membrane -bound immunoglobulin, or rosetting with sheep erythrocytes, ox erythrocytes coated with rabbit immunoglobulin G, or ox erythrocytes coated with rabbit immunoglobulin M and reacted with human serum partially de-complemented with zymozan, have been estimated for populations from human umbilical cord blood and the peripheral blood of babies aged one week and one month. These samples have been compared with values obtained from normal adult volunteers and eighteen newborn infants, measured during and after a suspected infective episode.

The dynamic change in those lymphocytes from normal infants staining for both IgM and IgD is shown, demonstrating that the number of circulating cells of this typeapproaches adult levels by the age of one month. Falls in the levels of all three types of rosetting lymphocyte were demonstrated over the same period. A drop in the number of lymphocytes forming sheep erythrocyte rosettes, and particularly those binding C3b-coated ox erythrocytes, in those babies suspected of infection showed a correlation with acute septicaemia or viraemia.

SPECULATION

The fall in the sub-population of lymphocytes forming all three types of rosettes, with increasing age, is felt to reflect the sudden antigenic challenge experienced by the newborn infant after birth. In particular, the dramatic fall in those lymphocytes bearing immunoglobulins M and D may mark the selection of virgin antigen-sensitive B-lymphocytes in response to antigens. The fall in the proportion of lymphocytes binding to C3b-coated ox erythrocytes in infected infants may indicate either that the lymphocyte C3b receptor becomes blocked by free C3b released as a consequence of complement activation, or, more plausibly, that there are circulating antigen/antibody complexes bearing fixed C3b in these babies. Finally, it should be noted that a fall in any lymphocyte population in peripheral blood, particularly of cells binding sheep erythrocytes, may be more closely related to a segregation of those cells within the lymphoid system rather than a failure to express the relevant receptor.

INTRODUCTION

A series of studies have been carried out to observe sub-populations of peripheral blood lymphocytes obtained from infants in the first month of life. Fluorescent antisera and rosetting techniques were used to differentiate between various sub-populations of lymphocyte and alterations in their proportion with increasing age and infection were sought.

The sub-population discriminators examined were total human immunoglobulin (total Ig), the human immunoglobulin classes G, A, M and D (IgG, IgA, IgM and IgD) and lymphocyte populations bearing receptors for sheep erythrocytes (E-rosettes), receptors for Fc of rabbit IgG (EA-rosettes) or receptors for the C3b component of complement (EAC3b-rosettes).

Studies on the spontaneous and induced blast responses of these lymphocytes and their sub-populations were carried out simultaneously with these measurements, which will be described in a subsequent paper.

MATERIALS AND METHODS

Blood Sampling

Samples of umbilical cord blood were collected at the time of delivery, with maternal permission, and defibrinated by rapid stirring with sterile orange sticks. Blood samples from babies aged one week were collected at the time of the routine Guthrie test, by heelprick. Blood samples from babies aged one month and free from obvious clinical infection were obtained when they were admitted for routine investigation, by removing the cell pellet from heparinised blood samples, taken for relevant plasma estimations. Blood samples from infants who were suspected of neonatal infection were obtained in a similar way. The volumes of blood obtained varied from 1.5ml to 3ml, but averaged 50ml for cord blood samples.

Lymphocyte Purification

Defibrinated or anti-coagulated blood was placed in a sterile universal container with 300mg carbonyl iron/20ml blood and mixed for 35 minutes at 37°C. The universal was then drawn through the jaws of a powerful magnet to remove phagocytic cells. The supernatant blood was removed and mixed with an equal volume of sterile saline and layered onto a mixture of 12 parts 9% Ficoll (Winthrop Laboratories) and 5 parts Hypaque (Pharmacia). This discontinuous gradient was centrifuged at 500g for 25 minutes, the cell-rich interface removed and washed three times in Eagles Minimal Essential Medium (MEM) (Wellcome Reagents Limited), buffered with hepes (Sigma Chemical Company)[2]. The lymphocyte concentration was adjusted to 2.5×10^6/ml.

Anti-Immunoglobulin Antisera

Antisera to human IgG, IgA, IgM and IgD were raised in sheep by immunisation with polyclonal and monoclonal immunoglobulins obtained from normal volunteers and from patients with multiple myeloma respectively. Injections were carried out using first Freund's complete and then Freund's incomplete adjuvant (Difco) and antibody titres boosted by repeated bleeding and intravenous antigen injection. Finally the sheep were exsanguinated via the carotid artery. The sera were fractionated on ion exchange resin columns to yield a 'slow' IgG moiety, which was digested with pepsin[12] to obtain a $F(ab')_2$ preparation as previously described[13].

The $F(ab')_2$ antisera were conjugated to fluorescein or rhodamine using modifications of conventional isothiocyanate methods as previously described[1,11]. The conjugates were freed of unbound fluorochrome by further column fractionation, isosmolar fractions were obtained, which were rendered class-specific for different immunoglobulins by purification on affinity columns[5] to which either IgG or IgM had been linked. The antisera were shown to be class-specific by radial immuno-diffusion in agar and by immuno-electropheresis. Ratios of fluorescein to protein were 1.8:1, while ratios of rhodamine to protein were 1:1, approximately[18].

Fluorescent Staining and Immunoglobulin Resynthesis

50 microlitres of the lymphocyte suspension under test, at a concentration of 2.5×10^6/ml, were placed in siliconised glass tubes on ice and appropriate dilutions of the fluorescent antisera were added. These dilutions had been estimated previously by titration against normal adult peripheral blood lymphocytes: that dilution two $\log_2$ above the point at which staining ceased to be specific was selected.. The mixture of cells and fluorescent antiserum was left on ice for 45 minutes, with occasional mixing, and the cells subsequently washed three times in MEM-hepes. The pellet of cells was re-suspended, dropped onto a siliconised slide, covered with a siliconised coverslip and the edges of the coverslip sealed with molten wax. Slides were kept in a moist box at 4°C, before counting the proportion of fluorescent cells under a Leitz Ortholux fluorescence microscope.

The re-synthesis of surface membrane-bound immunoglobulin was examined by staining the cells before, and at intervals after, treatment with 0.2mg/ml trypsin (Sigma Chemical Company). The fluorescent antiserum was affinity-purified anti-light chain (both kappa and lambda), produced and tested as described earlier. Fluorescent staining was carried out on lymphocyte samples before trypsinisation, immediately after and 3, 8, 24 and 30 hours later. Removal of surface membrane-bound immunoglobulin was achieved by incubating lymphocytes in the presence of trypsin for 30 minutes and then washing three times in MEM-hepes. Culture was continued in MEM-hepes and samples removed at intervals for staining.

E, EA and EAC3b-Rosettes

For E-rosettes, fresh ACD anticoagulated blood from a Scottish Blackface sheep, blood group ii, was washed three times in MEM-hepes and set up as a 2% suspension in the same medium. Equal 50 microlitre volumes of this preparation and a suspension of the lymphocytes under test were mixed, centrifuged at 250g for seven minutes and placed on ice. 10 microlitres of fetal calf serum was run down the side of each tube and the mixture left on ice for one hour at least.

To make EA-rosettes, ox erythrocytes were washed three times in complement fixation diluent (CFD) and made up as a 2% suspension in the same buffer. To 0.5ml of this suspension was added an equal volume of an appropriate sub-agglutinating dilution of rabbit anti-ox erythrocyte IgG. This mixture was incubated for 30minutes at 37°C, washed twice in CFD and finally in MEM-hepes. Equal 50 microlitre volumes of sensitised ox erythrocytes and the lymphocyte suspension under test were mixed, centrifuged at 250g for 7 minutes at 4°C and counted.

In making EAC3b-rosettes, ox erythrocytes were sensitised, this time with rabbit anti-ox erythrocyte IgM, as described above. 0.5ml of a 2% suspension of these sensitised cells were incubated with 0.5ml of normal human serum, partially decomplemented by previous incubation with zymozan at 37°C, and the reaction halted after 2 minutes by the addition of 5mg Antrepol (Suramin, Bayer). These cells were washed twice in CFD and finally in MEM-hepes. 50 microlitres of a 2% suspension of ox cells thus coated in C3b were added to an equal volume of the suspension of lymphocytes under test and the mixture centrifuged at 250g for 7 minutes at 4°C. The cell pellet was then incubated at 37°C for 30 minutes.

For each rosette preparation, the cell pellet was re-suspended and the proportion of rosetted cells counted in a haemocytometer. With E-rosettes, only those lymphocytes binding eight or more sheep erythrocytes were considered positive, to reduce inter-experimental error.

Statistical Comment

It is usual to express data on lymphocyte surface markers in terms of a percentage mean, with one or two standard deviation or a standard error. These types of analyses may be invalid, where there is heterogenous variance in the numerator. In these studies, 200 cells were always counted as the numerator. By applying a Poisson distribution and considering the total population counted as a fraction of the whole pool, as well as the number of positive cells, it is possible to derive a consistent standard deviation from the mean. Ideally, one would prefer to be able to count very much larger numbers of cells and derive a fiducial limit by the application of Feiller's Theorem. It may prove possible to achieve this by the use of automated rosette counting.

RESULTS

Table 1 shows the percentages of lymphocytes forming E-, EA- and EAC3b-rosettes in umbilical cord blood and in the blood of infants aged one week and one month. Values obtained from adult peripheral blood lymphocyte samples are included for comparison. Table 11 displays the values for class-specific and total immunoglobulin staining in the same groups.

Table 111 summarises recently published values for the proportions of lymphocytes in umbilical cord blood belonging to various sub-populations. There have been no previously published data showing the simultaneous evaluation of all the sub-population discriminators studied here.

Figure 1 follows the recovery of surface membrane-bound anti-light chain immunofluorescence before, and at intervals after, trypsinisation.

DISCUSSION

There is a fall in the proportion of lymphocytes forming E-, EA- and EAC3b -rosettes in the first month of life. This fall is most marked between one week and one month of age and is greatest for EA-rosettes. Lymphocyte sub-populations in cord blood compare with adult control values.

There is a similar fall in the proportion of B- lymphocytes staining for total immunoglobulin, largely accounted for by the fall in the number of cells carrying IgM and IgD. It may be that this reflects the commitment of virgin antigen-sensitive B-lymphocytes under the heavy antigenic challenge which occurs post-natally.

It follows that there has been a rise in the number of circulating lymphocytes bearing none of the surface discriminators measured here. The use of anti-B- or anti-T-lymphocyte antisera might allocate these cells to a particular sub-population, although they could be non-lymphocytic monocytes.

Examination of Table 111 shows that the values given here are low for immunoglobulin-bearing cells, but in close agreement with those given by Frøland and Natvig[8]. The proportion of cells bearing IgM and IgD is below the others shown. However, since the antisera were $F(ab')_2$ and affinity-purified, non-specific staining was at a minimum and probably explains these differences. As far as E-rosettes are concerned, the values here agree well with those of Davis and Galant[6] and Hamburg and his colleagues[10]. In all three studies, only lymphocytes showing a high affinity for sheep erythrocytes were included, which accounts for the higher values given by other workers, who included less firmly bound rosettes as positive.

The estimation of surface membrane-bound immunoglobulin is fraught with technical difficulties: commonly, overestimation due to the presence of passively-bound immune complexes by the Fc receptor, or due to the presence of fluorescent complexes in the test antiserum, binding in the same way, when a whole immunoglobulin preparation is used [9]. The extent of the first problem was assessed by the trypsinisation experiment (Figure 1), which showed that all but 10%-15% of neonatal lymphocytes could re-synthesise immunoglobulin, whereas 40% of adult lymphocytes failed to do so. The second difficulty was overcome by the use of $F(ab')_2$ antisera, which cannot bind to the Fc receptor.

The presence of lymphocytes bearing both IgM and IgD was confirmed in this study, having first been demonstrated by Rowe et al[14]. Although the function of IgD is still unknown, Pernis[13] demonstrated that the injection of anti-human IgD into monkeys led to a rise in IgM followed by an overwhelming increase in IgG, up to as much as 100mg/ml. These data suggest that antigen-binding by IgD at the lymphocyte surface may act as a de-repression signal, leading to B-lymphocyte differentiation and, ultimately, immunoglobulin production.

Several interesting points are raised by the studies displayed in Tables 1V, V and V1. All the infants were selected for the study on the basis of strong clinical suspicion of infection. High values were almost only found during the recovery phase, with the exception of one surface immunoglobulin value for cells from a baby known to have suffered an intra-uterine virus infection (baby 15).

With these exceptions, the most severely ill babies scored highest. When a score of 2 is considered as a cut-off point, only baby 14 did not prove to have a serious infection. Furthermore, if babies 15 and 16, who had severe and life-threatening vral meningo-encephalitis, are excluded as well, all the remaining infants were septicaemic, two died (babies 1 and 8) but the remainder recovered after treatment with penicillin and gentamycin.

When individual values are examined, the best discriminators for septicaemic infants were E- and EAC3b-rosettes; the former were low in 4 of 7 infected infants and the latter low in 6 of 7 infected infants. Furthermore, EA-rosettes were low in 5 of 7 infected infants.

The association between a significantly low value for EAC3b-rosettes and septicaemia is of interest. It is possible that the presence of bacterial antigen-maternal antibody immune complexes bearing fixed C3b has blocked lymphocyte receptor sites, although it may be argued that cells bearing this receptor would have then left the circulation. In view of the difficulty of early and rapid diagnosis of neonatal infection, this estimation, which can be carried out in 2 to 3 hours, may prove helpful. Further studies on babies thought to be infected are to be undertaken.

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22. Address for reprints: Dr Colin Stern, Department of Immunology, Royal Post-Graduate Medical School, Du Cane Road, London W12 OHS, England.
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TABLE 1

PERCENTAGE LYMPHOCYTES FORMING E, EA AND EAC3b ROSETTES AT DIFFERENT AGES

ROSETTES	CORD BLOOD % N=36[a]	ONE WEEK % N=11	ONE MONTH % N=7	ADULT % N=54
E[b]	36.6[e] $\pm$ 14.8[f]	32.2 $\pm$ 16.8	22.5 $\pm$ 5.0	34.9 $\pm$ 12.9
EA[c]	20.0 $\pm$ 4.6	20.9 $\pm$ 4.1	10.4 $\pm$ 1.0	20.7 $\pm$ 4.6
EAC3b[d]	15.8 $\pm$ 4.0	14.6 $\pm$ 2.7	9.9 $\pm$ 0.8	16.3 $\pm$ 3.8

a: denotes the number of observations. b: E-rosettes. c: EA-rosettes. d: EAC3b-rosettes. e: mean of N observations. f: two standard deviations.

TABLE 11

CHANGES IN CLASS-SPECIFIC SURFACE MEMBRANE-BOUND IMMUNOGLOBULIN STAINING OF B-LYMPHOCYTES IN NEWBORN INFANTS WITH AGE, COMPARED WITH ADULT B-LYMPHOCYTES.

SURFACE MEMBRANE-BOUND IMMUN-OGLOBULIN	CORD BLOOD % N=36[a]	ONE WEEK % N=11	ONE MONTH % N=7	ADULT % N=54
TOTAL	12.3[b] $\pm$ 4.1[c]	11.6 $\pm$ 3.0	10.0 $\pm$ 2.7	16.5 $\pm$ 3.1
IgG	2.0 $\pm$ 1.4	3.0 $\pm$ 1.0	5.9 $\pm$ 1.8	13.7 $\pm$ 4.6
IgA	1.0 $\pm$ 1.0	1.0 $\pm$ 0.8	0.4 $\pm$ 2.7	0.8 $\pm$ 0.9
IgM	8.9 $\pm$ 3.1	6.7 $\pm$ 1.3	3.1 $\pm$ 0.6	1.8 $\pm$ 2.4
IgD	8.0 $\pm$ 3.3	6.4 $\pm$ 1.3	3.0 $\pm$ 1.2	1.5 $\pm$ 1.6
IgM + IgD[d]	7.4 $\pm$ 3.7	5.5 $\pm$ 1.3	2.4 $\pm$ 1.2	1.3 $\pm$ 4.7
IgD ALONE[e]	0.6 $\pm$ 0.8	1.2 $\pm$ 0.7	0.7 $\pm$ 0.8	0.2 $\pm$ 1.4
IgM ALONE[f]	1.6 $\pm$ 2.2	0.9 $\pm$ 0.8	0.5 $\pm$ 0.7	0.5 $\pm$ 3.3

a: denotes the number of observations. b: mean of N observations. c: two standard deviations. d: cells bearing IgM + IgD. e & f: cells bearing IgD or IgM only.

TABLE 111

COMPARISON OF SOME PUBLISHED VALUES FOR LYMPHOCYTE SURFACE MARKERS IN CORD BLOOD

STUDY	SURFACE MEMBRANE-BOUND IMMUNOGLOBULIN TOTAL Ig %	IgG %	IgA %	IgM %	IgD %	IgM + IgD[a] %	ROSETTES E[b] %	EA[c] %	EAC3b[d] %
VOSSEN & HIJMANS[19]	19.3[e]	5.8	0.1	14.1	19.0	13.7	ND	ND	ND
FRØLAND & NATVIG[8]	9.9	6.1	0.0	10.4	ND	ND	ND	ND	ND
CAMPBELL ET AL[3]	32.3	ND[f]	ND	ND	ND	ND	53.2	ND	ND
DAVIS & GALANT[6]	ND	ND	ND	ND	ND	ND	33.3	ND	ND
FERGUSON ET AL[7]	ND	ND	ND	ND	ND	ND	48.2	ND	ND
ROWE ET AL[14]	ND	ND	ND	13.0	14.6	12.5*	ND	ND	ND
ROWE ET AL[15]	ND	ND	ND	ND	13.8	ND	ND	ND	ND
SMITH ET AL[16]	ND	ND	ND	ND	ND	ND	53.4	ND	ND
HAMBURG ET AL[10]	20.4	5.2	1.5	13.6	10.7	ND	39.2	ND	ND
THIS PAPER	12.3	2.0	1.0	8.9	8.0	7.4	36.6	20.0	15.8

a: lymphocytes positive both for IgM and IgD. b: E-rosettes. c: EA-rosettes. d: EAC3b-rosettes. e: mean value. f: ND signifies estimation not done. *: value calcualted from authors' data.

Tables 1V and V show the values for rosette formation and for SmIg-staining lymphocyte sub-populations carried out on eighteen babies at the time of a suspected infection and either three or four days later. Any value for a particular sub-population, measured at the time of first suspicion of infection, which fell more than two standard deviations below the mean, scored one. The sum of these abnormal scores for each baby has been used as a potential discriminator between infected and un-infected infants. The final diagnosis on each baby, together with any pathogenic organism identified, has been listed in TABLE V1.

TABLE 1V

THE PERCENTAGES OF E, EA AND EAC3b-ROSETTES FOUND IN THE PERIPHERAL BLOOD LYMPHOCYTES POPULATIONS OF INFANTS AT THE TIME OF A SUSPECTED INFECTIVE EPISODE AND EITHER THREE OR FOUR DAYS LATER

SAMPLE	E-ROSETTES %		EA-ROSETTES %		EAC3b-ROSETTES %	
	ACUTE	LATE	ACUTE	LATE	ACUTE	LATE
1	13.0-	-	8.2-	-	6.6-	-
2	29.2	47.5	17.6	18.2	15.0	15.6
3	38.3	41.0	16.2	19.8	12.7	21.3+
4	42.9	42.4	20.9	12.0-	12.7	14.9
5	16.5-	29.9	12.7-	14.2-	16.9	9.8-
6	26.2	26.7	12.0-	13.7-	9.7-	9.6-
7	11.5-	27.9	12.6-	12.9-	9.2-	9.0-
8	16.0-	34.4	10.3-	14.8-	1.8-	12.4
9	30.3	30.9	18.3	13.6-	12.4	9.6-
10	33.8	30.5	18.8	17.6	15.4	13.9
11	37.9	30.7	19.0	17.0	14.5	13.5
12	28.4	32.3	14.0-	15.2-	9.6-	12.8
13	38.5	36.9	17.7	17.0	14.5	13.5
14	18.0-	43.3	19.5	15.1-	7.9-	12.5
15	15.6-	18.3-	20.4	17.5	6.5-	8.5-
16	16.0-	18.9-	18.7	16.9	6.6-	9.4-
17	36.2	32.3	17.6	15.8	12.1	18.3
18	22.4	26.3	23.5	16.3	8.5-	12.0

TABLE V

THE PERCENTAGES OF THE TOTAL LYMPHOCYTE POPULATION WHICH STAINED FOR DIFFERENT MEMBRANE-BOUND IMMUNOGLOBULINS WHEN REACTED WITH CLASS-SPECIFIC ANTISERA, TAKEN FROM THE PERIPHERAL BLOOD OF INFANTS AT THE TIME OF A SUSPECTED INFECTIVE EPISODE AND EITHER THREE OR FOUR DAYS LATER

SAMPLE	TOTAL Ig		IgG		IgA		IgM		IgD		IgM + IgD	
	ACUTE %	LATE %	ACUTE %	LATE %	ACUTE %	LATE %	ACUTE %	LATE %	ACUTE %	LATE %	ACUTE %	LATE %
1	6.6-	-	2.4	-	0.2	-	3.4-	-	3.2-	-	2.4-	-
2	15.4	18.2+	2.7	4.1+	1.4	1.2	9.9	11.6+	10.0	11.2+	8.6	9.9+
3	10.5	10.9	1.2	1.2	0.4	1.2	8.4	8.1+	8.1	7.9	7.7	7.9
4	9.8	13.2	2.7	2.9	0.2	0.5	6.2	8.3+	6.0	8.7	5.3	8.2+
5	8.3	9.1	0.7	1.4	0.2	0.7	7.4	6.2	7.2	5.9	8.0	5.1
6	9.4	11.0	0.5-	1.7	0.5	1.0	7.8	7.9	7.4	6.9	6.8	6.5
7	12.3	12.7	1.0	2.6	0.2	0.7	10.5	9.0+	9.9	9.0+	9.3	8.6+
8	7.3-	13.4	0.2-	3.3	0.2	1.7	6.4	8.0	6.1	7.5	5.6	7.1+
9	13.2	19.3	2.3	3.9	0.5	1.2	9.0	10.0+	8.6	12.4+	7.8	8.2+
10	12.0	11.2	1.7	2.1	1.2	1.2	8.0	6.9	7.1	6.6	6.0	5.6
11	11.0	11.4	2.1	2.7	0.7	1.0	7.6	6.7	7.7	6.4	7.1	5.4
12	12.8	13.3	2.6	3.0	0.7	1.0	8.6	8.0	7.8	7.5	6.9	6.6
13	11.1	11.1	1.4	2.2	0.7	1.0	8.7	7.9	7.6	7.2	7.3	7.8+
14	12.0	12.3	1.7	2.6	0.7	1.2	9.1	7.8	8.4	7.8+	7.9	7.1+
15	16.4	14.4	1.9	3.2	0.9	1.2	11.6	9.6+	12.0+	9.3+	10.0	8.9+
16	13.0	17.2+	1.7	5.0+	0.7	1.0	10.1	10.2+	9.3	9.3+	8.8	8.3+
17	12.5	12.7	2.6	3.3	0.7	1.4	8.5	7.5	7.9	6.8	7.2	6.3
18	6.9-	11.6	0.5-	4.1	0.2	0.7	5.7-	6.0	5.1	5.2-	4.6	4.4-

In Tables 1V and V, values above or below two standard deviations from the mean have been marked + or - respectively.. Acute samples were taken at the time of diagnosis, late samples either three or four days later.

TABLE V1

RANKING OF INFANTS SUSPECTED OF AN INFECTIVE EPISODE DURING THE FIRST WEEK OF LIFE

NUMBER OF VALUES TWO SD BELOW MEAN	SICK BABIES SAMPLE NUMBER AND DIAGNOSIS
0	2. Urinary tract infection (*Escherichia coli*). 3. Birth asphyxia, on ventilator, recurrent apnoea. 4. Milk aspiration, later found to have co-arctation of aorta. 9. Recurrent apnoea. Cord IgM 200mg/ml, cause not found. 10. Respiratory distress syndrome. 11. Early jaundice. 13. Milk aspiration (right lung). 17. Aspiration pneumonia (probably milk).
2	5. Pneumonia and septicaemia (*Staphylococcus aureus*). 12. Prolonged rupture of membranes (*β-haemolytic streptococcus* found in blood culture). 14. Prolonged rupture of membranes. 15. Viral meningo-encephalitis. 16. Viral meningo-encephalitis } twins
3	6. Septicaemia (*Listeria monocytogenes*), on ventilator, ABO incompatibility. 7. Meningitis (*Proteus vulgaris*).
4	8. Pneumonia and septicaemia (*β-haemolytic streptococcus*). † 18. Pneumonia and septicaemia (*Staphylococcus aureus*).
7	1. Pneumonia and septicaemia (*Pseudomonas aeruginosa*). †

In Table V1, all those values below two standard deviations (SD) of the mean at the time of the first sample have been added up. † signifies that the infant subsequently died. Where a pathogen was subsequently grown, the organism has been identified.

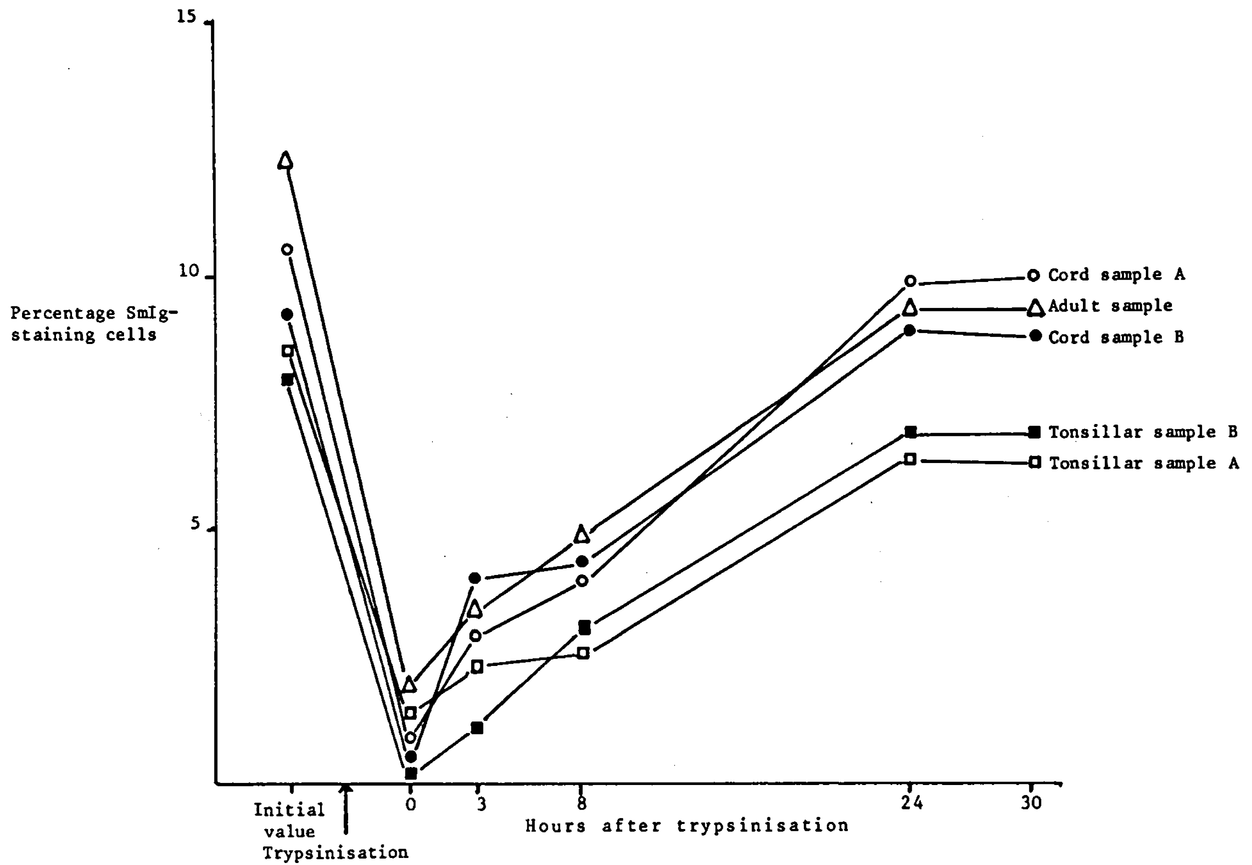

FIGURE 1. THE RECOVERY OF SURFACE MEMBRANE-BOUND IMMUNOGLOBULIN (SmIg) STAINING BY ADULT AND NEONATAL LYMPHOCYTES

Lymphocytes were obtained from five adult volunteers (mean values = △); two umbilical cord blood samples (mean values = ○ and ●) and two infants aged one week (mean values = □ and ■). Lymphocytes were stained with sheep anti-total human immunoglobulin antiserum before enzyme treatment (initial value) and at intervals after treatment.

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