THE FRAGILITY OF THE RED CELL TO HYPOTONIC SALINE IN INFANCY AND CHILDHOOD

BY

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A survey of the literature dealing with the resistance of the red blood corpuscle in infancy and childhood to hypotonic solutions of saline reveals a great divergence of opinion. Some authors (Goldbloom and Gottlieb, 1929; Cathala and Daunay, 1908; and Martha Wollstein, 1938) believe that the resistance of the red blood cell to hypotonic solutions of saline is diminished in the foetus and new-born child in comparison with what obtains in adult life; some (Mitchell, 1928) believe that there is no difference between early and adult life in this respect, whilst others again (Hornung, 1925; Whitby and Hynes, 1935; and Waugh, Marchant and Maughan, 1938) hold that the resistance is normal at birth and then increases during the first few days of life, reaching its maximum about the fourth day, after which it gradually returns to the normal adult level.

This diversity of opinion is not to be wondered at, since hardly two workers have employed the same method, and practically all have omitted to take into account factors which are now generally admitted to have a marked effect on the resistance of the red blood corpuscle.

The degree of haemolysis is determined either by noting the amount of haemoglobin liberated from the cells, or by observing the rate of disappearance of the red cells themselves. Those who practise the latter method, either by counting the number of cells in a unit volume of the mixture (Simmel, 1923; Whitby and Hynes, 1935; and Cormick, 1942) or by noting the amount of the centrifugalized deposit (Beebe and Hanley, 1935-6), dignify their particular method by calling it a 'quantitative test' in contrast to the former which they consider merely qualitative in character. Such a distinction is untenable. All methods are qualitative in their nature, in that it is the character of the reaction to hypotonic saline which is being observed, and at the same time they are all quantitative in that the degree to which haemolysis has taken place in any particular concentration is determined, whether this be judged from the depth of the supernatant-stained fluid, from the amount of the centrifugalized sediment composed of the corpuscles still unlysed, or from the calculated number of corpuscles still clearly discernable in the various saline concentra-

tions. The fact that a progressive increase in the process, with a fall in the saline concentration, is revealed by all methods surely demonstrates the quantitative nature of all the tests. There might be some justification for a claim that the different methods of detecting the process of haemolysis are not equally sensitive or equally exact, but that is not to suggest that any of them do not reveal the severity of the process relative to the degree of hypotonicity of the saline solution mixed with the blood. It is easier to detect slight changes in colour, particularly when dealing with lighter shades when differences of as small as 2 per cent. are easily appreciated than it is to detect slight differences in the numbers of red cells suspended in the fluid. Those familiar with routine blood counts are well aware that greater variations from day to day, and even in different specimens taken at the same time, are found in the red cell estimations than in those of the haemoglobin. Hence it would appear that the so-called **qualitative** test is the more accurate and more useful from a **quantitative** standpoint. These facts would seem to be of particular significance in deciding on the point at which haemolysis begins and in determining the mean corpuscular fragility, two of the most important landmarks in the recognition of variations in the process.

But apart from the above varying accuracy of these two types of estimation of haemolysis, it would appear that perhaps they reveal different stages in a process which is more complicated than is usually assumed. If this is so, then the two methods cannot be expected to give similar results. Much confusion has arisen in this connexion because of the crude idea which prevails regarding the result of the suspension of the red cell in a hypotonic solution of saline. The general conception would seem to be that in a hypotonic saline solution the red cell swells and ruptures with the liberation of its contents. That this is not what happens anyone can observe for himself by watching the effect of a drop of distilled water or hypotonic saline on a drop of blood in a wet preparation under the microscope. The red cells certainly swell but they do not rupture to permit of the escape of the haemoglobin. The haemoglobin would rather seem to diffuse through the intact cell membrane so that the cell becomes

pale and a mere ghost of its former state. Ultimately the cell structure does disintegrate but a proportion of these ghost cells persists and they can still be seen with careful focusing. Hence there are in truth two stages in the process, haemosmosis (escape of haemoglobin) and stromatolysis (disintegration of the cell).

This phenomenon had been noted apparently independently by some previous workers (Spiro, 1897; Rohonyi, 1916; Adair, Barcroft, and Bock, 1921; Brinkman and Szent-Gyorgi, 1924), but its true significance and importance in the estimation of haemolysis have been revealed in an ingenious experiment devised by Ladkin and Macfarlane (1945). By using the sediment of blood which had been lysed by dialysing against distilled water and observing under the microscope the effect of adding sufficient of a concentrated saline solution to bring the mixture up to 1 per cent. NaCl, Ladkin and Macfarlane state that 'a remarkable transformation is seen. Out of the ghost concentrate in which few outlines can be distinguished, there materializes cells which in shape, size and colour are normal red cells. In every case the change is accompanied by a marked decrease in volume.' These workers have also found that as much as 10 per cent. of the haemoglobin can be removed from these ghost cells which are not observable and thus calculable in the ordinary cell-count chamber, so that if a special point is to be made of the accuracy of any particular method, it is the one which depends on estimating the number of unlysed cells rather than the one which notes the amount of haemoglobin diffused into the medium which is the less accurate. On these grounds it might be surmised that the method of counting the cells would tend to overestimate the severity of the process, and this is indeed what is observed from a comparison of the findings of Creed (noting the escape of haemoglobin) and those of Whitby and Hynes (noting the disappearance of red cells).

It is remarkable, however, how few of the workers studying the question of the fragility of the red cell in infancy and childhood, whatever the method employed, have taken into consideration certain extrinsic factors which have long been known to modify the resistance of the cell. Although it has long been known that the CO₂ content of the blood has a definite effect so that venous blood is more fragile than arterial blood, with capillary blood occupying an intermediate position, yet in not one of the above-mentioned investigations was this factor taken into consideration. Some workers used venous blood and others capillary blood so that, other things being equal, it could not be expected that similar results would be obtained. It may be for this reason that the increased fragility in the new-born was reported by some authors because the conclusions were drawn from the examination of cord blood, or from blood obtained by puncture of the longitudinal sinus.

Another factor of considerable importance in assessing the findings is the relative amounts of

cells and plasma. The greater the relative amount of the salt-containing plasma to cells, the higher will be the ultimate saline concentration of the mixture. Dacie and Vaughan (1938) have shown that by adding one drop of blood of 20 per cent. packed-cell volume and one drop of blood of 60 per cent. packed-cell volume to 1 c.c. of saline dilution of 0.38 per cent. a difference of 4 per cent. in the final saline concentration results, being 0.398 per cent. with the former and 0.382 per cent. with the latter. For this reason there will seem to be an increased resistance of the red cell in anaemia, and for the same reason it would seem not improbable that some authors may have concluded that the red blood cell in the new-born is less resistant than during adult life because during the neonatal period of life the packed-cell volume is habitually nearer 60 per cent. than the normal adult level of 45 per cent.

Material and method

The subjects of the present investigation were all apparently normal children and were inmates of the Maternity Department and of the Children's Department, The Radcliffe Infirmary, Oxford.

The foetal blood was obtained from the umbilical cord immediately after birth and before pulsation had ceased. From the younger infants blood was obtained from a scalp vein or longitudinal sinus and from the older children from an arm vein. The blood was invariably collected in heparinized containers as this was found to be the only way in which haemolysis could be prevented before treatment with the hypotonic saline. Oxalate was found almost invariably useless for this purpose, particularly in the case of foetal blood, so that any studies of this question in the blood of the foetus and the newly-born in which oxalate has been the anti-coagulant employed are viewed with much scepticism.

As it was believed that it is easier to differentiate between shades of colour than between varying numbers of cells per unit volume, it was decided to investigate the question by noting the rate of diffusion of haemoglobin into the saline diluent. Moreover, as it is particularly easy to differentiate between colours when they are faint, this method would seem to have the distinct advantage of enabling the observer to note the start of the process, as also the 'median corpuscular fragility,' departures of which from the normal are the most constant features of conditions in which the fragility of the red cell is modified. In order to eliminate all possible disturbing extrinsic factors the method of Creed as modified by Dacie and Vaughan (1938) was employed. By this method the blood is collected by venepuncture in heparinized containers and, from the haematocrit reading, the proportion of cells to plasma is brought to the ratio of 45 and 55 respectively. This mixture is thereafter thoroughly oxygenated by exposure to the air. This is attained by gently revolving a thin layer of the blood in a small flask. The dilutions of the saline solution employed were also as recommended by Dacie and Vaughan, differing from each other by 0.02 per cent.

Results

In all, the blood of 98 children has been examined, with the following age distribution:—thirteen during foetal life, twenty-two during the first week of life, fifteen during the second week, eight during the third week, six during the fourth week, eleven during

FIG. 1.—Saline concentration at which haemolysis begins according to age.

the second month, five during the third month, seven varying between four and six months of age, four from seven to twelve months and seven during childhood.

Details of the findings in these 98 cases are given in the appendix, a general survey of which shows on

the whole remarkably little variation. For a better comparison, however, the results are presented in the form of three graphs.

The start of haemolysis. In fig. 1 the saline concentration at which haemolysis begins is plotted according to the age of the child, and from this it is seen that there is a slight but definite difference between the results obtained during foetal life and those obtained during post-natal life. During foetal life haemolysis begins with hypotonic saline dilution varying between 0.52 and 0.44 per cent., but most frequently between 0.48 and 0.46 per cent., whereas after birth haemolysis never starts with dilutions of more than 0.44 per cent., unless during the first two or three days of life. Creed gives the average dilution with which haemolysis begins in the adult as 0.44 per cent., so that it would appear that during foetal life there is a slightly diminished resistance and after birth a slightly increased resistance.

Median corpuscular fragility. In fig. 2 the saline concentration at which 50 per cent. haemolysis occurs is plotted according to age. Here again it is seen that there is a slight but definite difference between foetal blood and blood obtained after birth. In cord blood 50 per cent. haemolysis is found to occur with saline concentrations varying between 0.33 and 0.43 per cent. but chiefly with concentrations of more than 0.35 per cent., whereas after birth 50 per cent. haemolysis is noted to occur with saline concentrations of less than 0.35 per cent. This difference between foetal and post-natal life is well displayed in the following table giving the average concentrations at which 50 per cent.

FIG. 2.—Median corpuscular fragility in relation to age.

haemolysis (median corpuscular fragility) was noted at different ages. In this table is given the average

Age ..	Foetus	1/52	2/52	3 and 4/52	-6 mth.	-1 yr.	1 to 12 yr.	Adult
M.C.F.	0.382	0.337	0.33	0.338	0.335	0.336	0.338	0.35

of eleven estimations of mean corpuscular fragility in the case of the adult made during the same period of study. In those eleven adults the mean corpuscular fragility ranged between 0.33 and 0.38. Creed gives the range in the normal adult between 0.342 and 0.387 per cent. saline, so that again there is revealed during childhood a slight increase in resistance of the red cell to hypotonic saline and during foetal life, on the contrary, a slight decrease in resistance.

Complete haemolysis. The determining of complete haemolysis is a matter of no little difficulty

observations made during post-natal life and in addition a curve of the average degree of haemolysis with the same saline concentrations as found by Creed in the adult. This chart shows in a striking fashion the differences already mentioned, viz.:—the diminished resistance in the foetus and increased resistance during infancy and childhood. The ranges are quite separate in their lower portions referring to the start of haemolysis, but they overlap slightly in that section of the graph representing more complete haemolysis. Creed's curve for the adult occupies an almost medial position.

Conclusions

(1) All methods of estimating the resistance of the red blood cell to hypotonic solutions of saline are both qualitative and quantitative.

(2) The only method permitting of trustworthy comparisons is one in which the CO₂ concentration and the relative proportion of cells and plasma are brought to a uniform level.

(3) Creed's method alone fulfils these conditions.

(4) Using Creed's technique (as modified by Dacie and Vaughan), foetal blood shows a slightly diminished resistance to hypotonic saline and the blood of the infant and child a slightly increased resistance to hypotonic saline.

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FIG. 3.—Range of haemolysis in foetal and post-natal life.

because the comparison of colours of the depth of shade which prevails at such a concentration of haemoglobin is not easy. Moreover, in view of the work of Ladkin and Macfarlane, it would seem unlikely that 100 per cent. of the haemoglobin ever diffuses into the saline solution as amounts up to 10 per cent. remain adsorbed to the corpuscular membrane. It is, however, useful to view a picture of the complete process, and in fig. 3 there is depicted the complete range of haemolysis at the different saline concentrations as observed in the examples of foetal blood examined, with a similar graph of the

APPENDIX—LIST OF CASES EXAMINED

C.B.=cord blood.

tr.=trace. ft. tr.=faint trace. v. ft. tr.=very faint trace.

O=no haemolysis.

No.	Age	Haemat- crit per cent.	Per cent. haemolysis												Median corpus- cular fragility	
			0-26	0-28	0-30	0-32	0-34	0-36	0-38	0-40	0-42	0-44	0-46	0-48		0-50
1	C.B.	64		95	80	70	45	35	15	15		7	O			0.33
2	C.B.	53	100	90	80	70	55	45	35	25		12	5			0.35
3	C.B.	62	90	90	90	80	75	70	65	50	40	30	12	2	O	0.40
4	C.B.	56	100	100	100	85	65	55	45	30	15	10	tr.	O		0.37
5	C.B.	57	100	100	95	95	80	65	55	40	12	5	v. ft. tr.	O		0.39
6	C.B.	67	100	100	100	90	90	75	65	55	45	45	18	7		0.42
7	C.B.	55	100	100	95	85	75	70	55	50	42	35	15	6		0.40
8	C.B.	59	98	90	80	65	55	45	35	25	15	10	4	O		0.35
9	C.B.	60	95	90	90	80	70	65	50	40	26	22	6	v. ft. tr.		0.38
10	C.B.	59	100	100	100	95	92	85	75	62	55	45	20	5		0.43
11	C.B.	58	95	90	80	70	60	55	45	35	15	12	3	v. ft. tr.		0.375
12	C.B.	57	100	95	95	95	85	78	70	65	58	45	15	8	5 v. ft. tr.	0.43
13	C.B.	68	100	88	80	68	55	40	18	18	7	7	ft. tr.	O		0.35
14	4 hr.	65	100	100	100	90	80	45	35	15	5	O				0.35
15	14 hr.	62	100	100	90	80	60	40	25	15	5	O				0.35
16	30 hr.	70					80	20	25			10	O			0.33
17	41 hr.	64	95	95	80	70	45	30	15	10	5	ft. tr.	ft. tr.	O		0.335
18	41 hr.	74	100	85	70	60	45	20		20	tr.	tr.	O			0.335
19	2 D. 7 hr.	70		95	80	70	60	45	5	tr.	O					0.35
20	2 D. 9 hr.	60	100	90	85	75	65	55	25	5	5	ft. tr.	v. ft. tr.	v. ft. tr.		0.36
21	2 D. 11 hr.	55	100	95	75	60	35	15	tr.	O						0.33
22	2 D. 16 hr.	58	95	80	70	55	50	20	10	8	5	O				0.325
23	3 D.	62	95	85	75	55	45	25	15	v. ft. tr.	v. ft. tr.	O				0.33
24	3 D. 1 hr.	60		95	80	70	60	35	5	O						0.35
25	3 D. 1½ hr	51	100	100	65	45	30	12		tr.	tr.	O				0.31
26	4 D. 6 hr.	62	95	80	65	55		15	O							0.32
27	4 D. 12 hr.	62		85	80	70	30	25	8	4	O					0.33
28	4 D. 20 hr.	54	95	85	75	65	50	25	15	12	tr.	O				0.34
29	5 D. 14 hr.	54	100	100	90	80		25		tr.	tr.	O				0.34
30	5 D. 18 hr.	64	95	75	60	50	25		10	ft. tr.	ft. tr.	O				0.34
31	6 D.	56	100		70	45	25		7	tr.	O					0.32
32	6 D. 2 hr.	62		95	90	70	60	40	15	15						0.35
33	6 D. ½ hhr	57	95	90	75	65	55	25	15	tr.						0.34
34	6 D. 13 hr.	65	100	100	95	75	70	45	25			O				0.365
35	6 D. 14 hr.	43		90	70	60	35	20	12	5	tr.	ft. tr.	O			0.325
36	7 D. ½ hr.	61	90	85	80	55	45	25		5	tr.	O				0.335
37	8 D. 1 hr.	67		90	65	35	35	25	15	5	tr.	O				0.315
38	8 D. 1 hr.	55	95	90	80	60	45	15	5	ft. tr.	ft. tr.	O				0.33
39	9 D. 2 hr.	52	85	65	55	45	35	15	10	tr.	ft. tr.	O				0.31
40	9 D. 3 hr.	56	95	80	70	55	45	20		tr.	ft. tr.	O				0.33
41	9 D. 3 hr.	58	85	70	55	45	25	5	ft. tr.	ft. tr.	ft. tr.					0.31
42	9 D. 12 hr.	52	100	95	85	70	45	15	6	3	v. ft. tr.	v. ft. tr.	O			0.335
43	9 D. 20 hr.	54	95	90	75	45	35	15	12	ft. tr.	v. ft. tr.	O				0.315
44	10 D. 1 hr.	59	100	90	70	70		40	35	10	10	O				0.34
45	11 D. 17 hr.	52	95	85	75	55	45	15	12	ft. tr.	ft. tr.	O				0.33
46	12½ D.	59	100	85	85	60	50	40	15	O						0.34
47	13 D. 6 hr.	61	100	100	90	90	75	55	35	15	5	O				0.365
48	13 D. 19 hr.	62	90	85	75	60	45	15	10	5	3	O				0.335
49	13 D. 22 hr.	53	100	90	85	60	45	15	5	tr.	ft. tr.	O				0.33
50	14 D.	56	100	95	80	70	50	15	8	5	tr.	O				0.34
51	14 D.	64	95	90	85	75	50	25	15	12	8	ft. tr.	O			0.34
52	14½ D.	43	85	75	65	45	25	5	v. ft. tr.	v. ft. tr.	O					0.315
53	15½ D.	55	90	90	80	70	55	10	3	O						0.345
54	16½ D.	55	100	95	85	75	55	25	15	ft. tr.	O					0.345
55	18½ D.	59	95	75	60	50	38	25	18	5	tr.	tr.				0.32
56	19 D.	51	100	90	85	75	65	25	15	5	tr.	v. ft. tr.	O			0.35
57	19½ D.	61	95	85	75	65	50	25	10	5	v. ft. tr.	O				0.34
58	21 D.	38	100	90	75	60	30	15	5	O						0.33
59	21 D.	49	95	85	65	55	40	10	5	v. ft. tr.	v. ft. tr.	O				0.315
60	22 D.	50	100	95	75	65	50	25	5	ft. tr.	ft. tr.	O				0.335
61	23 D.	48	100	95	90	85	70	30	5	tr.	ft. tr.	O				0.35
62	24 D.	56	100	85	75	60	50	10	5	v. ft. tr.	v. ft. tr.	O				0.34
63	25 D.	44	100	100	80	75	75	25	20	ft. tr.	ft. tr.	O				0.34
64	26 D.	50	100	100	100	90	60	55	50	25	10	tr.				0.37
65	31 D.	43	100	90	85	75	55	15	5	v. ft. tr.	v. ft. tr.	O				0.345
66	33 D.	46	90	75	55	40	15	5	v. ft. tr.	v. ft. tr.	v. ft. tr.	O				0.305
67	5/52	33	100	90	75	65	50	5	ft. tr.	v. ft. tr.	v. ft. tr.	O				0.34
68	5/52	35	100	90	75	65	55	35	15	5	tr.	O				0.35
69	5/52	42	100	100	100	90	70	35	25	tr.	ft. tr.	O				0.35
70	6/52	40	100	100	90	80	60	50	8	5	tr.	O				0.355
71	6/52	26	100	90	85	75	50	25	ft. tr.	ft. tr.	ft. tr.	O				0.34
72	6/52	33	90	70	55	45	30	ft. tr.	ft. tr.	O						0.31
73	6/52	34	95	90	85	75	65	30	15	ft. tr.	ft. tr.	O				0.35
74	7/52	28	100	90	85	80	60	25	15	5	ft. tr.	ft. tr.	O			0.345
75	7/52	27	100	95	90	75	55	25	8	tr.	v. ft. tr.	O				0.345
76	8/52	32	100	100	95	85	65	45	25	5	O					0.36
77	8/52	35	100	100	95	80	70	15	tr.	tr.	O					0.35
78	9/52	30	100	90	70	45	25	10	tr.	ft. tr.	O					0.315
79	9/52	33	100	90	85	75	60	25	8		3	O				0.35
80	10/52	25		90	60	35	30	15	5	2	tr.					0.31
81	12/5	38	100	95	80	40	40	8	tr.	tr.	O					0.32
82	13/52	37	100	100	90	80	35	10	tr.	tr.	O					0.325
83	13/52	31	85	75	60	50	35	15	10	tr.	O					0.32
84	16/52	38	100	95	85	70	60	35	25	5	ft. tr.	O				0.345
85	17/52	43	95	90	80	65	55	5	ft. tr.	v. ft. tr.	v. ft. tr.	O				0.345
86	5/12	44	94	80	50	65	15	tr.	tr.	ft. tr.	O					0.315
87	5/12	37	100	100	85	65	45	25	15	tr.	v. ft. tr.	O				0.335
88	7/12	35	100	90	70	50	15	5	tr.	ft. tr.	ft. tr.	O				0.32
89	8/12	38	100	100	90	85	65	45	25	5	O					0.355
90	11/12	31	100	100	100	90	80	65	35	25	15	3	O			0.37
91	3 yr.	36		90	70		15			5	O					0.33
92	3½ yr.	36		90	80	30	15		tr.	tr.	ft. tr.	ft. tr.	O			0.33
93	3½ yr.	33		70	45				8		2	O				0.33
94	8 yr.	37	100	100	100	90	80	35								