J. clin. Path. (1951), **4**, 221.

COMBINED FRAGILITY TEST FOR CONGENITAL HAEMOLYTIC JAUNDICE

BY

S. VARADI

From the Department of Pathology, City General Hospital, Sheffield

(RECEIVED FOR PUBLICATION MAY 4, 1950)

In congenital haemolytic jaundice or familial acholuric jaundice increased osmotic fragility is by no means always present. Many cases, otherwise typical, have been shown to have normal fragility (Lommel, 1913 ; Dawson, 1931 ; Hansen and Klein, 1934 ; and others), and Gänsslen (1937) estimates that these may constitute 10% of the total. In some cases where a normal fragility is found at first a slight increase of fragility may sometimes be encountered if the test is repeated on several occasions. On the other hand, patients who have previously shown grossly increased fragility may show normal or nearly normal values during haemolytic crises. Even when demonstrated, however, increased fragility is not diagnostic of congenital haemolytic jaundice, for many acquired haemolytic (haemolysinic) anaemias also show it.

It was thought that a study of the fragility distribution by the latest photo-electric methods might reveal abnormalities which would be characteristic for congenital haemolytic jaundice even when the "minimum osmotic resistance" was normal or nearly normal. An opportunity to test this possibility came when a family of 12 members, five of whom had reticulocyte counts greater than 5% in the absence of anaemia, was encountered. As controls, four normal subjects, three with simple iron deficiency anaemia, two with iron deficiency anaemia of pregnancy, and one with spherocytic anaemia occurring during pregnancy, were studied. Of the 12 members of the family, only five (four affected and one normal) could attend the laboratory: the others were visited at their homes, up to 170 miles away, and the samples brought by public transport to the laboratory. Thus a delay of up to 24 hours occurred before they were tested. Control samples were treated in the same way, and it was found that blood from individuals with congenital haemolytic jaundice showed a marked increase in fragility after standing for 24 hours, while that of normal and other anaemic subjects showed a much smaller increase, about 1/5 to 1/10 of that shown by the blood from congenital haemolytic jaundice.

Methods and Results

The haemoglobin content was determined by King's method standardized by haemin. Red cell counts were made in a dilution of 1/200, and 2×80 small squares were counted in a Bürker's chamber. Reticulocytes were determined by a wet method, and Price-Jones curves by projection (Humble and Belyavin, 1948) ; 200 cells were counted. Fragility

was tested by Discombe's (1948) modification of Hunter's method. All blood was heparinized.

The family tree is shown in Fig. 1, and haematological data in Tables I and II. A few fragility curves are given in Figs. 2–8. Control experiments showed that mechanical damage during transport was negligible.

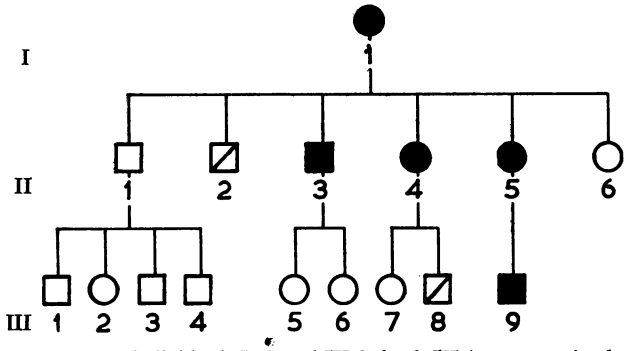

FIG. 1.—Individuals II 2 and III 8 dead, III 1 not examined. Circles = females, squares = males. Dark figures = affected individuals.

The three cases of congenital haemolytic jaundice examined immediately after venepuncture all showed normal values of mean cell fragility (M.C.F.), and, though the standard deviation was considerably increased, the minimum osmotic resistance was within the limits of normal. After 24 hours the M.C.F. had risen by about 0.2% NaCl, and the standard deviation had doubled, so that the osmotic resistance reached the grossly abnormal values expected in congenital haemolytic jaundice. None of the control cases showed such an increase, the increase in M.C.F. being 0.21% NaCl and the standard deviation showing only a slight change. It was interesting to find that the spherocytic anaemia of pregnancy differed so completely from that of congenital haemolytic jaundice.

Discussion

Many workers have observed that the blood of patients with congenital haemolytic jaundice undergoes an abnormally quick spontaneous haemolysis on standing (Lüdke, 1918 ; Meulengracht, 1922 ; Gripwall, 1938 ; Ham and Castle, 1940a ; Dacie, 1941 ; Lloyd, 1941 ; Caroli, Étévé, Paraf, and Robineau, 1949). Dacie noted that the speed of spontaneous haemolysis in congenital haemolytic jaundice only was associated with increased fragility, but that this was not true in acquired haemolytic anaemia. Three hypotheses have been suggested to account for this fact: first, the action of lysolecithin (Bergenhem and Fåhraeus, 1936 ; Gripwall, 1938) ; second, the abnormal shape of the cells (Ham and Castle, 1940a) ; and third, a structural defect in the cell (Lloyd, 1941).

Singer (1940) showed that the cells in congenital haemolytic jaundice were more susceptible to lysis by " lysolecithin " than were other cells, and claimed thus to differentiate the haemolytic anaemias.

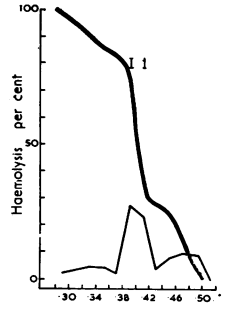

Fig. 2

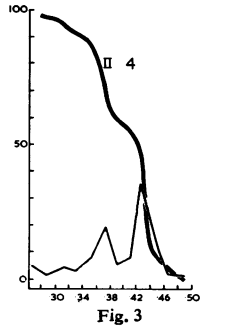

Fig. 3

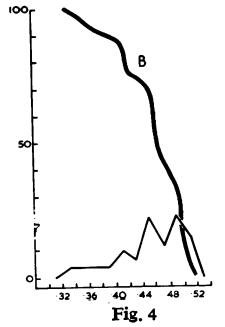

Fig. 4

per cent NaCl

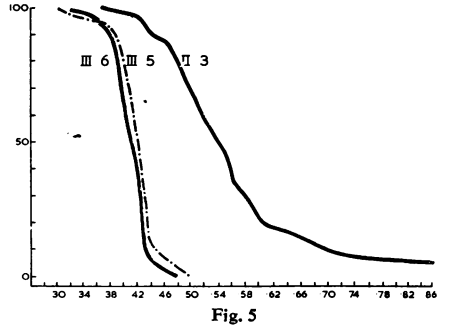

Fig. 5

FIGS. 2–8.—The lower curves represent the fragility distribution, constructed

Caroli *et al.* (1949) showed that the normally spherocytic and osmotically fragile erythrocytes of the goat underwent spontaneous haemolysis no more rapidly than did normal human cells, thus showing that spherocytosis alone had nothing to do with spontaneous haemolysis, this observation being confirmed for human subjects with acquired spherocytosis by Dacie (1941).

Ham and Castle (1940a and b), Emerson, Shen, and Castle (1946), Emerson, Shen, Ham, and Castle (1947), and Young (1947) observed that red cells incubated at 37° C. had an increased osmotic fragility especially marked in congenital haemolytic jaundice. This was also the case where fresh cells did not exhibit a significant increase of fragility (Young, 1947). This modified fragility test appeared to be specific for congenital haemolytic jaundice in Young's series.

All these observations can be reconciled on the hypothesis that the red cell of congenital haemolytic jaundice is structurally abnormal, and that on standing it deteriorates more rapidly than normal cells. This abnormality can be demonstrated by the "lysolecithin" test, by determination of fragility after standing 24 hours, or by a study of the rate of spontaneous haemolysis. Increased osmotic fragility itself is a function of the degree of spherocytosis (Haden, 1934), and when present in fresh cells has nothing to do with any other structural abnormality; but when blood is allowed to stand, the fundamental structural abnormality of the red cells in congenital haemolytic jaundice causes a slow decrease in cell integrity, which can be detected after a few hours by the change in osmotic fragility and spontaneous haemolysis. This deterioration affects the whole red cell population, and can be detected earlier and with greater certainty by fragility measurements than by the observation of spontaneous haemolysis. Fragility measurements at 0 and 24 hours after taking blood are much simpler than the "lysolecithin" test, and might be referred to as a "combined fragility test."

Summary

Congenital haemolytic jaundice can occur without clinical manifestations, whereas changes in fragility are inconstant and often indistinguishable from those occurring in other forms of anaemia.

When blood from a patient with congenital haemolytic jaundice is stored for 24 hours at temperatures between 20 and 37° C. the fragility increases greatly, the mean cell fragility increasing by 0.15–0.2% NaCl and the standard deviation being doubled.

The increase in the mean cell fragility of blood from normal subjects and subjects with other anaemias is only about one-tenth as large.

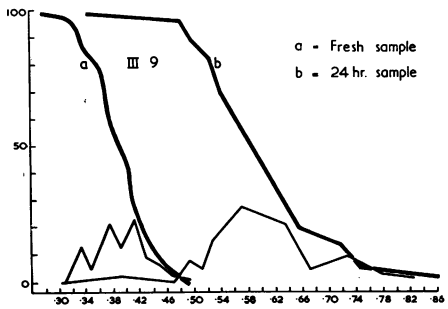

Fig. 6

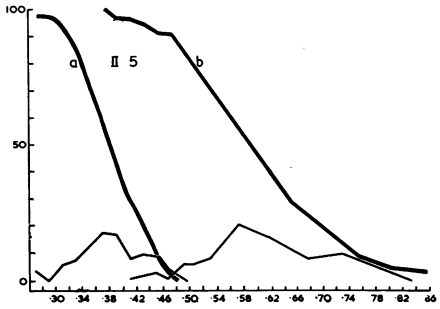

Fig. 7

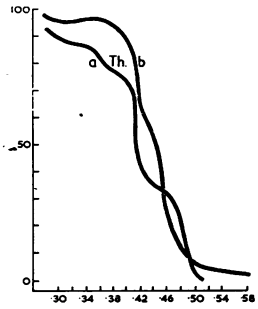

Fig. 8

per cent NaCl

from the corresponding curves using the method of haemolytic increments.

Singer's "lysolecithin" test and the combined fragility test appear to be diagnostic of congenital haemolytic jaundice. The latter is more convenient.

The combined fragility test is likely to be of value in studying "healthy carriers" and in cases where the clinical manifestations are slight.

It is my pleasant duty to thank Dr. K. J. G. Milne for the permission to use the data of his patient (III 9).

References

Bergenhem, B., and Fåhraeus, R. (1936). *Z. ges. exp. Med.*, **97**, 555. Quoted by Gripwall.
Caroli, J., Étévé, J., Paraf, A., and Robineau, R. (1949). *Sang*, **20**, 367.
Dacie, J. V. (1941). *J. Path. Bact.*, **52**, 331.
Dawson of Penn (1931). *Brit. med. J.*, **1**, 921, 963.
Discombe, G. (1948). *J. Path. Bact.*, **60**, 315.
Emerson, C. P., Shen, S. C., and Castle, W. B. (1946). *J. clin. Invest.*, **25**, 922.
—— —— Ham, T. H., and Castle, W. B. (1947). Ibid., **26**, 1180.
Gänsslen, M. (1937). *Klinische Fortbildung*, ed. Cobet, R., and Gutzeit, K., vol. 4, p. 607. Berlin.
Gripwall, E. (1938). *Acta med. scand.*, Suppl. 96.
Haden, R. L. (1934). *Amer. J. med. Sci.*, **188**, 441.
Ham, T. H., and Castle, W. B. (1940a). *Trans. Ass. Amer. Phys.*, **55**, 127.
—— —— (1940b). *Proc. Amer. phil. Soc.*, **82**, 411.
Hansen, K., and Klein, E. (1934). *Dtsch. Arch. klin. Med.*, **176**, 567.
Humble, J. G., and Belyavin, G. (1948). *J. clin. Path.*, **1**, 77.
Lloyd, T. W. (1941). "On the Aetiology of Acholuric Family Jaundice." Oxford University Thesis.
Lommel (1913). *Dtsch. Arch. klin. Med.*, **109**, 174. Quoted by Gänsslen.
Lüdke, H. (1918). *Münch. med. Wschr.*, **65**, 1098. Quoted by Dacie.
Meulengracht, E. (1922). *Der chronische hereditäre hämolytische Ikterus*. Leipzig. Quoted by Gripwall.
Singer, K. (1940). *Amer. J. med. Sci.*, **199**, 466.
Young, L. E. (1947). *N. Y. St. J. Med.*, **47**, 1875.

APPENDIX

Case Notes of Family

Case I 1.—Woman, aged 83; one of 11 children, none of whom had had anaemia, jaundice, or gallstones; one nephew had had jaundice. At 72, biliary colic and obstructive jaundice, when Hb was 68%, icterus index 110, and fragility normal. Recovery without operation. Spleen not palpable now.

II 3.—Man, aged 55. At the time of the investigations no symptoms attributable to congenital haemolytic jaundice. A year later (the fragility on fresh blood was estimated in another hospital) the spleen became just palpable.

II 4.—Woman, aged 54. Always delicate; for nine years bilious attacks without jaundice; complaints of pains in the back. One child died of hydrocephalus aged $7\frac{1}{2}$ months. Spleen not palpable but enlarged radiologically.

II 5.—Woman, aged 49. "Gall bladder trouble" or "colitis" at 16, 21, and 24 years; attacks of gnawing epigastric pain at 34 years; enlarged spleen noted at 37 years; cholecystectomy at 42 years; recurrence of pain and obstructive jaundice; fragility increased, haemolysis occurring at 0.5% NaCl and above. Spleen one fingerbreadth below costal margin.

III 9.—Man, aged 20. At age 18 albuminuria; at 19 football accident produced pigmented scar. Brown patches on anterior aspect of both legs. Liver and spleen two fingerbreadths below costal margin; W.B.C. 15–25,000 per c.mm., platelets 5–800,000 per c.mm. Marrow culture showed hyperplasia especially of erythroid series; leuco-erythrogenetic ratio 0.6.

The serum bilirubin levels of these patients were 0.9, 0.9, 1.1, 2.3, and 1.4 mg.% respectively. No increased urobilinogenuria was demonstrated.

TABLES I AND II

HAEMATOLOGICAL DATA

Subject	Hb (g.%)	R.B.C.	M.C.V.	Retic. (%)	Price-Jones Curve				M.C.T.	T/D Ratio
					Mean	S.D.	Mode	Microcytes %†		
I 1	12.6	3.91	97.2	10.0	6.91	±0.57	6.5	42.5	2.60	0.38
II 1*	15.4	5.15	95.1	0.4	6.99	0.49	6.0	24.0	2.48	0.35
3*	15.0	4.9	99.0	8.0	7.23	0.48	7.0	13.0	2.41	0.33
4	13.6	4.0	91.3	12.1	6.86	0.42	7.0	39.0	2.47	0.36
5	12.6	4.1	92.0	8.8	6.94	0.45	7.0	28.0	2.43	0.35
6	13.2	4.77	88.0	0.5	7.28	0.16	7.5	12.5	2.12	0.29
III 2*	14.1	5.33	84.4	0.2	7.40	0.46	7.5	7.0	1.96	0.26
3*	15.5	5.92	84.5	0.6	6.99	0.46	7.0	28.5	2.2	0.32
4*	15.0	5.82	85.9	0.3	7.19	0.46	7.0	14.5	2.12	0.29
5*	14.5	4.6	96.7	0.8	7.25	0.49	7.5	12.0	2.35	0.32
6*	14.5	4.77	96.4	0.2	7.53	0.49	7.5	5.0	2.17	0.28
7	15.5	4.62	91.0	1.6	7.09	0.46	7.0	21.5	2.31	0.32
9	16.3	5.58	90.0	5.6	6.96	0.46	7.0	31.5	2.37	0.34
Case M	10.4	4.21	78.4	0.7	—	—	—	—	—	—
Lo	7.8	4.93	59.2	3.5	6.58	0.62	6.5	65.0	1.74	0.26
K	8.4	4.25	—	0.6	—	—	—	—	—	—
Le	9.4	4.58	76.0	—	6.82	0.64	7.0	47.5	2.08	0.31
B	6.4	3.87	63.3	3.0	6.05	0.56	6.0	91.0	2.20	0.36
Th	10.4	3.52	99.1	7.2	6.80	0.78	7.0	46.5	2.73	0.4

* 24-hour sample, smears from fresh blood.
† Smaller than 6.7 μ.

Subject	Fresh Blood			24-hour Blood		
	M.C.F.	S.D.	Minimum Osmotic Resistance	M.C.F.	S.D.	Minimum Osmotic Resistance
I 1	0.410	0.047	0.50			
II 1				0.428	0.020	0.48 tr.
3			0.48 tr.	0.527	0.070	0.70
			0.52			
4	0.420	0.042	0.48 tr.			
5	0.384	0.046	0.48 tr.	0.578	0.101	0.75
6	0.425	0.025	0.46			
III 2				0.415	0.026	0.48 tr.
3				0.443	0.032	0.50 tr.
4				0.420	0.025	0.48 tr.
5				0.420	0.025	0.48
6				0.412	0.025	0.46
7	0.398	0.026	0.48 tr.			
9	0.389	0.045	0.48 tr.	0.583	0.086	0.70
Case M	0.387	0.038	0.44			
Lo	0.422	0.069	0.50			
K	0.379	0.068	0.50			
Le	0.428	0.040	0.50			
B	0.460	0.047	0.50			
Th	0.420	0.056	0.50	0.441	0.038	0.62
D	0.408	0.023	0.48 tr.	0.429	0.027	0.48 tr.
H	0.404	0.023	0.46	0.418	0.026	0.48 tr.
Da	0.415	0.024	0.46			
P	0.400	0.020	0.46			

tr. = trace of haemolysis.

The Morbid Anatomy of Tuberculosis in Old Age. ROULET, F. (1950). *Ann. Méd.*, **51**, 69.

In 270 necropsies carried out at the University of Basle on subjects over 60 years old, frank evidence of tuberculosis was present, although it was the cause of death in only 90 cases. The active focus was found in 245 cases around an old calcified or ossified nodule in the tracheo-bronchial lymph nodes; The lungs in these cases revealed no active primary focus, though an old, calcified, inactive lesion was often discovered. It is argued that active tuberculosis in old age is frequently caused by reactivation of an old lesion in the lymph nodes around the bifurcation of the trachea, possibly due to the chronic inflammation of the lower trachea and main bronchi, which is extremely common in old people. Tuberculosis of the tracheo-bronchial nodes frequently leads to complications: of the 245 cases of tracheo-bronchial-node infection, there was also frank tuberculous infection of the lungs in 35: in 12 cases there was a generalized haematogenous spread either from a focus in the tracheo-bronchial nodes or from an alternative site of active infection; in 13 cases there were foci both in the tracheo-bronchial nodes and elsewhere, but no generalized spread; and in 25 cases haematogenous spread had occurred with tracheo-bronchial nodes as the only possible primary source. In the remaining 160 cases the infection was confined to the tracheo-bronchial nodes.

Speaking generally, miliary tuberculosis develops more rapidly in the old than at other ages. The appearances are classical, though the tubercles may be rather large—as big as a pea or a hazel nut. In the whole series there were 16 cases of miliary spread amongst the 43 cases of frank pulmonary tuberculosis. Tuberculosis of the serous cavities is not uncommon in the elderly. Pulmonary tuberculosis may be of the chronic apical fibro-caseous type with cavities, or of the ulcerative caseous type of acute tuberculosis, the frequent occurrence of which in old people may possibly be due to reduced general resistance and antibody response.

Glomerulonephrosis. A Morphologic Manifestation of Renal Cortical Ischemia in Toxic Oliguria and Lower Nephron Nephrosis. FRENCH, A. J. (1950). *Arch. Path.*, **49**, 43.

The histopathological findings in the kidneys of 20 specially selected cases of glomerulonephrosis are described, special stress being laid on the glomerular changes. This study was undertaken in the hope of explaining the anuria or oliguria which accompanies lower nephron nephrosis (" tubular " or " toxic " nephritis), in which condition, on physiological grounds, there should be polyuria if a tubular lesion alone be present. For this purpose it was essential to eliminate cases of primary or secondary renal disease, eclampsia, and cardiovascular hypertension.

The changes found in the glomeruli, tubules, and medulla in sections of the kidneys (mostly stained with haematoxylin and eosin) are tabulated. Glomerular ischaemia, together with thickening of the capillary walls, was found in every case. The presence of granular material staining grey-blue in the glomerular spaces and proximal tubules was noted, this being regarded as the precursor of the protein casts seen in the distal convoluted tubules in all except one of these cases. *R. B. T. Baldwin.*

Corrections.—Dr. Varadi writes: I shall be obliged if you will print the following corrections to my article " Combined Fragility Test for Congenital Haemolytic Jaundice " (*J. clin. Path.*, **4**, 221). On page 222 in paragraph 3 read 0.021% instead of 0.21% NaCl, and, on page 224 paragraph 2 of the Appendix, marrow puncture instead of culture.

We much regret that in the description of a cell-suspension mixer by Dr. Matthews (*J. clin. Path.*, **4**, 383) the pipettes were described as 20 ml. and 50 ml. These should be 20 c.mm. and 50 c.mm.