Blood Cell Enzymes in Trisomy E (18) Syndrome*

MARJORIE A. BAUGHAN†, ROBERT S. SPARKES‡, DONALD E. PAGLIA,
and MIRIAM G. WILSON

From the Departments of Medicine and Pediatrics, UCLA School of Medicine, and the Department of Pediatrics, USC School of Medicine, Los Angeles, California, U.S.A.

Trisomy E (18) syndrome is associated with an extra number 18 chromosome, and though many of these patients can be readily recognized by their constellation of congenital defects, a chromosome analysis is usually required for confirmation. Because chromosome analysis still remains a fairly slow, expensive, and tedious examination, one purpose of the present study is to evaluate blood cell enzymes as a potential alternative approach to laboratory verification of clinical diagnosis. Secondly, it is believed that such studies in affected patients could aid in genetic linkage and chromosome mapping studies through correlation of changes in gene products, i.e. enzymes, with the chromosomal derangement. The results and interpretation of several blood cell enzyme studies in 5 patients with the trisomy E syndrome are presented.

Material and Methods

Five infants with clinical and chromosomal trisomy E syndrome were studied. The pertinent clinical findings which indicate this diagnosis in each patient are listed in Table I. There was no reason to suspect Rh or ABO incompatibility at the time of birth, but this was not further evaluated in any of the patients. The controls were approximately age-matched patients in hospital, who were not anaemic and did not have a chromosomal disorder.

Routine haematological studies were performed by standard methods. Chromosome analyses were performed on peripheral blood according to the method of Moorhead *et al.* (1960), or of Arakaki and Sparkes (1963). The activities of several erythrocyte and leucocyte enzymes, particularly of the Embden-Meyerhof and pentose phosphate pathways, were determined. These included hexokinase, glucosephosphate isomerase, phos-

phofructokinase, fructosediphosphate aldolase, glyceraldehydephosphate dehydrogenase, triosephosphate isomerase, phosphoglycerate kinase, phosphoglyceromutase, phosphopyruvate hydratase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase, and glutathione reductase. They were assayed as previously noted by Tanaka, Valentine, and Miwa (1962), Koutras *et al.* (1964), Schneider *et al.* (1965), and Valentine *et al.* (1967).

The leucocyte alkaline phosphatase was determined biochemically by the method described by Valentine and Beck (1951), and histochemically by a modification of the procedures described by Wachstein (1946) and Wiltshaw and Moloney (1955).

Results

The chromosome analyses in all five patients showed trisomy E (18); the findings did not suggest mosaicism in any patient. Routine haematological findings are summarized in Table II, which also includes control values from Wintrobe (1961), and Nelson (1964). The values for our control patients and those from the literature show no significant differences. These studies were not done on patient C.C., and only partially accomplished on patient N.G. Reticulocyte counts in the controls were within normal limits; patient L.R. had a normal reticulocyte count; G.H. had a mild reticulocytosis; and C.E. had a moderate reticulocytosis which was greater than the physiological reticulocytosis usually seen in an 8 to 10-week infant. All of the trisomic patients evaluated had slightly higher mean corpuscular volumes (MCV) than our age-matched controls and those in the literature. The red blood cell count on C.E. was slightly depressed. In the trisomy E patients, there did not appear to be any significant alteration in the white cell counts or differentials which were similar to the age-matched controls, except for N.G. who showed a lower leucocyte count. The percentage of neutrophils with two or more nuclear projections was similar in the trisomy E patients and age-matched controls.

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† Address: Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024, U.S.A.

‡ Reprint requests should be sent to R.S.S.

TABLE I
CLINICAL FINDINGS IN FIVE TRISOMY E (18) PATIENTS

Clinical Findings	Patients				
	C.C.	L.R.	G.H.	C.E.	N.G.
Age at examination, and sex	2 dy. (M)	16 dy. (F)	4 wk. (F)	9 wk. (F)	9 wk. (F)
Birthweight (g.)	2140	1990	2590	2200	1395
Gestation period (wk.)	40	43	43	42	40
Low-set malformed ears	+	+	+	+	+
Prominent occiput	+	+	+	+	+
Micrognathia	+	+	+	+	+
Congenital heart disease	+	+	+	+	+
Typical hand and finger posture	+	+	+	+	+
Simian lines	+	+	+	+	+
Digital arches	?	+	+	+	?
Rocker-bottom feet	+	+	+	+	+
Short sternum	+	+	+	+	+
Polyhydramnios	+	?	?	?	?
Small placenta	+	?	+	+	+
Failure to thrive	+	+	+	+	+
Meckel's diverticulum	—	?	+	—	+

Results of the 15 erythrocyte and leucocyte glycolytic and pentose pathway enzyme assays are shown in Tables III and IV, respectively. Enzyme activities are expressed as micromoles of substrate converted per minute per 10^{10} cells at 37°C ., except for hexokinase, glucosephosphate isomerase, and glucose-6-phosphate dehydrogenase, which are expressed as micromoles of reduced nicotinic adenine dinucleotide phosphate (NADPH) generated per minute per 10^{10} cells at 37°C . The leucocyte alkaline phosphatase is expressed as milligrams of phosphorus liberated per hour per 10^{10} leucocytes. The leucocyte alkaline phosphatase index by the histochemical method is derived by grading the intensity of the stain precipitation in the cytoplasm on a scale from 1 to 4 in each neutrophil: 100 neutrophils are graded and the total summed score constitutes the index.

Evaluation of the erythrocyte enzymes (Table III) indicates that, with the exception of one patient, the hexokinase activity in the trisomy E patients is considerably higher than in the age-matched controls, and does not fall towards the normal adult values with increasing age as in the controls. However, a slight to moderate reticulocytosis is present in some of these patients compared with normal controls, and this enzyme is usually disproportionately raised in very young cells. The values for several enzymes in both the trisomy E patients and the controls tend to be higher than the normal adult values, but the enzyme activities in the trisomy E patients are, in general, slightly higher than in the age-matched controls. An unexplained low value for phosphofructokinase is noted in N.G.

The leucocyte enzyme values in both the trisomy E patients and controls tend to be similar to the adult controls for most enzymes (Table IV). In contrast to the erythrocytes, the leucocyte hexo-

kinase increased to normal adult values with increasing age in the trisomic patients. In general, the leucocyte enzyme values found in the trisomy E patients are similar or slightly decreased compared to the age-matched controls. There is no apparent reason for the slightly low values for the alkaline phosphatase assays in the age-matched controls from age 2½ weeks to 11 weeks. Further, the low values in G.H. for glucose-6-phosphate dehydrogenase, phosphofructokinase, and phosphoglyceromutase, and the low value in C.C. for phosphopyruvate hydratase are unexplained, but, since this pattern is not seen in the other four trisomic patients, it is probably not related to the trisomic state.

Discussion

One purpose of the blood cell enzyme studies in the trisomy E (18) patients was to attempt localization of specific genes to the number 18 chromosome. This approach is based on two generally accepted genetic concepts. The first is that genes control cell metabolism through the formation of enzymes, the 'one gene—one enzyme' concept. The second is the 'gene dose effect' in which each allelic structural gene contributes quantitatively to the gene product (Harris, 1964). Normally, there are two allelic genes at an autosomal locus in humans. Thus, two genes determine the enzyme activity which normally can be considered to be 100%, with 50% contributed by each member of the allelic gene pair. In the trisomic state, the genes of the affected chromosome are present in triplicate. Based on the 'gene dose effect', each of the 3 allelic genes theoretically contribute 50% of normal activity resulting in 150% total activity or 50% above normal in the trisomic state. Therefore, the activity of certain enzymes may reflect the number of genes controlling

TABLE II
PERIPHERAL BLOOD HAEMATOLOGICAL VALUES

Age	Subjects	Hb (g./ 100 ml.)	Packed Cell Volume (%)	Red Cells $\times 10^6$	Reticu- lyocytes (%)	Mean Corpus- cular Volume (cu. microns)	Mean Corpus- cular Hb (μ g.)	Mean Corpus- cular Hb Concen- tration (%)	White Cells $\times 10^3$	Band Neutro- phil (%)	Polymor- phonu- clear Neutro- phil (%)	Eosino- phil (%)	Lympho- cyte (%)	Monocyte (%)
2 dy.	Control (B.G.V.)	20.8	63	6.03	3.7	104.4	34.5	33.0	14.2	1.5	41.5	7.5	35.0	14.5
2-4 wk.	Normal††	19.0	54	5.10	3.0	105.0	37.0	35.0	21.0	5.0	55.0	5.0	20.0	15.0
	Trisomy-E (L.R.)	15.0	46	4.44	0.5	103.6	33.7	32.6	15.6	—	34.0	1.0	58.0	7.0
	Trisomy-E (G.H.)	11.3	37	3.58	2.6	103.3	31.6	30.3	10.5	3.5	28.5	1.5	61.5	7.0
	Controls (J.B., J.J.)	12.3	37	3.68	0.9	101.0	33.2	33.2	12.5	3.8	31.5	3.8	53.5	7.4
8-9 wk.	Normal††	14.0 $\pm$ 3.3	42 $\pm$ 7.0	4.70 $\pm$ 0.9	0.5 - 1.5	90.0	30.0	33.0	11.0	0.0	36.0	3.0	53.0	8.0
	Trisomy-E (N.G.)	9.8	38	—	—	—	—	—	7.1	—	—	—	—	—
	Trisomy-E (C.E.)	10.1	31	3.25	5.6	95.3	31.5	32.6	14.6	—	36.5	0.5	50.5	12.5
	Controls (J.L., R.O., D.C.)	10.2	32	3.87	1.9	84.0	26.3	31.5	12.2	0.2	28.2	3.0	62.3	6.3
	Normal††	12.2 $\pm$ 2.3	36	4.50 $\pm$ 0.7	0.5-1.5	80.0	27.0	34.0	9.5	0.0	35.0	3.0	55.0	7.0

* Wintrobe (1961, pp. 92, 105).

† Nelson (1964, p. 1007).

TABLE III
ERYTHROCYTE ENZYME STUDIES*

Subjects	Age	Glucose-6- phosphate Dehydro- genase	Phospho- glucose Dehydro- genase	Glutathi- one Reduc- tase	Hexo- kinase	Glucose- phos- phate Iso- merase	Phospho- fructo- Kinase	Fructose- diphos- phate Aldolase	Glycer- aldehyde- phos- phate Dehydro- genase	Triose- phos- phate Iso- merase	Phospho- glycerate Kinase	Phospho- glycero- mutase	Phospho- pyruvate Hydra- tase	Pyruvate Kinase	Lactate Dehydro- genase
<i>E-Trisomy</i>															
C.C.	2 dy.	4.53	2.37	4.36	0.66	15.30	4.10	2.80	47.80	247.50	35.50	14.00	13.90	3.60	94.00
L.R.	16 dy.	7.41	3.32	3.76	0.47	27.40	—	1.90	59.70	215.00	33.90	13.65	12.90	3.72	72.80
G.H.	4 wk.	6.07	3.28	4.23	0.47	29.40	7.00	1.17	52.40	195.00	34.20	13.30	13.30	3.60	91.60
N.G.	9 wk.	4.88	2.52	4.21	0.61	24.0	3.50	1.40	38.40	217.00	31.90	9.58	7.80	4.75	60.10
C.E.	9 wk.	4.46	2.23	3.57	0.77	21.50	8.10	2.00	35.10	172.00	27.90	11.40	10.40	4.70	67.60
<i>Controls</i>															
B.G.V.	2 dy.	7.39	2.57	3.62	0.45	16.40	5.60	2.20	44.80	266.00	38.80	9.22	13.21	5.35	70.50
J.B., J.J.	2-4 wk.	5.98	2.99	3.67	0.44	19.10	5.85	1.43	38.45	235.00	35.90	11.45	13.00	4.81	83.80
J.L., R.O., D.C.	9-11 wk.	4.32	2.48	3.76	0.38	16.00	6.00	1.90	38.15	192.70	23.80	9.55	5.95	3.47	72.10
<i>Adult Mean</i> (10)		2.93	2.05	1.66	0.24	13.80	6.00	1.23	28.50	195.00	22.60	7.32	4.50	2.74	60.90
<i>S.D. (Adult)†</i>		(0.22)	(0.35)	(0.22)	(0.04)	(1.90)	(0.80)	(0.15)	(2.00)	(19.00)	(2.90)	(0.92)	(0.81)	(0.47)	(9.80)

* Units of activity defined in text.

† $\pm$ 2 standard deviations.

enzyme formation and thus suggest localization of the gene to the trisomic chromosome. This probably is too simple a concept since it is known that both genetic and non-genetic modifiers can alter the expression of a gene, and it is not yet known whether a 'gene dose effect' is operative in a human autosomal trisomic state. Regulatory genes, which have been demonstrated in some micro-organisms but not yet in man, are also not considered in this concept. Nevertheless, the study of enzyme activity in patients with chromosome abnormalities does represent one possible approach for localizing genes to certain chromosomes.

The slight increase in most enzyme activities in our patients and controls compared to adult values is in keeping with similar findings in newborns recorded by Oski and Naiman (1966). However, except for erythrocyte hexokinase which is notably raised in 4 of the 5 patients with trisomy E, the activities of the erythrocyte and leucocyte enzymes are essentially similar between these patients and the age-matched controls. Considerable reservation is in order regarding the interpretation of the high hexokinase as being due to a triple dose of a gene located on chromosome 18. For example, hexokinase is usually disproportionately higher in reticulocytes compared with other enzymes known to be raised in reticulocytes. Hexokinase is raised in two of our patients with reticulocytosis and in two in whom there are insufficient haematological data. The one subject in which the hexokinase was not raised as compared to the control was not anaemic and had no increased reticulocytosis. In addition, considerable variation in reticulocyte counts and mean cell age normally exists from infant to infant in this age-group and could influence the results in a limited study. A variety of other factors in this age-group, which could also account for a younger mean cell age and give similar results, include hypoxia, early clamping of the cord, foeto-maternal haemorrhage, late third trimester bleeding, or possibly a haemolytic process. Though the maternal history is incomplete in most of our trisomic cases, there is no positive history to implicate these possibilities. Unpublished data from our laboratory indicate that the hexokinase activity seen in the trisomy E patients tends to be higher than that seen in other patients with comparable reticulocyte counts. These latter preliminary observations were made primarily in adult subjects with various types of haemolytic anaemias and thus may not be comparable with the subjects of this report. Despite these reservations regarding the significance of high red cell hexokinase activity in patients with trisomy

18, it seems that further studies of this enzyme are warranted.

The wide variation of normal leucocyte enzyme values makes the recognition of any significant alteration in these cells difficult. Nevertheless, many of the leucocyte enzymes seem slightly lower in the trisomic patients than in the controls. The leucocyte hexokinase tends to be normal or low in both the 2-day-old patient and the age-matched control, but is close to normal adult values in the older patients.

The chromosome analysis of each patient was done only on lymphocytes and not specifically on the cells on which the enzyme analyses were performed. In the presence of the typical clinical syndrome with no evidence of chromosomal mosaicism, however, it is assumed that the cells examined for enzymes also contained the extra chromosome. Further, it is assumed that all five patients have the same chromosomal trisomy 18, which is compatible with the chromosome analysis and is supported by the typical clinical findings in the patients (Table I).

The problem of selecting biologically age-matched controls for patients of young age, as in our trisomic patients, needs consideration. First, at least three of the trisomic patients were born past the 40th week of pregnancy; and secondly, it is not clear whether the chronological age of trisomic patients is directly comparable biologically to the chronological age of a control patient. Without other guide lines, however, control patients were selected based on a comparable chronological postpartum age.

The only other report of blood cell enzyme studies in patients with trisomy 18 are those of Nadler, Inouye, and Hsia (1966) who evaluated four enzymes in nine patients and controls. Galactose-1-phosphate uridyl transferase, the only red cell enzyme studied, was found to have normal activity. The leucocyte enzyme analyses showed a normal glucose-6-phosphate dehydrogenase, a reduced acid phosphatase, and raised alkaline phosphatase activities. Because ages of the patients and controls in their group are not recorded, comparison with our results is difficult. However, in two of our youngest patients the leucocyte glucose-6-phosphate dehydrogenase activity was low.

Among the routine haematological parameters measured, only the mean corpuscular volume was higher in the trisomic patients. Though this may be related to reticulocytosis seen in some patients, it may more directly reflect the trisomic state through some mechanism such as embryonic hydration which has been postulated by Penrose

TABLE 1
LEUCOCYTE ENZYME

Subjects	Age	Glucose-6-phosphate Dehydrogenase	Phosphogluconate Dehydrogenase	Glutathione Reductase	Hexokinase	Glucose-phosphate Isomerase	Phosphofructokinase	Fructose diphosphate Aldolase
<i>E-Trisomy</i>								
C.C.	2 dy.	188	190	64	13	639	—	—
L.R.	16 dy.	321	147	53	43	2005	—	84
G.H.	4 wk.	125	64	66	42	1877	27	80
N.G.	9 wk.	276	133	118	—	—	—	71
C.E.	9 wk.	240	82	84	44	1555	73	89
<i>Controls</i>								
B.G.V.	2 dy.	263	104	83	25	1101	72	101
J.B., J.J.	2½-4 wk.	273	89	103	60	1317	97	93
J.L., R.O., D.C.	9½-11 wk.	259	112	82	62	1368	42	91
<i>Adult Mean</i> (10)		274	119	53	40	1449	77	78
S.D. (adult)†		(34)	(11)	(10)	(10)	(626)	(25)	(24)

* Units of activity defined in text.

† ± 2 standard deviations.

(1967) for dermatoglyphic patterns in human aneuploid conditions.

Summary

Erythrocyte and leucocyte enzyme assays were performed on five trisomy E (18) patients and normal age-matched controls. No evidence was detected locating the gene for any of the 15 enzymes studied on autosome No. 18, though further studies on erythrocyte hexokinase seem warranted. Thus far, the enzymes studied do not indicate their

utility in the diagnosis of persons with this chromosomal syndrome.

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IV

STUDIES*

Glycerol-dehydrophosphate Dehydrogenase	Triose-phosphate Isomerase	Phosphoglycerate Kinase	Phosphoglyceromutase	Phosphopyruvate Hydratase	Pyruvate Kinase	Lactate Dehydrogenase	Alkaline Phosphatase	Leukocyte Alkaline Phosphatase Index
1182	3916	505	312	287	1225	2024	—	—
1834	5852	726	534	589	1463	1956	31	—
1128	2609	476	45	590	1341	1802	—	—
—	2968	—	—	467	785	—	—	—
1168	2953	652	411	561	1130	1850	38	96
1193	5526	696	320	580	1023	1724	76	—
1236	3189	662	329	601	1802	2313	9	19
1010	3561	505	392	428	1419	1882	9	32
1039	2749	449	400	519	1373	1594	23	30
(176)	(312)	(75)	(39)	(95)	(222)	(335)	(12)	—

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