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Comparison of Mitotic Growth Rates of Human Capillary Whole Blood Cultured in a Variety of Media

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Subsequent to the short-term culture technique for peripheral blood leucocytes described by Moorhead, Nowell, Mellman, Battips, and Hungerford (1960), several other techniques utilizing small amounts of whole blood have been described. Those of Edwards (1962) and Frøland (1962) have been extremely useful in cytogenetic investigations of newborn infants and young children. The satisfactory working of these techniques required efficient separation of the leucocytes from the red blood cells before starting the culture. These techniques, apart from failing to give complete separation of the red and white cells, did not permit culture of all the leucocytes contained in the blood sample. By culturing samples of heparinized whole blood all the leucocytes present could be used.

Three recent techniques published by Arakaki and Sparkes (1963), Robinson, Bishun, Rashad, and Morton (1964), and Dartnall and Gray (1965) exploited small amounts of whole blood cultured in a variety of media. These techniques utilized stored calf serum, chick embryo extract, human autologous and heterologous serum, and the serum of the mother for the culturing of her baby's blood. The present note is designed to compare the mitotic growth rates of human whole blood grown in a variety of media utilizing Phytohaemagglutinin M as the mitotic stimulant.

Materials and Methods

Three microblood cultures were set up from each of 55 subjects ranging from newborn infants to adults of 86 years of age. Two drops of capillary whole blood ob-

tained by a finger- or heel-stab were cultured in each of three micro-cultures using the following as culture media.

(a) T.C.199 (Glaxo)	3 ml.	
Pooled human AB serum ..	2 ml.	(microculture C)
(b) T.C.199	3 ml.	
Pooled human AB serum ..	1 ml.	
Chick embryo extract	1 ml.	(microculture D)
(c) T.C.199 alone	5 ml.	(microculture E)

The mitotic growth rates of each culture were assessed by determining the mitotic index which was defined as the percentage of dividing cells observed in a total examined. The preparation of the slides and further details of the calculation of the mitotic indices have been described by Bishun, Morton, and Froggatt (1965).

Results

The results obtained from the two groups of subjects are shown in Tables I and II. These results were assessed statistically by using the square root of each reading as the means of measurement. The analysis of variance for Table I (shown in Table III) showed that there was a difference between methods ($p < 0.001$), but no difference between subjects ($p > 0.05$). The 't' test between methods (Table IIIA) showed that method C (AB serum and T.C.199) was slightly better than method D (AB serum, T.C.199 and C.E.E.) ($0.10 > p > 0.05$), and much better than method E (T.C.199 alone) ($0.01 > p > 0.002$). Method D was only slightly better than method E ($0.10 > p > 0.05$).

The analysis of variance for Table II (shown in Table IV) showed that there was a difference between methods ($0.01 > p > 0.001$) and also subjects ($0.025 > p > 0.01$). The 't' test between methods (Table IVA) showed that method C was better than methods D ($0.05 > p > 0.02$) and E ($0.02 > p > 0.01$), and methods D and E were equally good ($p > 0.25$).

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TABLE I

FIRST GROUP OF TRIALS: NEWBORN-1 YEAR OLD

Subjects	Age (yr.)	Chromosome Constitution	Mitotic Indices Cultures		
			C	D	E
N/49/64	NB	46/XX	2	13	1
N/50/64	NB	46/XY	2	2	3
N/51/64	NB	46/XY	9	7	6
N/52/64	NB	46/XX	1	1	1
N/53/64	NB	46/XY	3	9	9
N/54/64	NB	46/XX	17	6	9
N/55/64	NB	46/XY	3	1	1
N/56/64	NB	46/XY	2	1	1
N/57/64	NB	46/XX	2	1	2
N/58/64	NB	46/XY	1	1	1
N/59/64	NB	46/XX	1	1	1
N/7/64	NB	46/XX	16	10	3
N/69/64	NB	46/XX	3	3	3
N/109/64	NB	47/XY trisomy 21	5	3	5
N/117/64	NB	46/XY	20	4	4
N/21/64	8/12	46/XX	10	2	2
N/28/64	1	46/XY	5	3	2
N/45/64	6/52	—	2	2	0
N/60/64	8/12	46/XX	8	2	3
N/10/65	8/12	47/XY trisomy 21	10	3	7
N/94/64	33/366	47/XY trisomy 21	8	10	5
N/95/64	3/52	46/XY + fragment	10	25	5
N/95/64	2/52	46/XY + fragment	20	14	6
N/3110/64	3/12	46/XY + fragment	5	2	5
N/113/64	1/52	46/XX	20	10	5
N/118/64	2/52	46/XX	10	3	1
N/120/64	2/12	46/XY	2	2	0
N/124/64	2/12	46/XY	2	0	15
N/138/64	3/12	46/XX trisomy 21	20	16	4
N/2/65	3/12	46/XX	25	25	6
Average mitotic indices			8.1	6.1	3.7

NB = Newborn.

The subjects in Table II were divided into groups according to sex (13 males and 12 females) and age (14 between 2 and 21 years, and 11 between 30 and 86 years) before applying the 't' test as a means of explaining the difference in subjects. This test shows (Table IVB) that the sex ($p > 0.25$)

TABLE II

SECOND GROUP OF TRIALS: 2-86 YEARS

Subjects	Age (yr.)	Chromosome Constitution	Mitotic Indices Culture		
			C	D	E
N/63/64	20	46/XX	0	1	2
N/64/64	19	46/XX	1	1	1
N/65/64	21	46/XX	10	2	10
N/66/64	20	46/XY	2	0	1
N/25/64	2	46/XX	30	6	2
N/107/64	11	46/XY	3	1	1
N/106/64	14½	46/XY	11	1	1
N/115/64	2	47/XX trisomy 21	3	2	5
N/9/65	30	46/XX	19	10	14
N/52/63	21	46/XX	10	5	5
N/53/63	11	46/XY	6	3	3
N/54/63	11	46/XY	6	3	3
N/55/62	18	46/XX	3	0	3
N/64/63	2	46/XY	3	3	3
N/76/64	83	46/XY	9	6	6
N/125/64	2	46/XY	14	23	1
N/77/64	63	46/XY	2	2	2
N/78/64	84	46/XY	2	2	2
N/79/64	86	46/XY	6	6	2
N/80/64	67	46/XY	2	1	2
N/82/64	75	46/XX	2	2	1
N/83/64	77	46/XX	3	1	1
N/84/64	81	46/XX	2	1	2
N/85/64	78	46/XX	3	2	1
N/86/64	71	46/XX	4	3	1
Average mitotic indices			6.2	3.6	2.8

TABLE IIIA

T TEST BETWEEN METHODS (for Table I)

Methods	Difference Between Means	Standard Error of Difference	t'	Probability
C - D	0.38	0.22	1.7	0.10 > p > 0.05
C - E	0.93	0.32	2.9	0.01 > p > 0.002
D - E	0.55	0.31	1.8	0.10 > p > 0.05

Degrees of freedom = 29.

TABLE III
ANALYSIS OF VARIANCE

Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio (F)	Probability
Between cultures	14.96	2	7.48	7.48/0.13 = 57	p < 0.001
Between subjects	97.04	29	3.34	3.34/0.13 = 25	p > 0.05
Residual	7.80	58	0.13		
Total	119.80	89			

TABLE IV
ANALYSIS OF VARIANCE

Variation	Sum of Squares	Degrees of Freedom	Mean Value	Variance Ratio (F)	Probability
Between methods	6.50	2	3.25	3.25/0.54 = 6.02	0.01 > p > 0.001
Between subjects	27.70	24	1.15	1.15/0.54 = 2.2	0.025 > p > 0.01
Residual	26.10	48	0.54		
Total	60.30	74			

TABLE IVA

'T' TEST BETWEEN METHODS (for Table II)

Methods	Difference Between Means	Standard Error of Difference	't'	Probability
C - D	0.59	0.28	2.12	0.05 > p > 0.02
C - E	0.82	0.29	2.78	0.02 > p > 0.01
D - E	0.23	0.23	1.00	p > 0.25

Degrees of freedom = 24.

and age ($p > 0.25$) factors did not significantly contribute to the difference in subjects.

Discussion

Robbins (1964) suggested that all cultured cells required one or more substances derived from serum protein for survival and growth. The active component was apparently not the protein as such but a relatively small molecule which is either a polypeptide or a substance bound to the protein released from it, and may be growth-limiting *in vivo*. It appeared that cellular growth *in vivo*, and perhaps *in vitro*, was dependent as yet on unknown factors that actively stimulate cell growth. These factors may be present in human AB serum.

The results indicated that method C, in which AB serum and T.C.199 were used in the proportions of 3:2, was the best culture medium for culturing capillary whole blood. When the serum was excluded, as in method E, or replaced partially by another constituent as in method D, the mitotic growth rates were significantly lowered. This was suggestive of a growth-promoting factor in human AB serum as suggested by Robbins (1964).

The fact that some growth had been observed in microcultures where only T.C.199 (culture E) was present does not invalidate these tentative conclusions. A certain amount of serum in two drops of whole blood was present and might have supplied an inadequate amount of the necessary growth factors. These cultures (E) gave approximately the same mitotic index as those in which 1 ml. C.E.E. was substituted for 1 ml. AB serum (culture D). The larger amount of AB serum present in culture C would supply more of the

TABLE IVB

'T' TEST FOR AGE AND SEX (for Table II)

Degrees of Freedom	Factor	Difference Between Means	Standard Error of Difference	't'	Probability
23	Sex	1.01	1.5	0.7	p > 0.25
23	Age	1.00	1.52	0.7	p > 0.25

growth factors than would be present in either of the two other cultures.

This deficiency of AB serum factors in cultures D and E was reflected in the lower mitotic indices obtained with them as compared with culture C.

The age and sex factors were shown not to give significantly different results for each culture. Such differences as were observed between subjects may be explained as experimental errors.

Summary

Three microblood cultures, using capillary blood obtained from a finger or heel-stab, were set up from each subject from two separate age-groups.

It was shown that the bloods cultured in AB serum and T.C.199 gave a significantly higher growth rate (measured by the mitotic index) than those cultured in AB serum, T.C.199, and C.E.E. (chick embryo extract) or T.C.199 alone.

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