Assessment of Two Macromethods and Three Micromethods of Culturing Human White Blood Cells for Chromosome Analysis

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Three variations of the micromethod, previously described (Robinson, Bishun, Rashad, and Morton, 1964) for the culture of human white blood cells (WBCs) from capillary blood of newborn infants, have been used on peripheral venous blood of adults. The reliability of the micromethods has been assessed by comparing the mitotic indices for these methods with those obtained for two macromethods of culturing WBCs from venous blood as described below. The mitotic index is calculated for each method as the percentage of dividing cells seen in a total of 1,000, or more, cells. The present results do not include work done on the capillary blood of adults, the results of which will be reported later.

Materials and Methods

Macromethods. In each case, 20 ml. peripheral venous blood, usually obtained from the antecubital veins, were withdrawn into a dry-heat sterilized syringe. 10 ml. of this blood were immediately discharged directly from the syringe, after removal of the needle, into each of two heparinized bottles (Evans Medical), the caps replaced after flaming, and the bottles gently shaken. The treatment of the bloods differed from this point onwards. One bottle was allowed to remain at room temperature and the blood was used for one macromethod and for the three micromethods. The second bottle was immediately placed in a flask containing ice and left to cool for half an hour. This was used for the second macromethod. All cultures were incubated without replacement of the air by another gas.

Macromethod A. Peripheral venous blood at room temperature was allowed to stand for one to one and a half hours. The supernatant plasma, usually about 1–1.5 ml., was drawn off and put under sterile conditions into a culture bottle. 3 ml. of T.C. medium 199 (Glaxo) together with three drops of Phytohemagglutinin P (Difco) were added and the whole incubated for 70 hours.

Macromethod B. Eight drops of Phytohemagglutinin M (Difco) were added to the cooled venous blood after half an hour, and the bottle was shaken and replaced in the cooled flask for another five minutes. The blood was then centrifuged at 600 r.p.m. for seven minutes. The supernatant plasma was withdrawn, an equal amount of T.C. medium 199 was added, and the mixture was incubated for 70 hours.

Micromethods. In each case two drops of heparinized venous whole blood kept at room temperature and three drops of Phytohemagglutinin M (Difco) were added to the culture medium immediately after returning to the laboratory, and incubated for 70 hours. The three culture media were made up as follows:

Micromethod C. 4 ml. T.C. medium 199, and 1 ml. human AB serum.

Micromethod D. 3 ml. T.C. medium 199, and 1 ml. human AB serum. 1 ml. chick embryo extract (C.E.E.) prepared from 10-day incubated hen eggs.

Micromethod E. 5 ml. T.C. medium 199 alone.

After Treatment. 'Colcemid' (0.1 ml. of a 0.001 % solution per ml. of total fluid volume) was added to each culture after 70 hours of incubation, and incubation was continued for a further two and a half hours. Harvesting was carried out as previously described (Bishun, Morton, and McLaverty, 1964).

Results

Since the chromosome morphology and spreads were fairly uniform from all cultures, the mitotic indices may be used as a fairly reliable means of assessing the comparative growth rates in the various cultures. Table I shows the mitotic indices for each method for 24 patients (14 females (46/XX), 2 mosaic females (46/XX, 45/XO), 7 males (46/XY), and one Klinefelter male (47/XXY)), ranging in age from 2 to 72 years.

The percentages of successful mitotic indices obtained by the five methods (A to E) on blood from 24 subjects in Table I were transformed to their square-root, and compared by (a) analysis of

TABLE I

COMPARISON OF MITOTIC INDICES FOR FIVE METHODS OF CULTURING HUMAN WHITE BLOOD CELLS

Culture No.	Patient's Age	Chromosome Constitution	Mitotic Indices (No. of dividing cells per 100 cells)				
			Macromethods		Micromethods		
			A	B	C	D	E
N/91/64	72	46/XY	2	10	5	7	8
N/92/64	18	46/XX	5	1	2	1	2
N/93/64	24	46/XX	5	10	5	10	5
N/96/64	71	47/XXY	3	15	3	5	4
N/98/64	17	46/XX } 45/XO } mosaic	10	20	5	6	10
N/99/64	13	46/XX	10	20	2	3	2
N/100/64	18	46/XX	3	5	10	5	6
N/102/64	20	46/XY	20	5	5	10	5
N/104/64	18	46/XY	10	15	10	3	5
N/105/64	28	46/XX } 45/XO } mosaic	10	15	3	2	3
N/108/64	47	46/XX	10	15	10	4	5
N/112/64	28	46/XX	3	4	28	4	2
N/116/64	29	46/XX	15	25	8	5	3
N/121/64	45	46/XY	10	10	15	5	3
N/126/64	4	46/XY	15	20	10	3	2
N/127/64	2	46/XY	10	10	8	2	1
N/128/64	34	46/XX	2	17	15	10	3
N/129/64	24	46/XX	3	8	3	2	3
N/130/64	22	46/XX	3	15	10	5	2
N/131/64	45	46/XX	5	10	8	3	3
N/133/64	21	46/XY	12	15	10	10	3
N/134/64	19	46/XX	5	14	8	7	2
N/135/64	17	46/XX	10	10	10	5	3
N/136/64	34	46/XX	8	15	10	8	2
Total			189	304	203	125	85
Average mitotic indices			7.9	12.7	8.5	5.2	3.6

TABLE II

ANALYSIS OF VARIANCE ON THE TRANSFORMED (SQUARE-ROOT) VARIATE

Source of Variation	Sums of Squares	D.F.	Mean Square	Variance Ratio	Probability
Between methods	36.7823	4	9.1956	16.5003	$p < 0.001$
Between subjects	19.0226	23	0.8271	1.4841	$0.10 > p > 0.05$
Residual	51.2723	92	0.5573		
Total	107.0772	119			

TABLE III

STUDENT'S *t* TEST BETWEEN MEANS OF TRANSFORMED VARIATES FOR EACH METHOD

Methods	Difference Between Means	Standard Error of Difference	<i>t</i>	Probability
A-B	-0.7675	0.2561	2.9969	$0.02 > p > 0.01$
-C	-0.0996	0.2520	0.3952	$0.70 > p > 0.60$
-D	0.4750	0.2163	2.1960	$0.05 > p > 0.02$
-E	0.8679	0.2064	4.2049	$p < 0.001$
B-C	0.6679	0.2598	2.5708	$0.02 > p > 0.01$
-D	1.2425	0.2254	5.5124	$p < 0.001$
-E	1.6354	0.2159	7.5748	$p < 0.001$
C-D	0.5746	0.2207	2.6035	$0.02 > p > 0.01$
-E	0.9675	0.2110	4.5853	$p < 0.001$
D-E	0.3929	0.1667	2.3569	$0.05 > p > 0.02$

Note: Degrees of freedom = 46 throughout.

variance (Table II), and (b) Student's *t* test between all possible pairs of means (Table III). Table II shows that there is a significant difference between methods ($p < 0.001$) but not between subjects ($0.10 > p > 0.05$). Table III shows that method B was the most successful, method E the least successful, methods A and C equally successful, and both in turn better than D.

Conclusions

The best results are obtained where the total amount of blood is large and is cooled immediately after withdrawal from the subject. When only small amounts of blood are readily available, micro-culture method C, in which T.C. medium 199 and

AB serum are used, gives as good results as macro-method A, in which the blood is not cooled before culture.

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