Differential Loss of Cellular Constituents in Cytocentrifuge Preparations[1]

Preparations suitable for the cytochemical population analysis[2,3] of cultured human lymphocytes present certain technical difficulties, particularly if the cells are derived from suspension cultures. In an attempt to alleviate one of the major technical difficulties – lack of cell adhesion to glass slides – a cytocentrifuge technique similar to that described by WATSON[4] has been examined. Slides prepared by this technique contained properly adhered cells, and were satisfactory for routine light microscopy and cytochemical analysis. However, in order to exclude possible quantitative changes resulting from loss of cellular material from such cells during preparation in a cytocentrifuge[5], several cell cultures were prepared for cytochemical analysis by cytocentrifugation directly onto coverslips in parallel with the classical 'drop'method[6] and examined for specific alterations in dry mass and Feulgen-DNA.

Materials and methods. The human lymphocytic cells used have been described elsewhere: CCRF-CEM cells[7], CCRF-RKB cells[8], and EB-3 cells[9]. CCRF-CEM-ST cells were derived directly from the lymphomas developed in neonatal Syrian hamsters implanted with CCRF-CEM cells[10]. Non-leukemic lymphocytic cells derived directly from the inguinal lymph nodes of Swiss mice (ML) and Syrian hamsters (HL); and L-929 mouse fibroblasts[11] grown in monolayer cultures in EAGLE's basal medium supplemented with 10% whole fetal calf serum as described by EAGLE[12] were used as controls.

In the 'drop' method, an aliquot of cell suspension with the desired number of cells was placed on Bürker hemocytometer coverslips (0.3 mm thickness) and the drop allowed to stand 10 min during which time the cells settled and adhered to the glass surface, and then the preparations were fixed in 1:1 (V/V) acetone-ethanol for 1 h at room temperature. In the cytocentrifuge technique aliquots of the same cell suspensions were centrifuged for

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Table I. Mean per cell dry mass values (arbitrary interferometric units)

Cell population	Cells prepared by 'Drop' method $\bar{X}$[1]	(n)[2]	Cytocentrifuge method $\bar{X}$	(n)	Change in cytocentrifuge preparations (%)
CCRF-CEM	8.54	(100)	6.10	(100)	−28.6
CCRF-CEM	10.50[a]	(54)	8.48	(64)	−19.2
CCRF-CEM	8.35	(30)	6.58	(30)	−21.2
Pooled CCRF-CEM	9.08	(184)	6.95	(194)	−23.5
CCRF-RKB	8.68	(100)	5.55	(100)	−36.1
CCRF-RKB	8.38	(75)	5.72	(75)	−31.7
CCRF-RKB	8.57	(50)	6.02	(50)	−29.8
Pooled CCRF-RKB	8.55	(225)	5.71	(225)	−33.2
EB-3	9.55[a]	(100)	4.98	(100)	−47.9
EB-3	7.41	(65)	4.69	(75)	−36.7
EB-3	6.98	(30)	4.62	(30)	−33.8
Pooled EB-3	8.44	(195)	4.82	(205)	−42.9
HL	2.18	(100)	1.74	(100)	−20.2
HL	2.22	(100)	1.70	(100)	−23.4
Pooled HL	2.20	(200)	1.72	(200)	−21.8
CCRF-CEM-ST	12.11	(100)	8.36	(100)	−31.0
CCRF-CEM-ST	14.03	(100)	9.24	(100)	−34.1
Pooled CCRF-CEM-ST	13.07	(200)	8.80	(200)	−32.7
ML	2.67	(100)	1.52	(100)	−43.1
ML	2.19	(88)	1.25	(80)	−42.9
ML	2.49	(30)	1.53	(30)	−38.5
Pooled ML	2.45	(218)	1.42	(210)	−42.0
L-929	48.50	(100)	30.90	(100)	−36.3
L-929	38.50[b]	(100)	25.50	(100)	−33.7
Pooled L-929	43.50	(200)	28.20	(200)	−35.2

[a] Partially synchronized population; predominance of G-2 cells in populations. [b] Partially synchronized population; predominance of G-1 cells in population. [1] $\bar{X}$, average dry mass value. [2] (n), number of cells analyzed.

Table II. Mean per cell Feulgen-DNA values (arbitrary photometric units)

Cell population	Cells prepared by 'Drop' method $\bar{X}$ [c]	(n) [d]	Cytocentrifuge method $\bar{X}$	(n)	Change in cytocentrifuge preparations (%)
CCRF-CEM	11.20	(96)	12.00	(90)	+ 7.1
CCRF-CEM	20.92[a]	(54)	19.96	(50)	− 4.6
CCRF-CEM	13.35	(30)	13.65	(30)	+ 2.2
Pooled CCRF-CEM	14.61	(180)	14.48	(170)	− 0.9
CCRF-RKB	15.57	(100)	14.00	(100)	−11.2
CCRF-RKB	13.86	(74)	14.01	(75)	+ 1.1
CCRF-RKB	15.05	(50)	13.91	(50)	− 7.6
Pooled CCRF-RKB	14.97	(224)	13.98	(225)	− 6.6
EB-3	12.54	(100)	11.25	(100)	−10.3
EB-3	15.00[a]	(54)	13.76	(73)	− 8.3
EB-3	14.32	(30)	13.59	(30)	− 5.1
Pooled EB-3	13.55	(184)	12.49	(203)	− 7.8
HL	5.40	(98)	5.54	(91)	+ 2.5
HL	4.80	(100)	5.10	(100)	+ 6.3
Pooled HL	5.10	(198)	5.31	(191)	+ 4.1
CCRF-CEM-ST	18.77	(100)	17.61	(100)	− 6.2
CCRF-CEM-ST	22.50	(75)	20.01	(75)	−11.1
Pooled CCRF-CEM-ST	20.37	(175)	18.64	(175)	− 8.5
ML	7.57	(99)	7.00	(85)	− 7.5
ML	8.08	(52)	8.48	(50)	+ 4.9
ML	6.47	(30)	6.47	(30)	± 0.0
Pooled ML	7.53	(181)	7.35	(165)	− 2.4
L-929	46.23	(100)	46.80	(97)	+ 1.2
L-929	42.75[b]	(100)	47.82	(100)	+11.9
Pooled L-929	44.49	(200)	46.59	(197)	+ 4.72

[a] Partially synchronized population; predominance of G-2 cells in population. [b] Partially synchronized population; predominance of G-1 cells in population. [c] $\bar{X}$, average Feulgen value. [d] (n), number of cells in sample.

10 min at 1000 rpm directly onto coverslips in a Shandon cytocentrifuge and fixed as above.

In each experiment, parallel slides prepared by each of the 2 methods were hydrolyzed in 1 N HCl for 9 min at 60 °C and stained in leuco-Schiff reagent for 1 h at room temperature for Feulgen-DNA content[13].

Feulgen-DNA content was determined on individual cells at 546 nm in the high resolution, rapid-scanning microspectrophotometer described by LOMAKKA[14]. Total dry mass was determined on individual cells in duplicate unhydrolyzed preparations in the high resolution, rapid-scanning microinterferometer described by LOMAKKA[15].

Results and discussion. For all cell lines, the mean dry mass values per cell for cells prepared by the cytocentrifuge method were less than those prepared by the 'drop' method (Table I), clearly indicating a loss of dry mass during cytocentrifugation. The Feulgen-DNA mean values for cytocentrifuged cells were both above and below those values for the 'drop' preparations and were in each case within the 10% error intrinsic in the analytical measuring devices indicating that there was no substantial loss of Feulgen-DNA for the cell lines analyzed (Table II). These results support the contention that cytocentrifugation causes a loss in material (probably cytoplasmic constituents) from cells prepared for cytochemical analysis. It is of interest that the losses in dry mass observed in the cytocentrifuged cells varied with the different kinds of cells, and may be related to the nature of the cell membrane.

Zusammenfassung. Eine quantitative mikroskopische Analyse von Zellen, die man bei 1000 U/min zytozentrifugiert hatte, zeigte, dass die Zellen Proteinbestandteile des Zytoplasmas verlieren, während der DNS-Gehalt des Kerns unverändert bleibt. Untersuchungen von 8 verschiedenen Zellarten ergaben, dass die beobachteten Verluste spezifisch von der Zellart abhängen und mit der Struktur der Zellmembran zusammenhängen könnten.

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