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Evaluation of the efficacy of mitogens in buffalo leucocyte culture*

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Peripheral blood leucocyte culture technique was successfully used for karyological studies in buffaloes by Ulbrich and Fischer (1967). The efficacy of various techniques to obtain a consistantly large number of good metaphase spreads from the buffalo lymphocytes was studied by Rathnasabapathy and Ganesh (1980) and Rathnasabapathy and Venkatraj (1982), and the method involving TC 199 medium, phytohemagglutinin and 72 hour culture was found to be superior.

According to Thiagarajan *et al.* (1989) RPMI 1640 was best suited medium yielding higher mitotic index and poke weed mitogen was better than phytohemagglutinin for buffalo lymphocytes

The main objective of this study was to find out the best combination of mitogens for buffalo lymphocyte culture.

Materials and Methods

Sixty blood samples were collected from buffaloes found in Kerala in centrifuge tubes containing heparin sodium at a concentration of 65 IU per ml of blood formed the material for the study. The effect of three mitogens viz. (i) phytohemagglutinin (PHA) 7μg/ml (SIGMA),(ii) pokeweed mitogen (PWN) 3.5 μg/ml (SIGMA) and (iii) combination of PHA and PWN (3.5μg/ml and 1.75 μg/ml) respectively (SIGMA) were compared together with the effect of incubation time, ie. from the seeding to the time of addition of colcemid (2μg/ml) to medium, of 68 hours, 69 hours, 70 hours and 71 hours after the onset of culturing. Mitotic drive and mitotic index were used as the criteria to assess the efficacy of these treatments.

A randomised block design was adopted with the mitogens as treatments and time of addition of mitotic arrester as blocks. Samples were alloted to any one of the treatments or blocks randomly.

The basal medium was RPMI 1640 (SIGMA) and the composition of the medium was as follows.

RPMI 1640 dried powder SIGMA	1000 mg
Sodium bicarbonate MERCK	75 mg
Mitogen (PHA, PWM or PHA+PWM) SIGMA	1 ml
Penicillin solution (5000 IU/ml)	0.5 ml
Double distilled water	98.5 ml

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pH of the medium was adjusted to 6.8 and the reconstituted medium was distributed to screw capped vials five millilitre each. To each culture vial two ml of autologous plasma and 9 drops of well mixed blood from 21 G needle were added.

Results

From the table 1 it can be seen that mitotic drive and mitotic index were higher when the combination of PHA and PWM was used as mitogens. Analysis of variance revealed that the difference due to the mitogens was significant. The mixture of PHA and PWM was significantly superior to PHA, avd PWM occupied the intermediary position.

In assessing the interaction of mitogen and incubation time it was found that the effect is significant for mitotic drive. The combination of PHA and PWM and an incubation time of 71 hours was found to yield better results, as evidenced by the highest mitotic drive of 47.64±0.97. PWM at an incubation time of 70 hours was second with an average mitotic drive of 47.54±1.74. As regards to mitotic index no meaningful results were obtained while the interaction of different mitogens and incubation time were considered.

Discussion

From the table 1 it could be seen that mitotic drive and mitotic index were found to be higher when the combination of PHA and PWM was used. The phenomenon may be due to the differential property of the two mitogens to stimulate mitosis. Halnan (1989) reported that PHA mainly stimulates T lymphocytes while PWM stimulates B lymphocytes. Combination

Table 1. Effect of mitogens and incubation time on mean mitotic drive and mean mitotic index

Incubation Time (hrs)		Mitogens		
		Pokeweed	Phytohemag-glutinin	Combination
68	Mitotic drive	38.67±1.53	38.32±1.01	38.72±1.81
	Mitotic index	1.60±0.30	1.60±0.40	2.800.8±0
69	Mitotic drive	37.38±3.25	34.74±2.73	44.24±1.84
	Mitotic index	2.40±0.93	1.60±0.24	2.70±0.49
70	Mitotic drive	47.54±1.74	36.90±0.90	46.50±1.33
	Mitotic index	3.80±1.36	1.80±0.37	2.80±1.25
71	Mitotic drive	39.72±2.15	39.05±1.14	47.64±0.97
	Mitotic index	2.20±0.41	2.00±0.45	4.40±0.91

* n=5 in each treatment

** In analysis of variance differences due to mitogens was significant for both mitotic drive and mitotic index. There was significant interaction of mitogen and incubation time for mitotic drive.

of these two mitogens might have stimulated both T and B lymphocytes resulting in large proportion of lymphoblasts and metaphase cells.

Among the mitogens, PWM was superior to PHA in stimulating mitosis in buffalo lymphocytes. This finding is in agreement with that of Thiagarajan *et al.* (1989) in which PWM yielded higher mitotic index with little or no hemagglutination.

Incubation time was found to influence the mitotic drive and mitotic index. In the present study, the duration of culture ideally suited for buffalo lymphocytes is found to be 71 hours when a combination of PWM and PHA was used and 70 hours when PWM alone was used.

According to Halnan (1989) records over twenty years on the cultures from large animals indicated that there is much difference in the incubation time be 40-48 hours for two day cultures or 60-74 hours for three day cultures.

Summary

Comparative assessment of the mitogens and their combination based on mitotic drive and mitotic index revealed that the combination of PHA and PWM was significantly superior to PHA in inducing mitosis of buffalo lymphocytes. PWM has an intermediary mitotic drive and mitotic index. The combination of PHA and PWM at an incubation time of 71 hours exhibited the best results followed by the interaction of PWM and an incubation time of 70 hours.

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