our knowledge, is not on record for the genus *Phaseolus*. In view of the controversial opinions expressed the present observations are significant and may serve as taxonomic pointer in delimiting species. The occurrence of alternative germination in the genus *Phaseolus* thus raises phyletic problems and until further work is carried out it would be premature to regard a given *Phaseolus* species as primitive or advanced merely on the germination criteria alone.

FIGS. 1–3. Fig. 1. *Phaseolus vulgaris* showing epigeal seedlings. Figs. 2–3. *P. angularis* and *P. multiflorus* showing hypogeal seedlings.

N.B. Longitudnal striations on cotyledons of epigeal cotyledon can be seen while the hypogeal cotyledons are smooth.

We are grateful to the Directors of the various Agricultural Research Stations, for kindly providing the seed material of various species investigated and to Prof. U. B. S. Swami, for giving us facilities and encouragement.

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AN IMPROVED AND SIMPLE LEUCOCYTE CULTURE TECHNIQUE FOR CHROMOSOMAL PREPARATIONS FROM ALBINO RAT

RODENTS have been widely used for many biomedical investigations but their use for cytogenetic studies is not so extensive. One reason may be the lack of reliable tissue culture techniques, *in vitro*, for yielding a large number of cells at metaphase. The media for leucocyte cultures described by Nichols and Levan[1,2] (1961, 1962) and Ford and Woollam[3] (1963) have led to hemolytic reactions in our laboratory resulting in poor cell growth. The new method developed in this laboratory is a modification of the techniques described by William and Ray[4] (1965) and Sankar and Giesler[5] (1973). The technique reported here has certain advantages over existing ones. The medium, TC 199 has been employed in place of other media and Phytohemagglutinin–P, a more powerful agglutinating agent, has been added to the medium instead of Phytohemagglutinin–M. This has resulted in a considerable increase in the number of cells at metaphase and as a result, a minimum of 10 slides can be prepared from each tube. Furthermore the use of diluted foetal calf serum and very dilute sodium citrate, for hypotonic treatment[5], has been omitted. Treatment with Hank's solution followed by a brief incubation in 1% sodium citrate has been used instead. Another advantage of this technique is its adaptability, by suitable modifications, for culturing leucocytes from other rodents as well. For culturing leucocytes, the whole blood can also be used without going through the procedure of separating leucocytes but we prefer leucocyte separation for obtaining optimum cultures. The minimum mitotic index obtained was 30.

The blood was drawn into a 10 ml sterilized syringe, by cardiac puncture, rinsed with heparin. 1 ml of plasma containing $1{\cdot}2 \times 10^6$ leucocytes/ml was added to the culture tubes (Corning, 30 ml) having the prewarmed (37° C) culture medium. The medium included TC Medium 199 (Difco) 10 ml ; 1 ml of a solution of Penicillin–G (1000 units) and Streptomycin sulfate (1000 mcg) (Sigma) ; 1·80 ml L-Glutamine (Sigma) 100 μ moles/ml in saline ; 0·02 ml of Phytohemagglutinin-P (Difco) and 200 I.U. Heparin (Koch Light Laboratories). The cultures were incubated for

72 hours at 37° C. The pH of the medium was kept between 7·5 and 8·0 during the incubation period by loosening the cap by 1/3 turn daily.

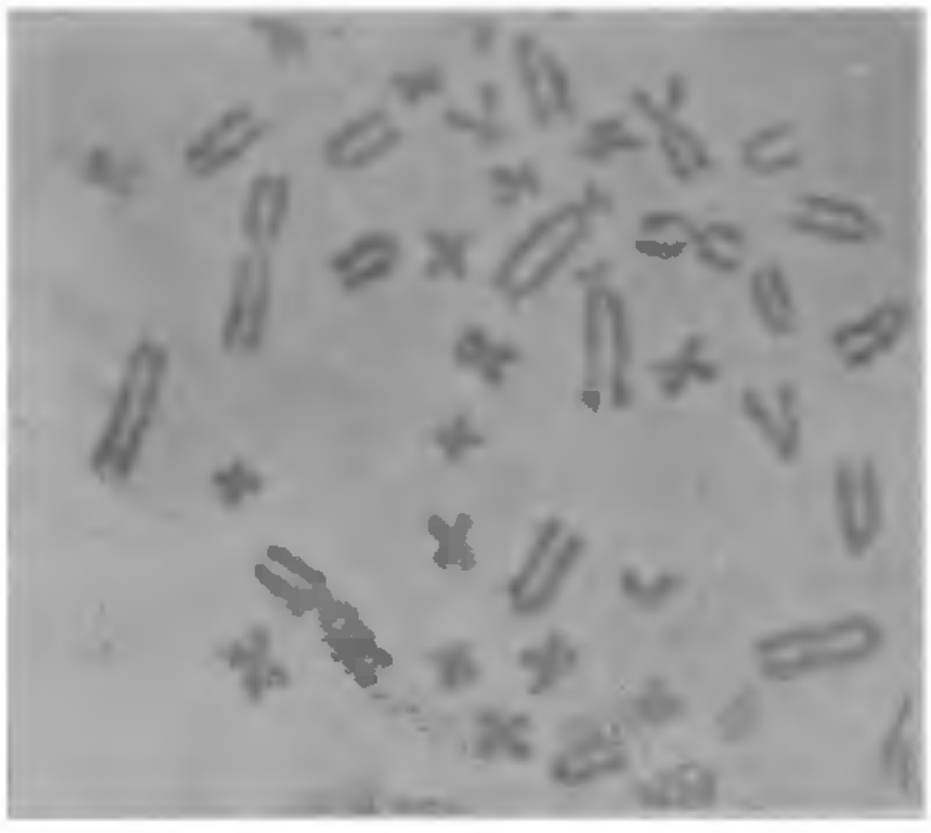

FIG. 1. Metaphase chromosomes from albino rat leucocytes cultured *in vitro*, (× 2,300) photographed on a Kodak Micro–File (panchromatic) Film using Carl Zeiss 'Ergavil' Research Microscope with Automatic Photomicrography Equipment.

0·2 ml of Colchicine (4 mcg/ml, Rousel Uclaf) was added to the tubes 2 hours prior to the harvesting of the cells. The tubes were then centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and the cells were suspended in 5 ml of prewarmed (37° C) Hanks solution. The tubes were again centrifuged at 1000 rpm for 5 minutes. The supernatant was aspirated with a pippet. The cells were resuspended in 3·5 ml of prewarmed (37° C) 1% sodium citrate solution and the suspension was incubated at 37° C for 10 minutes and then centrifuged at 800 rpm for 6 minutes and the supernataut vas discarded. The cells were resuspended in 1 ml of cold acetic acid-methanol fixative, for each ml of the original solution, for 10 minutes at room temperature. This suspension was then centrifuged at 800 rpm and 2 more changes of the fixative, after 15 minutes in each, were given. The cells were finally suspended in 1·5 ml of the fixative. The slides were prepared by the flame drying technique and the preparations were stained with phosphate buffered Giemsa stain, pH 6·8.

Results

Sufficient number of metaphase spreads were obtained for each slide prepared by this technique for either karyotyping or chromosomal analyses by banding pattern technique. Over 125 cultures have been established in this way and quite satisfactory preparations were obtained for chromosomal study, (Fig. 1).

The authors are extremely grateful to Prof. and Mrs. S. Hurtig (France) for providing TC Medium 199 and Phytohemagglutinin–P as a gift. Sincere thanks are also expressed to Head, Department of Zoology, for providing necessary laboratory facilities.

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