Culture of Peripheral Blood Leucocytes of the Golden Hamster.* (28631)

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The method of chromosome analysis based on the peripheral blood leucocyte culture technique of Moorhead *et al.*(1) has gained wide acceptance for human studies and has also been successfully applied to a variety of animals. However, attempts to culture leucocytes from the peripheral blood of the golden hamster, both by this method and by a modification which employs 6% dextran for leucocyte separation(2), have consistently failed. The success of peripheral blood culture depends to a large extent on adequate separation of competent leucocytes, while the efficacy of separation may be a function of viscosity. These considerations led to a trial of newborn calf serum and it was found that good leucocyte separation was effected by this agent. Supplementing the growth medium with calf serum, instead of with the isologous plasma which is customarily used for human leucocyte culture, yielded satisfactory growth. Mitotic activity reached its height on the sixth day, which is approximately double the time observed with human leucocyte cultures. The resistance of the golden hamster to colchicine necessitated an appropriate increase in the concentration of this agent to secure mitotic arrest.

The present report describes a method of blood leucocyte culture for chromosome analysis in the golden hamster based on these modifications of the classical technique of human leucocyte culture(1).

Materials and methods. Separation and culture of leucocytes. Blood (4-6 ml) was withdrawn by cardiac puncture from an anesthetized hamster by means of a 10 ml disposable syringe moistened with preservative-free heparin (1,000 units/ml) and was thoroughly mixed with newborn calf serum (Hyland) to give a total volume of 10 ml. The mixture was then centrifuged at 1000 rpm (200 g) for 10 minutes and the supernatant, together with any buffy layer, was transferred to another tube and was centrifuged at 1400 rpm (425 g) for 10 minutes. The supernatant was discarded and the cells were suspended in a volume of culture medium equal to twice the original volume of blood. The culture medium consisted of medium 199 (Hyland) with 20% calf serum, penicillin (200 units/ml) and streptomycin (200 γ/ml). The cells were incubated at 37°C with phytohemagglutinin in tightly capped tubes with occasional gentle agitation. At 24 hours the pH was adjusted to 7.0-7.2 with 7.5% sodium bicarbonate; particularly acid cultures received in addition 1-2 ml of culture medium with phytohemagglutinin.

Harvesting. On the sixth day, colchicine (0.2% in Ringer's solution) was added (0.1 ml/1 ml of culture) to give a concentration of 0.5×10^{-3} M and incubation was continued for 3 hours. Apart from a somewhat longer period of hypotonic treatment (incubation for 9 minutes and centrifugation for 5 minutes), the remaining stages of the procedure necessary for chromosome preparations were identical to those described for use with cultured human leucocytes(1).

Results and discussion. Cultures have been prepared from 17 adult hamsters of both sexes. In 4, the overall mitotic index on day 6 lay between 1% and 5%, with correspondingly numerous technically acceptable spreads available for analysis (Fig. 1 and 2). Although good leucocyte growth occurred in cultures prepared from 6 hamsters, the degree of mitotic activity was much less and only a few analyzable spreads were present on each slide. Cultures from 7 hamsters were recorded as failures, leucocyte growth being either inadequate for the appearance of mitoses or absent altogether.

Among the advantages that accrue from the use of calf serum to promote leucocyte separation are the convenience afforded by the ready availability of calf serum and the

* Supported by a research grant from the Division of General Medical Sciences, NIH, USPHS.

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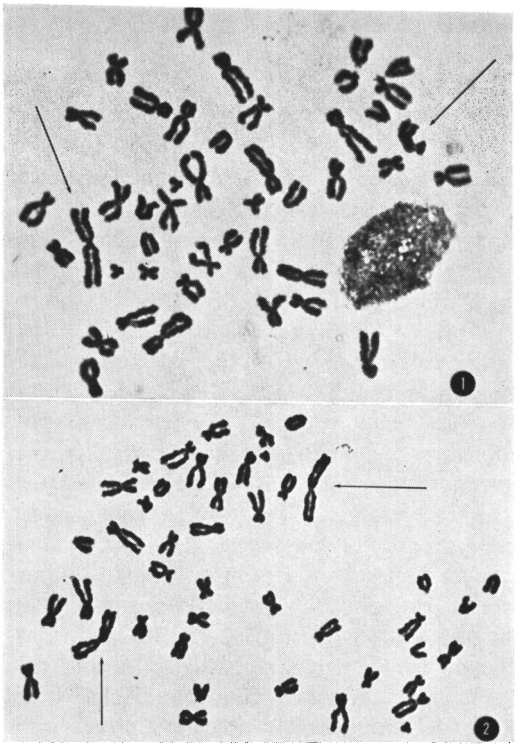

FIG. 1. The 44 chromosomes of a cultured leucocyte of a male golden hamster. Arrows indicate the large metacentric X chromosome and a terminal submetacentric chromosome which is a contender for the Y chromosome. Lightly stained with orcein and photographed with phase contrast. × 1000.

FIG. 2. Chromosomes of a cultured leucocyte of a female golden hamster. The X chromosomes are indicated by arrows. × 1000.

ease of handling the augmented volume of leucocyte suspension. The necessity to remove the separating agent by washing was avoided as calf serum was used to supplement the culture medium.

As with other species, the use of phytohemagglutinin appears necessary to stimulate mitotic activity.

The high concentration of colchicine required to arrest mitosis confirms the observations of Midgley, Pierce and Dixon(3) that the resistance of the golden hamster to this drug is associated with equivalent cellular unresponsiveness.

Chromosomes prepared by the blood method appear very similar to those obtained by tissue culture techniques(4). A noteworthy feature was the presence of faint although distinct satellites on the largest pair of acrocentric chromosomes (autosome pair 16) and occasionally also on the smaller pairs. In man, leucocyte preparations have proved particularly favorable for the study of satellites (5) and the same phenomenon may apply to the hamster, although satellites have been clearly depicted in tissue culture material(6).

Summary. A method is described for the separation of peripheral blood leucocytes of the golden hamster with calf serum, and for their short-term culture employing medium 199 supplemented with calf serum. Mitotic activity was adequate for chromosome preparations by established techniques.

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Received July 12, 1963. P.S.E.B.M., 1963, v114.