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How long after being collected can blood still be cultured for chromosomal studies in the tropics?*

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To examine how long after their collection samples of peripheral blood could be successfully cultured for cytogenetic analysis in tropical countries, we determined the mitotic index in cultures of blood stored for up to 7 days in summer and winter. The results indicate that chromosomal analysis is successful if blood is stored for up to 2 days in summer or for up to 4 days in winter before initiating culture. Storing the blood in tissue culture medium rather than in heparin solution did not significantly improve the mitotic index.

Determination of the chromosomal constitution of an individual usually involves growing peripheral blood lymphocytes in a tissue culture medium containing phytohaemagglutinin and analysis of cells in mitosis (*1*). In developing countries, there are only a few laboratories where chromosomes can be studied. It is therefore desirable to determine the maximum interval between the collection of blood in a remote area and its transportation to a genetic laboratory for a successful outcome in chromosomal analysis. Blood stored for a prolonged period at −80 °C in dimethyl sulfoxide or in culture medium has yielded ample mitotic cells on culture (*2*). Also bone marrow stored for 3 days at 4 °C has been successfully cultured (*3*). However, procedures that involve refrigeration are impractical in developing countries. Chromosomal aberrations have been successfully studied in cultures of heparinized whole blood stored at various temperatures for up to 168 hours and then exposed to radiation (*4*).

The present study had the following objectives: to examine the effect of storage of blood on the growth of lymphocytes; to evaluate whether preservation of blood in culture medium increased the rate of successful cultures for chromosomal analysis; and to recommend procedures for storing blood for such analysis in tropical countries.

Materials and methods

Samples of venous blood were drawn from 12 normal subjects (6 males and 6 females, aged 20–40 years). Six subjects were studied in summer and six in winter. A 5-ml sample of blood from each subject was collected in tubes containing 100 units of heparin[a] and divided equally into two aliquots: one containing heparin solution only (group A) and the other (group B) containing heparin, 5 ml of Eagle's minimum essential medium,[b] and 10% fetal calf serum.[c] Both aliquots were stored at room temperature (summer, 33 ± 2 °C; and winter, 20 ± 2 °C). Cultures were set up in duplicate on the day of collection (day 0), and after 48, 96, and 168 hours of storage. Briefly, 0.2 ml of blood from samples in group A, and 0.6 ml of blood mixed with culture medium from samples in group B, was added to 5 ml of Eagle's minimum essential medium containing Earle's salts,[b] with added glutamine, 10% fetal calf serum, and phytohaemagglutinin P.[d] After 72 hours the cultures were terminated and slides prepared. The slides were coded and screened independently by two investigators. From each culture, wherever possible, a minimum of 500 cells were analysed and the number of cells in mitosis counted.

Student's *t*-test was employed to compare the outcome under various storage conditions, using EPISTAT software.[e]

Results

Cultures from males and females. In terms of the frequencies of mitotic cells analysed during summer or winter and for storage in heparin or in culture medium (data not shown), there were no significant differences between the outcomes of cultures of blood from males or females. Hence, for further analysis the data for both sexes have been combined.

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[a] Biological Evans. [b] Gibco. [c] Seralab. [d] Difco.

[e] Available from: T.L. Gustafson, 1705 Gattis Road, Round Rock, TX 78664, USA.

Cultures in summer versus winter. The rate of successful culture (taken to be the availability of analysable metaphases) was greater during winter than summer (Table 1). For samples stored for 48 hours, all 12 study cultures were successful in both summer and winter. For the blood stored for 96 hours, 10 out of 12 cultures (83.3%) were successful in winter; in summer, the success rate was 7 out of 12 (58.3%). However, these differences were not statistically significant. After 168 hours' storage, 10 out of 12 cultures were successful in winter, while none was successful in summer.

The mean mitotic index (MI) values for different cultures in summer and winter are summarized in Table 1. These were greater in winter than in summer; however, the difference was not statistically significant ($P > 0.05$).

Storage in heparin versus culture medium. After storage for 48 or 96 hours (combined data for summer and winter, Table 2), the MI was slightly higher for blood stored in culture medium (group B) than for blood stored in heparin (group A). However, the difference was not statistically significant ($P > 0.05$).

Storage period. The combined data for groups A and B for summer and winter showed significant differences between cultures stored for 0 or 96 hours ($P = 0.026$) and between those stored for 48 or 96 hours ($P = 0.0047$).

Discussion

Schrörs has shown that storage of blood samples at 4 °C for 5 days produced a twofold increase in MI compared with that of fresh blood (*5*). Further storage up to day 15 resulted in a decrease in MI. Our data are not comparable with those reported by Schrörs, since we did not refrigerate the samples. In our study, blood collected in heparin as well as in culture medium could be stored successfully enough at room temperature to give metaphases for up to 2 days in summer and 4 days in winter. Sharma & Das observed haemolysis after 72 hours' storage at room temperature and after 48 hours' at 37 °C. No haemolysis was observed with any of our samples; however, we took the precaution of avoiding vigorously shaking them before initiating the culture.

Although storage in culture medium produced a marginally greater number of mitotic cells in both winter and summer for the storage periods tested in this study, the differences were not statistically significant. Since the culture medium has to be stored refrigerated, its use in remote areas where power supplies are erratic is not feasible. Our results show that, for subsequent cytogenetic analysis, storage of blood in heparin is as good as that in culture medium.

Table 1: **Success rate and mitotic index of blood stored for various times in summer or winter before being cultured**

	Storage time (hours)			
	0	48	96	168
No. of successful cultures				
Summer (n = 12)	12	12	7	–
Winter (n = 12)	12	12	10	10
Mitotic index (mean ± S.D.)				
Summer (n = 12)	4.06 ± 2.46	4.56 ± 2.14	2.57 ± 2.56	–
Winter (n = 12)	6.86 ± 2.16	6.03 ± 1.57	4.11 ± 2.35	3.14 ± 1.67

Table 2: **Mitotic index (mean ± S.D.) of blood stored in heparin or culture medium for different times, in summer and winter**

	Storage time (hours)			
	0	48	96	168
Group A, n = *12*[a]				
Summer + winter	5.46 ± 2.53	5.15 ± 2.38	2.49 ± 1.89	3.16 ± 1.88
Group B, n = *12*[b]				
Summer + winter	5.46 ± 2.54	5.43 ± 1.35	4.20 ± 2.69	3.11 ± 1.43

[a] Stored in heparin.
[b] Stored in culture medium.

We therefore recommend that blood samples collected in heparin in remote areas of tropical countries reach a genetic laboratory within 48 hours of collection in summer and within 96 hours in winter. Absolute asepsis is necessary during the collection of the blood, and precautions similar to those for withdrawing blood for bacterial cultures should be enforced. The blood samples can then be sent at ambient temperature to a genetic laboratory, taking care to avoid shaking them during the journey. Adherence to these guidelines can lead to successful chromosomal analysis on cultures of such blood.

Résumé

Combien de temps après avoir été recueilli du sang peut-il encore être cultivé pour étude chromosomique sous les tropiques?

Nous avons cherché à savoir si une analyse cytogénétique pouvait être réussie lorsque des échantillons de sang étaient transportés dans un laboratoire de génétique sans être réfrigérés. Des échantillons de sang périphérique de 12 sujets ont été recueillis et conservés à température ambiante pour examiner l'effet de la conservation sur la multiplication des lymphocytes, et également pour savoir si la conservation du sang dans un milieu de culture augmenterait la proportion de cultures réussies pour analyse chromosomique.

L'étude a été effectuée en été et en hiver pour savoir s'il existait des variations saisonnières dans la multiplication des lymphocytes après conservation. Aucune différence significative n'a été observée, pour des temps de conservation différents, entre le sang conservé sur héparine et celui conservé en milieu de culture. La proportion de cultures réussies et l'indice mitotique étaient plus élevés en hiver qu'en été, mais la différence n'était pas statistiquement significative. Cependant, une différence significative a été observée aussi bien en été qu'en hiver entre les indices mitotiques d'échantillons conservés pendant 0 ou 96 heures.

Il est recommandé que, dans les pays tropicaux, les échantillons de sang parviennent à un laboratoire de génétique dans les 48 heures qui suivent leur recueil en été, et dans les 96 heures en hiver. Pour les analyses cytogénétiques faites ultérieures, le sang conservé sur héparine est aussi satisfaisant que celui conservé en milieu de culture.

References

1. **Moorehead, P.S. et al.** Chromosome preparations of leukocytes cultured from human peripheral blood. *Experimental cell research*, **20**: 613–616 (1960).
2. **Nagone, Y. et al.** Preservation of whole blood for chromosome analysis. *Cytogenetics and cell genetics*, **33**: 254–255 (1982).
3. **Martin, P.K. & Rowley, J.D.** The effect of refrigeration of bone marrow and peripheral blood on cytogenetic analysis. In vitro *cellular and developmental biology*, **22**: 387–391 (1986).
4. **Sharma, T. & Das, B.C.** The effect of storage of blood on the yield of X-ray-induced chromosome aberrations and spontaneous sister chromatid exchanges. *International journal of radiation biology*, **45**: 151–158 (1984).
5. **Schrörs, E.** Transformation capacity, cell cycle and storage of human T lymphocytes: a comparison of two culture media. Paper presented at the *10th Meeting of the Gesellschaft für Umwelt-Mutationen, Erlangen, Germany, 25–27 March 1987*. See: *Mutation research*, **182**: 290 (1987) (Abstract No. 33).