THE USE OF THYMUS AND SPLEEN IN THE DEMONSTRATION OF CHROMOSOMES POSTMORTEM IN FOETUSES AND INFANTS

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INTRODUCTION

SINCE the majority of severe malformations in the human are fatal either at or shortly after birth, investigation of the karyotypes of still births and neonatal deaths must now form an integral part of perinatal pathology.

The culture of human fibroblasts has been, until recently, the recognised procedure for the investigation of chromosomes postmortem (Lejeune, Turpin and Gautier, 1959; Harnden, 1960). The disadvantage of this method lies in the high degree of technical skill required, the time consuming nature of the procedure and the necessity, if used on any scale, for proper tissue culture facilities.

The purpose of this communication is to demonstrate that the use of thymus and spleen as a source of cells for culture offers, in certain circumstances, a simpler alternative.

MATERIALS AND METHOD

T.C.M. 199.—As supplied by Glaxo Ltd.

Serum.—Pooled human serum group AB inactivated for 1 hr. at 56°

Colcemid.—1 mg./ml. (Ciba) diluted 1 : 6 with distilled water.

Hypotonic solution.—Phsophate buffered saline diluted 1 : 4 with distilled water.

Fixative.—Absolute ethanol (3 parts) + glacial acetic acid (1 part).

Glassware.—All glassware is cleaned with Haemosol and rinsed thoroughly with deionized water. It is then coated with silicone (Repelcote, Hopkins and Williams); it is essential that any traces of acid left after siliconing are removed by rinsing in deionized water. The glassware is then sterilized in a hot air oven.

Plastic containers.—Disposable sterile plastic universal containers (20 ml.) supplied by Henleys Medical Supplies Ltd.

Phytohaemagglutinin.—As supplied by Burroughs Wellcome Ltd.

The thymus or spleen, from the foetus or infant is removed as aseptically as possible and placed in a sterile container of T.C.M. 199. Until further processing can be carried out, storage is maintained either in a refrigerator at 4° or where this is not readily available in a vacuum flask containing ice.

The tissue is minced, with pointed scissors and the resultant mush transferred to a conical flask containing an excess of T.C.M. + 20 per cent human AB serum. The amount of this growth medium varies with the amount of tissue available. The flask is shaken by hand for several minutes and allowed to stand until the tissue fragments have settled, generally 2–3 min. The supernatant is decanted in aliquots of 10 ml. into sterile universal bottles or sterile plastic containers.

The culture is then set up by the following method. Phytohaemagglutinin (0·2 ml.) is added to each 10 ml. of culture. The bottles are incubated at 37° for 70 hr. after which time 0·2 ml. of Colcemid is added to each. After a further 2 hr. incubation the cultures are transferred to 15 ml. conical centrifuge tubes and spun at 500 r.p.m. for 5 min. at 4°. The supernatant is poured off, and replaced by hypotonic solution prewarmed to 37°. The cells are suspended in this hypotonic solution and left for 1 hr. at 37° and then centrifuged

for 5 min. at 500 r.p.m. The supernatant is decanted leaving one drop in which the cells are resuspended by gentle agitation. Fixative is now added drop by drop, agitating between each drop, until a total volume of 2 ml. is reached. The volume is then made up to 5 ml. and the tube centrifuged as before. The supernatant is again discarded except for one drop and in this the cells are resuspended. Fresh fixative is added, slowly with agitation, to a total volume of 5 ml. and the cells left in this fixative for 10–15 min. The cells are spun down and resuspended in a few drops of fixative. Smears are made by dropping 1–3 drops of cell suspension on to clean slides, previously cooled in the refrigerator, draining off the surplus fluid and drying the smear in a spirit lamp flame.

RESULTS AND DISCUSSION

Cultures of thymus and spleen have been carried out on a wide range of foetuses and infant.

In the foetus the gestational age varied from 19–40 weeks. Below 19 weeks gestation thymus and spleen were in themselves too small to yield sufficient cells for culture. However, on the basis of a limited number of cases it has appeared that successful cultures can be obtained from a mixed culture of the two glands. Although in the smaller foetuses explant cultures of pericardium and lung can yield satisfactory chromsome preparations in 3–5 days, thymus and spleen as a combined culture is always a useful adjunct.

In foetuses from 20–40 weeks gestation and in infancy, satisfactory cultures can be obtained from the thymus and/or spleen. In infants we have obtained satisfactory cultures up to the age of 1 year.

Several factors affect the culture results.

Primary infection.—The tissues are more likely to be infected in older infants, the spleen more so than the thymus.

Death-necropsy interval.—The longest death-necropsy interval after which satisfactory cultures have been obtained was 76 hr. Generally the most satisfactory results have been obtained within 48 hr. of death. In connection with this, refrigeration as soon after death as possible appears to have been of considerable advantage. In the case of the macerated foetus, we have never obtained a satisfactory culture.

Storage of cell suspensions prior to incubation.—When it has proved inconvenient to set up the cultures immediately after necropsy we have stored the cell suspension at 4° for up to 15 hr. Compared with control cultures set up immediately after necropsy, refrigerated cell suspensions show fewer mitoses.

Concentration of cell suspension.—Since in dealing with necropsy material many factors such as the unknown percentage of viable cells affect the number of mitoses obtained in culture, it has proved impossible to define what is the optimum concentration of cells. For this reason we have generally used a much higher concentration of cells than that recommended by Baker and Atkins (1963).

This method of culturing thymus and spleen has in our experience provided a definite diagnosis of an abnormal karyotype in a number of cases found at post-mortem to have multiple congenital abnormalities (Bain and Gauld, 1963). It has likewise failed to show any recognisable chromosomal abnormality in various other congenital abnormalities inconsistent with survival, *e.g.* absence of trachea.

Illustrations (Figs. 1 and 2) have been chosen to illustrate the quality of metaphase chromosome preparations which can be obtained with this technique. We feel that the wider use of cultures of thymus and spleen should enable chromosomes studies to be undertaken postmortem in foetuses and infants as readily as

they can be undertaken in the living by the use of the blood culture technique of Moorhead, Nowell, Mellman, Balipps and Hungerford (1960).

SUMMARY

A technical method has been described whereby satisfactory chromosome preparations may be obtained in foetuses and infants postmortem. It has been shown that the application of this technique is limited solely by the state of the tissue available. The relative simplicity of the procedure should enable it to be undertaken in the average hospital laboratory.

Satisfactory cultures have been obtained in foetuses from 19–20 weeks gestation onwards and in infants up to the age of 1 yr.

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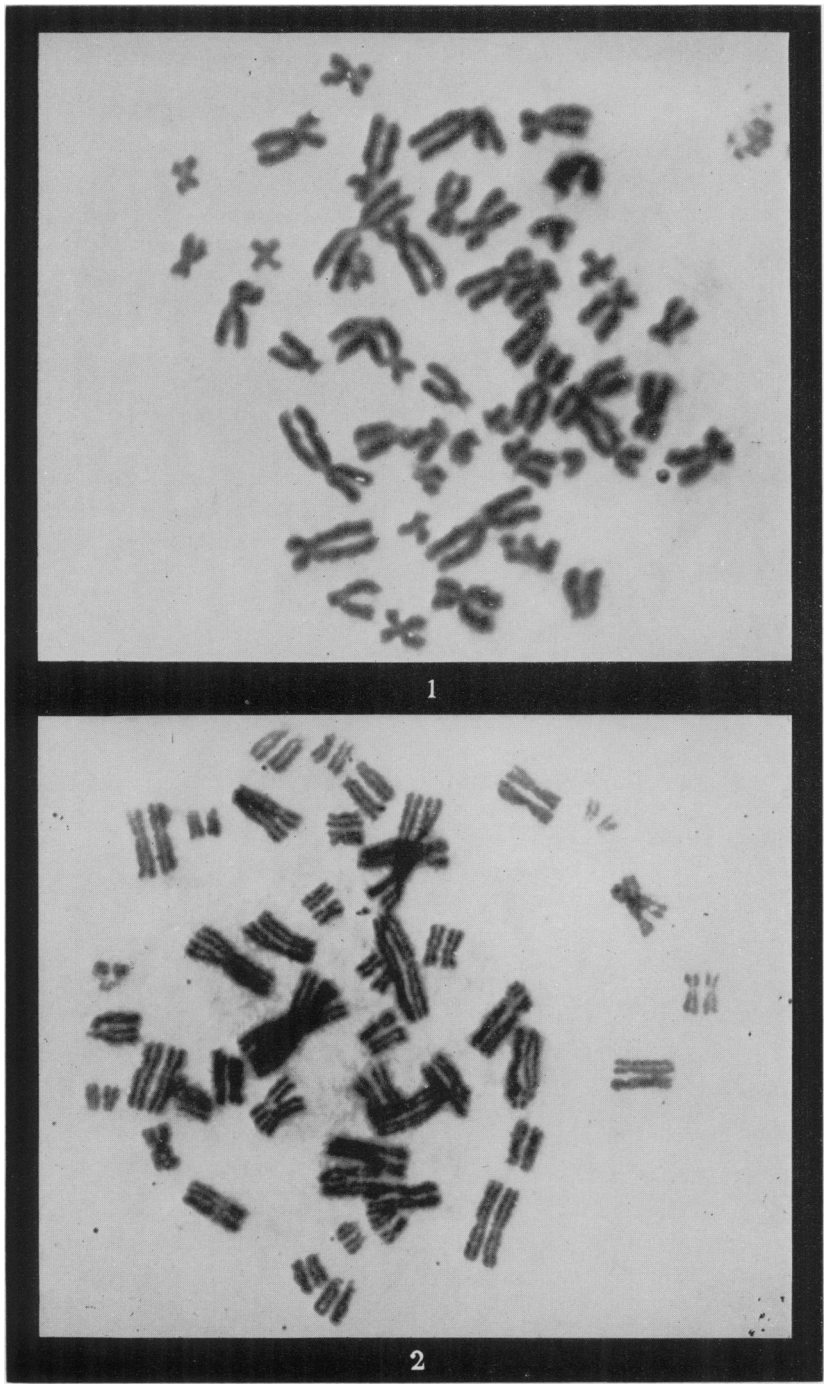
REFERENCES

BAIN, A. D. AND GAULD, I. K.—(1963) *Lancet*, ii, 304.
BAKER, M. C. AND ATKINS, N. B.—(1963) *Ibid.*, i, 1164.
HARNDEN, D. G.—(1960) *Brit. J. exp. Path.*, **41**, 31.
LEJEUNE, M. J., TURPIN, R. AND GAUTIER, M.—(1960) *Rev. Franc. Etudes clin biol.*, **5**, 406.
MOORHEAD, P. S., NOWELL, P. C., MELLMAN, W. J., BATIPPS, D. M. AND HUNGERFORD, D. A.—(1960) *Exp. Cell Res.*, **20**, 613.

EXPLANATION OF PLATE.

FIG. 1.—Chromosomes showing Trisomy 17 from the thymus culture of a 10 month old infant.
FIG. 2.—Chromosomes showing endoreduplication from the spleen culture of a 30 week gestation foetus.



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