Preliminary Communications

Chromosomes in Short-term Cultures of Lymphoid Tissue from Patients with Reticulosis

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We have recently described a method for the short-term culture of lymphoid tissue for chromosome studies (Baker and Atkin, 1963). Basically, this is a modification of the peripheral leucocyte culture technique of Moorhead *et al.* (1960). We have now applied this technique to lymph-node tissue from two patients with lymphosarcoma and two with Hodgkin's disease. In all these cases (which were untreated at the time of biopsy) chromosomal abnormalities were found. In contrast, no abnormalities were found in lymph-node tissue from a patient with untreated chronic lymphatic leukaemia or in non-malignant lymph nodes from 12 patients.

Brief details of these cases are given below. Wherever possible, a number of cultures were set up for varying periods of time, with and without the addition of phytohaemagglutinin M (Difco). Most of the non-malignant lymph nodes grew only if phytohaemagglutinin was present in the culture medium, but several of the lymph nodes from patients with reticulosis grew equally well without phytohaemagglutinin. However, it is not possible to be dogmatic about the effect of phytohaemagglutinin or the optimum duration of culture.

CASE REPORTS

Case 1: Lymphosarcoma.—Woman aged 51. Tissue from a cervical lymph node was cultured for one, two, and three days. There were plentiful mitoses in two-day and three-day cultures, and slightly fewer in one-day cultures. There was no difference between

cultures with and without phytohaemagglutinin. In all the cultures two classes of metaphases were present: (1) with normal karyotypes, and (2) with abnormal karyotypes (modal number, 46) including an easily recognizable marker chromosome (Fig. 1). Of the metaphases 50–80% had abnormal karyotypes. In *uncultured* material (pretreated by short incubation in the presence of Colcemid and hypotonic saline) there were fewer well-spread metaphases, but it could be seen that about half the metaphases had the marker chromosome. A peripheral leucocyte culture from this patient showed no chromosomal abnormality.

Case 2: Lymphosarcoma.—Woman aged 57. A total of 427 countable metaphases were found in cultured material from a cervical lymph node. The majority had 46 chromosomes and an apparently normal karyotype; in addition there were hyperdiploid cells with 47–51 chromosomes (most frequently 48 or 49). The proportion of hyperdiploid cells varied in different cultures. One-day cultures showed good growth with and without phytohaemagglutinin, with approximately equal numbers of diploid and hyperdiploid cells. Two-, three-, and four-day cultures with phytohaemagglutinin showed good growth with a decreasing proportion of hyperdiploid cells (24%, 9%, and 7% respectively); corresponding cultures without phytohaemagglutinin showed virtually no growth. Karyotype analysis of hyperdiploid cells showed extra medium-sized or large submetacentric chromosomes (Fig. 2).

Case 3: Hodgkin's Disease.—Woman aged 53. Cultured lymph-node tissue contained (1) cells with 46 chromosomes and apparently normal karyotypes and (2) hypertriploid cells (mostly with 70–75 chromosomes). There was moderate growth in one-day cultures with and without phytohaemagglutinin. In two-, three-, and four-day cultures there was good growth with and without phytohaemagglutinin, but cultures without phytohaemagglutinin had relatively more hypertriploid cells. A five-day culture with phytohaemagglutinin had mainly cells with normal karyotypes and only a few hypertriploid cells, and a six-day culture with phytohaemagglutinin (resuspended in fresh medium on the fifth day) had only normal metaphases.

Case 4: Hodgkin's Disease.—Man aged 30. Analysis of cultured tissue from an axillary lymph node has not yet been completed, but three-day cultures with and without phytohaemagglutinin have yielded a bimodal distribution with (1) diploid cells (70–80%) and (2) hyperdiploid cells (20–30%) with 48 or 49 chromosomes.

Case 5: Chronic Lymphatic Leukaemia.—Man aged 63. Tissue from a cervical lymph node was cultured. One-day to five-day cultures with phytohaemagglutinin showed good growth, maximal on the fourth day; but of the parallel cultures not containing phytohaemagglutinin only one (a five-day culture) showed growth. No chromosomal abnormality was found in any of the cultures.

Our findings on the lymph-node material from the patient with chronic lymphatic leukaemia are in agreement with reported findings on blood cultures and direct marrow preparations in this condition, which have so far failed to reveal any consistent chromosome abnormality (although it is still possible that there are significant chromosome changes which have yet to be uncovered) (Nowell and Hungerford, 1964).

Apart from the leukaemias, chromosome studies in the reticuloses are relatively scanty. There have, however, been reports of abnormal karyotypes in lymphosarcoma (Tjio *et al.*, 1963; Sandberg *et al.*, 1964), Hodgkin's disease (Ricci *et al.*, 1962; Spriggs and Boddington, 1962; Galan *et al.*, 1963), and myelomatosis (Bottura, 1963; Castoldi

FIG. 1.—Case 1. Lymphosarcoma. Abnormal karyotype (46 chromosomes), including the marker chromosome (arrow).

FIG. 2.—Case 2. Lymphosarcoma. Karyotype of cell with 50 chromosomes.

et al., 1963; Lewis *et al.*, 1963). These reports, together with the findings in the four cases cited above, may be added to the growing weight of evidence that in malignant disease as a whole cell-lines are present which, at least at the time when the disease is manifest clinically, very often have grossly abnormal chromosome complements.

We have shown that chromosomal abnormalities in lymph nodes can be revealed in short-term cultures. Quite probably, techniques not involving culture procedures may reveal a higher proportion of abnormal cells. However, the mitotic index may be low in "direct" preparations, in comparison with cultures where mitoses can be accumulated by the application of colchicine over a period of several hours; thus culture techniques may have practical advantages.

The non-malignant glands that we have examined include an axillary gland showing reactive hyperplasia, removed as a possible recurrence of a reticulum-cell sarcoma for which the patient (a woman aged 24) had had radiotherapy to the opposite axilla eight years previously. Growth was obtained both with and without phytohaemagglutinin; no chromosomal abnormality was found. Six apparently uninvaded glands removed at operation for carcinoma of the cervix uteri (two cases), corpus uteri (two cases), vulva, and penis showed good growth in cultures of two to five days' duration to which phytohaemagglutinin had been added, but no growth in parallel cultures without phytohaemagglutinin. Three-day cultures of a lymph node showing active tuberculosis yielded a moderate number of metaphases with phytohaemagglutinin but none without. Good growth was also obtained from enlarged glands removed from four other patients for diagnostic purposes; on histological examination these glands showed only chronic inflammatory changes. Three showed good growth only in the presence of phytohaemagglutinin, but the fourth grew equally well with and without phytohaemagglutinin. No significant departure from the normal diploid karyotype was found in any of these cases, apart from changes attributable to ionizing radiation (some of the metaphases showing chromosome breakage and reunion) in

three of the patients with uterine carcinoma who had completed a course of radium treatment two to six weeks previously.

A more detailed report on these cases is being prepared.

SUMMARY

Chromosomal abnormalities were found in short-term cultures of lymph-node tissue from two patients with lymphosarcoma and two with Hodgkin's disease; on the other hand, no abnormalities were found in cultures of a lymph node from a patient with chronic lymphatic leukaemia or of non-malignant lymph nodes from 12 patients. In some instances (including most of the non-malignant glands) growth was obtained only in the presence of phytohaemagglutinin.

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Medical Memoranda

Gastroschisis

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In the literature there is much confusion concerning the classification of congenital abnormalities of the anterior abdominal wall. Moore and Stokes (1953) have helped considerably to clarify the position. They clearly distinguish between three groups of congenital eviscerations. (1) Omphalocele (exomphalos), which is an umbilical-cord anomaly and the most common. The essential features consist of herniation of the viscera into the base of the umbilical cord, a covering membranous sac or the presence of ruptured remnants, and insertion of the umbilical cord into the sac. (2) Intussusception of the ileum through a persistent omphalo-mesenteric duct. (3) Gastroschisis—this word, freely translated from the Greek, means "belly cleft." The principal features of this rare condition are: the defect in the abdominal wall is extra-umbilical, there is no membranous sac covering the eviscerated mass of intestines, and the umbilical cord is inserted normally into the abdominal wall and is not involved in the evisceration.

Duhamel (1963), in a paper on the embryology of the abdominal wall, also clearly distinguishes between gastroschisis and omphalocele.

The defect in the abdominal wall in gastroschisis is on the right side of the umbilicus in 80% of cases (Moore, 1963) and usually the small intestine and part of the large intestine and stomach are eviscerated. Malrotation is said to be common. Bernstein (1940) first described the appearance of the intestine as markedly thickened, oedematous, darkly injected, and usually matted together with gelatinous material.

CASE HISTORY

A male child was admitted on 21 January 1964 six hours after delivery in another hospital. The infant was four weeks premature and weighed 4 lb. 4 oz. (1,930 g.). A previous pregnancy, in 1959, resulted in a male child which was alive and well. The present pregnancy and labour were uneventful apart from slight foetal distress during the second stage of labour.

On admission the general condition of the infant was good. There was a defect 2.5 by 1 cm. to the right of the umbilicus through which the whole of the small intestine and part of the stomach