able saving of time and the ease with which technicians screened the sections.

The false-positive rate is no higher than with the Papanicolaou smear method. Review of the final diagnosis in these cases shows that the errors of judgment occur in essentially similar conditions. They correspond to the false-positive cases analysed by Umiker (1957) in that they include chronic respiratory infection and other conditions associated with squamous metaplasia and hyperplasia of columnar epithelium.

At present the sputa submitted are almost exclusively from patients presenting with symptoms of respiratory-tract disease. According to Long (1963) it is in the early stages of bronchial carcinoma, before the bronchus is obstructed, that malignant cells are most likely to be found in the sputum. Cytodiagnosis is therefore most rewarding in precisely those cases where the chance of cure is greatest. It would be a logical extension of the service to examine sputa from symptom-free members of the population at risk who can provide specimens of true sputum, not merely saliva. A limiting factor is of course laboratory space and technicians' time. In this respect the three-step paraffin-section routine can contribute to expanding the scope of sputum cytodiagnosis.

Summary

A comparative study of two methods of cytodiagnosis of sputum is reported. These are (1) paraffin section following fixation according to Sirtori (1957), and (2) the routine Papanicolaou smear.

The results shows a clear superiority in the paraffin-section method over the Papanicolaou smear. Out of the 424 patients examined, 135 had bronchial carcinoma: of these 64% were correctly diagnosed by the paraffin-section method (one section only). Examination of two additional deeper sections improved this figure to 80%. By contrast the Papanicolaou smear (two smears) achieved an accuracy of only 55%.

Various aspects of the methods are discussed; and the conclusions are that the paraffin-section method, though longer in processing, is quicker in examining and reporting and gives clear cellular morphology commensurate with easy identification and accurate diagnosis.

The paraffin-section method is recommended for use in a busy laboratory. Further, it could well be an acceptable technique for use in large-scale surveys of the population at risk.

I wish to thank Dr. John C. Dick for helpful advice; Mr. John Sandison for screening specimens; and the technical staff of the pathology department of Stobhill General Hospital. I am grateful to Mr. P. S. Waldie for the photomicrographs and to Miss Helen E. Scott for secretarial assistance.

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PHYTOHÆMAGGLUTININ IN RELATION TO BURKITT'S TUMOUR

(African Lymphoma)

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Two laboratory strains of Burkitt's lymphoma have recently been isolated in Africa (Epstein and Barr 1964, Pulvertaft 1964). This paper presents evidence relating the Burkitt cell to lymphocytes which have been stimulated by bean extract (phytohæmagglutinin).

Technique

The collagen technique of Ehrman and Gey (1956) was used. Phase-contrast with collagen is slightly inferior to glass, and high powers cannot be used. Permanent preparations can be made by Wigglesworth's osmic acid/ethyl gallate method (Wigglesworth 1957).

The lymphocytes were in heparinised venous blood treated with phytohæmagglutinin (Wellcome).

Results

Burkitt cells are readily distinguishable from normal lymphocytes in size, granularity, lack of motility, rapid in-vitro lysis, and frequency of mitotic figures. A feature not previously recorded is nuclear folding: trefoil forms are common in freshly isolated cells, and bi-nuclear forms may be seen.

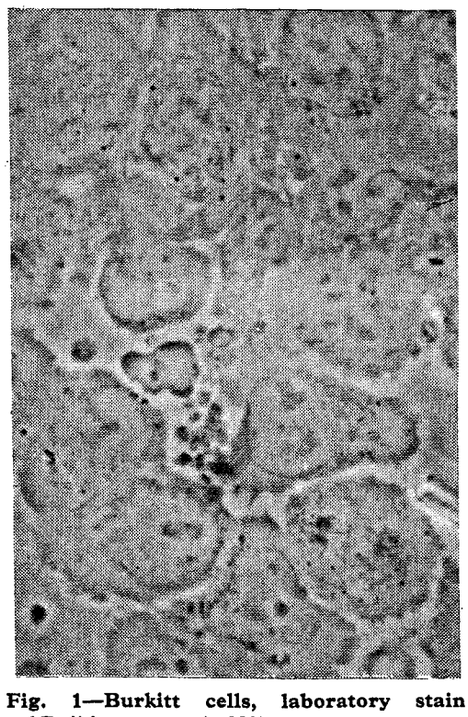

Fig. 1—Burkitt cells, laboratory stain 'Raji', on agar (×900).

The commonest source of the cells has been biopsy material from the jaw or cheek, but they have been found in many other places. In two cases where the cerebrospinal fluid showed pleocytosis the deposit was entirely of Burkitt cells. In two cases of gross ascites blood-stained fluid was obtained, with enormous numbers of Burkitt cells: this source has been neglected. Other sources have been lymph-nodes, stomach wall, breast, subcutaneous tissues, and thyroid.

Control cases included myeloid, monocytic, and lymphatic leukæmias, Hodgkin's disease, reticulosarcoma, multiple myelomatosis, adamantinoma and dental cyst, synovioma, osteogenic sarcoma, glioma, salivary tumours, Kaposi's tumour, and many carcinomata and sarcomata of soft tissues. All material was examined by tissue culture, and in no case were Burkitt cells seen. In Nigeria, neoplastic tissues, as well as thyroid tissue from goitres, show far fewer lymphocytes than similar material in London.

When grown in fluid cultures, Burkitt cells and phytohæmagglutinin-treated (transformed) lymphocytes grow in an identical way. They do not adhere to glass, and they grow only as a sediment, in lenticular aggregates, often with a central hollow, as in a doughnut. These aggregates are readily dispersed by agitation.

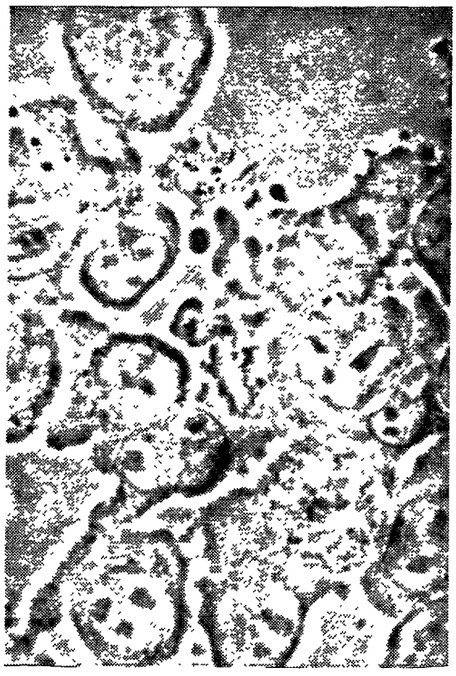

Fig. 2—Transformed lymphocytes on agar (×900).

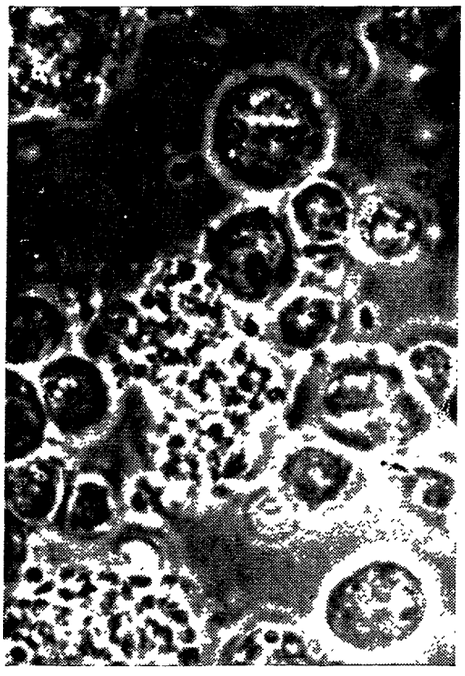

Fig. 3—Transformed lymphocytes, glass preparation, showing platelet aggregates (×900).

Fig. 4—Burkitt-cell colony on collagen, 48 hours. Laboratory stain 'Raji' (×90).

When the aggregates are squashed between coverslip and slide, the cells are indistinguishable (figs. 1, 2). But aggregates of transformed lymphocytes contain masses of aggregated platelets also (fig. 3). Time-lapse cinemicrography indicates that while at first only a few lymphocytes are transformed, in a week they all assume the new form.

Both Burkitt cells and transformed lymphocytes adhere immediately to collagen. Colonies of roughly circular outline are formed in both cases, from which serpiginous cells migrate very slowly. Collagen is not lysed during a 14-day period of observation (figs. 4, 5).

Burkitt cells assume very bizarre forms on prolonged culture (fig. 6). A shape like a spermatozoon with a long tail is common (fig. 7); elongated spindles are frequently formed, with long straight fine processes at each end of the nucleus.

The colonial form is identical in Burkitt cells and transformed lymphocytes, and is unique to these two cell types. Neither fibroblasts nor carcinoma-cell colonies are in any way comparable.

Discussion

Histologically, Burkitt tissue was recognised by its first students (O'Conor and Davies 1960) as a malignant lymphoma. In sections it is difficult to distinguish with certainty from retinoblastoma and neuroblastoma. But when embedded in agar and ester wax, and stained by Wigglesworth's method, its granular nature is often evident (fig. 8).

Preliminary tissue cultures of retinoblastoma indicate in three cases that the cells cohere in chains, which later often become circles (fig. 9). Neuroblastoma cells cultured, in Ibadan and in London, cohere in faceted

Fig. 5—Transformed lymphocyte colony on collagen, 48 hours (×90).

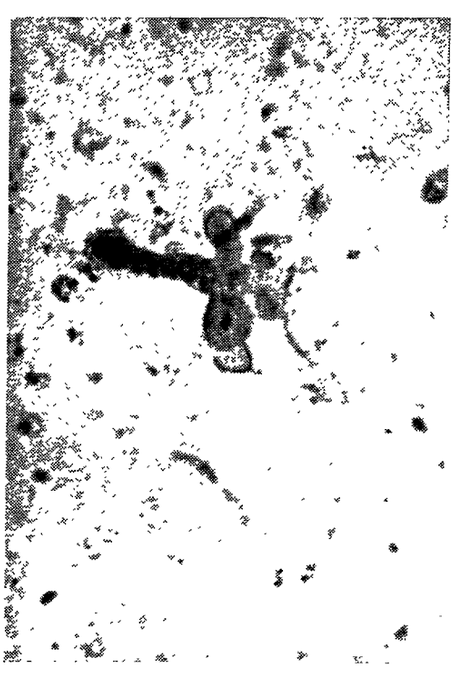

Fig. 6—Burkitt cell on collagen, stained Wigglesworth (×900).

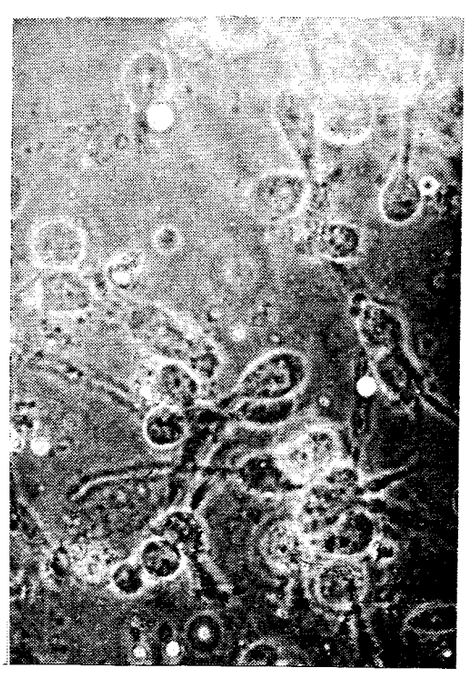

Fig. 7—Burkitt cells on collagen (×900).

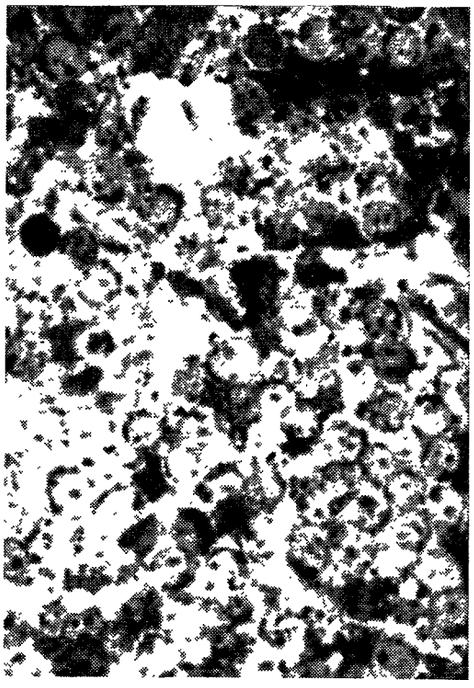

Fig. 8—Burkitt's tumour, deposit in scalp Agar and ester-wax embedding, stained Wigglesworth (×450).

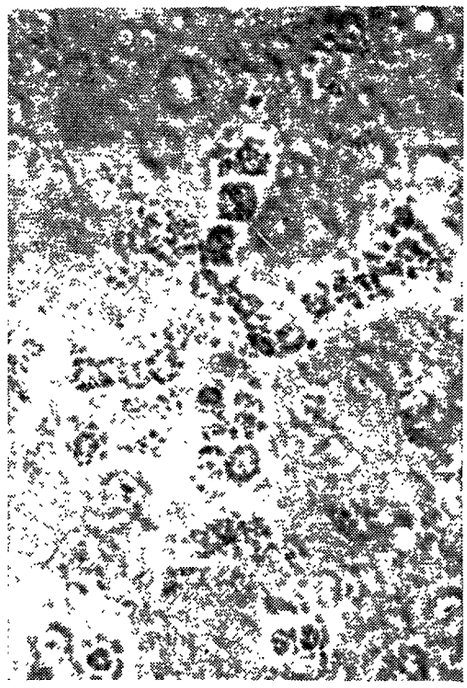

Fig. 9—Retinoblastoma, orbit. 4-day culture on collagen (×450).

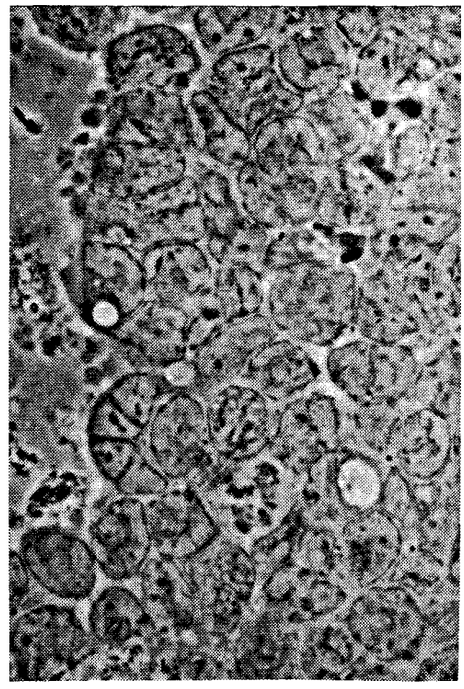

Fig. 10—Neuroblastoma, deposit in humerus. 4-day culture on agar (×900).

sheets (fig. 10). Both retinoblastoma and neuroblastoma cells adhere to glass. They can thus be distinguished cytologically from Burkitt cells.

The evidence here appears to identify the Burkitt cells with transformed lymphocytes. It provides no evidence of the transforming agent.

Lymphocytes from normal persons retain their normal chromosome pattern after stimulation by phytohæmagglutin. A preliminary report on the chromosome pattern in Burkitt's lymphoma (Jacobs et al. 1963) showed that it appeared normal in four cases, and abnormal in six. Investigations on these lines are in progress in this laboratory. Results are as yet inconclusive, but some comments on the possible relationship between a diet of beans and lymphocyte stimulation may be made.

In view of the shortage of animal protein in Africa, pulses of all sorts comprise a very significant proportion of the diet. In schools I have visited in Western Nigeria the midday meal usually consists of beans.

In many parts of the world " bean milk " is given to children as a substitute for animal milk. Cow's milk is not available in Western Nigeria; and in spite of the enormous numbers of goats, their milk is never consumed. In Western Nigeria, at least, beans are fed from birth to twins, premature infants, and babies born by breech presentation. On the other hand, many children dislike beans, owing to the prevalence of weevil spoilage. Pap made from contaminated beans contains adult and larval weevils, excreta, and in this moist tropical climate a great variety of moulds.

At present the most popular view of the cause of the tumour (Burkitt 1964) is that it may be a viral disease, spread by an insect vector. Its distribution in Africa in relation to rainfall and altitude is often stressed: it is unknown north of Kano. However, rainfall and altitude affect many factors besides insects, foodstuffs among them. In fact, north of Kano beans are not consumed.

The suggestion that consumption of beans might be associated with malignancy appears at first sight to be absurd. They are eaten in enormous quantities throughout the world, and it is unlikely that such an association, if it exists, should have passed unrecognised. Moreover, while Burkitt's tumour is not confined to Africa, it is elsewhere very rare, although beans are consumed in Asia more generally than in Africa. The immediate judgment is therefore that the Burkitt tumour manifests a reaction of the lymphocyte to a stimulating agent whereby it demonstrates its affinity with the transformed lymphocyte, and that there is no other relation. Analogy may be drawn with the close resemblance between a healing bone fracture and an osteogenic sarcoma. They are often tragically confused, and indeed trauma may initiate a sarcoma, particularly in childhood. But contemporary opinion holds that some agent additional to trauma must be involved.

The most we can do at present is consider what special conditions might apply to Africa. In the first place, the variety of bean consumed might be studied. Gross spoilage by insects and moulds must be remembered, as must local practices which may involve feeding children on beans at an unusually early age. An interesting feature is that Burkitt cells have not been found in human fœtal tissue cultures.

Summary

Evidence is presented showing a close similarity between Burkitt cells and human lymphocytes stimulated by bean extract. Their appearances are compared with those of retinoblastoma and neuroblastoma in tissue culture. In view of local traditions involving the feeding of infants on bean pap, the possibility of a relation between Burkitt's lymphoma and a diet of beans should not be neglected.

This work was financed entirely by a generous grant from the British Empire Cancer Campaign fund. All the technical work was done by my wife, Isobel Pulvertaft, in full-time and essential cooperation.

My thanks are due to Prof. G. M. Edington for hospitality and continued advice, and to all my colleagues in the department of pathology of the University of Ibadan. In particular, I wish to thank the surgeons and radiologists at University College Hospital, Ibadan, for their cooperation and tolerance, and the medical illustration unit for all prints.

References at foot of next page

IDIOPATHIC HÆMOCHROMATOSIS IN MENSTRUATING WOMEN

A Family Study, Including the Use of Diethylene Triamine Penta-acetic Acid

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IDIOPATHIC hæmochromatosis in young menstruating women is extremely rare; to our knowledge, it has been reported only five times (Roth and Gordon 1959, King 1962, Wasi and Block 1962, Milliken and Brown 1963, Luomanmäki and Helin 1964). This report is based on a study of a family which includes two such patients and five other women. In some members of the family the important problem of early accurate diagnosis of hæmochromatosis arises. A test recently investigated by Walsh et al. (1963), depending on the urinary excretion of iron after infusion of diethylene triamine penta-acetic acid (D.T.P.A.), was therefore employed, as well as hepatic biopsy and measurement of serum-iron.

Methods

Serum-iron and Urinary Iron

The serum-iron content was estimated by the method of Trinder (1956), and the iron-binding capacity by the method of Ramsay (1957). Urinary iron was estimated by digesting 2 ml. of urine with concentrated sulphuric acid, adding sodium hydroxide to attain a pH of 4·0, and then employing α, α-dipyridyl for colorimetric measurement. All apparatus used for these measurements, and for collection of blood samples and urine specimens, was rendered iron-free by soaking in normal hydrochloric acid solution for 30 minutes and rinsing with iron-free-glass distilled water.

D.T.P.A. Test

No dietary restrictions were made. The patient emptied the bladder, and the urine was discarded. A solution containing 1 g. of D.T.P.A. in 500 ml. of physiological saline was then infused intravenously over exactly 1 hour. All urine passed during the next 6 hours was collected, including that passed exactly 6 hours after the infusion was started.

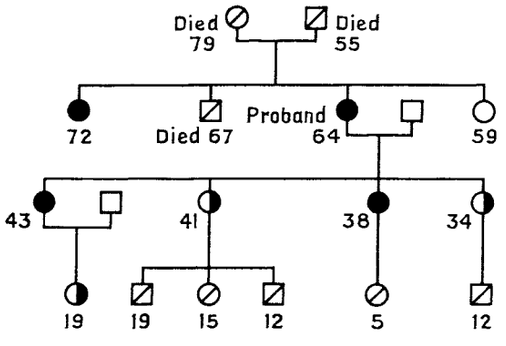
Case-reports

The *proband* presented in 1957, at the age of 57, with increasing lethargy, weight-loss, polyuria, and polydipsia for 8 months. Hepatomegaly and a generalised brownish skin pigmentation were noted. A glucose-tolerance test revealed diabetes mellitus, and a skin-biopsy specimen showed the presence of hæmosiderin and excess melanin in the basal layers of the epidermis. The serum-iron was 254 μg. per 100 ml., and the total iron-binding capacity (T.I.B.C.) was 95% saturated. Her menses were irregular and sparse until the age of 40, when she had heavy monthly losses until the menopause at the age of 45. From the age of 50 she had frequent epistaxes, and she was given a month's iron therapy by mouth for anæmia. She received no blood-transfusion or other iron therapy. The patient's diabetic state was controlled with insulin. A total of approximately 6·25 g. of iron was removed by venesections between 1957 and 1960, when the venesections were discontinued because they precipitated anginal pain. She survived a myocardial infarct in 1962. Her serum-iron is still raised (230 μg. per 100 ml., T.I.B.C. 280 μg. per 100 ml.).

Her mother died from heart-disease at 79, and her father died from an accident in his 50s. One maternal aunt had diabetes mellitus. Her brother had heart-disease and tuberculosis, and he died aged 67. She has two sisters. The elder is discussed in detail below. The *younger sister* shows no clinical abnormality, and her serum-iron and post prandial blood-sugar are normal. She had a hysterectomy at the age of 39 for menorrhagia. The proband's husband shows no clinical evidence of hæmochromatosis, and consanguinity is denied. We can find no environmental factor likely to cause or aggravate the disease in the family. All the patients are teetotal. The family is of European extraction.

The *elder sister* of the proband was examined as part of the family survey. She is a frail elderly woman with generalised brownish skin pigmentation, greatest on the exposed surfaces. The liver is palpable 10 cm. below the right costal margin, its edge is very firm, and the left lobe is prominent. Her serum-iron is 220 μg. per 100 ml. and the T.I.B.C. is 70% saturated. Her menstrual history is normal, and the menopause was at 50 years. Whereas the diagnosis of hæmochromatosis has not been proved by liver biopsy in this instance, it is considered almost certain in view of the clinical findings, the skin biopsy picture and the result of the D.T.P.A. test (see table).

The *eldest daughter* of the proband presented in March, 1963, aged 43, with recurrent furunculosis and symptoms of diabetes mellitus. She had a diffuse greyish-brown pigmentation of the skin, and a slightly enlarged firm liver. Investigations (see table) confirmed the diagnosis of idiopathic hæmochromatosis with cirrhosis and grade-4 iron deposition (grading as used by Scheuer et al. 1962). She has never received iron therapy or a blood-transfusion. Since the menarche at 13 years of age she has had regular 28-day menstrual cycles, using an average of twenty menstrual pads over the 5-day period. For the past 2 years her menses have been much heavier and somewhat irregular, at times continuing for up to 3 weeks. Also during these 2 years she has had repeated epistaxes, usually when menstruating. For the past 9 months she has had regular fortnightly venesections, each of 500 ml. Her hæmoglobin is now 12·8 g. per 100 ml., and her serum-iron is 220 μg. per 100 ml. Her diabetic state has been controlled with insulin. In December, 1963, her 6-hour urinary iron excretion during the D.T.P.A. test was 7·2 mg., indicating grossly excessive iron stores (Walsh et al. 1963).



The family tree.

Squares represent males, and circles represent females.

Blocked circles indicate complete disease, and half-blocked circles indicate grade-2 iron deposition on liver biopsy specimens.

Open squares and circles indicate normal members.

Crossed squares and circles indicate those members not examined.

Numerals indicate present ages or age at death.

The *third daughter* of the proband was found on routine investigation to have hæmochromatosis with asymptomatic diabetes mellitus (see table). She is a brunette with fair skin, and her liver is not palpable. Her periods began at the age of 14, they occur

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