correlation. Of the 95 patients, 91 had lymph-node involvement at death, widespread in 69 and more limited in 22. Of the four patients without node involvement one had lung infiltration persisting after treatment and three died with an acute terminal illness. Of these three, two had involvement of liver and spleen, but in one no sign of any tumour manifestation of Hodgkin's disease was left, and virtually no lymphatic tissue, the patient having been overwhelmed by infection (Smithers, 1959). This is the most nearly complete example of lymphoid depletion seen in our group.

Thymus

In any study of tumours involving what Medawar has called "immunologically competent cells," the thymus must be of special interest. Our three most likely cases of primary Hodgkin's disease of the thymus occurred in two women aged 23 and 33 and a man aged 24. All three had a thymectomy performed as their first treatment, one had a chain of nodes running up into the neck, but there was no sign of extension beyond the thymus in the other two. Thymic involvement was mentioned only rarely in our postmortem reports. Many

of the patients on whom necropsy was performed had mediastinal tumours, however, and it may have been difficult in some to know if the thymus was involved. Intrathoracic involvement outside the lungs was reported radiologically before treatment in 38% of our staged cases in the period 1945-64 (Table IV). Thymic involvement, primary or perhaps secondary, was confirmed in three patients, was suspected in another eight during life, was found at necropsy in two, and may well have been present in others. A few patients in whom a diagnosis of granulomatous thymoma (thymoma of Lowenhaupt) was made have not been included in this series owing to the dispute about its possible relation to Hodgkin's disease, which is not yet resolved.

TABLE IV.—*Pretreatment Radiological Evidence of Intrathoracic Tumour Outside the Lungs in 271 Patients with Staged Hodgkin's Disease Seen at the Royal Marsden Hospital, 1945-64*

Stage	No. of Patients	Patients with Intrathoracic Tumour Outside Lungs	Patients with Thymic Involvement	
			Suspected	Confirmed
I	67	7 (10%)	4	2
II	104	47 (46%)	3	1
III	39	20 (51%)	0	0
IV	61	28 (46%)	1	2*
	271	102 (38%)	8	5

* Confirmed at postmortem examination.

A higher incidence of thymic involvement than has been found in our series has been reported by others—for example, Marshall and Wood (1957) and Hoffbrand (1965). Nevertheless, experience refutes Thomson's (1955, 1956) idea that Hodgkin's disease is to be regarded as a primary tumour of the thymus. I opposed this suggestion at the time (Smithers, 1956), while listing some of the evidence for other connexions between the thymus and lymphoid tumours. It is interesting that our group of patients treated by thymectomy (3) or strongly suspected of thymic involvement during life (8)—all 11 of whom were irradiated—seems to have done well so far. The three patients operated on are all alive without having had any recurrence 2½, 3½, and 10½ years after their treatment. Of the eight patients who had radiological evidence strongly suggesting a thymic tumour during life and histological evidence of Hodgkin's disease on biopsy of a lymph node, one was not treated by us and has been lost sight of; six are alive 2½, 3½, 4, 5½, 10, and 15½ years after treatment; only one of these six living patients has had any recurrence—in an unirradiated axilla 10 years after treatment to the mediastinum. The eighth patient has died; he had no neck irradiation initially, had a recurrence in the neck nodes within nine months of treatment to the mediastinum, and died after repeated treatments nearly nine years later.

The relation between the thymus and radiation-induced lymphoma has long been established in laboratory animals (Kaplan, 1948, 1949). Whole-body irradiation can induce lymphomas transmissible to non-irradiated animals by thymic transplant after whole-body irradiation (Kaplan, 1950). Lymphomas can develop in non-irradiated thymic grafts placed in thymectomized irradiated mice (Kaplan and Brown, 1954; Law and Potter, 1958). Agents increasing thymic weight promote the induction of lymphoid tumours, and those reducing it prevent their occurrence or assist in their regression. The establishment in recent years of the thymus as a controlling influence in the development of immunological mechanisms—particularly through Miller's (1961, 1962) work on thymectomy in newborn mice—has added a new interest to the study of connexions between the thymus and lymphoreticular tumours.

Liver

Hodgkin's disease affecting the liver alone is rare; the liver is much more frequently involved as a terminal event than as a primary focus. Involvement of the liver by itself was reported

M. A. EPSTEIN *ET AL.* VIRUS IN LYMPHOBLASTS FROM BURKITT LYMPHOMA (p. 290)

All the figures are electron micrographs of thin sections of cultured lymphoblasts from a New Guinea Burkitt lymphoma. The cells were fixed in glutaraldehyde followed by osmium, embedded in epoxy resin, and contrast stained in the section with uranyl acetate.

FIG. 1.—Part of a nucleus bounded by its envelope (e) composed of the usual two membranes with perinuclear space between; some juxtanuclear cytoplasm with rough endoplasmic reticulum (er) lies on the extreme right. The nucleoplasm is of low electron opacity and contains immature virus particles (arrows) either empty or with dense central nucleoids. ($\times 34,000$.)

FIG. 2.—Detail of cytoplasm with altered mitochondria above, Golgi elements (g), and two immature virus particles about 75 m μ in diameter below and to the left; one of the particles lies close beside a membrane-bounded cytoplasmic space. ($\times 55,000$.)

FIG. 3.—Juxtanuclear cytoplasm with the double envelope of the nucleus just crossing the upper left corner of the field; free ribosomes are present throughout the cytoplasm together with some rough endoplasmic reticulum (er). On the right two immature virus particles can be seen in the process of budding into membrane-bounded cytoplasmic spaces. The particle above is close to a cytoplasmic membrane, causing it to bulge slightly, while the lower particle protrudes right into a cytoplasmic space whose limiting membrane now surrounds most of the particle's surface. ($\times 55,000$.)

FIG. 4.—Detail of cytoplasm between the nucleus on the left and the cell surface on the right. A mature virus particle (arrow) 115 m μ in diameter lies within a membrane-bounded cytoplasmic space; the particle is surrounded by its final additional outer membrane acquired from the cellular membrane as it matured by budding into the space from the cytoplasmic matrix (cf. Fig. 3). An altered mitochondrion (m) is also present. ($\times 55,000$.)

FIG. 5.—Part of a nucleus (on the left) and adjacent cytoplasm. In the centre of the field an immature virus particle is budding, in this case, through the inner nuclear membrane into the perinuclear space. At this stage the particle is partially enveloped by the inner nuclear membrane. ($\times 55,000$.)

FIG. 6.—Similar field to Fig. 5 with the nucleus again on the left. A virus particle lies in the perinuclear space. It is nearly mature, being almost fully surrounded by its final additional outer membrane derived from the nuclear membrane, but still remains attached at one point by a fine pedicle. On the right an altered mitochondrion can be seen. ($\times 55,000$.)

FIG. 7.—Similar field to Figs. 5 and 6. A mature virus particle enveloped by its final additional outer membrane, derived from nuclear membrane (cf. Figs. 5 and 6), lies free in the perinuclear space. There is abnormal reduplication of the nuclear envelope, and the mitochondria in the cytoplasm on the right are altered. ($\times 55,000$.)

FIG. 8.—Part of a degenerated lymphoblast. Mature virus particles (arrows) lie within membrane-bounded spaces in cytoplasmic debris. A large lipid body is present in the upper left corner of the field. ($\times 48,000$.)

Preliminary Communications

Virus in Cultured Lymphoblasts from a New Guinea Burkitt Lymphoma

[WITH SPECIAL PLATE]

Brit. med. J., 1967, 2, 290-291

Where Burkitt's lymphoma is abundant, as in tropical Africa (Burkitt, 1963) and New Guinea (ten Seldam *et al.*, 1966), its distribution appears to be determined by temperature and rainfall (Burkitt, 1962a, 1962b; Cooke, 1966, personal communication), and preliminary reports seem to indicate that these factors also operate in other tropical countries, such as Brazil (Luisi *et al.*, 1965) and Colombia (Beltrán *et al.*, 1966).

It is now generally accepted that the most likely explanation for this climate dependence is that an arthropod-borne onco-genic virus might play some causative part in the tropics (*Brit. med. J.*, 1966; *Lancet*, 1966), and because of this Burkitt's lymphoma has been extensively investigated virologically. Thus herpes simplex hominis has been isolated from about 9% of tumour samples (Woodall *et al.*, 1965; Simons and Ross, 1965), mycoplasma from various materials obtained from six patients (Dalldorf and Bergamini, 1964; Dalldorf *et al.*, 1966), and reovirus III from about 17% of tumours tested (Bell, 1967). In addition, a new unidentifiable virus, similar in morphology to herpes viruses but biologically (Epstein *et al.*, 1965c) and immunologically distinct from known members of the herpes group (Fink *et al.*, 1964; Henle and Henle, 1966; Henle *et al.*, 1966; Moore *et al.*, 1966), has been shown in cultured Burkitt tumour lymphoblasts from patients in Uganda (Epstein *et al.*, 1964a, 1964b, 1965a) and its presence has subsequently been confirmed in similar cultured Burkitt cells from patients in Nigeria (Stewart *et al.*, 1965; Rabson *et al.*, 1966) and the United States (O'Connor and Rabson, 1965).

In view of the possibility that this new, unusual virus from such an unusual tumour as Burkitt's lymphoma might be connected with the causation of the disease, it was thought important to determine whether it was also present in Burkitt tumours from an endemic area outside tropical Africa. The present preliminary communication reports the finding by electron microscopy of a similar virus in cultured lymphoblasts from a New Guinea case of Burkitt's lymphoma.

MATERIALS AND METHODS

Cultured Burkitt Tumour Cells.—A clinical case of Burkitt's lymphoma in New Guinea was confirmed histologically, and tumour material from the right maxilla was set up in vitro as described elsewhere (Pope *et al.*, 1967). Free-floating round lymphoblasts were cultured, closely similar in their mode of growth, morphology, and fine structure (Pope *et al.*, 1967) to all other strains of such cells grown previously from Burkitt lymphomas (Epstein and Barr, 1964, 1965; Pulvertaft, 1964; Epstein *et al.*, 1964b, 1965a, 1965b, 1966a, 1966b; Epstein and Achong, 1965; Stewart *et al.*, 1965; O'Connor and Rabson, 1965; Rabson *et al.*, 1966; Osunkoya, 1967).

Preparation of Cells for Electron Microscopy.—After 24 days in vitro the cells were fixed in glutaraldehyde followed by osmium, dehydrated, embedded in epoxy resin, and sectioned for electron microscopy by methods already reported (Epstein and Achong, 1965; Pope *et al.*, 1967).

OBSERVATIONS

Incidence of Cells with Virus.—In the electron microscope about 1 to 2% of the cells were found to carry formed virus particles (Special Plate, Figs. 1 to 8).

Structure and Maturation of the Virus.—Immature particles about 75 to 80 m μ in diameter, either empty or containing a central dense nucleoid, were present both in cell nuclei (Fig. 1) and in the cytoplasm (Figs. 2 and 3). These particles matured by budding at cellular membranes (Figs. 2 to 7), acquiring an additional covering from the membrane as they passed through (Figs. 3 to 7); the mature particles with this additional membrane measured 115-120 m μ in diameter (Figs. 4, 7, and 8) and their nucleoids about 45 m μ . Budding was observed in cytoplasmic spaces (Figs. 2 to 4), at the nuclear envelope (Figs. 5 to 7), and at the plasmalemma, and the mature particles were present therefore only in cytoplasmic vacuoles (Fig. 4), in the perinuclear space (Fig. 7), or just outside the cell surface.

Effect of Virus on the Cells.—The virus was seen both in characteristically altered intact cells (Figs. 1 to 7) and in cell debris (Fig. 8). Intact virus-containing cells showed decreased electron opacity of the nucleoplasm (Fig. 1), fragmentation and reduplication of the nuclear envelope (Fig. 7), mitochondrial changes (Figs. 2, 4, 6, and 7), and sheaves of electron-opaque altered spindle tubules; an exactly similar cytopathology has been fully described in other virus-bearing strains of Burkitt lymphoblasts (Epstein *et al.*, 1965c; Rabson *et al.*, 1966).

DISCUSSION

As regards both the immature and the mature particles the virus in cultured New Guinea Burkitt tumour cells (Figs. 1 to 8) is morphologically a member of the herpes group. In addition, the mechanism of its maturation by budding out through cellular membranes (Figs. 2 to 7) is characteristic of this group and exactly similar to that which has been established for herpes simplex itself, not only morphologically (Epstein, 1962) but also by the use of a dynamic functional marker (Epstein and Holt, 1963). However, the present virus appears to be consistently somewhat smaller than herpes simplex virus and differs from it radically in biological behaviour, since thorough investigation of the cells of both the original tumour and the cultured strain by inoculation into test tissue-culture systems and mice failed to reveal the presence of herpes simplex virus (Pope *et al.*, 1967). Tests for other agents, including reovirus and mycoplasma, were likewise negative (Pope *et al.*, 1967).

In size, morphology, mode of maturation, site within cells, the cytopathological changes it induces, and its biological behaviour, the virus reported here is indistinguishable from the virus carried by so many other in vitro strains of Burkitt lymphoblasts (Epstein *et al.*, 1964a, 1964b, 1965a; Stewart *et al.*, 1965; O'Connor and Rabson, 1965; Rabson *et al.*, 1966). Though the virus in cultured Burkitt tumour cells has sometimes been described as "herpes-like" because it is morphologically a member of the herpes group, using this term in its widest sense, it is biologically (Epstein *et al.*, 1965c) and immunologically (Fink *et al.*, 1964; Henle and Henle, 1966; Henle *et al.*, 1966; Moore *et al.*, 1966) different from known herpes viruses.

This new, unidentified herpes-type virus in cultured Burkitt lymphoblasts is thus quite distinct from the true herpes simplex virus isolated from a small number of Burkitt lymphomas (Simons and Ross, 1965; Woodall *et al.*, 1965). Ordinary herpes simplex virus is unlikely to be of aetiological significance since it has only been isolated from Burkitt tumours of the jaw with an incidence exactly comparable to the usual oral carrier rate; it has not been found in Burkitt tumours of any other anatomical site (Woodall *et al.*, 1965).

Similarly it is unlikely that the mycoplasma isolated from patients with Burkitt's lymphoma (Dalldorf and Bergamini, 1964; Dalldorf *et al.*, 1966) is of importance; it is more probably a commensal "passenger" in much the same way as is currently thought to be the case on the few occasions where it has been found in leukaemia (Sutton, 1966; Fallon, 1967; *World Medicine*, 1967).

Interest in the isolation of a widely prevalent virus such as reovirus III from some Burkitt lymphomas (Bell *et al.*, 1966; Bell, 1967) has recently increased after a claim that this virus may be associated in some way with a murine lymphoma (Stanley, 1966). In mice, neonatal reovirus III infection causes a runting syndrome; the inoculation of spleen cells from these runs into normal newborn mice results in turn in a runt disease, among the survivors of which a very few lymphomas have been observed (Stanley *et al.*, 1966b). Though reovirus III does not itself cause murine lymphomas (Stanley *et al.*, 1966a), if some connexion could in fact be established between this virus and the lymphomas in mice and an oncogenic role be established for it, then reovirus III might be of considerable relevance to the Burkitt tumour.

The aetiological significance of the virus reported here is likewise equivocal at the present time. It may of course prove to be a hitherto unknown passenger virus with a special predilection for the cells of Burkitt's lymphoma. It certainly cannot be regarded as a laboratory contaminant, since it has been found independently in many different centres by means of a variety of techniques. On the other hand, its almost constant association with Burkitt tumour lymphoblasts when these are cultured, its unusual biological and immunological features, and its presence now in numerous types of leukaemic and other malignant cell strains (Moore *et al.*, 1966; Rauscher *et al.*, 1966; Rauscher, 1966, personal communication) make it a highly important subject for intensive study. The present finding of this virus in Burkitt lymphoma cells from a region as self-contained and distant as New Guinea supports such a view; immunological investigations of the New Guinea example of the virus are currently being undertaken.

ADDENDUM.—Since this paper was prepared for publication we have found a morphologically identical virus in a second strain of cultured lymphoblasts from another New Guinea patient with Burkitt's lymphoma.

This investigation was supported in part by the British Empire Cancer Campaign for Research and aided by a grant from the

Jane Coffin Childs Memorial Fund for Medical Research. We are grateful to Mr. G. Ball for invaluable technical help.

M. A. EPSTEIN, M.A., M.D., D.S.C., PH.D., F.C.PATH.

B. G. ACHONG, M.D., B.CH., B.A.O.

Department of Experimental Pathology, Bland-Sutton Institute, the Middlesex Hospital Medical School, London W.1.

J. H. POPE, M.SC., PH.D.

Queensland Institute of Medical Research, Herston N.9, Brisbane, Queensland, Australia.

REFERENCES

- Bell, T. M. (1967). *Symposia Unio Internationalis Contra Cancrum*. In press.
- , Massie, A., Ross, M. G. R., Simpson, D. I. H., and Griffin, E. (1966). *Brit. med. J.*, **1**, 1514.
- Beltrán, G., Baez, A., and Correa, P. (1966). *Amer. J. Med.*, **40**, 211.
- Brit. med. J.*, 1966, **1**, 1043.
- Burkitt, D. (1962a). *Nature (Lond.)*, **194**, 232.
- (1962b). *Brit. med. J.*, **2**, 1019.
- (1963). In *International Review of Experimental Pathology*, edited by G. W. Richter and M. A. Epstein, vol. 2, p. 67. New York.
- Dalldorf, G., and Bergamini, F. (1964). *Proc. nat. Acad. Sci. (Wash.)*, **51**, 263.
- and Frost, P. (1966). *Ibid.*, **55**, 297.
- Epstein, M. A. (1962). *J. Cell Biol.*, **12**, 589.
- and Achong, B. G. (1965). *J. nat. Cancer Inst.*, **34**, 241.
- and Barr, Y. M. (1964a). *Lancet*, **1**, 702.
- , Zajac, B., Henle, G., and Henle, W. (1966a). *J. nat. Cancer Inst.*, **37**, 547.
- and Barr, Y. M. (1964). *Lancet*, **1**, 252.
- (1965). *J. nat. Cancer Inst.*, **34**, 231.
- and Achong, B. G. (1964b). *Path. et Biol.*, **12**, 1233.
- (1965a). *Wistar Inst. Symposia Monographs*, vol. 4, p. 69.
- (1965b). *Brit. J. Cancer*, **19**, 108.
- (1966b). *Ibid.*, **20**, 475.
- , Henle, G., Achong, B. G., and Barr, Y. M. (1965c). *J. exp. Med.*, **121**, 761.
- and Holt, S. J. (1963). *J. Cell Biol.*, **19**, 337.
- Fallon, R. J. (1967). *Brit. med. J.*, **1**, 111.
- Fink, M. A., Malmgren, R. A., Rauscher, F. J., Orr, H. C., and Karon, M. (1964). *J. nat. Cancer Inst.*, **33**, 581.
- Henle, G., and Henle, W. (1966). *J. Bact.*, **91**, 1248.
- Henle, W., Hummeler, K., and Henle, G. (1966). *Ibid.*, **92**, 269.
- Lancet*, 1966, **1**, 803.
- Luisi, A., Bertelli, A. de P., Machado, J. C., and de Freitas, J. P. A. (1965). *Rev. bras. Cirurg.*, **49**, 280.
- Moore, G. E., Grace, J. T., Citron, P., Gerner, R., and Burns, A. (1966). *N.Y. St. J. Med.*, **66**, 2757.
- O'Connor, G. T., and Rabson, A. S. (1965). *J. nat. Cancer Inst.*, **35**, 899.
- Osunkoya, B. O. (1967). *Symposia Unio Internationalis Contra Cancrum*. In press.
- Pope, J. H., Achong, B. G., Epstein, M. A., and Biddulph, J. (1967). Submitted to press.
- Pulvertaft, R. J. V. (1964). *Lancet*, **1**, 238.
- Rabson, A. S., O'Connor, G. T., Baron, S., Whang, J. J., and Legallais, F. Y. (1966). *Int. J. Cancer*, **1**, 89.
- Rauscher, F. J., Carrese, L. M., and Baker, C. G. (1966). *Cancer Res.*, **26**, 1176.
- Simons, P. J., and Ross, M. G. R. (1965). *Europ. J. Cancer*, **1**, 135.
- Stanley, N. F. (1966). *Lancet*, **1**, 961.
- , Papadimitriou, J. M., and Epstein, M. A. (1966a). *Brit. med. J.*, **2**, 767.
- , Walters, M. N.-I., Leak, P. J., and Keast, D. (1966b). *Proc. Soc. exp. Biol. (N.Y.)*, **121**, 90.
- Stewart, S. E., Lovelace, E., Whang, J. J., and Ngu, V. A. (1965). *J. nat. Cancer Inst.*, **34**, 319.
- Sutton, R. N. P. (1966). *Brit. med. J.*, **2**, 1496.
- ten Seldam, R. E. J., Cooke, R., and Atkinson, L. (1966). *Cancer*, **19**, 437.
- Woodall, J. P., Williams, M. C., Simpson, D. I. H., and Haddow, A. J. (1965). *Europ. J. Cancer*, **1**, 137.
- Wld Med.*, 1967, **2**, No. 9, p. 60.