the sensitivity of the method used or the stage of the illness at which the serum was tested. It has been shown that the antigen commonly disappears within a few weeks of the onset of the illness (Wright *et al.*, 1969). In our patients there was no correlation between the duration of symptoms and the presence of the antigen. The varying incidence of hepatitis-associated antigen in acute viral hepatitis may also indicate different epidemiological forms of the disease.

Krugman *et al.* (1967), and Prince (1968) believe that the antigen occurs only during serum hepatitis—that is, when infection is due to the long-incubation agent (MS-2). Contrary to previous belief Krugman *et al.* (1967) have shown that the infective agent of long-incubation-type hepatitis can be transmitted by oral ingestion of infected material. Prince *et al.* (1970) believe that this agent is mainly responsible for sporadic cases of hepatitis in adults in the U.S.A. The finding of the antigen in 54% of our cases of acute viral hepatitis may indicate that these patients had long-incubation-type hepatitis.

Before drawing conclusions from the apparent association of hepatitis-associated antigen with cirrhosis and primary hepatic carcinoma in our series several factors must be taken into consideration. Firstly, a 6% incidence of the antigen was found in apparently healthy blood donors. Secondly, liver disease is common in Kenya, and the possibility of a chance association cannot be excluded in the small numbers studied so far. Thirdly, patients with portal hypertension are likely to have received blood transfusion after haemorrhage from oesophageal varices. On this last point it would have been tempting to suppose that the increased incidence of hepatitis-associated antigen in these patients had resulted from transfusion of blood containing the antigen. In fact, there was no obvious relationship between blood transfusion and the presence of the antigen (Table II).

Hepatitis-associated antigen was present in 8 out of 39 patients with hepatic cirrhosis and 3 out of 22 with primary hepatic carcinoma. Alpha-fetoprotein was detected in the serum of 14 (64%) of the 22 patients with primary hepatic carcinoma, and the three patients with hepatitis-associated antigen were all alpha-fetoprotein positive. Though only two patients with primary hepatic carcinoma were known to have

Histological Diagnosis of Burkitt's Lymphoma: A Cautionary Tale

AVRUM Z. BLUMING, A. C. TEMPLETON

British Medical Journal, 1971, 1, 89-90

In 1969 the World Health Organization published a monograph listing histological criteria for the diagnosis of Burkitt's lymphoma. This was intended to help differentiate this neoplasm from the tumours with which it is most commonly confused: acute lymphoblastic leukaemia, acute myeloblastic leukaemia or the blastic phase of chronic myelogenous leukaemia, reticulum-cell sarcoma, and lymphocytic lymphosarcoma (Berard *et al.*, 1969). The diagnosis is facilitated when air-dried Romanovsky-stained imprint preparations are available for study (Wright, 1967). We recently saw a patient

Makerere University Medical School, Kampala, Uganda
AVRUM Z. BLUMING, M.D., Director, Lymphoma Treatment Centre
A. C. TEMPLETON, M.B., M.R.C.PATH., Senior Lecturer, Department of Pathology

TABLE II—Comparison of Occurrence of Hepatitis-associated Antigen in All Patients with That Found in Patients who had Not Received Blood Transfusion

Diagnosis	Total Series		Not Transfused	
	No. Tested	No. Positive	No. Tested	No. Positive
Cirrhosis	39	8 (20.5%)	32	8 (25%)
Primary liver cancer	22	3 (14%)	20	2 (10%)
Other conditions (see text)	89	5 (5.6%)	60	4 (6.6%)
Nairobi blood donors	200	12 (6%)	200*	12 (6%)

*It is assumed that none of the donors had received a blood transfusion.

cirrhosis, it must be acknowledged that without post-mortem examination cirrhosis cannot be excluded in the other patients. The numbers studied so far are small, but the results do suggest an association of cirrhosis and primary hepatic carcinoma with hepatitis-associated antigen.

Professor W. F. M. Fulton and Dr. A. T. T. Forrester kindly allowed us to study patients under their care. Dr. Forrester also gave valuable clinical assistance, and Professor Fulton gave advice on the preparation of the manuscript. Acknowledgment is also due to Professor H. M. Cameron for reviewing all liver biopsies, and to Dr. A. M. Prince for the original serum. This work was supported by Research Grants from the University of Nairobi, Nairobi, Kenya, and the Pfizer Corporation (E.A.) Ltd.

References

Blumberg, B. S., Gerstley, B. J. S., Hungerford, D. A., London, W. T., and Sutnick, A. I. (1967). *Annals of Internal Medicine*, **66**, 924.
Gocke, D. J., and Kavey, N. B. (1969). *Lancet*, **1**, 1055.
Jindani, A., Bagshawe, A., and Forrester, A. T. T. (1970). *East African Medical Journal*, **47**, 138.
Krugman, S., Giles, J. P., and Hammond, J. (1967). *Journal of the American Medical Association*, **200**, 365.
Okochi, K., and Murakami, S. (1968). *Vox Sanguinis*, **15**, 374.
Pitney, W. R. (1968). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **62**, 717.
Prince, A. M. (1968). *Proceedings of the National Academy of Sciences*, **60**, 814.
Prince, A. M. *et al.* (1970). *New England Journal of Medicine*, **282**, 987.
Sherlock, S., Fox, R. A., Niazi, S. P., and Scheuer, P. J. (1970). *Lancet*, **1**, 1243.
Wright, R., McCollum, R. W., and Klatskin, G. (1969). *Lancet*, **2**, 117.

in whom the histological differential diagnoses ranged much farther afield than other lymphoproliferative or myeloproliferative disorders before being recognized as Burkitt's lymphoma.

Case Report

A 4-year-old girl was admitted to the Lymphoma Treatment Centre on 19 September 1969 with a history of abdominal distension and paraplegia of three months' duration. Physical examination was remarkable only for two hard abdominal masses 10 by 12 cm, one arising from each iliac fossa, and flaccid paralysis of both lower limbs. At laparotomy on 21 September a greatly enlarged right ovary was excised; the left ovarian tumour was left in place. Frozen section of the mass showed a neoplasm made up of cells arranged in tubules and pseudorosettes. An imprint preparation showed small dark cells with little cytoplasm (Fig. 1), and there appeared to be a second population of paler, larger cells intermixed with them. A tentative diagnosis of neuroblastoma was suggested. A paraffin section prepared from tissue which had been placed in 10% formalin for 16 hours showed a malignant tumour whose cells were arranged in cords and clumps (Fig. 2). A tentative diagnosis of dysgerminoma was made, pending section of better fixed material. This tumour forms 4 to 10% of the neoplasms found in East African children (J. N. P. Davies, personal communication, 1969). A subsequent section of material from the edge of the block of tissue which had been in formalin for 64 hours showed all the features of Burkitt's lymphoma—that is, fat-containing phagocytic histiocytes interspersed among uniformly primitive lymphoblastic

FIG. 1—Imprint preparation from tumour showing tumour cells with rather less cytoplasm than usual; the nuclei appear more condensed and the chromatin is more clumped than in Burkitt's tumour cells which have dried rapidly. (Wright's stain $\times 1,000$.)

FIG. 2—First H. and E. section showing a malignant tumour with cells arranged in cords and clumps suggesting the appearance of a dysgerminoma.

cells whose nuclei approximated the size of the histiocytic nuclei (Fig. 3).

Two weeks after a single intravenous dose of cyclophosphamide (40 mg/kg) the mass in the left iliac fossa was no longer palpable.

Comment

This patient presented with clinical features typical of Burkitt's lymphoma. The pathologists were all aware of the clinical findings and had had extensive experience in the diagnosis of this tumour, so commonly seen in Uganda. In spite of this, incorrect histopathological diagnoses were suggested. A search for the source of these errors brought the following results.

Frozen Section.—The microtome blade used was dull and had distorted the tissue, producing the artefactual tubules and pseudorosettes. Even on the best frozen section, however, a definite diagnosis of Burkitt's lymphoma is next to impossible. This technique serves only to suggest other conditions or to exclude the diagnosis of lymphoma.

FIG. 3—Second H. and E. section showing histiocytic phagocytes interspersed among uniformly immature lymphoblasts, whose nuclei approximate the size of the histiocytic nuclei. These specimens were cut, stained, and photographed under identical conditions. ($\times 100$.)

Imprint Preparation.—The appearance of the cells in this type of preparation depends largely on the speed of drying. In a thick preparation, which will dry slowly, the cells appear smaller, the cytoplasm is less prominent, and the nuclei are darker, with more clumping of the chromatin than in a rapidly drying thin preparation. These features will be more pronounced in the centre of each area of cells and much more likely to be seen when the tumour is necrotic. In addition, the clear vacuoles of fat which are so striking in good imprints of Burkitt's lymphoma are much less obvious in the thicker, slow-drying preparations. The error made in this case was the result of insufficient appreciation of the extent of these changes, and, on review, more typical Burkitt lymphoma cells could be found in the extreme periphery of the slide. These problems are less likely to arise if the slide is placed on the tumour when making the imprint preparation rather than vice versa, as is usually done. This enables excess fluid to drain away from the slide rather than on to it.

First Paraffin Section.—Penetration of formalin into the centre of a large block of tissue proceeds slowly, while adequate fixation of the peripheral areas of the block may require only a few hours. To obtain the best results tissue should be left in formalin for 7 to 10 days (Drury and Wallington, 1967). The error in interpretation of this section can be attributed to selection of the tissue from the centre of the tumour rather than from the edge, and to consequent distortion of the partially-fixed tissue during processing. This artefact will be minimized if material is cut into thin (5-mm) slices with a sharp knife before immersion in formalin and fixed for 48 hours before sectioning.

This work was supported by contract Nos. PH-43-67-1343 and PH-43-67-47 from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

References

- Berard, C., O'Connor, G. T., Thomas, L. B., and Torloni, H. (1969). *Bulletin of the World Health Organization*, **40**, 601.
- Drury, R. A. B., and Wallington, E. A. (1967). *Carleton's Histological Technique*, 4th edn., p. 41. London, Oxford University Press.
- Wright, D. H. (1967). *Clinical Pediatrics*, **6**, 116.