The description of endocarditis in Q fever (Evans *et al.*, 1959) shows the importance of looking for evidence of infection by microbes other than bacteria.

Our difficulty in finding convincing cases of bacteriologically negative S.B.E. in a period of eight and a half years was surprising. In the M.R.C. series (Cates and Christie, 1951) there were 408 cases proved by blood culture and 34 others, from six centres, with negative cultures. The clinical features of those 34 strongly suggested bacterial infection, and vegetations were found on the heart valves of all 15 who came to necropsy. Other published series include the following proportions of cases in which no positive blood culture was obtained: Newman *et al.* (1954), 33% ; Wedgwood (1955), 10% ; Dormer (1958), 23%. Friedberg (1950) states that blood cultures may be persistently negative in 10–30% of cases. In our series there was one patient (Case 1) who resembled those described by Libman (1913). Here, unfortunately, the picture was confused by the fact that he had earlier had a course of streptomycin. There were four others, with negative blood cultures, who conceivably may have had S.B.E. ; in only two of them was the diagnosis considered likely, and in neither was it made with confidence.

Unless the technique of blood culture is satisfactory, a diagnosis of bacteriologically negative S.B.E. must be especially doubtful. The occurrence of cases with intermittently positive blood cultures should suggest a review of technique. Our method with large volumes of medium have been satisfactory, but smaller volumes of liquid broth (Stokes, 1955) should be as good. It is essential to use anaerobic as well as aerobic medium. The use of the two bottles of broth, as well as a plate culture, simplifies the distinction between significant organisms and contaminants. Skill in venepuncture, a careful aseptic technique at the bedside, and daily scrutiny of the cultures during incubation are important.

Any patient who is considered on clinical grounds to have S.B.E. must be given antibiotic treatment, even when a thorough bacteriological investigation fails to confirm the diagnosis. But before the diagnosis is finally accepted all the alternatives should be considered, especially if the response to treatment is inconclusive. Our difficulty in finding convincing examples of bacteriologically negative S.B.E. leads us to suggest that the condition is rarer than has been supposed.

Summary

Sixty-seven attacks of subacute bacterial endocarditis confirmed by positive blood culture are recorded. Brief reference is made to the clinical features, treatment, and progress in the first year after treatment. The bacteraemia was constant. The only sterile cultures were obtained from two patients who had previously had penicillin administered. Two further cultures were spoiled by contaminants. Provided no antibiotic has been given three blood cultures should suffice to isolate the infecting organism, and treatment can be begun before the results of the culture have been reported.

Only five possible examples of S.B.E. with persistently negative blood cultures were found. One died before any penicillin was given. There were no organisms in the vegetations found at necropsy. The four others were treated with a full course of penicillin and recovered: in two the diagnosis was doubtful and in two signs of embolism were unconvincing.

It is thought that S.B.E. with persistently negative blood culture is less common than has been supposed.

We are grateful to the physicians who allowed us to study their patients. Special thanks are due to Professor C. Bruce Perry, Dr. D. H. Davies, and Dr. J. E. Cates for their constructive criticism, and to Mr. L. J. Waller for technical help.

REFERENCES

Belli, J., and Waisbren, B. A. (1956). *Amer. J. med. Sci.*, **232**, 284.
Brewer, J. H. (1940). *J. Amer. med. Ass.*, **115**, 598.
Cates, J. E., and Christie, R. V. (1951). *Quart. J. Med.*, **20**, 93.
Christie, R. V. (1949). *Brit. med. J.*, **2**, 950.
Dormer, A. E. (1958). Ibid., **1**, 63.
Evans, A. D., Powell, D. E. B., and Burrell, C. D. (1959). *Lancet*, **1**, 864.
Friedberg, C. K. (1950). *Med. Clin. N. Amer.*, **34**, 769.
Keefer, C. S. (1937). *Ann. intern. Med.*, **11**, 714.
Libman, E. (1913). *Amer. J. med. Sci.*, **146**, 625.
—— and Friedberg, C. K. (1948). *Subacute Bacterial Endocarditis*, 2nd ed. Oxford Univ. Press, New York.
Newman, W., Torres, J. M., and Guck, J. K. (1954). *Amer. J. Med.*, **16**, 535.
Stokes, E. J. (1955). *Clinical Bacteriology*. Arnold, London.
Wedgwood, J. (1955). *Lancet*, **2**, 1058.

REED–STERNBERG CELLS IN THE PERIPHERAL BLOOD AND BONE-MARROW IN HODGKIN'S DISEASE

BY

STEPHEN VARADI, M.D.

Consultant Haematologist,

City General Hospital and Wharncliffe Hospital, Sheffield

Hodgkin's disease is, as a rule, associated with enlargement of lymph nodes which are either palpable or ascertainable by radiography. Splenomegaly is often found. The reticulum of the bone-marrow can also participate in the pathological process, and the involvement of the sternal marrow is then revealed by a circumscribed tenderness of the sternum and sometimes also by presternal oedema. There may or may not be accompanying constitutional symptoms and signs.

In the so-called abdominal form, in which one or several of the deep-lying abdominal lymph-node groups are the only ones to be enlarged to any extent, the diagnosis may on occasion be entirely unsuspected clinically.

Two cases of this latter type are reported: both presented as pyrexia of unknown origin with a macrocytic anaemia, leucopenia, and thrombocytopenia. In neither of the patients were the superficial and mediastinal lymph nodes enlarged, nor was the spleen palpable. In the first case, thought to be a perforated peptic ulcer, the diagnosis was revealed by the finding of Reed–Sternberg cells in the peripheral blood films ; in the second, an obscure macrocytic anaemia, the pathognomonic cells were identified in the marrow aspiration material.

Case 1

A 58-year-old labourer was admitted to hospital on February 21, 1956, with a history of abdominal tenderness and colic of one week's duration. He had apparently collapsed at work six weeks previously. He had lost weight and vomited the day before and on the day of his admission.

He was running a temperature of 103° F. (39.4° C.) (Fig. 1) and looked pale and emaciated ; B.P. 90/70. There were

a few scattered petechial haemorrhages on the skin, and the ankles were oedematous. The tongue was coated and dry. There were signs of consolidation over the right lower lobe and a marked generalized abdominal tenderness without guarding.

It was thought that he had a perforated peptic ulcer which had sealed itself off; this was confirmed by finding

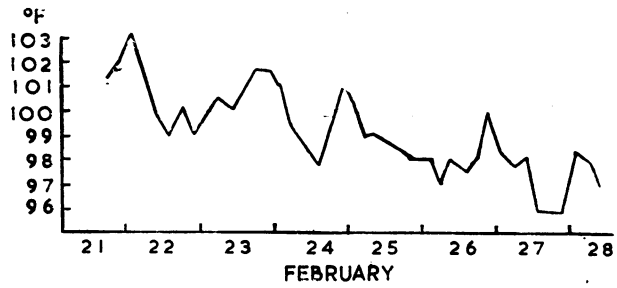
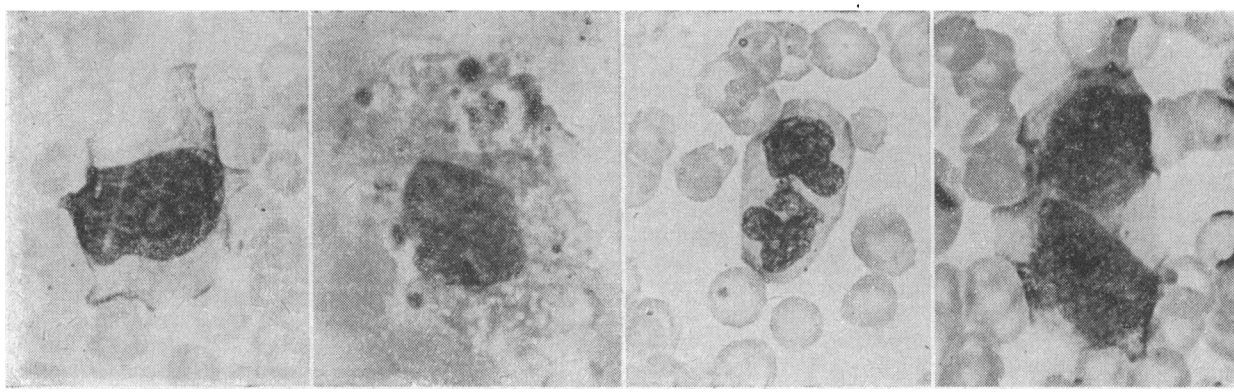


FIG. 1.—Case 1. Temperature chart.

air under the diaphragm. He was treated conservatively with penicillin, 500,000 units six-hourly. His condition, however, declined rapidly and he died on the eighth day after admission.

Post-mortem Examination (Dr. A. J. N. Warrack).—This showed the immediate cause of death to be lobar pneumonia. No actual ulcer was found, but pyloric and duodenal adhesions were compatible with the clinical diagnosis of perforated ulcer. Lymph nodes in the mesentery and mesocolon were enlarged, soft, and of grey-colour. No hepatomegaly or splenomegaly.

Histology.—The lymph nodes, spleen, liver, lungs, and bone-marrow (femur) showed invasion by malignant tissue composed mainly of bizarre, polymorphous cells, many of giant dimensions, with multiple nuclei and striking nucleoli. Capillaries in the lungs and lymph nodes and small arteries in the lungs were found to contain these cells (Figs. 8–10). The mesenteric lymph node examined showed a massive focus of autolysis, but there was plenty of surviving tissue with preservation of the Malpighian follicles. Between these the lymphoid tissue was replaced by a loose network of chiefly Dorothy Reed cells and large reticulum cells. The spleen showed foci of autolysis (necrosis of Malpighian bodies) and also small foci of Hodgkin tissue at the periphery of the Malpighian bodies, in the capsule, and, in a few places, in the trabeculae. The liver showed infiltration of the portal spaces, and giant cells were seen in the sinusoids. Histological diagnosis: Hodgkin's sarcoma (acute Hodgkin's disease).



FIGS. 2–5 (×880).—Case 1. Peripheral blood showing Reed–Sternberg cells of various sizes (25–55μ) and shapes. Figs. 2 and 3, with one nucleus. Fig. 4, with a nucleus of bizarre shape. Fig. 5, with two nuclei. The nucleoli are large and clearly visible in most cells. Fig. 3 shows phagocytosed particles.

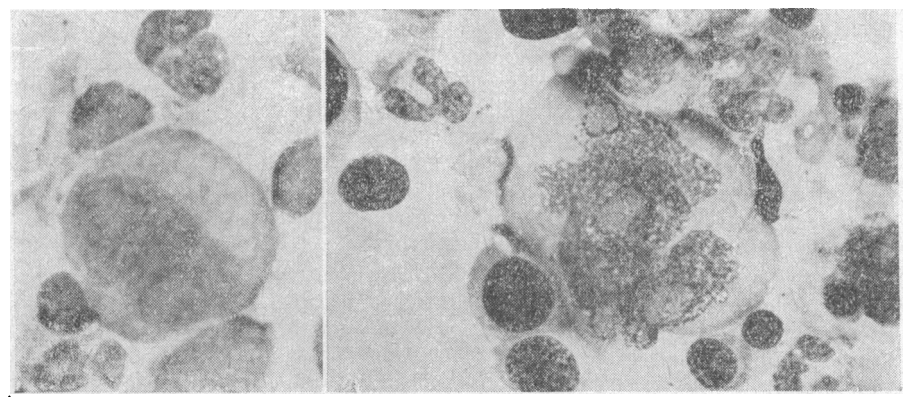
Laboratory Findings.—Blood counts (ranges from counts on five different days): Hb. 8.7–10.7 g./100 ml.; R.B.C. 2,470,000–2,970,000/c.mm.; reticulocytes, 0.8–2%; normoblasts, 0–2/100 W.B.C.; P.C.V., 29%, M.C.V., 117.4 cubic microns, M.C.H.C., 29%, M.C.H., 33.5 μμg.; W.B.C., 2,400–7,800/c.mm. (neutrophils 1,728–6,396, monocytes 96–325, lymphocytes 250–858/c.mm.). Giant histiocytes and Reed–Sternberg cells, 1 to 3% of white cells (Figs. 2–5).

Sternal Puncture. (February 23).—The leuco-erythrogenic ratio (Pontoni) was 1.42. About one-fifth of the nucleated red cells showed a megaloblastic nuclear pattern, giant neutrophils (segmented, band, and metamyelocytes) were numerous. The number of megakaryocytes was increased; many were very large and multisegmented, others having one single voluminous convoluted nucleus. In addition to a slight increase of various reticulum cells, some of which were phagocytic, typical Reed–Sternberg cells of various shapes and sizes were found scattered among other marrow elements (Figs. 6 and 7). Serum vitamin B_{12} level, 820 μμg./ml.

Case 2

A woman aged 69 was admitted to hospital on October 20, 1956, with a history of increasing fatigue for about a year, dyspnoea and swelling of ankles for six months, and flatulent dyspepsia for many years. In June–August, 1956, motions were more frequent than usual and appeared "frothy" (?). She had been having iron by mouth and vitamin B_{12} ("cytamen") injections for the previous 12 days.

On admission she was very pale and thin, with Paget's disease of the right femur, kyphosis, and rheumatoid



FIGS. 6 (×880) and 7 (×750).—Case 1. Bone-marrow: Reed–Sternberg cells (one in each figure), megaloid erythroblasts (in Fig. 7).

changes of the extremities. Jugular venous pressure was raised and the ankles were oedematous. There was a harsh systolic murmur, maximal at the apex. The lower half of the sternum was very tender on pressure. No other abnormalities were detected. It was felt that she might be suffering from steatorrhoea, and at first this diagnosis seemed to be supported by the haematological findings. The temperatures (Fig. 11), however, were unaccounted for until the discovery of Reed-Sternberg cells in the marrow aspiration.

The patient was treated with penicillin and folic acid by injections and was given transfusions. She died apparently of a cerebrovascular incident 11 days after admission.

Laboratory Findings.—Blood counts: a macrocytic anaemia was found with very large red cells, many of which contained Howell-Jolly bodies and/or Pappenheimer bodies (siderocytes + + +) (Fig. 12). Heinz bodies were also seen. Nucleated red cells, mainly with pyknotic nuclei, were numerous and reticulocytes were increased. Leucopenia (neutropenia) and thrombocytopenia were present. Hb, 4.5–5.6 g./100 ml.; R.B.C., 1,000,000–1,330,000/c.mm.; reticulocytes, 9.3–11%; nucleated R.B.C., 2,500–5,700/c.mm.; platelets, 6,000–9,000/c.mm; W.B.C., 1,700–3,500/c.mm. (neutrophils 350–780, monocytes 260–884, lymphocytes 816–2,210/c.mm.) (all figures are ranges of six blood counts); P.C.V., 20%; M.C.V., 150 cubic microns; M.C.H.C., 31.5%; M.C.D. >9μ.

Sternal Puncture (October 23 and 27).—Hyperplastic, mainly macronormoblastic erythropoiesis, with a number of intermediate megaloblasts and numerous iron granules in both nucleated and non-nucleated red cells. Leucopoiesis rather hypoplastic. L/E ratio 0.1. Platelet-formation reduced despite a numerical increase of megakaryocytes, many showing abnormal features such as unusually large size, with a great number of nuclear segments or a large convoluted nucleus occupying most of the area of the cell.

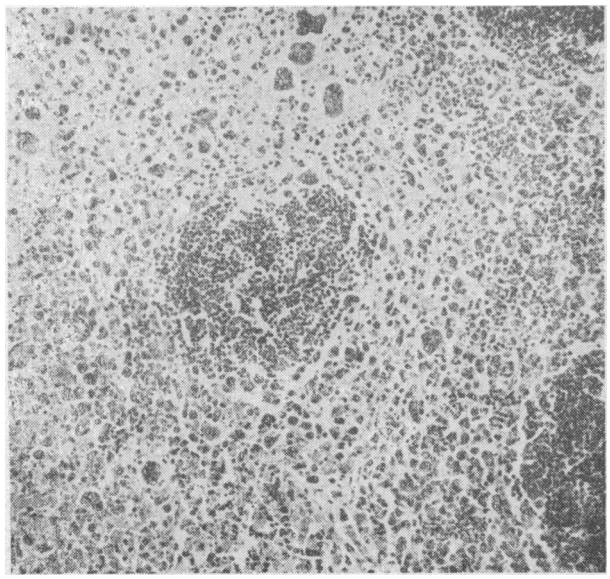


FIG. 8.—Case 1. Mesenteric lymph node. A follicle surrounded by tumour tissue with Reed-Sternberg giant cells. (×95.)

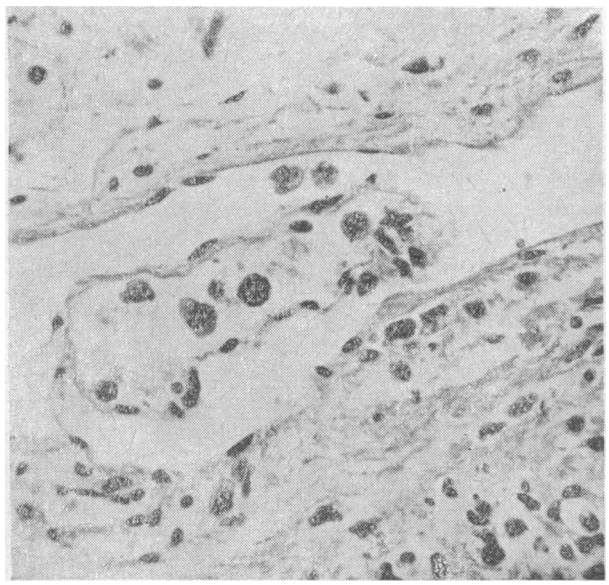


FIG. 9.—Case 1. Capillary on the periphery of the same lymph node containing reticulum cells and Reed-Sternberg cells (× 300.)

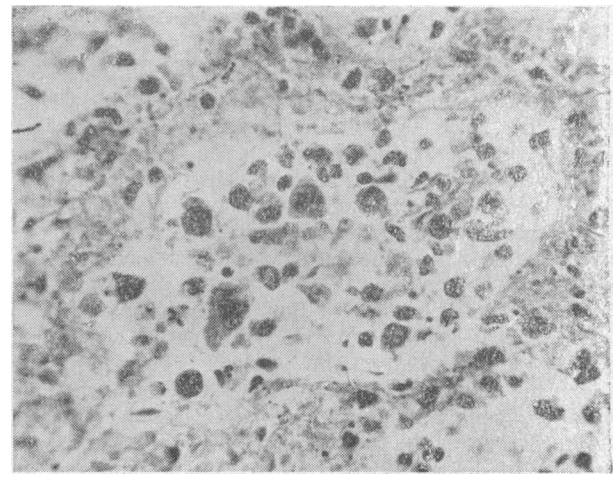


FIG. 10.—Case 1. Small artery in lungs filled with similar cells. (× 368.)

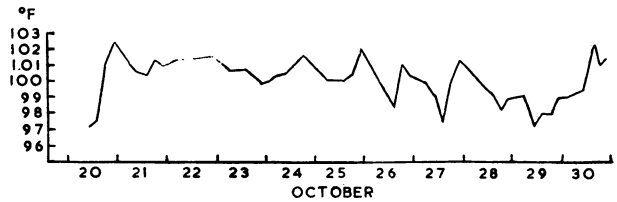


FIG. 11.—Case 2. Temperature chart.

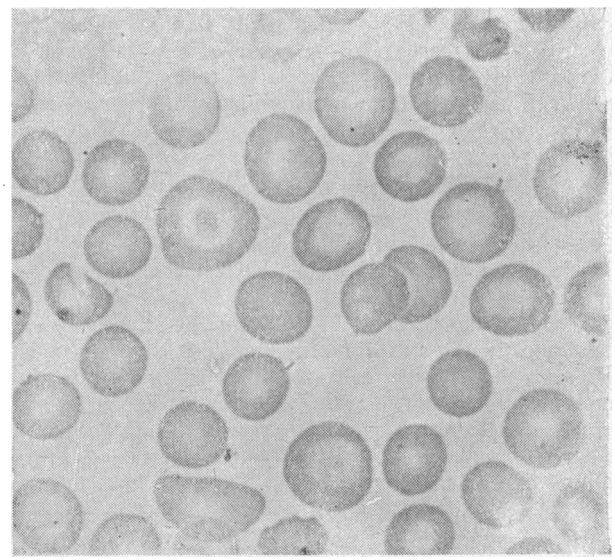


FIG. 12.—Case 2. Macrocytosis, Howell-Jolly and Pappenheimer bodies (peripheral blood). (× 1,150.)

Lymphocytes and reticulum cells increased in number. Reed–Sternberg cells present in some of the marrow smears on both occasions.

Biochemical Findings.—Fractional test-meal, free hydrochloric acid present. Serum alkaline phosphatase, 72 King–Armstrong units. All other liver-function tests normal.

Post-mortem Examination (Dr. Warrack).—Neither the liver nor the spleen (100 g.) were found to be enlarged and there was no marked lymphadenopathy. Histology confirmed the diagnosis of "lymphadenoma" (senile type), Reed–Sternberg type giant cells being present in portal, mesenteric, para-aortic, and axillary lymph nodes. Megakaryocytes were also seen in the para-aortic lymph node examined. The spleen showed loss of follicular pattern and a few patches of Hodgkin tissue with Reed–Sternberg cells.

Discussion

Reed–Sternberg Cells in the Bone-marrow

Until recently it was believed that haematology's contribution to the diagnosis of Hodgkin's disease was almost entirely non-specific (Limarzi and Paul, 1949). While this holds true in the great majority of cases for the peripheral blood, reports on the cytological demonstration of Reed–Sternberg cells in bone-marrow aspirations have been accumulating in the past few years. This is not surprising, considering the relatively frequent involvement of the bone-marrow as ascertained by post-mortem studies (Steiner, 1943). The specific cells were found almost exclusively at sites tender on pressure or slight percussion, while presternal oedema was often present (Varadi, 1938, 1955). It has been stated that it is in the acute forms of Hodgkin's disease that Reed–Sternberg cells are found in the aspiration material (Bayrd *et al.*, 1954). In eight personal cases the clinical duration of illness varied from just under 2 months to more than 54 months (see Table).

Duration of Illness in Relation to Sternal Puncture in Eight Cases of Hodgkin's Disease

Before	After
1 month 2 weeks	1 week (Case 1)
3 months 2 ,,	12 months
4 ,,	2 ,,
8 ,,	1 month 1 week
12 ,,	1 week (Case 2)
17 ,, (?)	3 months
36 ,, (?)	?
54 ,, (?)	?

In six followed-up patients whose bone-marrow had been found to be involved the survival periods ranged from 1 week to 12 months. As a whole it could be confirmed that cases with an acute or subacute febrile course were more likely to yield the diagnostic cells in smears of aspirated bone-marrow than the more chronic "cold" cases.

Reed–Sternberg Cells in the Peripheral Blood

The first reference to the presence of Reed–Sternberg cells in the peripheral blood was made by Isaacs (1944). Ludman and Spear (1957) showed photomicrographs of such cells in a case of known Hodgkin's disease. Jeanselme and Marchal (1926) were the first to show histologically (on post-mortem material) a "metastatic dissemination of giant cells described by Sternberg" in an acute malignant case of Hodgkin's disease, having demonstrated numerous emboli consisting of Sternberg cells in capillaries of lymph nodes, spleen, liver, pancreas, and lungs.

In Case 1, in which Reed–Sternberg cells were seen in the peripheral blood films, they were also found in capillaries of mesenteric lymph nodes and lungs, and in small pulmonary arteries. They must have crossed the pulmonary circulation to reach the peripheral blood. The invasive neoplastic type of the lesions is probably responsible for the cells making their appearance in the circulation. There is an analogy with lymphosarcoma, in which pathological cells often find their way into the peripheral blood, especially in advanced stages of the disease ("leucosarcoma").

The morphology of the Reed–Sternberg cells is typical of Hodgkin's disease. It permits a cytological differentiation of this disease, even in its sarcomatous form, from cases of polymorphic reticulosarcoma (Varadi, 1955). Cases of so-called Schilling type of monocytic leukaemia, in which the cell-type with its striking nucleoli is identical with the bizarre giant-cells of Hodgkin's sarcoma, may be considered the leukaemic form of Hodgkin's disease.

The Megakaryocytes

An increased number of megakaryocytes is the most frequent non-specific finding in the bone-marrow in Hodgkin's disease (Limarzi and Paul, 1949). This was also the case in both patients described. In addition, many of the megakaryocytes appeared to be pathological; they had voluminous folded nuclei, and in a few of them the peripheral zone of the cytoplasm was vacuolated. As a whole, platelet-forming cells were reduced in number and formation of platelets of unequal size, some very large ones, could be observed. The large blue well-demarcated nucleoli of the Reed–Sternberg cells allowed them to be clearly distinguished even from the pathological megakaryocytes.

It is by no means surprising, however, that, based on histological appearances, Hodgkin's disease was at one time thought to be a megakaryocytoma originating in the bone-marrow (Medlar, 1931). In fact, the impression gained from the bone-marrow cytology is that both the megakaryocytes (megakaryoblasts) and Dorothy Reed cells are developing from a common ancestor, a young reticulum cell, which is probably also the maternal cell of the granulocytes and monocytes. Both megakaryocytes and Reed–Sternberg cells can be seen amidst the cells of the white and red series, often in close proximity to each other. It is possible that the stimulus which causes the reticulum cells to develop into Reed–Sternberg cells also leads to increased megakaryocyte formation.

Macrocytic Anaemia

While anaemia, normochromic or hypochromic, is fairly common, especially in the advanced or generalized stages and in the acute form of Hodgkin's disease, macrocytic anaemia (meaning megaloblastic or mixed megalo-normoblastic erythropoiesis) occurs but rarely.

Megaloblasts are known to occur in malignant conditions affecting the erythron (erythraemic or erythroleukaemic myelosis), in pure leukaemias, and also in some cases of carcinomatosis of the bone-marrow (secondaries of prostatic carcinoma, unpublished personal observation). The vitamin B_{12} levels are normal or increased in these conditions, and it is thought that megaloblastic erythropoiesis is probably brought about by a lack of folic acid available in the erythron. Essential metabolites of nucleic-acid synthesis are perhaps used up by the pathological cells proliferating in the bone-marrow. Such an explanation could account for the partly megaloblastic erythropoiesis and for the giant metamyelocytes, and perhaps also for the defective platelet-formation in Case 1. Impairment of the

intestinal absorption through obstruction of the lacteals by mesenteric lymph nodes is also a possible contributory factor.

The explanation of the unusual haematological findings in Case 2 is not easy. In this case, in addition to macrocytosis and an increased reticulocyte count, there were numerous nucleated red cells, mainly with pyknotic nuclei, Howell–Jolly bodies, Pappenheimer bodies, and a number of Heinz bodies in the peripheral blood; the bone-marrow contained a smaller number of intermediate megaloblasts. Such findings occur sometimes in idiopathic steatorrhoea with atrophy of the spleen, which in turn is thought to be due to folic-acid deficiency (Nieweg and Arends, 1953). In this case the red-cell anomalies could have been due to the small and pathological spleen. In view of the patient's condition it was impossible to carry out tests confirming the presence of folic-acid deficiency.

Macrocytic anaemia with Howell–Jolly bodies and target cells was observed in a further case of febrile subacute Hodgkin's disease in which Reed–Sternberg cells were found in the bone-marrow and the spleen was small and atrophic.

Summary

Two cases of unusual presentation of Hodgkin's disease are reported: one appeared to be an acute abdomen, the other a possible case of steatorrhoea. No lymphadenopathy was demonstrable clinically or radiologically in either case and the spleen was if anything atrophic. In the first, a case of acute Hodgkin's disease, Reed–Sternberg cells were circulating in the blood, and their discovery in the blood smears led to the diagnosis. In the second case the diagnosis was reached from the sternal puncture carried out for the purpose of investigating a macrocytic anaemia.

Both patients had macrocytic anaemia, leucopenia, and thrombocytopenia. The haematological findings are discussed.

It is suggested that cases of pyrexia of unknown origin presenting with the above haematological picture be carefully scrutinized for the presence of sternal tenderness.

My thanks are due to Mr. F. Masina and Dr. J. Flint for permission to use their patients' case-notes. I am indebted to Dr. A. J. N. Warrack for post-mortem reports and access to material. I am grateful to Mr. C. Lambourne and Mr. R. Brookes for the photomicrographs.

REFERENCES

Bayrd, E. D., Paulson, G. S., and Hargraves, M. H. (1954). *Blood*, **9**, 46.
Isaacs, R. (1944). *Med. Clin. N. Amer.*, Chicago No., p. 211.
Jeanselme, E., and Marchal, G. (1926). *Ann. Méd.*, **20**, 1.
Limarzi, L. R., and Paul, J. T. (1949). *Amer. J. clin. Path.*, **19**, 929
Ludman, H., and Spear, P. W. (1957). *Blood*, **12**, 189.
Medlar, E. M. (1931). *Amer. J. Path.*, **7**, 499.
Nieweg, H. O., and Arends, A. (1953). *Blood*, **8**, 175.
Steiner, P. E. (1943). *Arch. Path. (Chicago)*, **36**, 627.
Varadi, S. (1938). *Sang*, **12**, 106.
—— (1955). *Brit. J. Haemat.*, **1**, 184.

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C

GLUCOSE TOLERANCE IN DYSFUNCTIONAL UTERINE BLEEDING AND IN CARCINOMA OF ENDOMETRIUM

A PRELIMINARY REPORT

BY

FRED BENJAMIN, M.B., Ch.B., M.R.C.O.G.

From the Department of Obstetrics and Gynaecology, University of Capetown

" and now remains
That we find out the cause of this effect,
Or rather say, the cause of this defect,
For this effect defective comes by cause."
Hamlet, II, ii.

Investigations have been carried out in the gynaecology department of the Groote Schuur Hospital, Capetown, to determine the value of glucose-tolerance studies in cases of abnormal uterine bleeding of both benign and malignant origin. Studies were made of patients at the climacteric as well as in other age-groups. The results have been correlated in each case with the histological picture of the endometrium. Some interesting and significant findings have come to light, so that, though the work is still in a preparatory stage, it is considered that a preliminary report is warranted.

Several investigators have found that the incidence of diabetes mellitus and of impaired glucose tolerance is high in cases of carcinoma of the body of the uterus. Way (1954) reported that 29% of such patients had frank diabetes mellitus, and a further 43% showed a " pre-diabetic type of glucose-tolerance curve." Similar figures were found by Garnet (1958), and the findings of many other writers confirmed this abnormality, though most figures are not as striking as those of Way and of Garnet (Hertig and Sommers, 1940; Scheffey *et al.*, 1943; Moss, 1947; Palmer *et al.*, 1949; Louw, 1958; Noble and Attwood, 1958). Other workers, however, have failed to find a positive association between diabetes mellitus and carcinoma of the endometrium. Such reports include those of Marble (1934), Smith (1941), Jacobson (1948), and a recent one from Vander (1959); the latter found an incidence of 5.6% of diabetics, which is not higher than the incidence of diabetes in the general population of the same age and sex. The conflicting reports must in part, at least, be due to the lack of careful and adequate controls in the same age-groups; and few of these cases had been fully investigated by glucose-tolerance tests.

In view of the divergent reports, we first investigated patients with carcinoma of the body of the uterus, together with control subjects of the same age-groups. The work was then extended to embrace all cases with abnormal uterine bleeding, and especially those with benign glandular hyperplasia of the endometrium. So far the following groups have been studied by carrying out glucose-tolerance tests in each case and by correlating the results with the histological picture of the endometrium: (1) One hundred random women of 45 years of age and over who were not suffering from cancer of the endometrium or benign glandular hyperplasia. (2) Fifty cases of cancer of the endometrium. (3) Fifty patients who were proved by histological examination of the endometrium to have benign glandular hyperplasia.