CYCLOPHOSPHAMIDE IN HODGKIN'S DISEASE AND RELATED DISORDERS

BY

J. Q. MATTHIAS, M.B., B.S., F.F.A. R.C.S. M.R.C.P.
Senior Registrar

J. J. MISIEWICZ, M.B., B.S., B.Sc.
Senior House Officer

AND

RONALD BODLEY SCOTT, M.A., D.M. F.R.C.P.
Physician

St. Bartholomew's Hospital, London

The value of the nitrogen mustards in the treatment of Hodgkin's disease and related disorders has been firmly established for many years (Gilman and Philips, 1946; Wilkinson and Fletcher, 1947). The limiting factor in their use has been their toxic action upon the bone-marrow and other tissues. These undesirable side-effects can be modified by the substitution of various radicals on the nitrogen atom. In this paper we report our experiences in the treatment of Hodgkin's disease and other tumours of lymphoreticular tissue with a cyclic nitrogen mustard phosphamide ester, cyclophosphamide (" cytoxan," " endoxan ").

Pharmacology

The pharmacological properties of the nitrogen mustards depend upon their β-chloroethyl groups (Goodman and Gilman, 1955). They are unstable in aqueous solutions: for instance, in the case of the methyl-*bis*(β-chloroethyl) analogue, intramolecular cyclization with the formation of biologically active quaternary immonium compounds occurs within a few minutes (Fig. 1). The biological activity of these drugs springs from their ability to attack nucleoproteins by combining with such of their component radicals as carboxyl, phosphoryl, or amino groups (Ross, 1953); it bears a direct relation to the rate of chloride dissociation (Fig. 1), which can be reduced by lowering the basicity of the nitrogen atom.

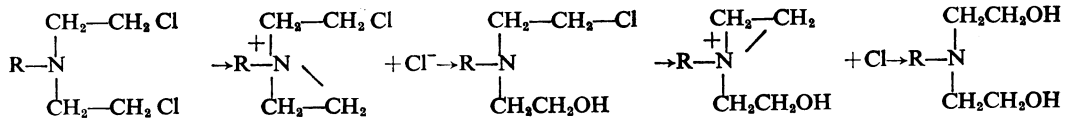
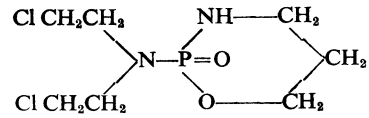


FIG. 1

Cyclophosphamide, the derivative we have studied, was synthesized in 1958 (Arnold *et al.*, 1958a, 1958b) with the aim of preparing an inactive " transport " form of nitrogen mustard which would be activated only on reaching the site where its therapeutic effect was required. Malignant cells are known to be rich in phosphatases and phosphamidases; it was postulated that a nitrogen mustard phosphamide might well be split by these enzymes to an active form within the tumour. In addition, the P=O linkage of the phosphamide molecule lowers the basicity of the nitrogen atom and thus reduces the ionizability of the chloride (Arnold *et al.*, 1958b).

Cyclophosphamide is a cyclic nitrogen mustard phosphamide ester N_1N-*bis*(β-chloroethyl)-N^1-O-propylene phosphoric acid ester diamide with the following structure:



Unlike HN2 (mustine), it is stable in aqueous solution at 4° and 37° C. and is inactive *in vitro*, as shown by the viability of cell cultures in media containing it (Brock and Wilmanns, 1958). Further, the rate of chlorine-ion liberation is appreciably lower than that of other nitrogen-mustard derivatives. Its therapeutic index is high and no toxic action was observed on the liver or kidney, although a leucotoxic effect was evident. It proved effective in the rat against Yoshida ascites sarcoma, Walker 256 carcinoma, Jensen sarcoma, and DS carcinoma (Arnold *et al.*, 1958a; Brock, 1958; Burkert, 1958). In mice, cyclophosphamide was shown to be more active than HN2 against advanced L 1210 leukaemia as well as being effective against L 1210/AG-R leukaemia resistant to 6-mercaptopurine (Lane, 1959; Lane and Kelly, 1959).

Method

Of the 45 patients treated with cyclophosphamide, 17 had Hodgkin's disease, 3 lymphosarcoma, 1 reticulum-cell sarcoma, 1 lymphoid follicular reticulosis, 14 myelomatosis, 5 chronic lymphocytic leukaemia, 1 polycythaemia rubra vera, and 3 other tumours.

No particular criteria were employed for its use. Most patients received an initial intravenous course, beginning with 100 mg. and continuing at 200 mg. daily up to a total of 2 to 4 g. The drug was prepared by dissolving 200 mg. in 10 ml. of sterile water shortly before injection. The intravenous course was usually followed by the oral administration of 100 to 150 mg. daily in 50-mg. (1 tablet) doses. No *a priori* limits were set to the total amount administered to any one patient, many patients receiving long-term maintenance treatment, which varied from 100 to 150 mg. a day. The drug was temporarily withdrawn when satisfactory response was achieved or when toxic side-effects occurred. Frequent blood examinations were made on in-patients. In out-patient practice, a complete blood count every two or three weeks gave adequate control provided initial leucocyte levels were satisfactory.

The effects of treatment were judged by the patient's subjective impressions, such as diminution of pain or malaise, and by objective criteria. By objective improvement is meant control of fever, a diminution in tumour mass or hepatosplenomegaly, a gain in weight, a significant alteration in the peripheral blood picture or marrow cytology, or a return of the blood chemistry to normal. Change in any one of these parameters would be classified as objective improvement. Leucopenia is defined as a total white count of less than 4,000 cells/c.mm., and thrombocytopenia as less than 150,000 platelets/c.mm. Some patients were leucopenic before treatment began. In these, leucopenia is recorded as being due to the cyclophosphamide if the existing white count was halved.

Results

Hodgkin's Disease. — The diagnosis had been established histologically in all the cases. The 17 patients in this group received cyclophosphamide varying in quantity from 2,100 to 22,050 mg. (average 9,500 mg.). The results are shown in Table 1, Cases 1–17. Fifteen patients (88%) experienced subjective improvement after medication. Signs of objective improvement were present in 11 (65%). Side-effects occurred in 8 instances (47%) but were mild and did not require withdrawal of the drug, except in Case 5, in which the peripheral white count fell to 600 cells/c.mm. for a time. This patient had widespread disease in the terminal phase and died less than a month after the end of treatment. It is perhaps significant that this was the only case which developed a leucopenia in this group. No patient became thrombocytopenic.

In Case 1 the patient was bedridden and required frequent heroin for alleviation of pain due to spinal deposits. After cyclophosphamide it became possible to wean him from opiates. He became ambulant and relatively symptom-free for a time, but relapsed four months later.

In Case 2 the patient gained 11 lb. (5.5 kg.) in weight. Recalcification of osteolytic lesions in the skull and ribs is shown in Figs. 2 and 3. Subcutaneous nodules on the head became impalpable. This patient's symptoms returned soon after discontinuation of maintenance therapy. They have been partially controlled by resumed administration of the drug. Case 9 presented twice with a large palpable abdominal mass; response to cyclophosphamide has been prompt on both occasions. One patient (Case 17) was emaciated, profoundly anaemic, and febrile, with splenic enlargement and a mass of nodes in the left axilla. Six months later she was able to enjoy a holiday in San Francisco, having gained 2 st. (12.7 kg.) in weight and being free from all symptoms.

Myelomatosis.—This group comprises 14 patients. The results are shown in Table I, Cases 18 to 31. The dose of cyclophosphamide varied from 1,250 to 15,700 mg. (average 7,600 mg.). Six patients (43%) were subjectively improved; two were difficult to assess. Five patients (36%) showed objective signs of improvement as evidenced, for example, by regression of hypercalcaemia (Cases 19 and 26) and reduction in the proportion of plasma cells in the marrow from 50% to 23% (Case 24). This patient was relatively free of symptoms and did not notice any subjective change. Platelet counts in Cases 27 and 28 were low before cyclophosphamide was given. Cases 21 and 22 showed a rise in the platelet count from thrombocytopenic to normal levels. 11 (79%) cases showed leucopenia: this is in contrast to the findings in the Hodgkins group. The fall in the peripheral white count was particularly severe and prolonged in Case 18, who received cyclophosphamide and irradiation therapy at the same time. Thrombocytopenia was present in four patients (29%), but two of these had low counts originally.

TABLE I

Case No.	Sex and Age	Diagnosis	Duration of Disease	Previous Treatment	Dose of Cyclophosphamide (mg.)	Side Effects	Whether Improved	
							Subjectively	Objectively
1	M 28	Hodgkin's disease	4 yr.	Trimustine. DXR. HN2	6,000	—	+	+
2	M 43	,, ,,	1 ,,	Chlorambucil. DXR.	17,000	Mild alopecia. Transient vomiting	+	+
3	M 30	,, ,,	2 ,, 6 m.	DXR. HN2	2,100	Nausea	+	+
4	F 28	,, ,,	2 ,, 6 ,,	—	12,600	—	+	−
5	M 22	,, ,,	3 ,,	Prednisolone. DXR. HN2	3,150	Transient leucopenia	+	+
6	M 30	,, ,,	7 ,,	,, ,,	14,950	Transient nausea	+	+
7	F 30	,, ,,	2 ,, 2 ,,	DXR.	5,100	Leucopenia	+	−
8	F 39	,, ,,	6 ,,	Mannomustine. DXR. Prednisolone	22,050	—	+	+
9	M 27	,, ,,	4 ,, 9 ,,	DXR.	8,400	Transient nausea	+	+
10	M 22	,, ,,	4 ,,	,,	4,500	—	±	+
11	M 48	,, ,,	5 ,,	Cobalt therapy. DXR. HN2	4,500	Mild alopecia. Reduction in beard growth	+	+
12	M 17	,, ,,	2 ,,	DXR	10,000	—	+	+
13	M 43	,, ,,	2 ,, 6 ,,	Busulphan	9,450	—	+	−
14	F 18	,, ,,	1 ,,	—	5,250	—	+	−
15	F 27	,, ,,	5 ,,	—	6,250	—	−	−
16	F 27	,, ,,	2 ,,	—	14,700	—	+	−
17	F 23	,, ,,	4 ,,	DXR. Chlorambucil	15,600	Mild alopecia	+	+
18	F 52	Myelomatosis	1 ,, 9 ,,	DXR. Phenamidine	6,400	Leucopenia	−	−
19	F 43	,,	3 ,, 6 ,,	Transfusions	6,500	Leucopenia. Thrombocytopenia	+	+
20	M 51	,,	5 ,, 6 ,,	DXR	11,100	—	+	−
21	M 61	,,	1 ,, 4 ,,	DXR. Prednisone. DD4450. Transfusions	4,200	Leucopenia	±	−
22	F 46	,,	1 ,,	—	6,950	,,	−	−
23	F 67	,,	4 ,,	—	8,600	,,	±	−
24	M 54	,,	3 ,,	—	9,950	,,	−	+
25	M 50	,,	1 ,,	—	3,000	,,	+	−
26	F 53	,,	1 ,,	—	11,000	Thrombocytopenia. Leucopenia. Mild alopecia	+	+
27	F 47	,,	3 ,,	—	2,400	Leucopenia. Thrombocytopenia	−	−
28	M 61	,,	4 ,,	—	1,250	,, ,,	−	−
29	M 41	,,	1 ,,	DXR	15,700	—	+	+
30	M 38	,,	3 ,,	M&B 938. DXR	14,800	—	+	+
31	F 62	,,	2 ,,	DD4450	4,500	Leucopenia. Mild alopecia	−	−
32	M 53	Lymphosarcoma	3 ,,	CB1348. DXR	1,100	Thrombocytopenia	−	−
33	F 51	,,	2 ,,	DXR	8,250	Mild alopecia	+	+
34	F 64	,,	7 ,,	,,	5,400	—	+	+
35	F 52	Reticulum-cell sarcoma	1 ,,	—	2,900	—	+	−
36	M 70	Lymphoid follicular reticulosis	4 ,,	Chlorambucil. DXR	2,850	—	+	−
37	F 63	Chronic lymphocytic leukaemia	3 ,,	—	1,050	—	−	−
38	M 59	,, ,,	1 ,, 6 ,,	Mannomustine	6,300	—	+	+
39	M 56	,, ,,	3 ,,	—	4,900	—	+	+
40	M 63	,, ,,	4 ,,	Chlorambucil	3,700	Leucopenia	+	−
41	M 62	,, ,,	3 ,,	,,	20,450	Thrombocytopenia	±	+
42	F 48	Polycythaemia rubra vera	1 ,,	32 P	4,200	,,	+	+
43	M 59	Ca. bronchus	3 ,,	—	1,050	—	−	−
44	M 48	Ca. rectum	5 ,,	Surgery	3,000	—	−	−
45	M 45	Liposarcoma	3 ,,	DXR. CB1348	3,000	—	+	−

Lymphosarcoma. Reticulum-cell Sarcoma. Lymphoid Follicular Reticulosis.—Of three patients with lymphosarcoma, one failed to improve. The other two, however, improved subjectively and objectively (Cases 33 and 34), although one exhibited a mild alopecia. The patient suffering from reticulum-cell sarcoma felt better after cyclophosphamide, as did the one with lymphoid follicular reticulosis. Neither showed objective signs of improvement. Dosage in the above conditions varied from 1,100 to 8,250 mg. No leucotoxic effects were observed, but one patient (Case 32) became thrombocytopenic.

Chronic Lymphocytic Leukaemia.—Three of five patients responded as shown by diminution of lymphadenopathy, decrease in size of the liver and spleen, and a fall in the peripheral lymphocyte count. Evidence for marrow remission is not available at the moment. One patient (Case 40) died of bronchopneumonia and his leucopenia was probably a terminal event. One patient (Case 41) has bronchial carcinoma as well as lymphocytic leukaemia. This thrombocytopenia is mild and the white count has been well controlled over seven months by a maintenance dose of cyclophosphamide.

Polycythaemia Rubra Vera.—This patient (Case 42) received 4,200 mg. of cyclophosphamide, the P.C.V. falling from 62% to 53% and the haemoglobin from 17.6 to 51.7 g./100 ml. The neutrophilia was likewise controlled. The platelet count was reduced, but as it had been low beforehand the significance of this is doubtful.

Other Tumours.—Two cases of carcinomatosis, one of bronchial and one of rectal origin, were treated with no effect. One case of widespread liposarcoma received cyclophosphamide with temporary subjective remission. One patient with bronchial carcinoma as well as chronic lymphocytic leukaemia (Case 41) has not shown any clinical or radiological evidence of spread of his cancer over seven months since cyclophosphamide was started.

Discussion

Cyclophosphamide has proved satisfactory in clinical usage. The amount given daily has been similar to that used by the German workers (Gross and Lambers, 1958). In contrast, Coggins *et al.* (1959) employ a loading dose of 7.5 mg./kg. body weight for six days followed by oral or intravenous administration sufficient to keep the white-cell count at 1,000–2,000 cells/c.mm. Alternatively, successive single injections of 45 to 100 mg./kg. body weight were used.

Most patients in the present series were given maintenance treatment of 100 to 150 mg. of cyclophosphamide daily following upon the initial course of the drug, and several patients (for example, Cases 2, 8, 16, 33, and 41) have been taking it regularly for

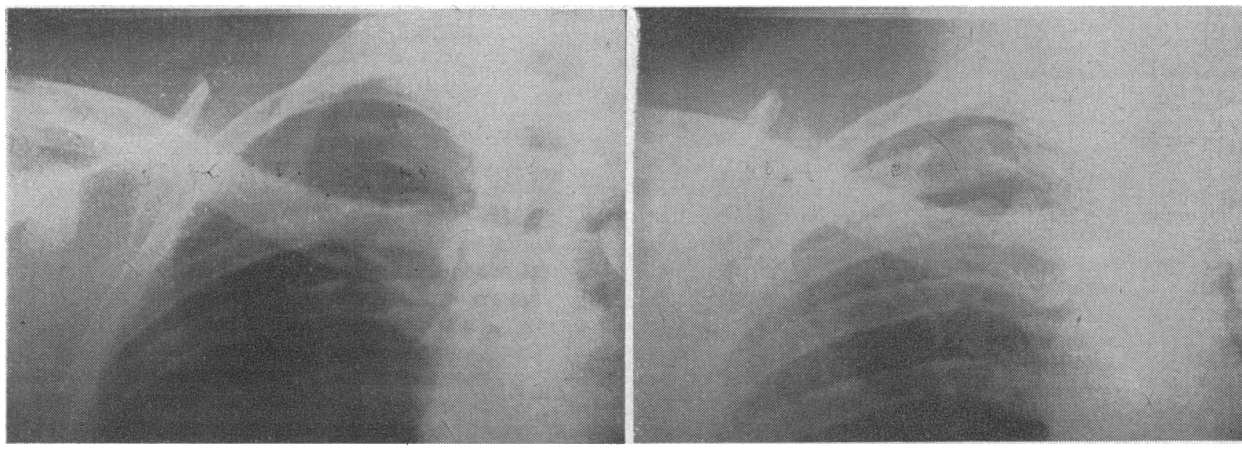


FIG. 2.—Case 2. Recalcification of Hodgkin's deposits in the third rib after treatment with cyclophosphamide. Radiographs taken on January 1 and July 30, 1960.

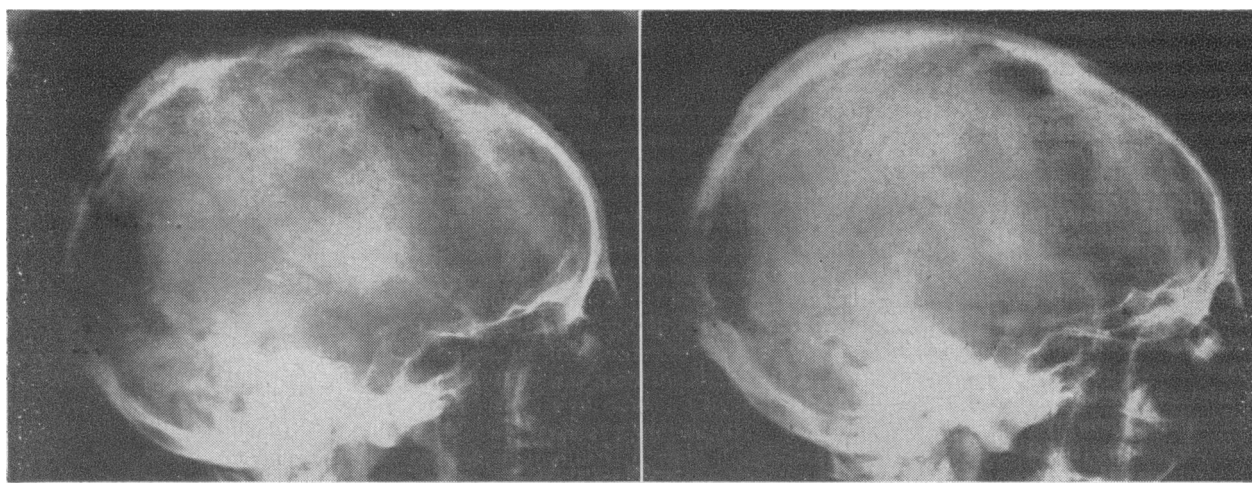


FIG. 3.—Case 2. Recalcification of Hodgkin's deposits in the skull after treatment with cyclophosphamide. Radiographs taken in February and July, 1960.

periods varying from four to seven months with beneficial effect. Some patients who experienced a return of symptoms when maintenance treatment was stopped obtained further relief by restarting cyclophosphamide. Somewhat similar conclusions were reached by Larionov (1956) using novoembichin.

Side-effects following upon the administration of cyclophosphamide have been inconspicuous in the present series. Alopecia has not proved troublesome, no venous thromboses occurred at the site of injections, and nausea and vomiting were infrequent, One male patient noticed a reduction in beard growth. In general, side-effects such as alopecia, sterile cystitis, gastro-intestinal disturbances, and transient fall in the haematocrit are met with more often when amounts in excess of 200 mg. of cyclophosphamide are given daily. Thrombocytopenia is said not to occur even at the higher levels of dosage (Haar *et al.*, 1960). In four of our patients, however, low platelet counts were found after treatment; two of these were suffering from myelomatosis.

The fact that of the 17 patients suffering from Hodgkin's disease 11 (65%) were objectively improved and that 15 (88%) benefited subjectively (Table II)

TABLE II

Diagnosis	No. of Cases	No. Improved	
		Objectively	Subjectively
Hodgkin's disease	17	11	15
Lymphoid follicular retic.	1	0	1
Lymphosarcoma	3	2	2
Reticulum-cell sarcoma	1	0	1
Myelomatosis	14	5	6
Chronic lymphocytic leukaemia	5	3	3
Polycythaemia rubra vera	1	1	1
Other tumours	3	0	1
Total	45	22 (49%)	30 (67%)

deserves note. Similar figures were obtained in other series (Gross and Lambers, 1958; Petrides and Moncke, 1958; Brichta *et al.*, 1958). Improvement may be expected 10 to 14 days after the beginning of treatment, the control of fever being a notable feature.

It is encouraging to find a 36% objective improvement rate in myelomatosis. Case 26 shows a particularly good remission, the serum calcium being reduced from 18.6 to 10.5 mg./100 ml. This patient gained 11 lb. (5.5 kg.) in weight; her haemoglobin rose from 6.2 to 9.2 g./100 ml. and has been maintained at the latter level. 11 (79%) cases of myelomatosis showed leucopenia during treatment, in constrast to the other groups treated.

Partial remission of chronic lymphocytic leukaemia was achieved in three out of five of our patients. However, Haar *et al.* (1960) could find evidence of marrow remission in only one out of their five cases that responded to treatment with cyclophosphamide.

Cyclophosphamide has been tried in a wide variety of other malignant tumours with variable effect (Lemke *et al.*, 1959; Baumann 1959; Coggins *et al.*, 1959). In one series (Coggins *et al.*, 1959), 18 out of 47 "evaluable" cases responded, the average duration of remission being two months. No improvement occurred in our three patients.

Summary and Conclusions

45 patients with malignant disease were treated with cyclophosphamide. 42 suffered from diseases of the haemopoietic or reticuloendothelial systems, three had disseminated malignant disease of other types.

Cyclophosphamide has proved a valuable addition to the chemotherapeutic drugs available for the treatment of malignant diseases of the haemopoietic and reticuloendothelial systems. It was found particularly effective in Hodgkin's disease, lymphosarcoma, chronic lymphocytic leukaemia, and myelomatosis, and it promises well in the treatment of polycythaemia rubra vera.

Side-effects are minor, although some depression of the white-cell count usually accompanies effective therapy. Maintenance treatment is safe, worth while, and simple, as the drug can be administered in tablet form. White-cell-count control is essential. Usually, while patients are on maintenance therapy, a count every three to four weeks will suffice.

We thank Dr. J. M. Simister, of Ward, Blenkinsop and Co., Ltd., for supplies of cyclophosphamide.

REFERENCES

Arnold, H., Bourseaux, F., and Brock, N. (1958a). *Nature (Lond.)*, **181**, 931.
—— —— —— (1958b). *Naturwissenschaften*, **45**, 64.
Baumann, E. (1959). *Medizinische*, **1**, 659.
Brichta, G., Kühböck, J., and Reimer, E. E. (1958). *Wien. Z. inn. Med.*, **39**, 306.
Brock, N. (1958). *Arzneimittel-Forsch.*, **8**, 1.
—— and Wilmanns, H. (1958). *Dtsch. med. Wschr.*, **83**, 453.
Burkert, H. (1958). Asta Nachrichten, No. 18.
Coggins, P. R., Ravdin, R. G., and Eisman, S. H. (1959). *Cancer Chemother. Rep.*, **3**, 9.
Gilman, A., and Philips, F. S. (1946). *Science*, **103**, 409.
Goodman, L. S., and Gilman, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 1416.
Gross, R., and Lambers, K. (1958). *Dtsch. med. Wschr.*, **83**, 458.
Haar, H., Marshall. G. J., Bierman, H. R., and Steinfeld, J. L. (1960). Cancer Chemother. Rep., No. 6, p. 41.
Lane, M. (1959). *J. nat. Cancer Inst.*, **23**, 1347.
—— and Kelly, Margaret G. (1959). *Proc. Amer. Ass. Cancer Res.*, **3**, 35.
Larionov, L. F. (1956). *Brit. med. J.*, **1**, 252.
Lemke, J., Stange, H. M., and Rumphorst, K. (1959). *Landarzt*, **20**, 724.
Petrides, P., and Moncke, C. L. (1958). *Mitt. Ges. Bekämpf. Krebskr. Nord-Westfall.*, **2**, No. 6.
Ross, W. C. J. (1953). *Advanc. Cancer Res.*, **1**, 397.
Wilkinson, J. F., and Fletcher, F. (1947). *Lancet*, **2**, 540.

MEGALOBLASTIC MADNESS

BY

A. D. M. SMITH, M.B., M.R.C.P., B.Sc.
Medical Registrar, Central Middlesex Hospital, London

The occurrence of mental symptoms in association with pernicious anaemia has been known for many years and been commented upon by various authors (McAlpine, 1929; Samson *et al.*, 1952; MacDonald Holmes, 1956; Wiener and Hope, 1959). Langdon (1905) drew attention to a group of cases in which nervous and mental symptoms preceded the onset of anaemia and described a great variety of neurotic and psychotic manifestations. Latterly (Wiener and Hope, 1959) the extreme variability of the symptoms has been stressed, and it is obvious that anything from a mild mood disorder to grossly psychotic behaviour may be encountered. Epilepsy, urinary and faecal incontinence in the absence of overt spinal lesions, dysphasia, and confusional states are also mentioned (MacDonald Holmes, 1956). Furthermore, it is well recognized that vitamin-B_{12} deficiency may give rise to optic atrophy, and it has been shown that tobacco amblyopia may be cured by administration of this vitamin (Heaton *et al.*, 1958).

The exact nature of the defect in cerebral metabolism which is produced by deficiency of vitamin B_{12} is not