accelerated destruction of abnormal red-cell precursors within the marrow, secondary to inefficient erythropoiesis (Finch *et al.*, 1956; Witts, 1961) and the release of cellular enzymes into the surrounding plasma. On the other hand, the enzyme concentrations of the megaloblastic marrow tissues were, with the exception of one with minimal change, greater than those of the normoblastic marrows (Fig. 2). It is unlikely that the increased lactate dehydrogenase concentration of the megaloblastic marrows can be fully explained by the increased proportion of immature red cells present in these marrows. Immature red cells of normoblastic marrows have a larger complement of glycolytic enzymes than mature cells, but the lactate dehydrogenase concentration is the same irrespective of the stage of maturity (Marks *et al.*, 1958; Bernstein, 1959).

FIG. 2.— α -Hydroxybutyrate dehydrogenase (H.B.D.) activities of marrow plasma related to marrow cellular activities in normoblastic and megaloblastic marrows.

The alternative explanation for the increased lactate dehydrogenase and α -hydroxybutyrate dehydrogenase content of megaloblastic marrows is an increased enzyme content of the individual erythroid cells, as suggested by Heller *et al.* (1960b),

and that this increased content reflects greater metabolic activity or failure of utilization or both. The available evidence indicates that during the development of megaloblastosis in patients with vitamin-B₁₂ or folic-acid deficiency the abnormal red-cell precursors acquire a higher concentration of certain enzymes, including lactate dehydrogenase and α -hydroxybutyrate dehydrogenase, than their normal counterparts. This increased enzyme content associated with an accelerating destruction rate of the cells causes the release of large amounts of enzyme protein into the marrow plasma. The rising lactate dehydrogenase and α -hydroxybutyrate dehydrogenase activities in peripheral blood serum reflect the rising marrow plasma activities.

Summary

The raised serum lactate dehydrogenase and α -hydroxybutyrate dehydrogenase activities in patients with megaloblastic erythropoiesis secondary to folic-acid deficiency are derived from high concentrations in the marrow plasma. According to available evidence these high concentrations in turn result from the increased destruction within the marrow of abnormal red-cell precursors with a high enzyme content.

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Natulan in Management of Late Hodgkin's Disease, Other Lymphoreticular Neoplasms, and Malignant Melanoma

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Bollag and Grunberg (1963) described the tumour-inhibitory effects of methyl-hydrazine derivatives. As a result one of these agents, Natulan (1-methyl-2-*p*-(isopropylcarbamoyl) benzyl-hydrazine chlorhydrate), has been undergoing clinical trials. Mathé, Berumen, Schweisguth, Brule, Schneider, Cattani, Amiel, and Schwarzenberg (1963) described their experience in treating 44 patients, and concluded that it was "of considerable value in the treatment of Hodgkin's disease." Sicher and Backhouse (1963) confirmed the benefits of this

drug, but did not think it should yet replace more firmly established cytotoxic agents.

Clinical Material and Methods of Administration

There were 40 patients in this series, of whom 33 had lymphoreticular disease: Hodgkin's disease, 24 cases; lymphosarcoma, 5 cases; mycosis fungoides, 2 cases; Brill-Symmers disease, 1 case; reticulum-cell sarcoma, 1 case; melanomatosis, 6 cases; and carcinomatosis (primary unknown), 1 case.

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All the patients with lymphoreticular disease had features of generalization and all had had extensive previous radiotherapy and treatment with at least one other cytotoxic drug. The patients with malignant melanoma and carcinoma also had widespread disease beyond any useful treatment with surgery, radiotherapy, or intra-arterial chemotherapy.

Natulan was given by mouth as a 50-mg. capsule. In view of the frequent occurrence of nausea, it is recommended that only one capsule is given on the first day, but that the dose is gradually increased over the course of a few days to six capsules (300 mg.) daily in divided doses. When nausea occurred it could usually be controlled by stopping at a lower dose, say 200 mg. daily, or by giving an anti-emetic such as chlorpromazine (25 to 50 mg. daily) concurrently. A dose of 300 mg. daily was continued until a remission was obtained or until the effect on the peripheral blood count necessitated a reduction of the dose to a maintenance level, commonly of the order of 100 to 200 mg. daily. The drug was freely administered to out-patients, and a weekly or fortnightly blood count was usually quite sufficient to control the dosage.

Intravenous injection does not prevent nausea (Sicher and Backhouse, 1963), but may be an acceptable alternative means of administration to in-patients.

Results of Treatment

Hodgkin's Disease.—There were 24 patients (15 men and nine women) with this diagnosis. The youngest was aged 6 years and the eldest 70, and the diagnosis was confirmed histologically in all cases. Fifteen of these patients had a remission lasting one month or more. In all 15 there was improvement in well-being; pruritus was controlled in five cases; backache was relieved in four cases; and cough, dyspnoea, and dysphagia associated with mediastinal deposits were relieved in two cases. One patient mentioned an increase in her libido. With regard to objective evidence, lymph nodes became measurably smaller in six cases (for example, see Figs. 1 and 2), the haemoglobin level rose by 10% or more in four cases, splenomegaly became less in three cases, pyrexia abated in two cases, and abdominal distension became less in two cases. The average duration of remission was 3.3 months (including four patients in remission

at the time of writing), and the longest, so far, is six months. In all cases it was possible to compare the effect of Natulan with at least one previous chemotherapeutic agent. The only distinct pattern which emerged was that there was no obvious "cross-resistance" with the alkylating agents or vinblastine.

FIG. 2.—Repeat radiograph of same patient as in Fig. 1 during treatment with Natulan to show marked, though incomplete, regression of the enlarged mediastinal nodes.

Other Lymphoreticular Disease.—Of the five patients with generalized lymphosarcoma, one died within a few days of taking the drug, two have shown temporary subjective improvement without objective evidence of remission, and two have shown both subjective and objective improvement. One of these latter patients, whose haematological data are summarized in Fig. 3, has achieved a worth-while remission with increase

FIG. 1.—Radiograph of the chest of a 37-year-old woman with Hodgkin's disease to show mediastinal lymphadenopathy.

FIG. 3.—Haematological effects of Natulan on a 40-year-old man with generalized lymphosarcoma. The patient's well-being improved, subcutaneous deposits regressed, and bone pain was relieved. As can be seen, his haemoglobin level rose during treatment, but there was relatively little effect on the platelets or white cells. Just before death there was rapid increase in the size of his intrathoracic deposits.

in well-being, regression of subcutaneous lumps, relief of bone pain, and capacity to return to work for a period of five months.

Two patients with mycosis fungoides, one patient with Brill-Symmers disease (giant follicular lymphoma), and one patient with reticulum-cell sarcoma have had no benefit from this agent.

Melanomatosis and Carcinomatosis.—Six patients with melanomatosis are included in the series. The first patient treated has shown considerable, though incomplete, regression of widespread subcutaneous masses with marked improvement in well-being and relief of melanuria. In her case benefit has continued for over seven months. Unfortunately, only one of the other five has shown any response, and that has been short-lived.

A patient with generalized deposits of an undifferentiated carcinoma, from an undiscovered primary, who had previously had a complete remission on cyclophosphamide lasting for five months, failed to respond to Natulan.

Side-effects

Nausea.—With the additional complaints, in a few cases, of vomiting, anorexia, and a bad taste in the mouth, nausea was severe enough to stop treatment in three cases, though in one of these (the patient whose chest radiographs appear in Figs. 1 and 2) subsequent admission to hospital and the free use of chlorpromazine enabled the patient to take sufficient Natulan to induce a remission. After a few days' treatment nausea became much less troublesome, and she was able to continue on a maintenance dose as an out-patient for the three months' duration of the remission. In 11 cases the nausea was severe enough to warrant the use of an anti-emetic or reduction of the dosage. Three patients had mild nausea at the start of treatment, but required no help to overcome it. Twenty-three patients had no nausea at the beginning of treatment, but five of this group mentioned gastro-intestinal upset in the form of nausea, anorexia, or "indigestion" towards the end of the course. These symptoms cleared up as soon as treatment was stopped. There were no cases of diarrhoea.

Leucopenia.—The effect on the peripheral blood count was similar to that seen when using alkylating agents. Examples are shown in Figs. 3 and 4. There did not seem to be a selective effect on polymorphs or mononuclear cells. In this series the total white-cell count did not fall below 1,100/c.mm. in any patient, though some had total doses of 20 g. or more spread over four to six months. The lowest dose which produced a substantial fall in the white-cell count (from 4,800 to 1,200/c.mm.) was 6 g. given over three weeks, and in this patient the count rapidly returned to normal when the drug was discontinued. In most cases there was little effect on the platelet count, though in a minority it was reduced by about one-half during treatment. The only potentially serious case was the one shown in Fig. 4, in which the count fell from an initial level of 280,000/c.mm. to 55,000/c.mm. after five months' treatment and a total dose of 21 g. had been given. In this case there was no purpura or any other complication.

On the whole, the haemoglobin and red-cell count were unaffected by treatment, though in four cases where anaemia

was a feature of the disease there was a rise associated with the induction of remission.

Flush Syndrome.—After the drinking of wine this symptom was noted in five patients reported by Mathé *et al.* (1963). In the present series two patients complained of flushing of the face after taking a small alcoholic drink, and a third thought that Natulan caused marked potentiation of alcohol. These instances do not provide a reliable assessment of the possible incidence of this complication, because, latterly, all patients were warned to avoid alcohol during treatment.

Dry Mouth.—Two patients, both of whom had slight dryness of the mouth after x-ray therapy (in each case three years previously) to areas including the oral mucosa and salivary glands, complained of intensification of this symptom during treatment.

Rash.—Two patients developed a punctate erythematous rash on the legs, in both cases quite early in the course of treatment (soon after the daily dose of 300 mg. had been attained). In one patient the dose was dropped to 150 mg. daily and the rash cleared; in the other the rash persisted despite reducing the dose, and cleared only when the drug was discontinued after the induction of a remission.

Euphoria.—Elevation of mood, which they thought was more than could be accounted for by the sense of well-being associated with their remission, was mentioned by two patients.

Epilation.—A slight outfall of hair, possibly associated with Natulan treatment, was noticed by one patient.

Discussion

Natulan finds its main place in the management of generalized Hodgkin's disease. In this condition the proportion of remissions obtained, their quality, and their duration are comparable with those for alkylating agents and vinblastine.

Experience in the Christie Hospital, Manchester, between 1955 and 1960 with patients suffering from generalized Hodgkin's disease shows that of 67 patients treated with mustine (in a dose of 0.4 mg./kg. of body weight administered intravenously in two days), who had not had previous treatment with an alkylating agent, 39 (58%) went into remission. Again, since 1962, 45 patients with generalized Hodgkin's disease received treatment with vinblastine for the first time (27 of these had had previous treatment with an alkylating agent) and 29 (64%) obtained a remission. These figures of 58% for mustine and 64% for vinblastine are of the same order as the 62.5% (15/24) recorded for Natulan. It is of interest in this connexion that, since 1958, 30 patients in the Christie Hospital have had treatment with cyclophosphamide, having previously been treated with other alkylating agents (mustine, tretamine, or melphalan), and of these only 10 enjoyed a remission. This would suggest that, though perhaps there is some degree of "cross-resistance" between different alkylating agents, previous treatment with an alkylating agent or vinblastine does little to reduce the possibility of successful treatment with Natulan.

In the management of a case of generalized Hodgkin's disease use is likely to be made of one or more alkylating agents, vinblastine, and Natulan. As the likelihood of procuring a remission is similar for each, and as there is no evidence in favour of any particular sequence in their use, the order in which these agents are used is likely to be governed by considerations such as ease of administration (especially to out-patients) and freedom from side-effects. Excepting in those cases where nausea is very troublesome, Natulan is as easy and safe to use and as acceptable to the patient as, say, cyclophosphamide or vinblastine. Neither Natulan nor any other chemotherapeutic agent should, of course, replace radiotherapy in the management of localized Hodgkin's disease; for, while chemotherapy is never more than palliative, radiotherapy (Easson and Russell, 1963) is potentially curative.

FIG. 4.—Haematological effects of Natulan on a 28-year-old man with late Hodgkin's disease. The low platelet count and low white-cell count which developed after intensive treatment did not give rise to any complications.

With regard to other diseases, Natulan, as Mathé *et al.* (1963) suggest, may sometimes be of value in lymphosarcoma and may occasionally be worth trying in melanomatosis. The drug has not yet been used widely enough in other tumours to make an assessment.

Summary

A series of 40 patients treated with Natulan is presented, and an account given of the administration and side-effects of this drug.

Assessment of response to treatment suggests that Natulan has a place in the palliation of late Hodgkin's disease comparable with the alkylating agents and vinblastine. Further,

this drug still offers a good chance of obtaining a remission in cases refractory to the alkylating agents and vinblastine. Natulan may be of value in other lymphoreticular neoplasms such as lymphosarcoma.

Dr. A. M. C. Duffus, of Roche Products Limited, Welwyn Garden City, Hertfordshire, supplied the Natulan used in this trial.

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Preliminary Communications

Hepatic Impairment During Intake of Contraceptive Pills: Observations in Post-menopausal Women

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Eisalo *et al.* (1964) recently reported that liver dysfunction developed in 10 out of 12 post-menopausal women after only 14-29 days of treatment with an oestrogen (3-methoxy-17 α -ethynyl-oestradiol; mestranol) alone or in combination with a progestin (17 α -ethinyloestrenol). The patients varied in age from 52 to 80 years. The serum-transaminase levels (S.G.P.T. and S.G.O.T.) usually were elevated within 20 days of treatment. The S.G.P.T. rose as high as 1,900 units in one patient. It is worth noting that two of the patients had levels slightly above normal before starting the study. In four patients tested for bromsulphalein retention this was also found to be abnormal.

Palva and Mustala (1964) subsequently reported the cases of five patients aged 62 to 90, all of whom developed increases in S.G.O.T. within seven days of administering a closely related oestrogen (ethinyloestradiol). In addition, by the 14th day three of the five had an elevated serum-alkaline-phosphatase level and all five had an abnormal bromsulphalein retention.

By contrast, Swaab (1964) reported that in two large studies of women receiving medication for six months or more 342 S.G.O.T. determinations were well within the normal limits. However, these subjects were young (aged 21-35). A similar failure to find evidence of hepatic dysfunction was reported by Linthorst (1964) in 52 women aged 18-48 treated for 14-43 months, and by Rice-Wray (1964) in 56 menstruating women treated for 3-12 cycles. The possibility that liver impairment may be found only in menopausal women was suggested by these reports.

During the past two years a double-blind study (report in preparation) at this clinic has tested the possible value of the addition of a progestin to oestrogen in the substitution treatment of ovarian failure in the menopause. Until the above reports were published no attention had been paid to the possibility of hepatic damage, although none was suspected on clinical grounds. All available patients were then called in and tested for hepatic function.

METHODS

A total of 36 women were tested. All were menopausal; 32 had had hysterectomy. Their ages ranged from 42 to 77,

with a mean of 57. S.G.P.T., alkaline phosphatase, thymol turbidity, cephalin flocculation (read at 24 and 48 hours), and cholesterol measurements, along with a complete blood count, were made in each woman and repeated in several. All these tests were performed within a six-week period. All of the patients received mestranol in a dose of 0.075 mg. daily, either alone (in three instances) or in combination with 5 mg. of norethynodrel.¹ The drugs had been administered for 1 to 24 months, with a mean of 8.76 months, at the time of testing.

RESULTS

The results are summarized in the Table.

Liver-function Tests in 36 Menopausal Women Treated with Mestranol or Enavid

	No.*	Mean	Range	No. Abnormal	Normal Limits
Age (years) ..	36	57 $\pm$ 1.6	42-77		
Duration of therapy (months) ..	36	8.76	1-24		
Cephalin flocculation:					
24 hours ..	39	0.89	0-2	6	0-1
48 hours ..	30	1.2	0-3	4	0-2
Thymol turbidity (MacLagan units) ..	41	2.14	0.6-3.7†	(1)‡	0-4
S.G.P.T. (units) ..	41	13.6	1-59	(3)‡	5-35
Alkaline phosphatase (Bodansky units) ..	42	3.0	1.5-0	6	1.5-4
Cholesterol mg./100 ml. ..	43	253	139-285	12	150-250

* The number of some tests exceeds the number of patients because of duplicated tests.

† One value of 12.9 units omitted because it was believed to be spurious; it was 2.0 when checked.

‡ When repeated two of the three were normal.

The cephalin-flocculation test read at 24 hours was slightly abnormal in six individuals; no 4-plus readings were observed. Similarly, six individuals (all different from those mentioned above) had an elevated alkaline phosphatase, but in no instance was it more than a marginal increase of one unit. Only one thymol-turbidity test was abnormal. Upon repetition it was found to be normal. Two of the three abnormal S.G.P.T.s were normal upon being repeated.

In addition, all the complete blood counts were normal, a bromsulphalein performed in two was normal, bilirubin and fractionation was normal in three, and protein fractionation was normal in two. The S.G.O.T. was performed in seven women and the highest value was 26 units, the upper limit of normal being 40 units.

¹ Mestranol and Enavid were generously provided by G. D. Searle and Co.