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A Syndrome Resembling Adrenal Cortical Insufficiency Associated with Long Term Busulfan (Myleran) Therapy

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BUSULFAN (Myleran, GT-41, 1:4 dimethanesulfonyloxybutane) has been in use for over ten years in the treatment of chronic granulocytic leukemia.[1] This alkylating agent, which is a potent inhibitor of the growth of cells of the granulocytic series, is considered by many the treatment of choice in chronic granulocytic leukemia. In the great majority of patients with this disease, treatment with busulfan results in rapid symptomatic improvement, reduction in the leukocyte count to normal levels and marked diminution in the size of the spleen. The remissions thus obtained can be maintained for months or even years by the continued use of small doses of the drug.

Careful hematologic observation of these patients usually helps to avoid bone marrow depression, which is the most significant complication of busulfan therapy.[1-26] Rarely, however, bone marrow depression occurs even with small doses of the drug. Other complications which may be encountered during busulfan treatment are: amenorrhea,[4,9-11,15,17,21-23,26] hyperpigmentation of the skin,[1,8,10,11,13,14,17,23,27-31,47] gastrointestinal reactions,[8,28,31] hyperuricemia with renal stones,[22] testicular atrophy,[21] gynecomastia[11] and interstitial pulmonary fibrosis.[32] Table 1[1-31,33-47] summarizes the toxic effects of busulfan in the treatment of chronic granulocytic leukemia. With the exceptions of interstitial pulmonary fibrosis and myelotoxicity, these complications rarely necessitate interruption of drug therapy.

It is the purpose of this paper to describe a syndrome appearing in patients with chronic granulocytic leukemia who were treated with bulsulfan for protracted periods. This syndrome is characterized by hyperpigmentation of the skin, severe weakness, fatigue, anorexia, nausea and weight loss. In each of the patients to be described, as in a case briefly noted by Marinho and Martins,[31] the clinical features strongly suggested adrenal cortical insufficiency, but this diagnosis could not be substantiated by laboratory tests.

CASE REPORTS *(Tables 2–4)*

Case 1: J. G. (NECH 130–670): This 52-year-old white man was well until early 1957, when he developed increasing fatigability, night sweats, pallor, dyspnea, and abdominal

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Table 1.—*Review of Toxicity of Busulfan in Treatment of Chronic Granulocytic Leukemia*

Name	Number of cases	Bone marrow depression*	Thrombocytopenia	Hyperpigmentation	Amenorrhea	Miscellaneous
Galton[1,9-11]	42	3		3	4	Gynecomastia (2)
Nelson & Lowry[2]	5	1				
Schilling & Meyer[3]	19	1	1			
Turesson[4]	39	1	1		Several	
Unugur et al.[5]	35	1	2			
Haut et al.[6-8]	30	3	4	2		Vomiting (2) Diarrhea (1)
Hayhoe & Kok[12]	12	2				
Desai[13,14]	31	1		16		
Louis et al.[15,16]	32	1			4	Leukopenia (3)
Grieg[17,18]	34	1	2	2	3	Agranulocytosis (1)
Levin et al.[19]	6	1				
Miesch[20]	13	3	2			
Kenis et al.[21]	22	1	1		3	Degenerated testes (1)
Ducach et al.[22]	35	1			3	Renal Colic (5) Urticaria (2)
Wilkinson[23,24]	53	2	11	1	3	Agranulocytosis (4)
Hyman & Gellhorn[25]	21	1				
Bollag[26]	6	1			2	
Petrakis et al.[27]	11		3	3		
Ritz & Krim[28]	12			1		Nausea (2)
Spurr et al.[29]	11		4	1		
Vahlquist & Vuille[30]	5		1	1		
Marinho & Martins[31]	5			2		Diarrhea (1) E. nodosum (1)
Frost & Jackson[33]	10		1			
Videbaek[34]	7		1			Hemolysis (1)
Bethell[35]	31					
Blackburn et al.[36]	17					
Rundles et al.[37]	21					
Casford[38]	1					
Doctor et al.[39]	4					
Umegaki & Watanabe[40]	1					
Kurrle[41]	10					
Hansen[42]	33		2			
Saurez et al.[43]	3					
Bousser & Christol[44]	14					Leukopenia (1)
Frelick[45]	13		1			
Bernard et al.[46]	50		3			
Rieder[47]	94			7		Agranulocytosis (8)
Total	788	25	40	39	22+	Osteomyelosclerosis (5)

*Including Pancytopenia.

enlargement. The leukocyte count was 300,000/cu.mm. He was first seen by us in November 1957. Examination revealed the liver extending 5 cm. below the costal margin and the spleen reaching the pelvic brim. Laboratory studies included: hemoglobin 10.0 Gm.

Table 2.—*Historical Findings*

	Pigmentation	Fatigue	Gastrointestinal symptoms	Weight loss	Duration CML*	Duration busulfan*	Dosage busulfan (in mg.)	Status of leukemia
J. G.	Yes	Yes	Yes	15 lbs.	50	27	2,630	Remission
P. R.	Yes	Yes	Yes	40 lbs.	72	65	5,710	Relapse
M. M.	Yes	Yes	Yes	58 lbs.	17	11	820	Remission
A. G.	Yes	Yes	Yes	20 lbs.	54	46	3,330	Remission

*In months.

Table 3.—*Physical Examination and Hematologic Values*

	Blood pressure	Liver*	Spleen*	Heart size	Hgb. Gm. %	WBC/cu.mm.	% Blasts in peripheral blood	Platelets/cu.mm.	ESR (Westergren)
J. G.	120/60	2	1	Normal	13.0	8,900	0%	501,600	11
P. R.	90/60	3	1	Normal	11.4	15,250	16%	262,770	115
M. M.	100/60	0	0	L.V.H.†	11.2	8,450	2%	1,537,000	45
A. G.	100/60	4	0	Normal	11.1	16,600	1%	Normal	91

*Cm. below costal margin.

†Left ventricular hypertrophy.

Table 4.—*Laboratory Findings*

	FBS	Na	K	Cl	CO_2	Control* 17-OH	Control* 17-Keto	After ACTH* 17-OH	After ACTH* 17-Keto	Skin biopsy
J. G.	75	139	4.8	100	30	3.2	5.0	67	13.7	Melanosis
P. R.	105	144	3.6	95	37	4.75	8.5	20.3	21.6	Melanosis
M. M.	77	141	4.5	99	26	2.0	2.0	14.2	8.2	Melanosis
A. G.	62									

*Mg./24 hr.

per cent, leukocytes 332,800/cu.mm. with polymorphonuclear leukocytes 26 per cent, bands 24 per cent, metamyelocytes 29 per cent, myelocytes 13 per cent, myeloblasts 5 per cent, and basophils 3 per cent. Bone marrow aspiration revealed the characteristic findings of chronic granulocytic leukemia. He was given busulfan in an initial dosage of 8 mg. daily which was gradually decreased to maintenance levels 2½ months later when the leukemia was in remission. He continued taking busulfan and remained well until June 1959, when he developed anorexia, weight loss of 15 pounds, and hyperpigmentation of the skin. At this time, the leukemia was in complete remission. Anorexia persisted and he developed nausea, fatigue and irritability as well as increased pigmentation.

Upon hospitalization on November 15, 1959, examination revealed a diffuse brownish hyperpigmentation of the skin which did not involve the oral mucous membranes or palmar creases. The liver and spleen extended 2 cm. and 1 cm. respectively below their costal margins. Laboratory studies included hemoglobin 13.0 Gm. per cent, WBC 8,900/cu. ml. with polymorphonuclear leukocytes 70 per cent, bands 5 per cent, lymphocytes 12 per cent, monocytes 4 per cent, eosinophils 1 per cent, basophils 3 per cent, metamyelocytes 2 per cent, and myelocytes 3 per cent, platelets 501,600/cu.mm., and the bone marrow examination demonstrated no increase in myeloblasts. The blood urea nitrogen, sodium, potassium, chloride and carbon dioxide combining power were within normal limits. An upper gastrointestinal series and chest roentgenogram were normal. The 24-hour urinary excretion of 17 ketosteroids and 17 hydroxycorticosteroids before and after stimulation with 40 units of ACTH given intramuscularly every 12 hours for 48 hours was normal. A skin biopsy of a

hyperpigmented area revealed melanosis largely limited to the basal area of the epidermis. Busulfan was discontinued, but there was at first no improvement. In March 1960, because of recurrence of the manifestations of leukemia, irradiation therapy (850 r) was given to the spleen. This resulted in considerable temporary improvement. In August 1960, the patient developed fatigue, dyspnea, increased hepatosplenomegaly, anemia and leukocytosis of 325,000/cu.mm. and busulfan, 4 mgm. daily, was reinstituted on September 15, 1960. This resulted in an improvement in all aspects. Repeat studies of 24 hour urinary excretion of 17 hydroxycorticosteroids and 17 ketosteroids in December 1960, were normal. In January 1961, he was much improved and the hyperpigmentation had decreased. He was taking busulfan 4 mg. daily and his leukemia was in remission. However, in February 1961, weakness, anorexia, nausea, weight loss, diarrhea and increased hyperpigmentation of the skin recurred. The drug was again discontinued. At that time serum iron, carotene, fasting blood sugar, serum calcium and phosphorus levels were normal. The liver function tests were normal except for alkaline phosphatase levels of 9.6 and 11.0 Bodansky units. Urinary excretion of coproporphyrins and uroporphyrins was not increased, and the Watson-Schwartz reaction for porphobilinogen was negative. Glutathione content of the red cells, serum ascorbic acid, Schilling test, xylose tolerance test and roentgenograms of the upper intestinal tract, small bowel, colon and skull were within normal limits. The bone marrow showed no increase in myeloblasts. When last seen in April 1961, his appetite and strength was slowly improving.

Case 2: P. R. (NECH 138–015): This 45-year-old white man was well until January 1955, when he developed sub-acromial bursitis and a routine blood count revealed a leukocytosis of 120,000/cu.mm. He was asymptomatic except for mild fatigue and on examination the spleen filled the left upper quadrant and reached the umbilicus. Laboratory studies were as follows: Hemoglobin 11.7 Gm. per cent, leukocytes 121,500/cu.mm. with a differential count and bone marrow aspirate compatible with chronic granulocytic leukemia. He was given busulfan, 12 mg. daily, which was gradually reduced to maintenance levels, and by March 1955, he was in a satisfactory clinical and hematologic remission. He continued to take maintenance doses of busulfan and remained in remission. In January 1960, a generalized brownish hyperpigmentation of the skin, involving the neck, upper back and the abdomen below the waist line was found. He was asymptomatic, had no splenomegaly and laboratory studies were not remarkable. Fatigue and weight loss began during the summer of 1960. In early November 1960, he was exposed to inclement weather and developed a cough and increased fatigue which was followed by vomiting, weight loss of 40 pounds and mental confusion. Busulfan was discontinued on November 26, 1960, but he did not improve, and hospitalization was required on December 1, 1960. Physical examination revealed a dark brownish hyperpigmentation of the neck, face, shoulders, upper back and inguinal areas. Scars and nipples were dark but palmar creases and oral mucous membranes were not involved except for a hyperpigmented linear area on the gingiva. The blood pressure was 90/60 and he was very confused and disoriented. The liver was 3 cm. below the costal margin and the tip of the spleen was just palpable. Laboratory studies included: Hemoglobin 11.4 Gm. per cent, platelets 262,770/cu.mm., leukocytes 15,250/cu. mm. with polymorphonuclear leukocytes 14 per cent, bands 16 per cent, lymphocytes 27 per cent, monocytes 3 per cent, basophils 6 per cent, metamyelocytes 2 per cent, myelocytes 15 per cent, promyelocytes 1 per cent and myeloblasts 16 per cent. The percentage of myeloblasts ranged from 16–29 per cent during hospitalization. There was a proteinuria of .02–.03 Gm. per cent. A bone marrow aspiration revealed a dry tap, but a biopsy showed a hypercellular marrow containing many myeloblasts. The fasting blood sugar, serum sodium, potassium and chloride were normal. The carbon dioxide combining power ranged from 27–37 vol. per cent. The prothrombin time was 16 seconds (control 13 seconds), bromsulphthalein dye retention 25 per cent, serum albumin 2.2 Gm./100 ml. and serum globulin 3.0 Gm./100 ml, but transaminases, bilirubin, thymol turbidity, cephalin flocculation and blood ammonia were normal. The serum carotene was 67 μg./100 ml., serum iron 155 μg/100 ml. and cholesterol 220 mg./100 ml. The serum alkaline phosphatase was 29.5 Bodansky units, blood urea nitrogen 32 mg. per cent, creatinine 1.6 mg. per cent, serum

calcium 12.2 mg./100 ml. on two occasions and phosphorus 4.8 mg./100 ml. The 24 hour urinary excretion of calcium was 284 mg. Skull roentgenograms and cerebrospinal fluid examinations were negative. Numerous blood cultures were negative. Chest roentgenograms demonstrated atelectasis at the left base. Roentgenographic studies of the upper gastrointestinal tract, gall bladder and small bowel were negative. A neurologist felt that the patient had a metabolic encephalopathy of undetermined origin. Skin biopsy showed an atrophied epidermis with minimal hyperkeratosis and a moderate amount of melanin within the chromatophores of the corium (fig. 1). Urinary excretion of 17 ketosteroids and 17 hydroxycorticosteroids before and after ACTH stimulation were normal.* During hospitalization he ran a low grade fever of 99–101 F., remained confused, had polyuria (4–5 L. daily), and nausea and vomiting. The blood pressure ranged between 90/60 to 120/70. A trial of methionine, 3 Gm. daily, was given for 5 days without any apparent benefit. Prednisone, 100 mg. daily, was given because of hypercalcemia. He continued to deteriorate and expired January 12, 1961. Post-mortem examination disclosed a moderate infiltration of the adrenal glands with leukemic cells, but neither atrophy nor replacement of adrenal tissue was present. The small intestine was normal. Leukemic infiltrates were found in the liver, brain and spleen.

Case 3: M. M. (NECH 121-751)† This 66-year-old white woman was well until July 1958, when she developed ecchymoses, dyspnea and an ear infection. The blood counts were: hemoglobin 12.5 Gm. per cent, platelets 679,900/cu.mm., leukocytes 110,200/cu.mm. with polymorphonuclear leukocytes 16 per cent, bands 22 per cent, lymphocytes 3 per cent, eosinophils 3 per cent, basophils 12 per cent, myelocytes 17 per cent, metamyelocytes 25 per cent, and myeloblasts 2 per cent. Moderate hepatosplenomegaly was found and bone marrow examination was compatible with chronic granulocytic leukemia. Treatment with busulfan was begun with an initial dose of 8 mg. daily, and a maintenance dose of 2–4 mg. per day was achieved. There were satisfactory clinical and hematologic responses to this treatment and the patient was able to resume her usual activities. In September 1959, 13 months later, she complained of marked weakness, dyspnea, anorexia, and a weight loss of 58 pounds, which could not be accounted for by her leukemia. There was no diarrhea or vomiting. She was hospitalized and on physical examination appeared chronically ill and cachectic. The blood pressure was 100/60, and there was a generalized brownish hyperpigmentation of the skin most marked on the abdomen and extremities. The nipples were somewhat darker than normal, but there was no abnormal pigmentation of the mucous membranes. No hepatosplenomegaly or lymphadenopathy was present. The laboratory data were: hemoglobin 11.2 Gm. per cent, platelets 1,537,000/cu.mm., leukocytes 8,450/cu.mm. with polymorphonuclear leukocytes 56 per cent, bands 14 per cent, lymphocytes 8 per cent, monocytes 2 per cent, basophils 8 per cent, myelocytes 5 per cent, metamyelocytes 4 per cent, promyelocytes 1 per cent and myeloblasts 2 per cent. A bone marrow biopsy was hypercellular with an over-all pattern consistent with chronic granulocytic leukemia. The blood urea nitrogen, serum proteins, bilirubin, sodium, potassium, chloride, carbon dioxide content and fasting blood sugar were normal. The serum carotene was 25 μg./100 ml. and xylose tolerance test revealed a urinary excretion of 3.4 Gm. in 5 hours. Serum calcium was 10.2 mg./100 ml., phosphorus 3.4 mg./100 ml., alkaline phosphatase 1.8 Bodansky units and no evidence of osteomalacia on roentgenographic examination. Cultures of the sputum, blood and urine yielded no pathogens. A study of urinary 17 ketosteroid and 17 hydroxycorticosteroids before and after ACTH stimulation did not substantiate a diagnosis of adrenocortical insufficiency and there was no clinical response to physiologic doses (25 mg./day) of cortisone. Roentgenographic examinations of the chest showed a diffuse, fine, lace-like infiltrate throughout both lung fields. Pulmonary function studies were compatible with the syndrome of alveolar-capillary block. During these diagnostic studies the patient developed a fever varying between 101 F. to 102 F., rectally. No definite cause for the fever could be found. A lung biopsy demonstrated chronic interstitial pneumonitis

*The ACTH was kindly supplied by E. H. Sellmer of Wilson Laboratory, Chicago, Ill.

†This patient is case 2 of reference 32.

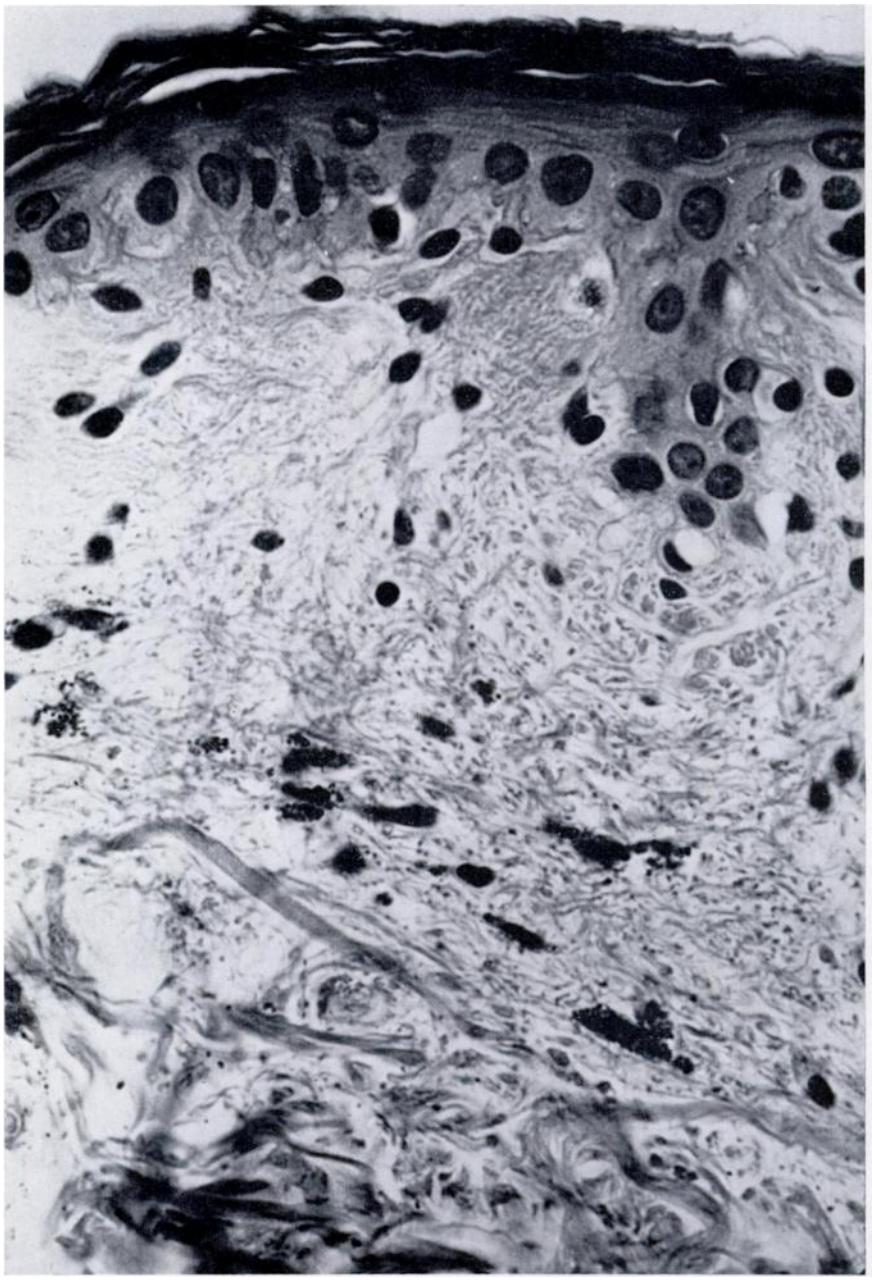

Fig. 1.—Section of skin showing a moderate increase of pigment containing chromatophores in the corium, atrophy of the epidermis and mild hyperkeratosis. X750.

with fibrosis, but no leukemia or vasculitis was found. Biopsy of the skin revealed an appreciable increase in pigment-containing macrophages of the corium. Busulfan therapy was discontinued and treatment with prednisone, 100 mg. per day, was initiated. The patient improved symptomatically and when seen two months later, all pulmonary symptoms had disappeared, but the weakness persisted, and a repeat chest roentgenogram showed

persistence of the pulmonary lesions. The hyperpigmentation was a little less prominent. In January 1960, she suddenly developed pulmonary edema and died at home. No postmortem examination was performed.

Case 4: A. G. (NECH 130–025)° This 66-year-old dark complexioned white woman was well until the spring of 1955, when she developed mild weakness, anorexia and anemia. She consulted her physician in January 1956, and he found the liver and spleen palpable 8 cm. and 9 cm. below the respective costal margins. The hemoglobin was 10.0 Gm. per cent and leukocytes 250,000/cu.mm. with a differential count compatible with chronic granulocytic leukemia. Busulfan, 4 mg. daily, was given and by March 1956, she was in a clinical and hematologic remission. She took small doses of busulfan and felt well until March 1958, when she complained of anorexia, nausea and dyspnea and was found to have hyperpigmentation of the face, neck, lower abdomen and nipples, but not of the oral mucous membranes. She was troubled with frequent upper respiratory infections and a chronic cough, but repeated chest roentgenograms were negative. She developed severe anorexia and occasional vomiting and was hospitalized in May 1959, because of abdominal cramps, diarrhea and weight loss. The serum iron was 83 μg./100 ml., calcium 10.8 mg./100 ml., alkaline phosphatase 7.4 units, prothrombin time 90 per cent, and bromsulphthalein retention 15 per cent in 45 minutes, but bilirubin, albumin, globulin, thymol turbidity, cephalin flocculation and transaminase values were normal. Roentgenographic studies of the upper gastrointestinal tract and small bowel were normal. No evidence of osteomalacia was seen. She gradually became weaker, more nauseated and continued losing weight, and in April 1960, developed epigastric pain. Hyperpigmentation of the skin had gradually increased during this time. Busulfan was discontinued in June 1960. She was hospitalized in July 1960, because of cough, chest pain and fever of 2 weeks duration. The liver was palpable 4 cm. below the costal margin but the spleen was not felt. Laboratory studies included: hmoglobin 11.1 Gm. per cent, leukocytes 16,600 with polymorphonuclear leukocytes 78 per cent, bands 3 per cent, lymphocytes 10 per cent, monocytes 6 per cent, metamyelocytes 1 per cent, myelocytes 1 per cent, and myeloblasts 1 per cent. Her leukemia was in remission and there was no evidence of a blast crisis. Chest roentgenograms revealed bilateral pneumonitis which gradually increased during hospitalization. Her condition deteriorated and she expired on July 22, 1960.

Discussion

Analysis of Results

In the past 18 months we have had the opportunity to observe four patients† who developed a similar group of symptoms and objective manifestations after continued therapy with busulfan. This syndrome consisted of hyperpigmentation of the skin, fatigue, weakness, weight loss, anorexia and nausea; from the clinical standpoint, it mimicked adrenal cortical insufficiency. The complete syndrome developed after treatment with busulfan for one to five years, and the total dose of drug varied from 820 to 5,710 mg. Three of the patients were in hematologic remission when the syndrome developed while the fourth was in a blast crisis when the condition was recognized. Although weakness, weight loss and anorexia may be present in patients with chronic granulocytic leukemia in relapse, such symptoms do not occur during remission. The patients in this study were under excellent control when the syndrome developed and thus the symptoms were on some basis other than the leukemia. In the fourth patient, hyperpigmentation of the skin was seen eleven

°We wish to thank Dr. Robert Goldstein for allowing us to report this case.

†Three other patients not reported here were also observed. Two were incompletely studied and, therefore, not included; another developed extreme hyperpigmentation simultaneously with "blast crisis" and porphyria. The latter case will be reported separately.

months prior to the onset of the blast crisis, and the weakness and weight loss began four months before the appearance of primitive cells in the peripheral blood. It does not appear, therefore, that the status of the leukemia played an important role in the development of the symptoms.

Fatigue and weakness were among the earliest symptoms. The weakness was at times profound and incapacitating; two of the patients remained in bed and were unable to carry out their daily activities. The fatigue and weakness improved when busulfan was discontinued in two patients, but persisted in the remaining two. These latter two expired shortly after busulfan was discontinued. Anorexia and weight loss were constant features of the syndrome. There was a marked distaste for meat in two of the patients. Weight loss was moderate in most instances, but two patients lost forty pounds or more. The other gastrointestinal symptoms included nausea and vomiting while diarrhea was present in two patients. A variety of gastrointestinal symptoms may appear as "side effects" of busulfan therapy. Under these circumstances, they occur early in the course of treatment and subside promptly on cessation of therapy. The gastrointestinal complaints of the present patients, however, had their onset after at least one year of treatment and usually persisted in spite of discontinuance of the drug.

The physical examination revealed hyperpigmentation as described below. The blood pressure was normal in three patients and the fourth had a mild hypotension. Slight hepatomegaly was found in three patients and slight splenomegaly was present in two instances.

Hyperpigmentation of the skin, which is indistinguishable from the melanosis of Addison's disease, may be the first sign of the syndrome to appear, and in one instance this abnormality preceded the appearance of the other symptoms by many months. The pigmentation was brownish, generalized and tended to be most pronounced on the trunk, face and hands. The mucous membranes were not involved except in one patient who had a linear deposit of pigment on the gingiva. In three of the patients the areolae of the breasts were hyperpigmented. Palmar creases were not hyperpigmented and scars were infrequently pigmented. There was gradual increase in pigmentation with continued administration of the drug. In one case hyperpigmentation slowly receded when the drug was stopped and recurred five months after it was resumed, indicating a distinct relationship between drug administration and pigmentation. Biopsy of pigmented skin was performed in three of the patients and showed melanosis, localized either in the basal layer of the epidermis, or in the chromatophores of the corium. There was no increase in the number of melanocytes when a split section of the skin was examined.* The dopa reaction was strongly positive, indicating that the pigment was melanin.* The pigment did not show a positive stain for iron. Sections of the skin were stained for the presence of SH (sulfhydryl) groups and although quantitation was difficult, it seemed that these hyperpigmented patients had some decrease in SH groups.*

Hyperpigmentation of the skin is almost never seen in patients with chronic

*We are indebted to Drs. G. Szabo, A. Quintanilla and W. J. Mitus for these studies.

granulocytic leukemia who have not received busulfan. A review of the literature disclosed only three cases in which generalized hyperpigmentation of the skin was seen in patients with this disease not receiving busulfan.[48,49] A fourth patient had hyperpigmentation, but she received Fowler's solution, and it was felt that it might have been responsible for the hyperpigmentation.[50] Hyperpigmentation, however, is not uncommonly seen in patients treated with busulfan, but without the development of other symptoms.[1,8,10,11,13,14,17,23,27-31,47] Marinho and Martins[31] described a patient with chronic granulocytic leukemia treated with busulfan who showed many of the clinical features of Addison's disease, but, as in our cases, none of the laboratory tests substantiated this impression.

Differential Diagnosis

The clinical features of these cases were difficult to distinguish from those of adrenal cortical insufficiency. In addition to the hyperpigmentation, weakness, gastrointestinal symptoms and borderline hypotension were present. On the other hand, there was no significant pigmentation of the oral mucous membranes, no calcific deposits in the pinnae, and no loss of body hair. The laboratory tests clearly excluded adrenal cortical insufficiency. In none of the cases was the heart judged to be small on roentgenographic study; hypoglycemia was not present; studies of serum electrolytes, including sodium, potassium, chloride and carbon dioxide combining power did not suggest adrenal insufficiency; and the urinary excretion of 17 hydroxycorticosteroids and 17 ketosteroids was not diagnostic of Addison's disease. A normal response in the urinary excretion of these steroids was obtained after stimulation with ACTH in all of the patients in whom this test was performed. Determination of urinary excretion of 17 hydroxycorticosteroids and 17 ketosteroids in an additional six patients who received long term busulfan therapy and developed hyperpigmentation without other symptoms showed no evidence of adrenal cortical insufficiency.

The possibility of hemochromatosis was excluded by the presence of normal serum iron levels and the absence of cirrhosis and diabetes. Idiopathic sprue was considered, but the diagnosis was not substantiated in any instance. Serum carotene levels were determined in several other hyperpigmented patients with chronic granulocytic leukemia receiving busulfan and all were normal. Abnormal concentrations of uroporphyrins, coproporphyrins or porphobilinogen were not found in the patient in whom they were determined. No evidence of other causes of melanosis such as vitamin deficiency, arsenic poisoning, Hodgkin's disease, pernicious anemia, Gaucher's disease, chronic infection, Whipple's disease, acromegaly, Cushing's syndrome, hyperthyroidism, cirrhosis or scleroderma was found. Only one of the patients was dark complexioned so racial pigmentation did not appear to play a role.

Mechanism of Drug Toxicity

The mechanism of the production of this syndrome is obscure. Hyperpigmentation occurs fairly commonly during busulfan therapy but development

of the complete syndrome described here is rare. Recent studies have reported either normal or borderline increased levels of melanophore stimulating hormone (MSH) in patients with chronic granulocytic leukemia in whom hyperpigmentation developed while taking busulfan.[14] Busulfan in concentrations ranging from 2 mg. to 16 mg. per ounce of hydrophilic ointment was applied twice daily to the arms of the authors for four weeks without any evidence of local hyperpigmentation. No direct effect of busulfan in darkening of frog skin was observed by Lerner.[51] It is of interest to note that the intraperitoneal injection of triethylene thiophosphoramide (Thio-Tepa), a sulfur-containing alkylating agent, into mice produces hyperpigmentation.[52] We have never observed either hyperpigmentation of the skin or the other manifestations of the present syndrome in patients undergoing long-term therapy with leukeran (chlorambucil) or other non-sulfur containing alkylating agents. One may speculate as follows: Melanin is formed by the enzymatic oxidation of tyrosine by tyrosinase.[53] Copper has been shown to be necessary for the activity of tyrosinase—at least in mammalian tissue such as mouse melanoma.[54] Organic sulfur-containing compounds such as glutathione or cysteine inhibit the action of tyrosinase, presumably by combining with copper.[54] Extracts of human epidermis will inhibit melanin formation but when iodoacetamide, a specific poison for sulfhydryl groups, is added, this inhibition of melanin formation by human epidermis is lost.[55] Busulfan reacts with the thiol group of glutathione and with reduced keratin.[56] Therefore, it does not seem unreasonable to assume that busulfan inactivates sulfhydryl groups in the skin by "dethiolation", thereby removing an inhibitor of tyrosinase. The formation of melanin may be accelerated with subsequent development of hyperpigmentation.

The reaction of busulfan with the thiol groups of cysteine, glutathione and proteins appears to account for the major action of the drug,[56] although a variety of other reaction sites have been reported earlier.[57,58] At the cellular level, this action is manifested by the inhibition of mitosis due to suppression of the formation of new deoxyribonucleic acid (DNA). Thus, Li demonstrated depressed uptake of P^{32} in the DNA fraction of leukemic granulocytes obtained from patients treated with busulfan.[59] Chromosomal disruption was observed in Walker carcinoma 256 of the rat[60] and in vicia faba seeds after treatment with busulfan.[61] Experimentally, busulfan has been shown to induce cataracts[62,63] and sterility[64] or damaged testes and ovaries[65] in rats. In view of these studies, the development of pigmentation, weakness and gastrointestinal symptoms in some patients with leukemia given long continued busulfan therapy is probably due to some profound metabolic defect induced by the drug on many different cells and tissues. Thus, this drug, which in many respects is the most useful of the chemotherapeutic agents used in chronic granulocytic leukemia and the least likely to induce severe reactions, is by no means without harm. As a cytotoxic agent, it has generalized effects but in particular there are effects upon the bone marrow, the gonads, and the skin. This study indicates a more subtle effect upon other systems of the body with resultant weight loss, gastrointestinal symptoms, and increasing weakness, mimicking a number of features of Addison's disease. Fortunately, the complete syndrome as described in

this study ordinarily occurs after one to five years of administration of the drug, and in only a small number of patients in whom the drug is given.

Therapy

The treatment of the syndrome is unsatisfactory. Busulfan was discontinued, of course, with variable success. In one patient (J. G.), all of whose symptoms and signs disappeared when the drug was discontinued, there was recurrence of all manifestations after the drug was again given for five months. In another patient not reported in this paper, discontinuance of the drug when marked pigmentation and moderate weight loss appeared was followed by a gradual reduction in pigmentation and a gradual increase in weight. Because the administration of a substance containing sulfhydryl groups such as glutathione or methionine might conceivably overcome the "dethiolation" effect of busulfan, one patient with a severe form of the syndrome (P. R.) was given a short course of treatment with methionine without improvement. However, the dose of methionine may have been too small and the duration of treatment too short. Further trials of this or another SH-containing material should be made. Thus, at this time, prophylaxis is the best form of therapy. It would seem that one should use as little busulfan as possible to keep the leukemia under control; whether or not intermittent rather than continuous therapy with busulfan is preferable cannot be stated from the present experience.

Summary

Four patients who developed a clinical syndrome characterized by hyperpigmentation of the skin, weakness, fatigue, weight loss, anorexia and nausea while on long-term treatment with busulfan were described. The etiology of the disorder, although clearly related to the busulfan therapy, is obscure, but may be related to "dethiolation".

Summario in Interlingua

Es describite le casos de quatro patientes qui disveloppava un syndrome clinic characterisate per hyperpigmentation del pelle, debilitate, fatiga, perdita de peso, anorexia, e nausea durante que illes esseva sub tractamento a longe vista con busulfan. Le etiologia del disordine—ben que clarmente relationate con le therapia a busulfan—es obscur. Illo implica possibilemente un processo de "dethiolation."

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