1132R

Triethylene Melamine in the Treatment of Neoplastic Disease

717191

By R. Wayne Rundles, Ph.D., M.D. and W. Bruce Barton, M.D.

REPOSITORY Oak Ridge Operations Records Holding Area
COLLECTION ~~Documents~~ 1944-94 Contracts AT-(40-1)-1040-1092
BOX No. 3 of 3 Bldg. 2714-H
FOLDER Contracts AT-(40-1)-1081 Duke Univ.

Reprinted from Blood, The Journal of Hematology
Vol. VII, No. 5, May, 1952
Printed in U.S.A.

1110818

Reprinted from Blood, The Journal of Hematology
Vol. VII, No. 5, May, 1952
Printed in U.S.A.

Triethylene Melamine in the Treatment of Neoplastic Disease

By R. Wayne Rundles, Ph.D. M.D. and W. Bruce Barton, M.D.*

TRIETHYLENE MELAMINE was discovered to have nitrogen mustard-like effects on experimental tumors and leukemia in mice late in 1949.[1,2] Clinical studies were soon undertaken. In the first series of patients treated with the chemical, Rhoads, Karnofsky and collaborators[3] found that temporary periods of improvement comparable to those obtained by injections of nitrogen mustard could be produced in patients with Hodgkin's disease, lymphosarcoma, chronic lymphatic and myelogenous leukemia, and in mycosis fungoides. While no new principle of tumor chemotherapy was involved, the discovery of the new compound seemed to represent a significant advance in practical management. Triethylene melamine, an inexpensive chemical, was found to be effective when given by mouth and did not produce the severe nausea and vomiting or the venous thromboses that sometimes complicate nitrogen mustard therapy. The need for repeated hospital admissions was reduced, and optimally spaced and sustained therapy rendered more feasible. Since a wide use of the new chemical may be anticipated, it is necessary to investigate its safety as a therapeutic agent, and to study its potentialities for prolonged as well as for immediate use, in comparison with and in conjunction with other agents of established value.

The effect of triethylene melamine on normal and neoplastic tissues appears to be identical to that of the nitrogen mustard compounds.[3] The action of the latter can be reviewed briefly. The ethylenimonium transformation products of the nitrogen mustards, which form as they are dissolved in body fluids, react with the functional groups of many cell proteins and enzyme systems, inhibit mitoses, produce cytologic alterations, distort cell function and may produce cell death. The tissues predominantly affected are those in which there is active cellular proliferation, particularly the lymphoid tissues and the bone marrow.

From the Department of Medicine, Duke University School of Medicine, and the Hematology Laboratory, Duke Hospital, Durham, North Carolina.

Submitted December 26, 1951; accepted for publication February 5, 1952.

The expense of this work was defrayed in part by a grant from the Atomic Energy Commission supporting a related project. Triethylene melamine was generously provided by Dr. J. M. Ruegsegger of Lederle Laboratories.

The authors are indebted to the many physicians who have referred patients, and to those who have assisted with the care of patients, particularly Drs. C. L. Rast, Jr., R. W. Willett, J. C. Muller, G. D. Chase, R. A. Shimm and Ladd W. Hamrick, Jr. Children were studied through the cooperation of the Department of Pediatrics. Mrs. Elaine Sullenberger, Mrs. Ella Schipke and Mrs. Betty Somers provided valued technical assistance.

* American Cancer Society Fellow.

1110819

The neoplasms especially benefited by nitrogen mustard therapy are those which arise from these tissues also.[5, 12]

Nitrogen mustard and ionizing irradiation are thought to produce similar patterns of tissue injury and delayed cell death.[5, 13] In the therapeutic use of the two agents anatomic considerations are of primary importance. Roentgen irradiation can be used with the greatest efficiency when the disease is anatomically well circumscribed. Nitrogen mustard therapy affects tissues in widespread anatomic sites on a chemical or functional basis. The effect of the two agents differs in two important respects. Nitrogen mustard appears to act more quickly than roentgen irradiation and the results are shorter lived. Injections must be repeated more frequently for sustained benefit. Bone marrow depression, which is often the limiting factor in the therapy of widespread neoplastic disease, subsides more quickly after the administration of nitrogen mustard and there is usually no cumulative injury after repeated courses of judicious therapy.[6]

In considering the clinical usefulness of the nitrogen mustards the conditions under which the chemotherapy has been given must be kept in mind. Treatment has usually consisted of a series of 4 to 6 intravenous injections, 0.1 mg. Kg. each, given in a period of a few days. Few patients have been treated except during periods of manifest disease activity, and often not until they had become unresponsive to persistent roentgen therapy. Nitrogen mustard, nevertheless, has been accepted during the last five years as a definite adjunct to irradiation therapy in the treatment of the malignant lymphomas and leukemias.[5, 8–12, 14, 15] It is regarded as especially valuable in Hodgkin's disease which has become refractory to irradiation therapy, and in patients with widespread disease with constitutional symptoms. It is perhaps the treatment of choice in those with spinal cord compression, bronchial obstruction, or lesions in the pulmonary parenchyma.

Among the theoretical concepts that influence present day treatment of neoplastic disease of the hemopoietic tissues is the thesis that neoplastic cells are scarcely more sensitive to the destructive effects of irradiation, or to nitrogen mustard compounds, than are the normal cells of their respective tissues of origin.[5, 6, 16] Block and Jacobson[17] have recently summarized widely accepted opinions that guide practical therapeutic management. These have evolved out of long experience with agents having little or no curative value, and only limited palliative potentiality, but capable of inflicting much harm. In their opinion the life span of an individual with one of these diseases is not prolonged by treatment, and cure is not to be expected. If the disorder is discovered during an asymptomatic stage, they believe that therapy should not be instituted until symptoms of disease activity develop. Efforts are then directed toward relieving the symptoms rather than in attempting to retard or arrest the course of the disease at an earlier and possibly more favorable stage. Block and Jacobson warn that "any attempt to 'burn' the disease out of the patient is not only doomed to failure on theoretical as well as practical grounds, but will invoke a series of complications that are more distressing than the disease itself." We may expect that as new therapeutic agents are developed, our theoretical concepts may become modified and the results expected from therapy viewed more optimistically.

1110820

The present report concerns 134 patients with malignant lymphomas, leukemia and other neoplastic diseases treated with orally administered triethylene melamine (TEM) during a period of 18 months (table 1). The immediate results of therapy are presented and discussed with particular reference to the long-term management of these disorders.

TABLE 1.—*Therapeutic Effect of Orally Administered Triethylene Melamine*

Disease	Effect on Disease Manifestations					
	Excellent	Good	Slight	None	Inconclusive	Total
Hodgkin's disease	9	5	4		7	25
Follicular lymphoma	1		1	1		3
Lymphocytic lymphoma	2	1	1	2	1	7
Lymphoblastic lymphoma	4	1	2		1	8
Reticulum cell sarcoma		1	1			2
Leukemia, chronic lymphocytic	12	6	5			23
Leukemia, subleukemic lymphocytic	4		7	3	2	16
Leukemia, acute lymphocytic			1	2		3
Leukemia, chronic granulocytic		4	2			6
Leukemia, myelogenous with panmyelosis			1	2		3
Leukemia, subleukemic granulocytic				1		1
Leukemia acute myelogenous			1	6		7
Polycythemia rubra vera					3	3
Multiple myeloma				2	1	3
Lymphoepithelioma, nasopharynx		2			1	3
Adenocarcinoma, oral cavity, with metastases				3		3
Carcinoma of bronchus				2		2
Carcinoma, renal				1		1
Teratoma, testis				1		1
Teratoma, mediastinum				1		1
Ovarian cystadenocarcinoma	1	2			1	4
Carcinoma, metastatic, source?				6		6
Hand-Schuller-Christian disease			1			1
Mycosis fungoides				1	1	2
Total						134

HODGKIN'S DISEASE

Triethylene melamine was regarded as a suitable agent for trial therapy in patients with disseminated Hodgkin's disease producing generalized symptoms, in those prone to develop new lesions frequently, and in those with disease in anatomic sites, such as the liver and bone marrow, in whom roentgen therapy is ordinarily not productive of good results. Twenty-five patients were treated. The effect of the chemical on the different manifestations of the disease, fever, weakness, lymph node enlargement, pruritis, anemia, etc., was rated as excellent in 9, good in 5, slight in 4, and inconclusive in 7 (table 1). The persistence of

1110821

local lesions after otherwise adequate response to TEM over a period of weeks or months was regarded as an indication for supplementary roentgen therapy. In studying the reasons for the variable effect of the chemical in individual patients, it appeared that good or excellent responses occurred characteristically in those who had had no previous treatment or who had developed fresh lesions after earlier roentgen therapy. In those in whom the response was slight or inconclusive the disease was very extensive anatomically, or had been present for a long time and subjected to prolonged therapy with other agents.

The effect of TEM on some of the common manifestations of Hodgkin's disease, its employment in different clinical situations and its effect on a rare complication, Hodgkin's disease with symptomatic or acquired hemolytic anemia, are illustrated by the following 3 cases.

FIG. 1.—[redacted] Response of a cervical tumor in Hodgkin's disease to TEM.

[redacted] This 14 year old colored school boy was first seen at Duke Hospital on October 25, 1950, complaining of a tumor on the side of his neck. Two years previously a small nodule had appeared in the left mid-cervical region and a mass gradually developed in the area. He regarded his general health as good, but his weight during this period fell from a maximum of 101 lbs. to 90 lbs. He had had no weakness, pruritis, chills or fever. On physical examination the only abnormal finding was a lobular, slightly tender mass under the angle of the left mandible measuring 12 x 13 cm. (fig. 1). Lymph nodes in other superficial areas were not enlarged. The spleen was not palpable.

The hemoglobin was 10.7 Gm. per 100 cc., WBC 11,250, RBC 4,600,000, hematocrit 37.8 per cent, and differential white blood count normal. An x-ray film of the chest showed a rounded prominence above the left hilum. A biopsy of the cervical mass showed the typical histologic features of Hodgkin's disease.

He was given 5 mg. of TEM daily for 3 days without side reactions, and then 5 mg. a week for 3 weeks. The tumor became considerably smaller (fig. 1). Because the disease appeared to be predominantly localized and adequate hematologic supervision was difficult, x-ray therapy to the cervical region was then advised. One year after his first visit he had gained 25 lbs. in weight and showed no evidence of disease.

1110822

[redacted] This 59 year old widow, admitted to Duke Hospital on November 29, 1950, had become ill with periodic weakness, cough and fever two years previously. A few months after the onset of symptoms, lymph nodes appeared along the left side of her neck and in the right axilla. During the ten months before admission she had had intermittent fever with chills, a severe nonproductive cough and pronounced weakness. Her weight fell from an average of 210 lbs. to 159 lbs.

Physical examination showed a pale, febrile, chronically ill woman with a harassing cough. Numerous cervical and axillary lymph nodes measured 2 to 3 cm. in length, and a large node in the margin of the right pectoralis major muscle measured 3.5 x 4 cm. The tip of the spleen was palpable.

The hemoglobin concentration was 10.2 Gm. per 100 cc., RBC 4,250,000, WBC 17,300, hematocrit 36.0 per cent, reticulocytes 1.5 per cent and platelets 600,000. The differential white cell count was segmented neutrophils 58 per cent, nonsegmented neutrophils 26 per cent, eosinophils 1 per cent, lymphocytes 11 per cent and monocytes 4 per cent. A roentgenogram of the chest showed pronounced widening of the upper mediastinum and areas of infiltration in the pulmonary parenchyma (fig. 2). Bronchoscopy showed no tracheobronchial abnormalities. A cervical lymph node was excised and the pathologic interpretation was Hodgkin's disease.

She was given 17.5 mg. of TEM in 4 days without adverse reaction. The cough subsided, the lymph nodes became smaller and the temperature normal. The leukocytosis persisted for several days and two additional 5 mg. doses of TEM were given.

FIG. 2.—[redacted]. Hodgkin's disease with mediastinal tumor and infiltration of pulmonary parenchyma before therapy.

At home she took 5 mg. of TEM a week for 5 weeks. Her health improved progressively, whereupon she decided to suspend therapy. Ten weeks later weakness, cough, mild chills and fever returned and gradually became worse.

She was re-admitted to the hospital on May 3, 1951. On physical examination she was again febrile but not acutely ill. The superficial lymph nodes were about half their previous size. The spleen was not palpable. Roentgen films of the chest showed less infiltration in the pulmonary parenchyma, but little, if any change in the mediastinum. TEM therapy was reinstituted. The fever and leukocytosis promptly subsided (fig. 3).

After returning home the second time she continued taking 5 mg. of TEM each week for nearly 4 months. She became stronger, gained weight and strength and had few complaints. Nine months after the first TEM therapy the superficial lymph nodes were reduced to about one-third their original size. There was further regression in the pulmonary parenchymal infiltration. TEM therapy was then suspended while the residual node enlargements were irradiated.

[redacted] This 13 year old colored boy was admitted to Duke Hospital on June 21, 1950, because of poor appetite, listlessness, weight loss, enlarged lymph nodes in the neck and periodic vomiting and fever of 5 months' duration. During his last week at home his family physician thought that he was jaundiced.

On examination he appeared lethargic, undernourished, and chronically ill. The temperature ranged from 37.4 to 38.4 C. There were numerous cervical and supraclavicular lymph nodes 3 to 4 cm. long. His spleen was palpable.

The hemoglobin concentration was 5.5 Gm. per 100 cc., RBC 2,760,000, WBC 5,000, hematocrit 21.0 per cent, MCV 76 cu. μ, reticulocytes 2.3 per cent and platelets normal in

1110823

number. The differential white blood count was normal except for a rare immature granulocyte. Bone marrow aspirated from the sternum was of high cellularity. The myeloid erythroid ratio was 1:1.

Tuberculin and histoplasmin skin tests were negative. The serum proteins were 7.3 Gm. per 100 cc., with albumin 2.4 Gm. and globulin 4.9 Gm. The blood bilirubin was 0.62 mg. per 100 cc.

X-rays of the chest showed widening of the upper mediastinum on the right and an opacity above the right hilum. A cervical lymph node was removed. Sections showed hyalinized fibrous connective tissue and pleomorphic cellular proliferation typical of Hodgkin's disease.

X-ray therapy was given to the cervical lymph nodes and to the mediastinum. His temperature became normal and his strength and appetite improved. After 12 days in the hospital he was able to return home. A re-examination two months after discharge was recommended but he failed to appear.

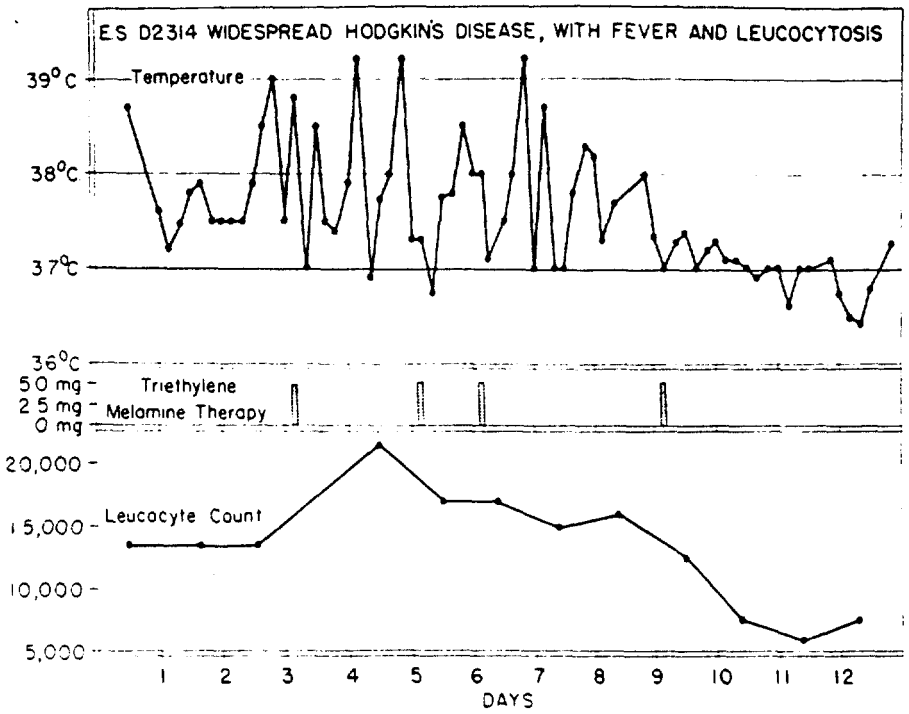

FIG. 3.—Hodgkin's disease. Response of temperature and leukocytosis to TEM.

On November 30, 1950, he was brought back to the hospital critically ill. He had appeared outwardly well until six days previously when signs of an upper respiratory infection and abdominal pain developed. Three days before admission his parents noted yellowish sclerae and dark urine. He rapidly became weak and began to vomit. A few hours before admission he became stuporous.

On physical examination he was thin, acutely ill, deeply jaundiced, and semicomatose. The blood pressure was 90/60, temperature 37.8 C. Lymph nodes 1 cm. long were palpable in the supraclavicular fossae bilaterally. The heart was enlarged and the pulse rapid. The abdomen was tense, but it was thought that the liver and spleen were enlarged.

Blood studies showed a hemoglobin concentration of 2.6 Gm. per 100 cc., WBC 33,300, hematocrit 8.8 per cent, reticulocytes 0.1 per cent and platelets enormously increased in number. The red blood cells could not be counted because of autoagglutination. In the stained blood films the great majority of the leukocytes were neutrophils with an occasional immature granulocyte. Bone marrow aspirated from the sternum was again extremely cellular. The myeloid-erythroid ratio was 1:10.

The urine was dark greenish-brown in color and contained large amounts of urobilinogen and bilirubin. The serum bilirubin was 11.4 mg. per 100 cc. The serum proteins were 8.4

1110824

Gm. per 100 cc., of which albumin comprised 2.7 Gm. and globulin 5.7 Gm. The thymol turbidity test was negative.

In preparing for blood transfusion the patient's red cells could not be typed because of pronounced autoagglutination at all temperatures. The antibody could not be removed by washing the cells repeatedly with saline, even up to a temperature of 40 C. (Dr. Ivan W. Brown). His serum agglutinated all available donors' cells in high dilution. He was transfused with Type O, Rh negative packed red cells from 2 pints of blood to which A and B substances had been added. He responded slowly to the transfusions. On the second day a limited exchange transfusion was performed, 2,000 cc. of blood being given and 1,660 cc. removed. Following this procedure a splenectomy was done. He recovered well from the operation and 4 days later was walking about the ward.

His spleen weighed 280 Gm. Sections showed the pleomorphic cellular proliferation characteristic of Hodgkin's disease, acidophilic "paraamyloid" deposit, vascular congestion, pronounced erythrophagocytosis and erythropoiesis. Lymph nodes present in the hilum of the spleen showed similar histologic changes.

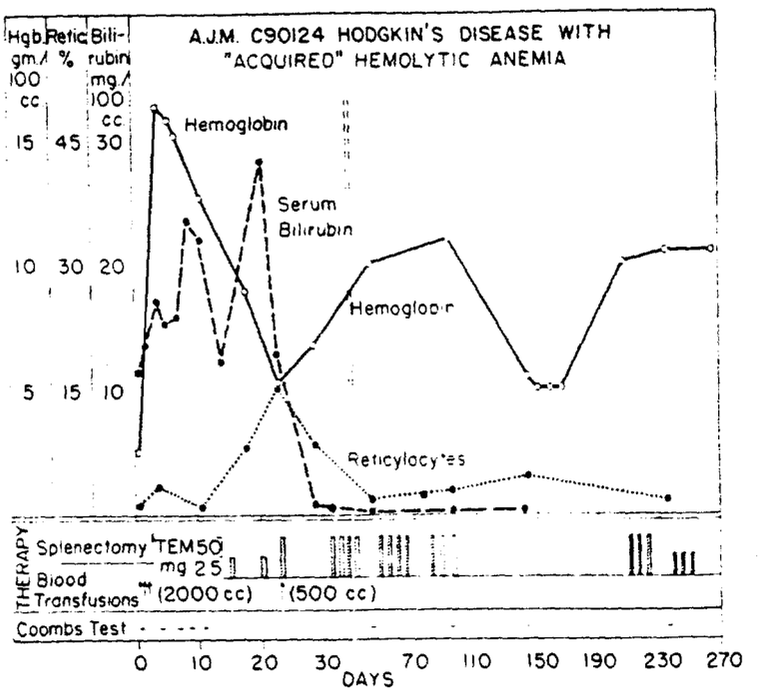

FIG. 4.—Subsidence of hemolytic anemia after TEM in patient with Hodgkin's disease.

Despite the early improvement, intense jaundice and fever up to 38-38.9 C. persisted (fig. 4). On the fourth postoperative day a 2 to 4 p.m. urine specimen contained 20 Ehrlich units of urobilinogen and a barium chloride strip test for bilirubin gave a 2+ reaction in a dilution of 1:10. On the thirteenth day a 2 hour urine specimen contained 67 Ehrlich units of urobilinogen. During the following week he became much worse, developed nausea, vomiting, diarrhea, and required intravenous fluids. The hemoglobin fell to 5.2 Gm. per 100 cc. and he was given a 500 cc. blood transfusion (fig. 4).

Red cells withdrawn soon after his admission to the hospital gave a strongly positive direct Coombs test. Cells removed on the fourth and seventh days gave a negative test, but the reaction was again positive on the ninth and eleventh days.

In view of the evidence of continued hemolysis and known activity of the Hodgkin's disease, 2.5 mg. of TEM were given on the fourteenth and twentieth hospital days, and 5 mg. on the twenty third day. After the third dose, fever and jaundice disappeared and he began to improve rapidly (fig. 4). On the twenty-eighth hospital day a 2 to 4 p.m. urine specimen contained 6.7 Ehrlich units of urobilinogen. Bilirubin was absent. He was able to return home.

1110825

During the next month he felt well and gained 8 Kg. in weight. Re-examination at that time showed that the enlarged lymph nodes had virtually disappeared. The hemoglobin concentration had risen to 10.0 Gm. per 100 cc. The urine contained no bilirubin and the urobilinogen was not elevated. The direct Coombs test was negative and autoagglutination could not be demonstrated even after trypsinization of the patient's red blood cells.

He continued to take 5 mg. of TEM per week at home for 8 weeks. Because adequate hematologic supervision could not be arranged therapy was then suspended. When he returned for a re-examination 4 months after leaving the hospital he had gained another 2 Kg. in weight and had few complaints. Blood counts, however, showed that his hemoglobin had dropped to 5.5 Gm. per 100 cc. The WBC was 1,725, RBC 2,010,000, hematocrit 19 per cent, reticulocytes 4.1 per cent and platelets 80,000. Bone marrow aspirated from the sternum was found to be almost acellular. Early erythroid and myeloid elements predominated and there were 18 per cent plasma cells. The blood bilirubin was normal. Two months later, without further therapy being given, the hemoglobin had risen to 10.0 Gm. per 100 cc. TEM was then resumed in smaller amounts. Sixteen months after his first hospital admission his general health appeared to be excellent. His hemoglobin remained in the neighborhood of 10.5 Gm. per 100 cc., but there was no sign of active Hodgkin's disease and no evidence of accelerated hemolysis.

In October, 1951, his hemoglobin concentration fell to 8.3 Gm. per 100 cc. The reticulocytes were 6.1 per cent and the Coombs test was negative. TEM therapy was suspended because of suspected depression of bone marrow function. On December 22, 1951, he was readmitted to the hospital, again acutely ill. He was icteric and the urine urobilinogen was greatly increased. The hemoglobin concentration was reduced to 2.9 Gm., and reticulocytes were 53 per cent. The Coombs test was positive. TEM therapy was resumed and there was subsidence of hemolytic activity.

Comment

The etiology of Hodgkin's disease, infectious, neoplastic, or otherwise, remains unsettled, and it is uncertain whether the disease is unicentric or multicentric in origin.[18-20] In the overwhelming majority of cases the apparent site of origin is in the cervical lymph nodes.[19] In those who die of the disease necropsy studies have shown with great frequency, however, involvement of internal nodes in which disease was not suspected during life.[18, 21]

The long survival of some patients with localized disease who are given surgical or radiologic treatment suggests that Hodgkin's disease may arise unicentrically.[20, 22, 23] The favorable results in this group of patients emphasizes the importance of early diagnosis, and amply justifies aggressive therapy in early cases, although the extent of the node involvement at the outset will often be underestimated.

Patients with localized disease are best treated with irradiation, care being given to deliver adequate dosage to all areas of disease while sparing neighboring tissues, particularly the bone marrow.[13] The frequency with which there are hidden foci, however, may explain the added benefit of whole body irradiation in these instances.[24] TEM, which can be used without producing cumulative tissue injury, may prove to be a more desirable agent for this purpose. Those with disease in many areas may respond well if they are first treated with TEM over a period of weeks or months until maximal improvement has occurred. Residual areas of disease should then be irradiated.

Most authors are of the opinion that the administration of nitrogen mustard in Hodgkin's disease will at best produce only a modest prolongation of life.[5, 12] The following patient, treated with nitrogen mustard injections at intervals

1110826

of 1 to 4 weeks over a period of 6 months, combined with local irradiation, obtained an unusually favorable result which has suggested to us the need for more persistent and aggressive therapy than generally given to those with rapidly recurring and extending disease, and gives some hope in fortunate instances of eradicating tissues exhibiting abnormal proliferative capacities.

[redacted] A 37 year old textile worker, was admitted to Duke Hospital on April 23, 1948, for the investigation of a painful tumor that had developed along the right side of his neck in a period of six weeks. He had noted, in addition, weakness and loss of weight.

On examination the tumor appeared to consist of a mass of lymph nodes, each about 1 to 3 cm. in length. A questionably enlarged node was palpated in the left axilla. The right tonsil was two and one-half times as large as the left. The remainder of the physical examination showed no abnormalities.

The peripheral blood examination showed no abnormalities except a leukocytosis of 13,300. The bone marrow was normal. X-ray films of the paranasal sinuses, skull, chest and abdomen showed no abnormalities. One of the cervical lymph nodes was removed and the pathologic interpretation was Hodgkin's disease.

Roentgen therapy to a total of 1550 r was given to the enlarged lymph nodes over a period of two weeks. They were soon smaller and in a few days he was able to leave the hospital. One month later, however, a mass developed in the right axilla and the right arm became edematous. X-ray therapy to the axilla was given. The following week he developed abdominal cramps. These became steadily worse until in a few days he had constant pain, abdominal distention and vomiting. He was re-admitted to the hospital. Bulging lymph nodes were present in both axillae. The upper abdomen was distended, peristaltic sounds were increased, and a mass was palpable in the periumbilical area. He was thought to have partial intestinal obstruction. X-ray therapy over the abdomen was started and in a few days the acute abdominal symptoms subsided but he remained generally ill.

Two months after his first visit the lymph nodes in the right axilla had again increased in size, and an exquisitely tender area had developed in the seventh rib on the right. Further roentgen therapy was given with temporary benefit.

On June 14, 1948, he was re-admitted to the hospital, "completely miserable." Enlarged cervical lymph nodes had reappeared. He had pain and stiffness about the right shoulder, edema of the right arm, and abdominal pain with occasional vomiting. An area over the third rib on the right was extremely tender. The consensus at this time was that his condition was hopeless and that with the rapidly advancing and recurring disease he might live for a few months.

In view of the poor prognosis, a plan of giving him nitrogen mustard therapy in the largest tolerated doses at intervals of 10 to 14 days, and continued for as long a period as possible was adopted. The following amounts were given:

1948		
7/16–19	4 injections, 0.1 mg./Kg. each	(28 mg.)
8/20	1 injection, 0.15 mg./Kg.	(10 mg.)
9/17	1 injection, 0.15 mg./Kg.	(10 mg.)
10/1	1 injection, 0.2 mg./Kg.	(14 mg.)
10/5	1 injection, 0.15 mg./Kg.	(10 mg.)
10/9	1 injection, 0.15 mg./Kg.	(10 mg.)
11/5	1 injection, 0.2 mg./Kg.	(14 mg.)
11/12	1 injection, 0.2 mg./Kg.	(14 mg.)
11/22	1 injection, 0.2 mg./Kg.	(14 mg.)
1949		
1/11	1 injection, 0.3 mg./Kg.	(20 mg.)
1/17	1 injection, 0.2 mg./Kg.	(14 mg.)
Total nitrogen mustard 2.0 mg./Kg.		**(158 mg.)**

1110827

After the first 4 injections of nitrogen mustard, the lymph nodes became smaller and a moderate fever subsided. The white blood count fell to 2,600 but soon rose to normal. His general condition improved so that he was able to continue therapy as an out patient. At the end of a month the enlarged lymph nodes had virtually disappeared and he had gained 10 lbs. in weight. He was able to resume work and in spite of later difficulties continued with full-time employment.

Six weeks after beginning the nitrogen mustard therapy he developed a painful tumor about the medial end of the right clavicle. The clavicular tumor became smaller and less painful 2 to 3 days after each injection of nitrogen mustard, but in another 7 to 10 days would recur. By November 22, 1948, the clavicular tumor had grown to measure 8 x 8 cm. A few days later the overlying skin ulcerated, and bony fragments were discharged. An abdominal mass again became palpable. Between November 27 and December 6, 1948, irradiation to a total dose of 1,000 r was given over the clavicular tumor and to the abdominal mass to a total dose of 900 r. In January, 1949, the ulcerated area overlying the clavicular tumor increased in size and further nitrogen mustard therapy was given. Shortly after this the ulcer healed, and all manifestations of the disease disappeared. He has been seen frequently during the following 3 years. There has been no evidence of disease reactivation at any time.

Follicular Lymphoma, Lymphocytic and Lymphoblastic Lymphoma, and Reticulum Cell Sarcoma

Twenty patients with malignant lymphomas of the above varieties were selected for trial therapy with triethylene melamine. Like those with Hodgkin's disease, all had widespread lymph node involvement. Abnormal leukocytes were not present in the circulating blood. Bone marrow aspirated in 18 patients was normal. Abnormal lymphoid cells were aspirated from an area of sternal tenderness in one patient, and in one instance the marrow was not examined.

Constitutional symptoms were uncommon during the early phases of the disorder, in contrast with those with Hodgkin's disease, and there was even less tendency for the lymphatic involvement to remain localized. The commonest presenting symptom was the appearance of one or more lymphoid tumors. Splenomegaly preceded the development of enlarged superficial lymph nodes in one patient. In 4 other instances the presenting sign was rectal bleeding due to infiltration of the lower bowel, edema of the legs with proteinuria, severe steatorrhea, and nonthrombocytopenic purpura, respectively.

In some respects the group was heterogeneous. Two 4 year old boys were included. Two of the adults with rapidly progressive disease died within 6 months of the appearance of their first symptom, in contrast to 5 patients who lived for 5 to 8 years while being given periodic irradiation therapy. Half of the group had had no treatment before being given TEM.

The overall therapeutic effect of TEM in this group was judged largely by the reduction in size of local tumors. The results were rated as excellent in 7, good in 3, slight in 5, none in 3 and inconclusive in 2 (table 1). The good to excellent results occurred predominantly in 2 groups: (1) patients with newly developed tumors, some of whom had been previously treated successfully with irradiation, and (2) patients given TEM as their initial therapy. Poor results occurred in those with rapidly extending or recurring disease, and in those with tumors causing severe pain, suggestive of local tissue invasion by the malignancy. In 3 of the patients in whom the drug apparently had little or no effect, gastro-intestinal symptoms and or leukopenia were not produced. The dose of the chemical, accordingly, might have been inadequate. No clear differentiation in clinical fea-

1110828

tures, or in the response to therapy, was discernible between those diagnosed as having lymphocytic lymphoma and those having lymphoblastic lymphoma. Two of the patients with follicular lymphoma who were little benefited were treated late in the course of their disease. One patient diagnosed as having reticulum cell sarcoma 4 years previously, had extensive abdominal disease with ascites at the beginning of TEM therapy and responded slowly over a period of months.

The following patient with lymphocytic lymphoma is illustrative of what was regarded as an excellent therapeutic result.

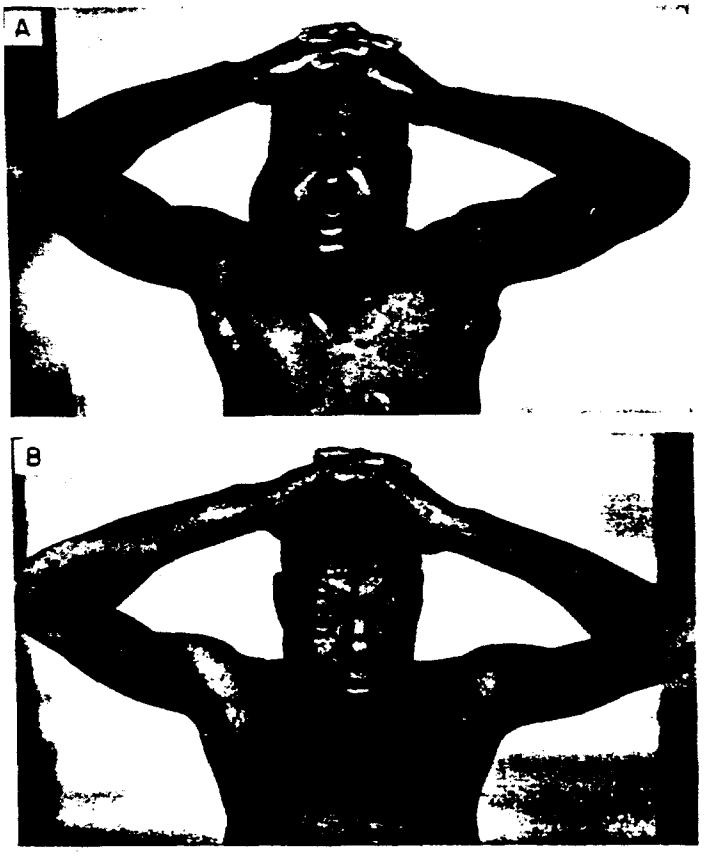

FIG. 5. — [redacted] Reduction in size of lymph nodes in malignant lymphoma after TEM therapy.

[redacted] This 50 year old colored preacher and farmer was admitted to Duke Hospital on September 25, 1950, complaining of tumor masses in his neck. A nodule had first appeared under the angle of the mandible on the right three or four years previously. This fluctuated in size for some months, but then began to enlarge steadily. About one year before admission masses began to appear on the left side of his neck, in the axillary, inguinal and epitrochlear areas. For 3 or 4 months he had had cough and dyspnea when lying supine, but he was able to continue with hard manual labor.

On physical examination there was massive lymph node enlargement in all superficial areas (fig. 5). The liver and spleen were palpable. The hemoglobin was reduced to 11.7 Gm. per 100 cc. The WBC was 7,800. No abnormal cells were present in the circulating blood. Marrow aspirated from the sternum was extremely cellular and contained no abnormal leukocytes. An x-ray of the chest showed no mediastinal or pulmonary parenchymal abnormality. Biopsy of a cervical lymph node showed dense lymphocytic infiltration with

1110829

obliteration of the node architecture. The pathologic interpretation was lymphocytic lymphoma.

He was given 5 mg. of TEM daily for 4 days. With the fourth dose he developed anorexia and mild nausea that lasted for several hours. Six days after the first TEM was given the WBC had fallen to 3,400, but soon rose again to normal. The lymph nodes at this time were considerably softer and possibly smaller.

On re-examination two months later, the superficial lymph nodes were definitely smaller. He was advised to take two additional 5 mg. doses of TEM. A few weeks later he developed pneumonia, and when given a variety of medications became acutely disturbed and psychotic. He recovered when the administration of drugs was discontinued.

Four months after his first visit he regarded his general health as quite satisfactory. The lymph nodes were about one-fifth their original size (fig. 5). There were no other significant abnormalities. He was advised to continue taking periodic doses of TEM.

Comment

The therapeutic problems presented by patients with follicular, lymphocytic and lymphoblastic lymphoma, and reticulum cell sarcoma vary from those encountered in Hodgkin's disease to those in lymphocytic leukemia. Bulky tumors usually respond well to local roentgen irradiation. The widespread involvement of lymphoid tissue in most cases,[21] however, indicates the advisability of generalized therapy. TEM appears to be a most promising agent for this purpose.

Chronic Lymphocytic Leukemia

(A) *Leukemic Group*

Twenty-three patients having chronic lymphocytic leukemia with high leukocyte counts were treated with TEM. All patients with this disease were regarded as suitable for trial therapy. The disease was of recent onset in 11 of them and TEM was the first anti-leukemic therapy given. Most of the others had been given x-ray therapy earlier for disease of 18 months to 7 years duration. The therapeutic effect of TEM was judged by the degree to which the primary manifestations of the disease—lymphocytosis, lymph node and splenic enlargement and related symptoms—could be affected without producing or increasing depression of bone marrow function. The results were considered excellent in 12 patients, although in 2 of them pre-existing anemia due to bone marrow replacement was never overcome; good in 6, 2 of whom remained anemic; and slight in 5. In those to whom TEM was of least benefit, the leukemic process was advancing rapidly and the abnormal cells were more immature.

The following 4 case histories illustrate variation in effective TEM dosage, the production of a long remission in a patient treated for asymptomatic disease, hematologic improvement in a patient with leukemic marrow infiltration, and the failure to improve the anemia in 2 patients with leukemic marrow replacement.

[redacted] This 73 year old farmer was admitted to Duke Hospital on August 3, 1950, for the excision of an ulcerating carcinoma of the right cheek. Except for 4 or 5 attacks of transient precordial pain that had occurred on exertion during the previous two or three years, his general health had been good. Physical examination revealed a few superficial lymph nodes 0.5 to 1.0 cm. in length, moderate cardiac enlargement, and the liver and spleen to be palpable 3 to 4 cm. below their respective costal margins. Blood study showed a WBC of 127,000, 93 per cent of which were immature lymphocytes. The hemoglobin was 12.0

1110830

Gm. per 100 cc., hematocrit 38.8 per cent, reticulocytes 1.2 per cent and platelets normal in number.

The carcinoma was excised and the defect repaired.

TEM therapy was begun during his convalescence. Five mg. were given daily for 3 days (fig. 6). There were no side reactions. A month later the white blood count had fallen to 47,500. Two additional 5 mg. doses of TEM were prescribed. During the following weeks the WBC fell to a range between 8,000 and 13,000. The enlarged superficial lymph nodes disappeared and the liver and spleen regressed until they were not palpable.

Eight months after his first TEM therapy, he developed a prolonged upper respiratory infection and finally lobar pneumonia, with pneumococci type VII in the sputum. Despite intensive antibiotic therapy, pulmonary cavitation occurred. His convalescence was slow and required 4 weeks in the hospital.

On re-examination 16 months after his first therapy he appeared to have excellent general health. Physical examination showed no significant abnormalities. The only hematologic

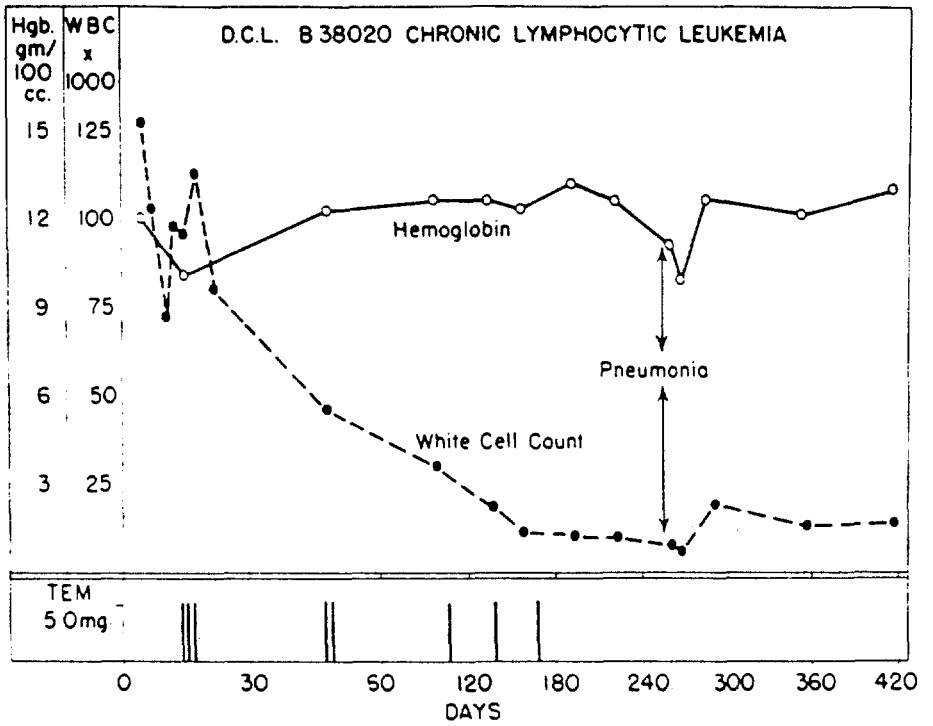

FIG. 6.—Hematologic response to TEM in a patient with asymptomatic lymphocytic leukemia.

abnormality was a small percentage of immature lymphocytes in the circulating blood, despite 8 months without anti-leukemic therapy.

[redacted]. This 39 year old colored farmer was first seen at Duke Hospital on October 3, 1947, complaining of frequent sore throats and enlarged "neck glands" for 3 years. On physical examination his tonsils were huge. Lymph nodes in the cervical, axillary and inguinal areas measured 1.0 cm. or more in length. The hemoglobin concentration was 15.0 Gm. per 100 cc., RBC 5,130,000, and WBC 17,000, of which 70 per cent were abnormal lymphocytes. Sections of a lymph node removed from the neck showed lymphocytic infiltration and obliteration of the node architecture.

He was given x-ray therapy to the superficial lymph nodes. On re-examination one month later the nodes were considerably smaller and the white blood count was 5,450.

He was not seen again for 3 years when he returned with the same complaints as before. On this occasion the superficial nodes measured up to 3 cm. in length. His spleen was palpable several cm. below the rib margins. The hemoglobin was 11.7 Gm. per 100 cc., RBC

1110831

4,610,000, hematocrit 38.0 per cent and WBC 126,000, of which 92 per cent were abnormal lymphocytes. Reticulocytes and platelets were normal in number. Marrow aspirated from the sternum, contained 93 per cent immature lymphocytes and very few myeloid or erythroid cells.

TEM therapy was instituted in doses of 2.5 to 5.0 mg. per day (fig. 7). There were no side reactions. After two months of therapy the nodes were much smaller, the spleen was not palpable, and the WBC ranged from 12,000 to 14,000. The hemoglobin subsequently rose to within range of normal. At 8½ months he considered his general health as entirely satisfactory. Re-examination of the bone marrow showed fewer lymphocytes and an abundance of erythroid and myeloid cells.

[redacted]. This 60 year old man was first seen on February 21, 1947, and followed closely for nearly 5 years. Six months before his first visit he had become ill with chest pain and fever. A few weeks later enlarged cervical lymph nodes appeared. An x-ray of his chest

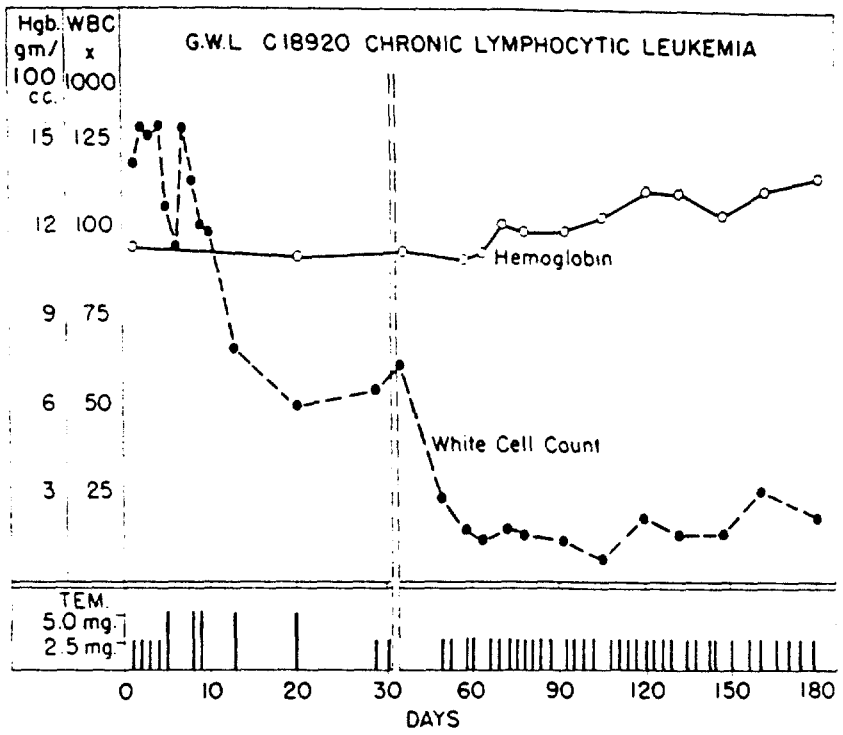

FIG. 7.—Hematologic response to TEM in a patient with chronic lymphocytic leukemia with mild anemia and lymphocytic bone marrow infiltration.

showed masses in both hila. Biopsy of a cervical node showed heavy lymphocytic infiltration and obliteration of the normal node architecture. He was referred to us for therapy.

Physical examination showed pronounced enlargement of the tonsils. The cervical and inguinal lymph nodes measured 1 to 2 cm. in length. The hemoglobin concentration was 13.5 Gm. per 100 cc., and the WBC was 53,000 of which 82 per cent were abnormal small lymphocytes. He was given x-ray therapy to the enlarged superficial and hilar lymph nodes. The former disappeared but the latter changed very little. The WBC remained moderately elevated. Additional roentgen therapy was given to the hilar areas two months later.

Eighteen months after his initial visit he developed chronic suppuration of the left tear duct. Physical examination showed no other relevant abnormalities. His hemoglobin was 11.5 Gm. per 100 cc. and the WBC 44,000. The predominant cells in marrow aspirated from the sternum were early lymphocytes. Erythroid and myeloid elements were greatly reduced. Whole body roentgen irradiation was administered. During the ensuing months the white blood count dropped to normal levels but his hemoglobin continued to fall.

He was re-admitted to the hospital two years after his first visit because of progressive

1110832

anemia. He was given 4 injections of nitrogen mustard intravenously, 0.1 mg. Kg. each during a period of 8 weeks. Three weeks after completing the therapy he became extremely weak and had mild generalized purpura. The WBC was found to be 4,000, the hemoglobin 5.2 Gm. per 100 cc., and the platelet count 60,000. Re-examination of the bone marrow showed it to be virtually acellular. After he was given 3 blood transfusions of 500 cc. each he became more comfortable and had no further bleeding.

During the following 17 months he required 2 to 4 blood transfusions every 4 to 6 weeks to maintain comfortable blood levels. At the end of this time the superficial lymph nodes enlarged again, the spleen became palpable and the WBC rose to 48,000 to 57,000.

TEM therapy was instituted with caution and continued for over a year. With an average dose of 2.5 mg. per week the superficial lymph nodes disappeared, the spleen regressed in size, and the white count was held within range of normal. He did not develop thrombocy-

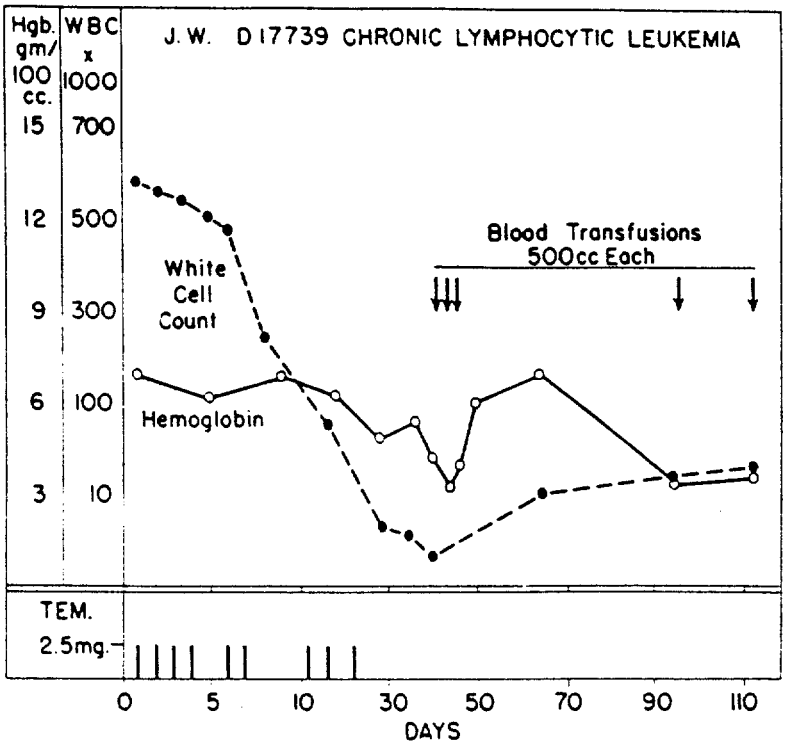

FIG. 8.—Persistence of anemia after TEM therapy in patient with chronic lymphocytic leukemia with leukemic marrow replacement of long standing.

topenia or hemorrhagic manifestations. Blood transfusions, however, continued to be required at regular intervals.

[redacted]. This 64 year old colored man was first seen at Duke Hospital on July 9, 1951. He had had gradually enlarging lymph nodes about his neck for some 18 months. For a year he had had weakness, ease of fatigue and dyspnea on exertion, and for 3 to 4 months his legs had been grossly edematous.

On examination large masses of lymph nodes were present in the cervical, axillary and inguinal regions. The liver edge was 10 cm., and the tip of the spleen 3 cm. below the rib margins. Hard, non-pitting edema involved the legs to the groins, and the external genitalia were edematous.

The hemoglobin concentration was 6.8 Gm. per 100 cc., RBC 3,030,000, hematocrit 21.0 per cent and WBC 587,000, 97 per cent of which were abnormal lymphocytes. The platelets were moderately reduced in number. Marrow aspirated from the sternum contained virtually nothing but abnormal lymphocytes.

He was given TEM in 2.5 mg. doses to a total of 22.5 mg. in a period of 2 weeks. The last 3 doses were probably given inadvisedly considering the precipitous fall in WBC

1110833

(fig. 8). The hemoglobin and red cell count continued to fall until blood transfusions were required. Satisfactory blood counts were still not maintained 6 months after the beginning of therapy.

Comment

Minot and Isaacs[25] emphasized in 1924 the grave prognostic significance of the development of anemia and or thrombocytopenia—indications of bone mar-

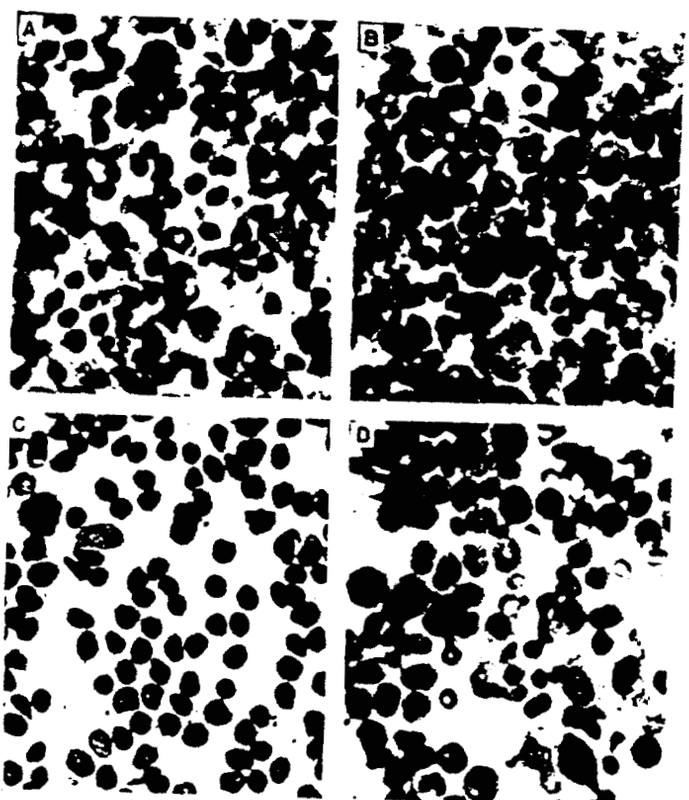

FIG. 9.—[redacted] A 49 year old farmer was found to have chronic lymphocytic leukemia in July, 1948. Enlarged superficial lymph nodes had been present for 6 months. The hemoglobin concentration was 14.0 Gm., RBC 4,300,000, hematocrit 38.0 per cent, and WBC 30,000. The bone marrow was normally cellular but 60 per cent of the cells were lymphocytes. He was given nitrogen mustard injections at intervals of 1 to 3 months for two and one-half years, and during this time enjoyed excellent health. His physical and hematologic status remained, however, substantially unchanged. He was then given TEM for a year, the average dose being 2.5 mg. every two weeks. During this period the lymph nodes became smaller, and WBC was held virtually within range of normal. Lymphocytic infiltration disappeared from the marrow.

A Photograph (X400) of marrow aspirated on March 29, 1950, showing reduced myeloid and erythroid elements, and lymphocytes 65 per cent.

B Photograph (X400) of marrow aspirated on November 23, 1951, showing numerous granulocytes and normoblasts, and lymphocytes 16 per cent.

[redacted]. This 45 year old physician, had had increasing sensitivity to cold for twelve years, and progressive anemia for five years. The hemoglobin was eventually reduced to 6.4 to 6.8 Gm. per 100 cc. He was found to have hypoalbuminemia and cryoglobulinemia. The bone marrow contained virtually nothing but primitive lymphoid elements.

C Photograph (X400) of marrow before treatment.

He was given TEM for 10 months. The hemoglobin concentration was then 11.2 Gm. per 100 cc. and the bone marrow was virtually normal.

D Photograph (X400) of marrow showing numerous granulocytes and normoblasts.

1110834

row involvement in the course of chronic lymphocytic leukemia. The important causes of disability and ultimate fatality in this disease, infection, anemia and hemorrhage are attributable in large measure to replacement of the bone marrow by infiltrating or proliferating lymphocytes. Large numbers of abnormal lymphocytes were present in the bone marrow in 19 of our 22 patients before TEM therapy was started. No examination was made in one instance. Tissues other than the bone marrow, such as the liver, heart muscle, etc. were less commonly the sites of serious leukemic damage.

A major object in the early treatment of patients with chronic lymphocytic leukemia, even in an asymptomatic stage, should be the prevention of bone marrow invasion and damage. There is little evidence that the irradiation of superficial lymph nodes or of the spleen delays or protects against bone marrow involvement. Whole body irradiation[24] or radioactive phosphorus therapy[26–28] may be of more value, especially when given in regularly spaced, individually titrated doses.[29] Once marrow replacement has occurred, as evidenced by granulocytopenia, anemia or thrombocytopenia and substantiated by the findings on direct marrow examination, therapy of any type entails greater risk and is often ineffective. All forms of irradiation under these circumstances, as well as nitrogen mustard as commonly used, have failed to improve bone marrow function significantly,[5, 9–11, 15, 24–29] or to reduce lymphocytic infiltration.[30]

The extent to which damage to the bone marrow and to other vital tissues can be prevented by sustained TEM therapy in any significant number of patients with chronic lymphocytic leukemia is still to be determined. Our observations thus far are encouraging. In the 2 patients in this series with normal bone marrow before therapy, and in the 10 with marrow infiltration but no anemia, neither persistent anemia nor thrombocytopenia has develpoed either as a result of therapy or of progression in the disease. In several instances, marrow that was once heavily infiltrated was later found to contain fewer lymphocytes or to be entirely normal (fig. 9).

Two of the 9 patients who had anemia with bone marrow infiltration and replacement at the outset had substantial hematologic improvement following TEM therapy. Four responded poorly to TEM, or died soon after it was started, without showing definite improvement in marrow function. In 3 patients the primary disease responded well to TEM, but anemia due to marrow replacement, which had been present for some months, persisted and continued to require repeated blood transfusions. It appears that bone marrow damage produced by leukemia becomes irreversible in the course of time.

Chronic Lymphocytic Leukemia

(B) *Sub-Leukemic Group*

Sixteen patients with chronic sub-leukemic lymphocytic leukemia were treated with TEM. The leukocyte count was normal or low in all cases. Abnormal lymphocytes were constantly present in the circulating blood. The disease appeared to be of less than one year's duration in 10 patients when treatment was begun. Five died within a year of onset of their symptoms. Examination of the bone marrow before treatment showed no abnormality in 3 patients, lymphocytic

1110835

infiltration in 1 who was not anemic and lymphocytic replacement in 11 who were anemic.

The response of this group to TEM was generally poor, in contrast to that of the group with chronic lymphocytic leukemia in which the leukocyte count was elevated. The therapeutic effects were excellent in 4, slight in 7, absent in 3 and inconclusive in 2. The less favorable results in this group seemed to be due to the basic severity of the disease, to the high incidence of bone marrow replacement and in some cases to delay in recognition and the initiation of treatment. The following case history illustrates an excellent therapeutic result in a patient whose prognosis would ordinarily be grave.

[redacted] This 30 year old white married woman was first seen on October 1, 1950. Two years previously she had developed nasal obstruction and nodular tumors about her upper eyelids. Orbital tissue was removed for pathologic study at another hospital and a diagnosis of lymphatic leukemia made. The orbital tumors disappeared following x-ray therapy and she became outwardly well.

Two months before her hospital visit she became weak and pale was was found to be severely anemic. She was given a total of 1500 to 2000 cc. of blood intravenously on three occasions with symptomatic relief, but the anemia recurred each time in a matter of 2 to 4 weeks.

Physical examination showed obesity, no jaundice, lymph nodes in the left axillary, epitrochlear and inguinal regions measuring up to 1 to 3 cm. in length and moderate splenomegaly.

The hemoglobin concentration was 10.2 Gm. per 100 cc., RBC 4,140,000, WBC 6,500, hematocrit 34.0 per cent and reticulocytes and platelets normal in number. The white cell differential count was neutrophils 23 per cent, nonsegmented neutrophils 10 per cent, metamyelocytes 1 per cent, eosinophils 2 per cent, monocytes 10 per cent and abnormal lymphocytes 55 per cent. Bone marrow aspirated from the sternum was highly cellular. The predominant cells were immature lymphocytes, but small islands of normal marrow tissue persisted. Urine and stool analyses, blood bilirubin concentration and the excretion of bromsulfalein dye were all normal. Serologic tests for syphilis and a direct Coombs test were negative.

She was given 3 doses of TEM, 5 mg. each, in 4 days. The lymph nodes became smaller. She returned home and during the following week anemia severe enough to require 2 blood transfusions recurred.

Twenty days after the first TEM therapy, the superficial lymph nodes had disappeared and the spleen was barely palpable. The hemoglobin was 6.8 Gm. per 100 cc., RBC 2,400,000, WBC, 1,800, hematocrit 19.5 per cent and platelets and reticulocytes normal in number. Re-examination of bone marrow showed a great reduction in total cellularity. Only a few lymphoid cells and a few early erythroid and myeloid elements were present in the aspirated bone marrow.

At home she improved rapidly and was soon feeling "wonderful." She was given about 10 mg. of TEM in small doses during the following 5 months. Re-examination 10 months after her first visit showed no significant physical abnormalities. The hemoglobin concentration was 13.5 Gm. per 100 cc., RBC 4,790,000, WBC 7900, hematocrit 44.0 per cent, reticulocytes 1 per cent and platelets normal in number. Twelve per cent of the leukocytes in the circulating blood were immature lymphocytes.

Leukemia, Acute Lymphocytic

Three patients with acute lymphocytic leukemia, 2½, 3 and 12 years old respectively were treated with TEM. The youngest child, treated with the chemical alone, obtained definite symptomatic benefit for a period of 8 to 10 weeks. Persistent leukopenia and increasing anemia associated with low marrow cellularity then made another type of therapy necessary. The second child, ill for

1110836

5 weeks, had severe anemia and thrombocytopenia when first seen. TEM given over a period of 2 months was of no symptomatic or hematologic benefit. The third child had had a malignant lymphoma excised from the scalp at the age of 8 years. Four years later he developed, in a few weeks time, multiple subcutaneous tumors, leukocytosis with large, immature lymphoid cells in the circulating blood. The bone marrow soon became infiltrated and replaced. The disease was rapidly fatal and its course was not altered by TEM therapy.

Leukemia, Granulocytic

Six patients with chronic granulocytic leukemia were given TEM therapy. One patient had untreated disease. The other 5 had been given urethane for 10 to 40 months, until incipient liver disease or intolerance to therapeutically adequate doses of the chemical made it necessary to change the plan of therapy. Three of the group responded quickly to TEM. Fever, bone tenderness, splenomegaly and granulocytosis subsided and the anemia began to regress. A fourth patient responded slowly, only after about 100 mg. of TEM had been given over a period of 6 months. The patient with previously untreated disease responded poorly and after 25 days remained ill, although a 50 per cent reduction in white blood count had occurred. She then responded satisfactorily to urethane.

Three patients with chronic granulocytic leukemia with variable degrees of erythroid and megakaryocytic overgrowth (panmyelosis) were treated without definite benefit.

A patient with chronic subleukemic granulocytic leukemia was given sufficient TEM to reduce the WBC for several weeks without altering the course of her disease.

Seven patients with acute granulocytic leukemia were treated with TEM. The chemical was effective in reducing leukocytosis during the period of its administration but noteworthy symptomatic or hematologic benefits were not produced.

Polycythemia Vera

Three patients with newly discovered polycythemia vera, all of whom had been subjected to phlebotomy until the erythrocytosis was controlled, were given TEM in an effort to prevent the return of excessive marrow activity. A substantially normal hematologic status was maintained for periods of 7, 12 and 14 months respectively. When therapy was suspended in the patient treated for 7 months relapse occurred within a few weeks.

Multiple Myeloma

Three patients with multiple myeloma were treated with TEM after their disease had become refractory to urethane. Depression of bone marrow function was produced in all of them without a definite effect on the growth of plasma cells being observed, although in one instance the amount of abnormal gamma globulin in the serum was slightly reduced.

Miscellaneous Neoplasms

Twenty-two patients with widespread tumors not primarily of hemopoietic origin were given TEM therapy (table 1). Worthwhile palliation was observed

1110837

only in those with lymphoepitheliomas arising from the nasopharynx and in those with papillary cystadenocarcinomas of the ovary. The following cases are illustrative.

[redacted] A 14 year old white boy, was referred to Duke Hospital on August 21, 1950. He had been well until 2½ months previously when a painless mass began to grow on the left side of his neck. A few weeks later a similar mass developed on the opposite side. He was examined by his family physician who found a tumor in the nasopharynx. A portion of the latter was taken for biopsy but the pathologic interpretation was in doubt.

On physical examination firm masses in the posterior cervical areas measured 5 x 7 cm. and 3 x 5 cm. (fig. 10). The nasopharynx was filled with a friable nodular tumor which obstructed the right posterior naris. Study of the peripheral blood and bone marrow disclosed no abnormality. A mento-vertex film of the skull showed irregularity in bone density and areas of destruction. Biopsy specimens were taken from the cervical and nasopharyngeal tumors. The pathologic interpretation was lymphoepithelioma with metastasis.

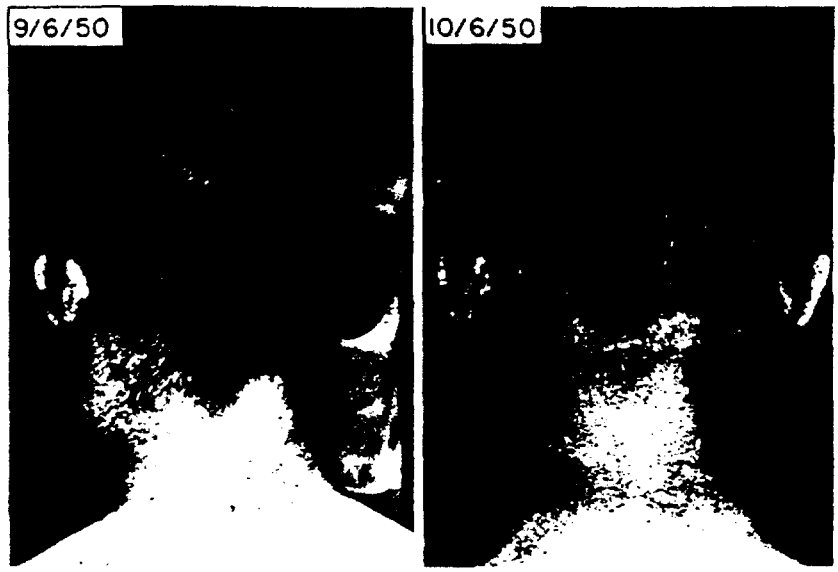

FIG. 10.—Reduction in size of cervical tumors after TEM in lymphoepithelioma of nasopharynx with metastases.

He was given 22.5 mg. of TEM over a period of 5 days without adverse reaction. One month later there was a conspicuous reduction in the size of the cervical metastases (fig. 10). Two 5 mg. doses of TEM were given during the following month but the masses became slightly larger. Roentgen therapy was then given to the nasopharynx and neck.

Three months after his first visit the mass on the left side of his neck measured 2 x 2 cm. while that on the right side had disappeared. Examination of the nasopharynx showed no abnormality. At home he took 2.5 to 5 mg. of TEM per week, adjusting the dose to maintain a slight leukopenia.

Seven months after his initial visit a tumor appeared in the frontal region. In three weeks it increased in size to measure 2.5 x 3.5 x 4.5 cm. An x-ray of the skull showed destruction of the bone underlying the tumor.

Roentgen therapy was administered to the tumor and TEM was suspended for a month. During this time he developed several enlarged cervical nodes, one of them in the left supraclavicular fossa measuring 3 x 3 cm. TEM was resumed in the largest amounts permitted by the blood counts and during the ensuing weeks all cervical tumors disappeared. Periodic doses of TEM were continued. Re-examination 18 months after he was first seen revealed no evidence of disease.

[redacted] This 14 year old school girl became aware of a mass in the lower abdomen

1110838

in May, 1951. She was operated upon and an ovarian tumor was partially resected. The pathologic diagnosis was papillary cystadenocarcinoma. She was given roentgen therapy for over 30 days and for 4 months enjoyed good health. She then developed chronic fatigue, dizzy spells and nausea. Examination disclosed hepatomegaly and a mass in the lower abdomen. Fluoroscopy of the chest showed elevation of the diaphragm on the right side. She became rapidly worse with chest pain and fever, and was admitted to Duke Hospital on October 23, 1951.

Physical examination showed signs of pleural effusion on the right, a greatly enlarged liver and a tender mass in the lower abdomen. The temperature ranged from 38 to 39 C. Hematologic study showed a normocytic, normochronic anemia with the hemoglobin concentration reduced to 9.9 Gm. per 100 cc. Serial roentgen films of the chest showed rapid progression in her disease (fig. 11). Thoracentesis was performed and about 400 cc. of bloody fluid removed.

Between the second and fifth hospital days she was given nitrogen mustard intravenously to a total dose of 0.45 mg. Kg. She improved and therapy was continued with TEM at home. Six weeks after her initial visit she had no complaints and was able to return to school. The abdominal mass was no longer palpable and the liver was normal in size. A roentgenogram of the chest showed considerable improvement (fig. 11). The hemoglobin concentration had risen to 10.8 Gm.

FIG. 11.—Serial chest roentgenograms of patient with metastases from an ovarian cystadenocarcinoma, showing increase in disease, and then regression following nitrogen mustard and TEM therapy.

DISCUSSION AND SUMMARY

The therapeutic effect of triethylene melamine (TEM) on neoplastic disease as reported by Karnofsky and collaborators[2] has been confirmed in 134 patients treated over a period of eighteen months. The discovery of this chemical has permitted significant advances in practical therapy. Its effectiveness when given by the oral route and its relative freedom from disturbing side reactions, in contrast to nitrogen mustard, make optimally spaced and sustained therapy feasible. Its generalized action renders it an agent suitable for the treatment of diseases which involve tissues in widespread anatomic areas.

As summarized above in reference to different disease entities, TEM has proved to be especially useful in the treatment of the chronic proliferative diseases arising from lymphatic tissue. Localized Hodgkin's disease and localized lymphomatous tumors continue to be best treated with roentgen irradiation.[3] Combined local and systemic therapy is probably indicated in the majority of patients. In managing diffuse nonlocalized disease, TEM is a promising agent which may compare favorably with whole body irradiation and P_{32}. When the bone marrow is involved, as in chronic lymphocytic leukemia, the beneficial effect of TEM administration appears to surpass that of any other agent.

Although TEM may suppress the growth of normal bone marrow constituents to a very pronounced degree, its therapeutic effect in the myeloid proliferative

1110839

diseases has been somewhat disappointing. In atypical and subleukemic granulocytic leukemia and in multiple myeloma the results have not been promising. The long term effect in polycythemia vera merits further study.[31] In the small number of patients with chronic granulocytic leukemia in this series, the results seem to be inferior to those achieved by urethane. To evaluate the comparative merits of different therapeutic agents in this disease will require a large-scale cooperative study.[32]

In acute leukemia, rapidly progressing malignant lymphomas, and in most nonhemopoietic tumors TEM therapy has been of little value. Worthwhile palliation has been observed in nasopharyngeal lymphoepithelioma and in ovarian papillary cystodemocarcinoma with metastases. Additional study of the effect of TEM on these tumors is indicated.

The critical problem in TEM therapy is the administration of therapeutically adequate amounts of the chemical while avoiding the serious hazards of overdosage. The correct dose cannot be forecast but must be determined empirically for each patient. The effective intravenous dose is one-fourth to one-half that of nitrogen mustard, or in adults 2 to 3 mg. daily for 2 to 3 days.[3, 33] Oral doses range from these amounts upward. The effective total dose of TEM during the first 1 to 3 weeks of treatment averages 15 to 25 mg. About 1 patient in 10 will develop temporary depression of bone marrow function when given as little as 8 to 12 mg. of TEM in a span of 5 to 7 days. A few may take 10 to 15 mg. per week almost indefinitely with little result. The reaction of an individual patient to given amounts fortunately remains relatively constant, except that increased sensitivity will occasionally develop after it has been used for some weeks or months.

The considerable variation in effective dose that occurs in different patients does not appear to be related to the type of disease they may have. Greater caution should be exercised, however, in those with pre-existing bone marrow damage. The chemical decomposes when in contact with organic materials, and in an acid medium. Differences in dosage requirements may possibly be due to variations in gastric acidity or to the facility with which the chemical is absorbed. Tablets containing TEM should be given to patients in a fasting state with water, and perhaps with a buffering alkali, to minimize decomposition before absorption has taken place.[3]

The initial oral dose of TEM should not exceed 2.5 mg. If this amount is well tolerated for 1 to 2 days, the dose may be increased to 5 mg. Although slight loss of appetite or nausea is a common occurrence, the appearance of severe anorexia, nausea, vomiting, or diarrhea indicates overdosage, either from excessively large single doses or from a cumulative effect. The WBC should be determined before each dose is given. When the prevailing count falls abruptly, it is imperative that the administration of the drug be suspended immediately until the hematologic status becomes stabilized. The full effect of a given dose may not be manifest for 10 to 14 days. Anemia or thrombocytopenia rarely develops during prolonged therapy without antecedent depression of the leukocyte count. In the present group of patients serious depression of marrow function resulted from overdosage of TEM in 10 instances. This usually subsided fully

1110840

and spontaneously in 2 to 6 weeks, but it may have hastened the death of 2 patients.

Harmful effects of TEM can be avoided only by carefully regulating the amount of the chemical given. If the difference between the toxic and therapeutic doses could be increased by the administration of a compound like cysteine the safety and utility of the chemical would be enhanced.[34, 35]

Once the initial effects of TEM have been produced and the approximate dose established for a given patient, the problem of maintaining a therapeutic effect and preventing relapse arises. The action of TEM, like that of nitrogen mustard, is transitory. Long remissions in the diseases that respond to this chemical are scarcely to be expected after brief periods of therapy. In most patients continued evidence of disease activity make it necessary to give maintenance doses at intervals no greater than 1 to 2 weeks. TEM seems to be an agent that is well adapted for sustained therapy, apparently differing in this regard from another nitrogen mustard compound (R 48) that has been effective when given by mouth.[36] Prolonged remissions have been maintained in some of our patients with doses as small as 1 to 2.5 mg. per week. Others have required as much as 5 mg. twice a week.

How long the administration of TEM should be continued without interruption to obtain the best long-term results in different disease entities is a matter for further study. Our policy has been to maintain treatment for at least 5 to 6 months, even though signs of disease activity may disappear sooner, before therapy is suspended and evidence of relapse awaited. Many patients will probably require the administration of TEM at close intervals, indefinitely.

Deleterious effects from sustained therapy, other than temporary depression of bone marrow function, have not been observed. Patients treated for many months have maintained their optimal body weight, have not shown undue susceptibility to infection and have not developed abnormalities in serum protein constituents.

The availability of a chemotherapeutic agent such as TEM, which can be used with reasonable convenience and safety without cumulative damage to normal tissues, suggests the need for reconsidering some currently accepted opinions regarding the long term management of the chronic proliferative diseases arising from lymphatic tissues. It would appear desirable to suppress the objective manifestations of disease continuously, even in the absence of clinical symptoms, and to prevent, if possible, the development of complications such as bone marrow damage. The extent to which health and longevity can be prolonged by this means is a matter for continued study.

Conclusions

1. Triethylene melamine (TEM) is an important addition to the chemotherapeutic agents useful in the treatment of malignant disease, particularly Hodgkin's disease, malignant lymphoma, chronic lymphocytic leukemia, lymphoepithelioma of the nasopharynx and papillary cystadenocarcinoma of the ovary.

2. TEM may be used advantageously in conjunction with local roentgen therapy, and in situations in which whole body irradiation or P_{32} has been used.

1110841

3. TEM may be the agent of choice in treating chronic lymphocytic leukemia, especially in patients with leukemic infiltration or replacement of the bone marrow.

4. The effective dose of TEM when given by mouth varies greatly from patient to patient. With adequate precautions it can be used without undue risk.

5. TEM appears to be an agent well suited for sustained therapy without the production of cumulative damage to normal tissues.

REFERENCES

[1] Lewis, M. R. and Crossley, M. L.: Retardation of tumor growth in mice by oral administration of ethylenimine derivatives. Arch. Biochem. *26:* 319, 1950.

[2] Burchenal, J. H., Crossley, M. L., Stock, C. C. and Rhoads, C. P.: The action of certain ethylenimine (Aziridine) derivatives on mouse leukemia. Arch. Biochem. *26:* 321, 1950.

[3] Karnofsky, D. A., Burchenal, J. H., Armistead, H. C., Southam, C. M., Bernstein, J. L., Craver, L. F. and Rhoads, C. P.: Triethylene melamine in the treatment of neoplastic disease. Arch. Int. Med. *87:* 477, 1951.

[4] Gilman, A. and Philips, F. S.: The biological actions and therapeutic applications of the β-Chloroethyl amines and sulfides. Science *103:* 409, 1946.

[5] Karnofsky, D. A.: Nitrogen mustards in the treatment of neoplastic disease, in: Dock, W. and Snapper, I.: Advances in Internal Medicine. Chicago, Year Book Publishers, 1950, vol. 4, p. 1.

[6] Block, M., Spurr, C. L., Jacobson, L. O. and Smith, T. R.: Histopathologic effects of nitrogen mustard therapy upon normal and neoplastic hemopoietic tissues. Am. J. Clin. Path. *18:* 671, 1948.

[7] Guzman Barron, E. S., Bartlett, G. R. and Miller, Z. B.: The effect of nitrogen mustards on enzymes and tissue metabolism. I. The effect on enzymes. J. Exper. Med. *87:* 489, 1948.

[8] Rhoads, C. P.: Nitrogen mustards in the treatment of neoplastic disease. J.A.M.A. *131:* 656, 1946.

[9] Jacobson, L. O., Spurr, C. L., Guzman Barron, E. S., Smith, T. R., Lushbaugh, C. and Dick, G. F.: Nitrogen mustard therapy. J.A.M.A. *132:* 263, 1946.

[10] Wintrobe, M. M., Huguley, C. M., Jr., McLennan, M. T. and de Carvallio Lima, L. P.: Nitrogen mustard as a therapeutic agent for Hodgkin's disease, lymphosarcoma, and leukemia. Ann. Int. Med. *27:* 529, 1947.

[11] —, McLennan, M. T. and Huguley, C. M., Jr.: Clinical experiences with nitrogen mustard therapy, in: Moulton, F. R.: Approaches to Tumor Chemotherapy. Washington, American Association for the Advancement of Science, 1947, p. 347.

[12] Dameshek, W., Weisfuse, L. and Stein, T.: Nitrogen mustard therapy in Hodgkin's disease. Blood *4:* 338, 1949.

[13] Guzman Barron, E. S., Dickman, S., Muntz, J. A. and Singer, T. P.: Studies on the mechanism of action of ionizing radiations. I. Inhibition of enzymes by x-rays. J. Gen. Physiol. *32:* 537, 1948–1949.

[14] Craver, L. F.: Recent advances in treatment of lymphomas, leukemias and allied disorders. Bull. New York Acad. Med. *24:* 3, 1948.

[15] Burchenal, J. H., Myers, W. P. L., Craver, L. F. and Karnofsky, D. A.: The nitrogen mustards in the treatment of leukemia. Cancer *2:* 1, 1949.

[16] Graff, W. S., Scott, K. G. and Lawrence, J. H.: The histologic effects of radiophosphorus on normal and lymphomatous mice. Am. J. Roentgenol. & Rad. Ther. *55:* 44, 1946.

[17] Block, M. and Jacobson, L. O.: Management of the lymphomas. M. Clin. North America Jan. 1951, p. 93.

[18] Jackson, H., Jr. and Parker, F., Jr.: Hodgkin's Disease and Allied Disorders. New York, Oxford University Press, 1947.

1110842

[19] Hoster, H. A., Dratman, M. B., Craver, L. F. and Rounick, H. A.: Hodgkin's disease, 1832–1947. Cancer Res. *8:* 1, 1948.

[20] Slaughter, D. P. and Craver, L. F.: Hodgkin's disease. Five-year survival rate; value of early surgical treatment; notes on four cases of long duration. Am. J. Roentgenol. & Rad. Ther. *47:* 596, 1942.

[21] Symmers, D.: Clinical significance of the deeper anatomic changes in lymphoid disease. Arch. Int. Med. *74:* 163, 1944.

[22] Craver, L. F.: Lymphomas and leukemias. The value of early diagnosis and treatment. J.A.M.A. *136:* 244, 1948.

[23] Peters, M. V.: A study of survivals in Hodgkin's disease treated radiologically. Am. J. Roentgenol. & Rad. Ther. *63:* 299, 1950.

[24] Medinger, F. G. and Craver, L. F.: Total body irradiation. Am. J. Roentgenol. & Rad. Ther. *48:* 651, 1942.

[25] Minot, G. B. and Isaacs, R.: Lymphatic leukemia; age incidence, duration, and benefit derived from irradiation. Boston Med. & Surg. J. *191:* 1, 1924.

[26] Reinhard, E. H., Moore, C. V., Bierbaum, O. S. and Moore, S.: Radioactive phosphorus as a therapeutic agent. A review of the literature and analysis of the results of treatment of 155 patients with various blood dyscrasias, lymphomas, and other malignant neoplastic diseases. J. Lab. & Clin. Med. *31:* 107, 1946.

[27] Lawrence, J. H., Low-Beer, B. V. A. and Carpender, J. W. J.: Chronic lymphatic leukemia, a study of 100 patients treated with radioactive phosphorus. J.A.M.A. *140:* 585, 1949.

[28] Diamond, H. D., Craver, L. F., Woodard, H. Q. and Parks, G. H.: Radioactive phosphorus. I. In the treatment of lymphatic leukemia. Cancer *3:* 779, 1950.

[29] Dargeon, E. E.: Titrated, regularly spaced radioactive phosphorus or spray roentgen therapy of leukemias. Arch. Int. Med. *87:* 329, 1951.

[30] Berlin, N. I. and Lawrence, J. H.: The changes in the bone marrow differential in chronic leukemia treated with P^{32} and Y^{90}. Acta. Med. Scandinav. *140:* 99, 1951.

[31] Iverson, K. and Meulengracht, E.: Oral treatment of polycythaemia vera with β-naphthyl-di-2-chlorethylamine. Brit. M. J. *2:* 510, 1951.

[32] Shimkin, M. B., Mettler, S. R. and Bierman, H. R.: Myelocytic leukemia: An analysis of incidence, distribution and fatality, 1940–1948. Ann. Int. Med. *35:* 194, 1951.

[33] Paterson, E. and Boland, J.: Trisethyleneimino-s-triazine in human malignant disease: Preliminary trial. Brit. J. Cancer. *5:* 28, 1951.

[34] Weisberger, A. S., Heinle, R. W. and Levine, B.: On the chemical structure of compounds capable of preventing leukopoenia induced by nitrogen mustard. J. Clin. Investigation *30:* 681, 1951.

[35] Brandt, E. L. and Griffin, A. C.: Reduction of toxicity of nitrogen mustards by cysteine. Cancer *4:* 1030, 1951.

[36] Matthews, W. B.: A trial of β-naphthyl-di-2-chloro-ethylamine (R18) in leukaemia, Hodgkin's disease, and allied diseases. Lancet *1:* 896, 1950.

1110843

Respiratory and Glycolytic Activities of Human Leukocytes in Vitro

GORDON R. McKINNEY, SAMUEL P. MARTIN, R. WAYNE RUNDLES AND ROSALIE GREEN. *From the Departments of Medicine and of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina*

Reprinted from JOURNAL OF APPLIED PHYSIOLOGY

Vol. 5, No. 7, January, 1953 *Printed in U. S. A.*

1110844

Reprinted from JOURNAL OF APPLIED PHYSIOLOGY
Vol. 5, No. 7, January, 1953
Printed in U.S.A.

Respiratory and Glycolytic Activities of Human Leukocytes in Vitro[1, 2]

GORDON R. McKINNEY,[3] SAMUEL P. MARTIN,[4] R. WAYNE RUNDLES AND ROSALIE GREEN. *From the Departments of Medicine and of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina*

THE METABOLISM OF MATURE LEUKOCYTES and the hemopoietic tissues from which they arise have received comparatively little study. Kempner (1) reviewed the early work on the metabolism of normal and leukemic leukocytes. Most investigators found the glycolytic rate of normal cells to be high in comparison with the oxidative metabolism, and lactic acid to be formed aerobically. Kempner found that lymphoblasts and myeloblasts from patients with leukemia differed in that the oxidative rate was high and the glycolytic rate was low. Valentine (2) has reviewed the more recent biochemical, histochemical and *in vitro* enzymatic studies on leukocytes in man.

A major handicap in the investigation of leukocyte metabolism has been the technical problem of obtaining adequate quantities of viable, uninjured leukocytes for study. The Formed Elements Group of Harvard University (3) has largely solved this problem by developing procedures for the separation of white cells from the other constituents of the blood without exposure to the harmful action of anticoagulants, wettable surfaces or excessive gravitational force. These procedures for obtaining and handling leukocytes were adopted in the present study of the *in vitro* metabolic activities of normal white blood cells. The work reported at this time concerns three metabolic activities of these cells: respiration, glycolysis and the utilization of carbohydrates. The effects of certain sugars, metabolic intermediates and inhibitors on these processes were studied.

METHODS

Approximately 300 ml. of blood were taken from healthy individuals into the Fenwal Blood Pack.[5] Phlebotomy was accomplished by the use of a nonwetting, laminar flow needle, and the blood passed through plastic tubing into a column containing a cation exchange resin (Dowex-50) which removed sufficient calcium to prevent coagulation. From the resin column, the blood passed into a plastic container.

Received for publication August 20, 1952.

[1] A portion of this work was presented at the 43rd annual meeting of the American Society of Biological Chemists, New York City, April 1952.

[2] This investigation was supported in part by research grants from the Atomic Energy Commission, Contract No. AT-(40-1)-1081; the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service; and Baxter Laboratories, Inc.

[3] Fellow of the American Cancer Society, recommended by the Committee on Growth, National Research Council.

[4] Markle Scholar in Medicine.

[5] Fenwal Laboratories, 47 Mellen Street, Framingham, Mass.

1110845

Rapid sedimentation of the erythrocytes was induced by the addition of one part of a 5 per cent dextran[6] solution in physiological saline to five parts of blood. After 30 minutes' sedimentation in the refrigerator, the supernatant plasma containing platelets and leukocytes was expressed into a siliconed (4) 250 ml. centrifuge tube. After centrifugation for 15 minutes at 50 × *g*, the supernatant plasma was decanted. The sediment was resuspended in one part of the residual plasma and five parts of a modified Hanks' solution.[7]

The white blood cell count of these preparations was adjusted to approximately 30,000 cells cu.mm., the great majority of which was segmented neutrophils. The ratio of erythrocytes to leukocytes was approximately 3:1. The number of platelets was negligible. Control experiments showed that the red blood cells did not materially contribute to the respiration and that the glycolytic activity of leukocytes was at least 100 times greater than the erythrocytes. Aliquots of this cell suspension were transferred by siliconed pipettes to siliconed Warburg vessels. To obtain accurately measurable gaseous exchange, it was necessary that a vessel contain at least fifty million cells. Buffer was added to bring the fluid volume to 3.0 ml. The center-well contained 0.2 ml. of 20 per cent KOH and a filter paper roll. Oxygen consumption was measured by Warburg's direct method for a 4-hour period with air as the gas phase. All values are expressed as μ moles per ten million white blood cells per hour.

In order to compare the metabolic activity of intact with broken cells, preparations of intact cells in Hanks' solution and plasma were centrifuged for 20 minutes at 400 × *g*. The cell mass was ground with alumina gel in a mortar and pestle, resuspended in the supernatant fluid and filtered through four layers of cheese cloth. Aliquots of this material were transferred to siliconed vessels and the oxygen consumption determined by Warburg's direct method. The volumes of oxygen are reported as percentages of variations from the control values.

Chemical Analyses. To determine lactate formation, and glucose, fructose and glycogen utilization, standard colorimetric procedures were employed except where modified by one of us (*R. G.*) as described below. At the end of the incubation period, the reaction mixtures were transferred to small tubes containing crystals of sodium fluoride.

Lactate. Protein-free filtrates were prepared by a modification of the Folin-Wu technique (5) and aliquots were taken for analysis according to the method of Barker and Summerson (6).

Glucose. A filtrate was prepared by another modification of the Folin-Wu method (5). Glucose was determined on this filtrate by the method of Somogyi (7).

Fructose. Fructose was determined on a 2.0 ml. aliquot of the glucose filtrate by the method of Roe as described by Umbreit (8).

Glycogen. Glycogen analysis was made by a composite modification of three methods (9-11). Since dextran gives the glycogen reaction the remaining traces of this carbohydrate were removed by dilution of 1.0 ml. of the reaction fluid to 10.0 ml. with 0.9 per cent NaCl in a 15-ml. centrifuge tube. The mixture was centrifuged

[6] The authors appreciate the generous quantities made available by Pharmacia, Ltd., Uppsala, Sweden. This dextran was pyrogen-free, had been fractionated twice and had an intrinsic viscosity of 0.46.

[7] Hanks' solution was prepared by diluting 10.0 ml. of stock solution A and 7.0 ml. of stock solution B to 100 ml. with sterile, nonpyrogenic distilled water supplied by Baxter Laboratories, Inc.; pH of the final working solution was 7.4. One liter of stock A contained 75.0 gm. NaCl, 7.5 gm. KCl, 1.0 gm. Na_2HPO_4, 1.2 gm. KH_2PO_4 and 0.5 gm. K_2HPO_4. One liter of stock B contained 10.0 gm. Na_2HPO_4. Nonpyrogenic solutions of the hexoses were used.

1110846

for 10 minutes at 350 X g and the supernatant fluid decanted. One milliliter of 0.9 per cent NaCl was added to the residue. Thorough mixing was obtained by drawing the material in and out of the pipette until it was homogeneous. From this stage, analysis of glycogen was made by the techinque of Good, Kramer and Somoygi (9).

Results

The oxygen consumption of leukocytes prepared and studied under the conditions described above was linear over a period of 4 to 8 hours. Table 1 shows the effect of the addition of glucose on the oxygen consumption of the cell suspension. The concentration of glucose in the control was less than 0.0011 M (20 mg. %). The addition of glucose to a final concentration of 0.0056 M (100 mg. %) reduced the oxygen consumption significantly, but fructose had no effect. The addition of glucose increased lactate production, i.e. aerobic glycolysis. The increment was proportional to the quantity of glucose added until a level of 0.0044–0.0056 M (80–100 mg. %) was reached, above which the glycolytic rate decreased. However, the addition of fructose did not increase glycolysis to the same degree as did glucose. Glucose and fructose both exhibited a sparing action on glycogenolysis, with the effect of glucose being

Table 1. Effect of glucose and fructose on respiration and carbohydrate metabolism of intact leukocytes

Final concent. of hexose	Q_{O_2}	$Q_{lactate}$	$Q_{glucose}$	$Q_{fructose}$	$Q_{glycogen}$
	μ moles	μ moles	μ moles	μ moles	μ moles[1]
Control (<0.0011 M)	−0.150± 0.007[2]	+0.266± 0.028	−0.021± 0.003		−0.018± 0.002
Glucose (0.0056 M)	−0.092± 0.006	+0.740± 0.107	−0.205± 0.022		−0.006± 0.003
Fructose (0.0056 M)	−0.148± 0.015	+0.469± 0.00	−0.022± 0.005	−0.081± 0.018	−0.015± 0.002

[1] Calculated as micro-molar equivalents of glucose. [2] Standard error of the mean.

greater. The addition of phosphorylated hexoses affected neither oxygen consumption nor lactate production, and analyses revealed no utilization.

The Q_{O_2} in 100 per cent and in 21 per cent oxygen with and without glucose was the same. Similarly, there was no significant difference in lactate production in the presence of 100, 21 and 0 per cent oxygen.

The effect of intermediates and inhibitors of the Krebs' cycle on intact and on broken cells is summarized in table 2. The absolute respiration of the broken cells was about one half that of the intact cells, while the glycolytic rate was one fifth. The only intermediate that produced a significant increase in the oxygen consumption of the intact cell was pyruvate. In the broken cells, however, succinate produced a marked increase in oxygen consumption while precursors of succinate had less pronounced effects. However, fumarate and malate, products of succinate oxidation, diminished the oxygen uptake of broken cell preparation. Cytochrome *c* increased oxidation only in broken cells. The effects of succinate and cytochrome *c* were additive. Of the various inhibitors, only KCN, *p*-chloromercuribenzoate, fluoroacetate and DNP had an effect on intact cells. With the broken cells, however, malonate produced a significant inhibition of respiration and this could be overcome by the addition of an equimolar concentration of succinate.

Acetate, citrate, α-ketoglutarate, succinate and malonate had no effect on aerobic

1110847

glycolysis and glucose utilization in intact cells. Table 3 shows that pyruvate increased lactate production and decreased glucose utilization. Fluoroacetate and KCN increased, while *p*-chloromercuribenzoate decreased both processes. Dinitrophenol increased the formation of lactate.

Discussion

The above findings confirm those of numerous earlier investigators regarding the high glycolytic rate of leukocytes (1). A particularly interesting feature of their metabolism corroborated by this study is the lack of an increase in this rate by incubation in lowered oxygen tension (1, 12, 13), a reaction different from that of other normal tissues. However, in preliminary experiments, leukocytes from patients with myelogenous leukemia have exhibited a low glycolytic rate in air which increased on exposure to a low oxygen tension.

Table 2. Effect of intermediates and inhibitors of the Krebs-cycle on respiration of intact vs. broken leukocytes

	Intact	Broken
Pyruvate	+12	−19
Acetate	−2	−7
Oxalacetate	+5	−14
Citrate	−2	−24
α-Ketoglutarate	+0	+31
Succinate	−3	−108
Fumarate	+4	−11
Malate	−2	−17
Glutamate	−1	+14
Cytochrome *c* (0.00005 M)	−3	−17
Succinate + cytochrome *c*	−4	−126
Malonate	−1	−43
Succinate + malonate	−2	−3
Fluoroacetate	−37	−46
KCN (0.0001 M)	−81	−75
p-Chloromercuribenzoate (0.0001 M)	−85	−83
Dinitrophenol (0.0001 M)	−30	−43

All final concentrations are 0.01 M, unless specified otherwise. Data represent percentage of change from the control in duplicate determinations in at least three experiments for each compound.

Glucose when added in physiologic concentrations increased the high glycolytic rate and reduced the oxygen utilization. Why the glycolytic process prevails rather than the oxidative is not understood. It is interesting, however, that glycolysis proceeds most rapidly when the glucose concentration is within the physiologic range of 0.0044–0.0056 M (80–100 mg. %). On the basis of changes in free energy ($-\Delta L$), the increase in lactate production which follows the addition of glucose may compensate for the drop in oxygen consumption. The total loss in free energy in both systems with and without glucose was essentially the same: 0.035 calories without glucose as opposed to 0.031 calories with glucose (14). The alterations in oxygen consumption and lactate production that occur with the addition of glucose are highly significant ($P < 0.01$). Since there was no observed depression of respiration by fructose, despite its utilization (table 1), it appears that this hexose affects the cells either differently or to a smaller degree than glucose.

1110848

This study has disclosed the existence of Krebs' cycle activity in the human polymorphonuclear leukocyte and indicates that the intracellular oxidation may pass through the cytochrome system (table 2). This activity may not be quantitatively similar to other tissues, but it would appear that there exists in leukocytes a final common pathway for oxidation qualitatively similar to that found in most tissues, even though these cells normally have a high rate of aerobic glycolysis.

The difference in metabolic response between intact and broken cells to intermediates and inhibitors may be related to cell permeability. It should be pointed out that in contrast to surviving tissue slices the response of these leukocytes to certain chemicals parallels the pharmacologic effect in the intact animal. An *in vivo* experiment connotes an intact animal while an *in vitro* system implies an isolated tissue or cellular constituent. Between these extremes lies the isolated but unaltered intact cell surviving in a milieu similar to its *in vivo* environment. Each cell evenly dispersed in its environment may react as a unit in response to chemical and other stimuli as it would in the living host. Considerable potentialities exist for further biochemical study of such preparations of isolated, uninjured cells surviving in their natural state, free from the homeostatic influences of the intact animal.

TABLE 3. EFFECT OF INTERMEDIATES AND INHIBITORS OF THE KREBS-CYCLE ON AEROBIC GLYCOLYSIS AND GLUCOSE UTILIZATION OF INTACT LEUKOCYTES

ADDITION FINAL CONCENTRATION	$Q_{lactate}$	$Q_{glucose}$
	μ moles	*μ moles*
Control	−0.728	−0.348
Pyruvate (0.01 M)	−0.861	−0.311
Fluoroacetate (0.01 M)	+0.865	−0.303
KCN (0.0001 M)	−0.002	−0.444
p-Chloromercuribenzoate (0.0001 M)	−0.020	−0.300
Dinitrophenol (0.0001 M)	−0.703	−0.370

All flasks contained glucose (final concentration, 0.0050 M). Data are means of duplicate determinations in two experiments.

SUMMARY

A method is described for obtaining leukocytes from the blood of human subjects in adequate quantities for *in vitro* metabolic studies without exposing the cells to anticoagulants, excessive gravitational force or wettable surfaces. Three metabolic activities of leukocytes were studied: respiration, glycolysis and the utilization of carbohydrates. The effects of certain sugars, metabolic intermediates and inhibitors on these processes were investigated.

Under the conditions of these experiments, increasing the glucose concentration from low levels to physiologic range increased lactate formation proportionately to the amount of glucose added. In the presence of added glucose, oxygen consumption diminished. Fructose did not increase lactic acid production comparably to glucose and was without effect on respiration. Both fructose and glucose reduced glycogenolysis. Low oxygen tension did not alter the high rate of aerobic glycolysis of mature leukocytes. The effect of metabolic intermediates and inhibitors indicates the existence of Krebs' cycle activity in the human polymorphonuclear leukocyte.

The authors gratefully acknowledge the technical assistance of Mrs. John W. Sullenberger and the helpful suggestions given by Dr. Frederick Bernheim, Duke University, and by Dr. James L.

1110849

Tullis, University Laboratory of Physical Chemistry related to Medicine and Public Health, Harvard University.

REFERENCES

1. KEMPNER, W. *J. Clin. Investigation* 18: 291, 1930.
2. VALENTINE, W. W. *Blood* 6: 843, 1951.
3. WALTER, C. W., E. S. BUCKLEY, JR. AND J. S. GIBSON II. *Separation of the Formed Elements, The Protein, Carbohydrate, Steroid, Peptide and other Components of Plasma.* Cambridge: Harvard University, July 11, 1950. Reprinted December 1951.
4. TULLIS, J. L. AND E. G. ROCHOW. *Blood* 7: 850, 1952.
5. FOLIN, O. AND H. WU. *J. Biol. Chem.* 38: 81, 1919.
6. BARKER, S. B. AND W. H. SUMMERSON. *J. Biol. Chem.* 138: 535, 1941.
7. SOMOGYI, M. *J. Biol. Chem.* 160: 69, 1945.
8. UMBREIT, W. W., R. H. BURRIS AND J. F. STAUFFER. *Manometric Techniques and Tissue Metabolism.* Minneapolis: Burgess 1949, p. 191.
9. GOOD, C. A., H. KRAMER AND M. SOMOGYI. *J. Biol. Chem.* 100: 485, 1933.
10. WAGNER, R. *Arch. Biochem.* 11: 249, 1946.
11. SEIFTER, S., S. DAYTON, B. NOVIC AND E. MUNTWYLER. *Arch. Biochem.* 25: 191, 1950.
12. GLOVER, E. C., G. A. DALAND AND H. L. SCHMITZ. *Arch. Int. Med.* 46: 46, 1930.
13. BIRD, R. M., J. A. CLEMENTS AND L. M. BECKER. *Cancer* 4: 1009, 1951.
14. DICKENS, F. In: *The Enzymes*, edited by J. B. SUMNER AND K. MYRBÄCK. New York: Academic Press, 1951, vol. 2, part 1, p. 629.

1110850

Reprinted from ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 46, No. 1, September, 1953
Printed in U.S.A.

Glyoxalase Activity in Human Leucocytes

Gordon R. McKinney

1110851

Glyoxalase Activity in Human Leucocytes[1]

From studies on the mechanism of aerobic glycolysis and the absence of the Pasteur reaction in human leucocytes *in vitro*, evidence pointed to a source of lactate outside the Meyerhof-Embden scheme. Methylglyoxal was considered as a possible precursor of the lactate. Subsequent experiments revealed that leucocytes contain a high degree of glyoxalase activity. In 1913, Dakin and Dudley (1) discovered glyoxalase in various tissues, including cells of whole blood, and in the same year Levene and Meyer (2) observed it in sterile exudate leucocytes of dogs. Various reports (3–5) would indicate that erythrocytes possess the major

[1] This investigation was supported by a research grant from the U. S. Atomic Energy Commission, Contract No. AT-(40-1)-1081.

1110852

fraction of blood glyoxalase activity. Employing more refined methods for the separation and handling of leucocytes (6), this report indicates that glyoxalase is present in the human leucocyte in an amount per cell unit greater than that of an erythrocyte.

Leucocytes were obtained from the blood of healthy individuals and were processed by a method previously described (6). Erythrocytes and leucocytes were exposed to the same centrifugal force. Concentrated cells were resuspended in a modified Hanks's solution, containing a bicarbonate buffer (cf. footnote 7,

TABLE I

Aerobic Glyoxalase Activity of Human Leucocytes and Erythrocytes in Vitro

Donor	Leucocytes		Erythrocytes	
	Control	Methylglyoxal[a]	Control	Methylglyoxal
1	1.31[b]	13.96	0.022	0.037
2	1.73	14.27	0.043	0.066
3	1.18	11.23	0.019	0.031
4	1.69	16.49	0.042	0.062
Mean	1.48	13.99	0.032	0.049
Standard error	±0.137	±1.078	±0.006	±0.008
Methylglyoxal–control	12.51		0.017	
Ratio of LGA:EGA[c]	735:1			

[a] Final concentration: 0.0056 *M*.

[b] Values calculated on the basis of microliters CO_2 produced by 10^7 cells/hr. during first hour, as described in the text.

[c] LGA = leucocyte glyoxalase activity;
EGA = erythrocyte glyoxalase activity.

Ref. (6): 5.0 ml. of 4.0% $NaHCO_3$ solution replaced 7.0 ml. of stock solution B), saturated with either 95% O_2–5% CO_2 or 95% N_2–5% CO_2 depending upon the desired experimental conditions. In the leucocyte suspension the ratio of erythrocytes to leucocytes was approximately 4:1, whereas the ratio in the erythrocyte suspension was essentially 2500:1. The leucocytes were largely segmented neutrophiles. Aliquots of these cell suspensions were transferred to organosilicon-coated Warburg vessels which were gassed with either of the above gas mixtures. Methylglyoxal, a product of Delta Chemical Works, was tipped in from the side arm; its final concentration was 0.0056 *M*. Cells were incubated for 5 hr. at 37°C. Glyoxalase activity was determined manometrically and was calculated on the basis of microliters of CO_2 produced by 10^7 cells/hr. during the first hour of incubation.

Data in Table I indicate that the glyoxalase activity was greater in the leuco-

1110853

cyte fraction. When the control values without substrate were subtracted from those obtained when methylglyoxal was present, the ratio of glyoxalase activity in leukocytes to that in erythrocytes was 735:1. Values in Table I were obtained by calculating the amount of CO_2 contributed by each cell type in the preparations, using two simultaneous equations, one derived from an erythrocyte to leucocyte ratio of 4:1, and the other of 2500:1. The lower glutathione content of erythrocytes as opposed to leucocytes[2] did not seem to account for the lesser activity of the former cells since exogenous glutathione did not alter the CO_2 production by erythrocytes. However, added glutathione did depress the rate of CO_2 evolution by leucocytes. The rates of CO_2 production did not increase upon incubation in N_2:CO_2.

From these data no statement can be made as to the significance of the enzyme in human leucocytes. If it is important in lactate production, it might explain the absence of the Pasteur effect in mature leucocytes, since glyoxalase activity was independent of oxygen tension. At this point no pathway is known for the formation of methylglyoxal, but it is considered possible that glyoxalase may play a key role in leucocyte metabolism. Experiments are in progress to test this hypothesis.

References

1. Dakin, H. D., and Dudley, H. W., *J. Biol. Chem.* **14**, 423 (1913).
2. Levene, P. A., and Meyer, G. M., *J. Biol. Chem.* **14**, 551 (1913).
3. Jowett, M., and Quastel, J. H., *Biochem. J.* (London) **27**, 486 (1933).
4. Cohen, P. P., and Sober, K. K., *Cancer Research* **5**, 631 (1945).
5. Denstedt, O. F., *in* Tullis, J. L., ed., Blood Cells and Plasma Proteins, p. 244. Academic Press, Inc., New York, 1953.
6. McKinney, G. R., Martin, S. P., Rundles, R. W., and Green, R., *J. Appl. Physiol.* **5**, 335 (1953).

Departments of Medicine and of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina

Gordon R. McKinney[3]

Received May 13, 1953

[2] Green, R., Chaudhuri, S. N., McKinney, G. R., and Martin, S. P., unpublished results.

[3] Fellow of the American Cancer Society, recommended by the Committee on Growth, National Research Council.

1110854

Effects of Glucose and Fructose on Aerobic Glycolysis of Human Leukocytes *in vitro*

by

Gordon R. McKinney, Samuel P. Martin and Rosalie Green

Departments of Medicine and Physiology and of Pharmacology, Duke University School of Medicine, Durham, North Carolina

Leukocytes were separated from the blood of healthy individuals without exposing the cells to wettable surfaces, anticoagulants or excessive gravitational force and suspended in plasma diluted 1:5 with Hanks' solution for metabolic studies.

The aerobic formation of lactate by intact cells was greater from glucose than from an equal concentration of fructose: 100 mg. per cent (final concentration) glucose yielded +0.740 (±0.107 s.e.) μ mols of lactate per ten million leukocytes per hour, whereas 100 mg. per cent fructose gave +0.469 (±0.06 s.e.) μ mols. Below physiologic levels lactate production was proportional to the amount of added glucose but above 80-100 mg. per cent the rate declined. However, lactate formation with fructose increased directly until the concentration reached 300 mg. per cent. When the two sugars were present together and the final concentration of each was 100 mg. per cent, the yield of lactate was equal to the sum of either alone. With broken cells aerobic glycolysis proceeded similarly with glucose or fructose, and the relationship of lactate production to increasing concentrations of either hexose was qualitatively similar to that of intact cells in the presence of glucose.

1110855

These data indicate the existence of two separate, non-competing mechanisms whereby glucose and fructose enter the normal human leukocyte. The greater utilization of glucose by the intact cell may indicate active absorption whereas fructose may diffuse passively into the cell.

111085b

The Effect of Bacterial Products and Hormones on Human Leukocytes

by

Samuel P. Martin, S.N. Chaudhuri, R. Green and Gordon R. McKinney

Department of Medicine, Duke University, School of Medicine, Durham, North Carolina

The metabolic behavior of human leukocytes and the alterations induced by bacterial products and hormones have been studied. The blood was collected by special techniques to prevent injury. Dextran was used for separation of the leukocytes.

These cells have an oxygen utilization less than liver, active, aerobic glycolysis, and are not inhibited by malonate. The latter appears to be a measure of an intact cell. On mechanical injury, the oxygen utilization decreased, glycolysis increased, and malonate inhibition was noted. With the endotoxin from N. intracellularis, P. aeruginosa, S. typhosi and S. marcesens, there was a marked increase in aerobic glycolysis with a decrease in oxygen consumption. This effect was elicited with 0.5-5.0 γ of pneumococcal polysaccharide and 0.05-0.005 γ of the endotoxins. In the experiments where glucose utilization was measured, a rise in sugar consumption occurred. The carbohydrate of type B K. pneumoniae stimulated oxygen uptake. Fifteen γ of cortisone caused a significant decrease in aerobic glycolysis in normal cells.

These data indicate that bacterial endotoxins may induce a reaction of injury in the leukocyte. The marked differences in

1110857

pattern produced by various agents correlate with their ability to incite disease. The increase in aerobic glycolysis and glucose utilization may account for some of the metabolic changes seen in infection. The opposite effect of cortisone may account for the lowered inflammatory response in cortisone-treated individuals.

1110858

Effect of Glucose, Fructose, and Insulin on the Leukocytes of Diabetics

by

Samuel P. Martin, Gordon R. McKinney and R. Green

Department of Medicine, Duke University
School of Medicine, Durham, North Carolina

Since diabetic subjects are prone to infection and the bacteriocidal capacity of their whole blood is low, a study was undertaken to compare the metabolic activities of white cells from diabetic and normal subjects. The blood was collected and handled in a non-wetting system. The red cells were separated by the dextran sedimentation method. The white cells were further concentrated by centrifugation and resuspended in a phosphate buffer for metabolic studies. Cells collected in this manner have good oxygen utilization and produce lactic acid in the presence of oxygen. In the normal leukocyte, this lactate production is increased by the addition of glucose and fructose. Glucose causes a sharp increase in lactate formation as the concentration of hexose is increased, while fructose produces a slow rise. At 100 mg. per cent hexose the rate of lactate production from glucose is 50.53±15 (S.D.) gamma per ten million cells per hour and with fructose is 34±9. In diabetic leukocytes, the situation is reversed, with glucose 38.6±13 gamma per ten million cells per minute and fructose 49.7±7. This data would indicate that leukocytes from diabetic subjects had impaired utilization of glucose and with this had an impaired lactic acid production.

1110859

The addition of insulin (0.1 u) with glucose returned the lactate production toward normal. Since many of the early chemical changes in areas of inflammation as well as bacteriocidal activity may be correlated with lactate production in tissue, this defect in diabetic leukocytes might lead to an alteration in inflammation and susceptibility.

1110860

Duke University
DURHAM
NORTH CAROLINA

School of Medicine
Department of Medicine
Reply to Undersigned

October 1, 1951

Manager,
Oak Ridge Operations Office
Atomic Energy Commission
Office of Research and Medicine
Post Office Box E
Oak Ridge, Tennessee

Dear Sir:

Enclosed you will find our first Progress Report on the Research project sponsored since January 1, 1951, by the Atomic Energy Commission on Metabolism of Human Bone Marrow, a revised summary of the scope and purpose of this project, and a proposed budget for the coming year.

Sincerely yours,

Wayne Rundles

Wayne Rundles, M.D.

WR:nb

Enclosures

CONTRACTS

1110861

October 1, 1951

Dr. Charles L. Dunham,
Chief, Medical Branch
Division of Biology and Medicine
United States Atomic Energy Commission
Washington 25, D. C.

Dear Doctor Dunham:

Enclosed you will find our first Progress Report on the research project sponsored since January 1, 1951, by the Atomic Energy Commission on Metabolism of Human Bone Marrow, a revised summary of the scope and purpose of this project, and a proposed budget for the coming year.

The initial work has proceeded slowly, but on the whole about as well as I expected.

Sincerely yours,

Wayne Bundles, M.D.

M:nb

Enclosures

cc with enclosures sent to:
Oak Ridge Operations Office
Atomic Energy Commission
Office of Research and Medicine
Post Office Box E
Oak Ridge, Tennessee

1110862

Progress Report,

Research Project Sponsored by the Atomic Energy Commission,

Metabolism of Human Bone Marrow, R. W. Rundles, M.D.,

Duke University School of Medicine, October 1, 1951.

The loss of professional assistants from our Laboratory early in 1951, occasioned by the needs of the Armed Forces, delayed the beginning of our study on the metabolism of the human bone marrow. Nevertheless, considerable progress has been made during the last few months in this intrinsically difficult investigation.

Nearly all past studies of tissue metabolism have utilized organs and tissues other than bone marrow. A standard of reference for the metabolic processes of bone marrow was considered necessary. It appeared that a satisfactory comparison could best be made with normal human leucocytes. The technical problem of obtaining adequate quantities of normal, living leucocytes has been overcome. From previous work it was apparent that contact with wettable surfaces, anticoagulants, strong centrifugal force, etc., would have to be avoided. All surfaces coming in contact with the cells should be coated with silicone, or be made of plastic. Coagulation should be prevented by ion exchange resin decalcification. A satisfactory blood donor kit incoporating these essentials has recently become available (Fenwal Laboratory) and has proved eminently suitable for our purposes. To separate red from white cells, the most satisfactory method has proved to be the addition of one part in twenty of 5% Swedish Dextran of specific viscosity 0.46, suspending the plastic container in a closed water filled vessel at 4°C, and allowing the erythrocytes to sediment. After the red cells have sedimented in a matter of 45-60 minutes the supernatent plasma, which contains a large percentage of

1110863

the leucocytes, can be forced into a collecting jar by increasing the water pressure surrounding the plastic bag. By centrifugation at 50-60 G., or less, one can obtain concentrated suspension of leucocytes, 20,000,000-30,000,000 per cc., relatively free of red blood cells. Experiments were performed regarding the effect on respiration of different media in which white cells were suspended. It was found that leucocytes were injured if they were suspended in protein-free electrolyte solutions, or in solutions containing only albumin. Most satisfactory rates of respiration were obtained with leucocytes suspended in plasma diluted with a balanced electrolyte solution. After respiratory measurements have been completed, the leucocyte protein content is determined by nitrogen analysis by the micro-Kjeldahl method. Satisfactory respiratory quotients have been obtained with leucocytes obtained and studied by these procedures.

The handling of bone marrow after aspiration was patterned on the above principles. Several methods for separating bone marrow particles from blood, utilizing differences in specific gravity, were explored with little success. Concentrated albumin solutions and gelatins were mechanically faulty, if not fatally injurious, to surviving cells. While a final method for separating marrow particles for metabolic studies has not been adopted, it appears at this time that placing the marrow in a plasma solution containing Dextran, followed by differential centrifugation will be satisfactory. The use of other red cell agglutinating agents, a phytohemagglutinin, a dextran with a molecular weight of about 440,000 and specific viscosity of 0.64, and a dextrin will be studied.

Warburg equipment has been assembled, calibrated and is in active use. A micro-Kjeldahl nitrogen analysis method is in

1110864

operation. Methods for measuring lactic acid production, aerob and anaerobic glucose utilization are being standardized. Filt paper chromatography methods for amino acid analysis are being up. Our hematology technicians have acquired skill in methods utilized in bone marrow examinations, cell counting, staining, etc. They have not had all of their time fully occupied on this project to date, but have helped maintain access to clinical material. They have, in addition, assisted with a research project begun earlier on the effects of Triethylene melamine in neoplastic disease. A publication dealing with our investigations in this field will be completed during the coming months, in which partial support by the Atomic Energy Commission will be acknowledged.

Personnel.

Gordon R. McKinney, Ph.D. Dr. McKinney finished his [redacted] He has worked full time on this project since July 1. He is supported this year by an American Cancer Society Fellowship.

George Chase, M.D. Dr. Chase finished [redacted] He was able to assist us with the early phases of this work from Jan.-June, 1951, before beginning his internship July 1, 1951.

Ray Shipke, M.D. Dr. Shipke finished his [redacted] Since July, 1951, he has assisted us in obtaining blood from donors, in preparing leucocyte suspensions, processing marrow specimens , and setting up chemical methods. He is now familiarizing himself with paper chromatographic methods which we

1110865

hope to be able to use for studying the utilization and incorporation into cellular protein of amino acids.

Mrs. Ella Hardy Shipke, a graduate medical technologist, has worked full time but is now working part time.

Miss Betty Simpson, a graduate medical technologist, was transferred to this project July 1, 1951, working full time.

Expenditures, (January 1, 1951 to September 15, 1951).

Capital equipment			$1,249.88
Salaries			
Dr. Gordon McKinney	$ 0.00	$ 0.00	
Dr. George Chase	200.00/mo.	1,080.00	
Dr. Ray Shipke	300.00/mo.	750.00	
Miss Betty Simpson	185/mo.	462.50	
Mrs. Ella H. Shipke	185/mo. 6 mos. 130/mo. currently	1,545.00	
			$3,837.50
Expendable Supplies			403.09
Travel			155.98
			$5,705.45
	University Overhead		456.44
	Total Expenditure		$6,161.89

Salaries at the above rate will continue throughout this year. It is estimated that $800-$1,000 will remain unexpended at the end of the year.

Summary Satisfactory progress has been made on this project so far and continued work should result in significant scientific advances.

Wayne Rundles, M.D.

Wayne Rundles, M.D.

11108bb

Summary of Research Project Sponsored by the Atomic Energy Commission,

Metabolism of Human Bone Marrow, R. W. Rundles, M.D.,

Duke University School of Medicine, October 1, 1951.

The bone marrow differs from most organs and tissues in that its main product is blood cells. Comparatively little study has been made of its metabolism. Mose investigators have studied the eosinophilic marrow of rabbits. The purpose of this project is to investigate the basic metabolic processes in human marrow, in normal individuals, in disease states and following exposure to chemical and other injurious agents.

In this study it seemed necessary to establish at the outset some standard of reference for bone marrow metabolic processes. The most satisfactory one appeared to be that of normal human leucocytes. A satisfactory method of obtaining the latter from donors in suitable quantity for metabolic study has been developed. Contact with wettable surfaces, use of anticoagulants, excessive centrifugation, and exposure to protein free solutions or solutions containing only albumin have to be avoided. Metabolic rates are calculated in terms of the total protein content of the cells in the suspension. Similar methods for handling aspirated bone marrow are being perfected.

A study of the rate of O_2 consumption, CO_2 production, glucose utilization, lactic acid production, and other metabolic processes under the conditions specified above is being undertaken.

1110867

October 1, 1951

Proposal for Extension of Research Project Supported by the Atomic Energy Commission.

1. Title. Metabolism of Human Bone Marrow.
2. Institution. Duke University School of Medicine.
3. Investigators. R. Wayne Rundles, Ph.D., M.D., Gordon R. McKinney, Ph.D., and others.
4. Scope and Present Status. Summarized in Progress Report dated October 1, 1951.
5. Outline of work to be undertaken. As before.
6. Material, Equipment, and Facilities. As before.
7. Scientific Personnel. See Progress Report.
8. Budget, proposed for January 1-December 30, 1952, appended.
9. Other responsibilities of investigators, etc. (as previously given).

Proposed Budget to be supplied by the Atomic Energy Commission, Jan. 1-Dec. 30, 1952.

Salaries:	Ray Shipke, M.D., professional assistant	$ 3,800
	Technicians	4,000
Capital equipment:		
	Laboratory benches, Cabinets, Warburg vessels and manometers, blood centrifuge equipment, etc.	1,000
Expendable equipment and supplies:		
	Chemicals, reagents, glassware, etc.	1,000
Communication and travel:		400
		10,000
	Institutional Overhead	800
	Total	10,800

Principal Investigator — Duke Hospital, Durham

Administrative Approval — Business Manager & Comptroller, Duke University

Duke University

1108968

December 30, 1954

Mr. S. W. Scott
Chief, Patent Branch
Oak Ridge Operation
U.S. Atomic Energy Commission
Oak Ridge, Tennessee

Re: Contract AT-(40-1)-1081

Dear Mr. Scott:

Your letter of December 15, 1954, regarding Contract AT-(40-1)-1081 has been received. On March 10 of this year, we completed as part of our final report, a Certificate of Reports, Notebooks and Inventions and relayed it on to Dr. Sam Shoup and others of your AEC offices. Since your copy is apparently the only one that has been misplaced, we are preparing a duplicate which is enclosed.

Since our last communications, however, the final reprints of our scientific publications under this contract have become available. We are enclosing 2 reprints for your office, and others have been mailed as indicated below.

If other questions arise as to this work, I shall be very glad to hear from you.

Very sincerely yours,

Wayne Rundles, M.D.

WR:mc

Enclosure
cc and enclosures: Chief, Medical Branch
Div. of Biology and Medicine
U.S. Atomic Energy Commission
Washington 25, D.C.

Dr. Sam Shoup
Oak Ridge Operations Office
U.S. Atomic Energy Commission
Oak Ridge, Tennessee

JAN 5 1955

D-6619
CONTRACTS 105
Dune

1110869

Reprinted from THE JOURNAL OF CLINICAL INVESTIGATION, Vol. XXXII, No. 11, pp. 1171–1174, November, 1953
Printed in U. S. A.

THE INFLUENCE OF GLUCOSE, FRUCTOSE, AND INSULIN ON THE METABOLISM OF LEUKOCYTES OF HEALTHY AND DIABETIC SUBJECTS[1, 2]

BY SAMUEL P. MARTIN, GORDON R. McKINNEY,[3] R. GREEN, AND CAROLINE BECKER[4]

(From the Departments of Medicine, of Bacteriology, and of Physiology and Pharmacology, Duke University School of Medicine, Durham, N. C.)

(Submitted for publication April 21, 1953; accepted July 8, 1953)

The *in vitro* influence of glucose, fructose, and insulin on human leukocytes of healthy and diabetic subjects was measured as a part of a study of the metabolic behavior of these cells, and an attempt was made to correlate metabolic alterations in leukocytes with resistance and susceptibility to infectious diseases. Previous workers have shown that leukocytes from alloxan diabetic rabbits are able to phagocytize but not destroy bacteria (1). Dubos has shown that lactate in the presence of low oxygen tension is bactericidal for *M. tuberculosis* (2). Human leukocytes produce lactic acid in the presence of oxygen, and the quantity is not changed markedly by decreased oxygen tension (3, 4). It has also been shown that these cells produced more lactate from glucose than from fructose, and that when the two sugars were added simultaneously the lactate production was additive. The effect of these hexoses and insulin was studied on leukocytes from normal and diabetic subjects. The responses after cell injury were also determined.

METHODS

Healthy subjects and subjects with moderately severe untreated diabetes mellitus were used as donors. The obese mild diabetics were excluded from this series. The blood was collected before breakfast. The white cells were prepared for study by a method previously described (4). This consisted of the processing of the cells in a non-wetting system without exposure to excessive gravitational forces. The blood was drawn from the cubital vein into a plastic bag[5] or siliconed syringe. Dowex 50 or heparin (0.02 mg. per ml.) was used as an anticoagulant. Dextran,[6] in a final concentration of one per cent, was added to induce rouleaux formation and rapid sedimentation of the red cells. The white cells in the supernatant plasma were concentrated by centrifugation for 7.5 minutes at 50 × G. The cells were resuspended by gentle agitation in a buffered saline solution.[7] Sufficient buffer was added to obtain a cell count of 30,000 to 50,000 cells per cu. mm. In order to study the broken cells, the resuspended preparations were counted and recentrifuged at 400 × G for twenty minutes. This cell mass was ground in a mortar with alumina gel, filtered through cheese cloth, and again suspended for metabolic studies. Alumina gel was used because this system caused destruction of 90 per cent of the cells. Previous studies showed this material prepared in this manner had Krebs cycle activity (4).

The cells or brei were added to an equal volume of the buffered solution containing the hexose. The final cell counts were from 15,000 to 25,000 per cu. mm. and a total of 45,000 to 75,000 cells per vessel. The reaction mixtures were placed in 25 ml. Erlenmeyer flasks in a constant temperature water bath (37° C.) and shaken at ten oscillations a minute for four hours. Before and after incubation, lactic acid determinations were done by the method of Barker and Summerson (5). The values were expressed as micromoles of lactic acid produced by ten million white cells in an hour. The methods of Somogyi (6) and Roe as described by Umbreit,

[1] This work is supported in part by a grant (G 3476) from the National Institutes of Health, Public Health Service, the Atomic Energy Commission Contract No. AT(40-1)1081 and the Baxter Laboratories, Inc.

[2] A part of this work was presented at the Southern Society for Clinical Investigation and American Federation for Clinical Research, January 29–30, 1953, New Orleans, La.

[3] Fellow of the American Cancer Society, recommended by the Committee on Growth, National Research Council.

[4] Public Health Service Post-doctorate Research Fellow.

[5] Fenwal Blood Pack, Fenwal Laboratories, Framingham, Mass.

[6] Pyrogen free dextran, intrinsic viscosity 0.46, obtained from Pharmacia Ltd., Uppsala, Sweden.

[7] Solution prepared by diluting 10 ml. of Stock A and 7.0 ml. of Stock B in 100 ml. sterile non-pyrogenic water. One liter of Stock A contains 75.0 Gm. NaCl, 7.5 Gm. KCl, 1.0 Gm. Na_2HPO_4, 1.2 Gm. KH_2PO_4, and 0.5 Gm. K_2HPO_4. Stock B contains 10.0 Gm. Na_2HPO_4 in one liter. The hexose solutions and non-pyrogenic distilled water were supplied by Baxter Laboratories, Morton Grove, Illinois.

D-6619

1110870

Burris, and Stauffer (7) were used for glucose and fructose analyses and the values were converted to the micromoles of glucose utilized by ten millon cells in one hour. In the usual experiment, the final concentration of insulin was 0.1 u per ml.[8] In a group of healthy and diabetic subjects, the response of the cells to insulin with 100 mg. per cent of glucose or fructose was measured.

RESULTS

The results of the experiments on intact and broken leukocytes from healthy subjects are graphically outlined in Figure 1. At low hexose concentration, the production of lactate was proportional to the concentration of the sugar. With glucose, there was a sharp increase with a peak at 50 to 80 mg. per cent hexose, depending on the total number of cells. As the concentration was increased above 100 mg. per cent, there was a decrease in lactate production. With fructose, the increase in lactate production was less rapid and remained linear until a concentration of 300 mg. per cent was reached. In the broken cells, the response to the two hexoses was qualitatively and quantitatively similar. When the sugars were added simultaneously, their effects were additive in the intact cell but not in the broken cell.

[8] Insulin obtained from Eli Lilly Co., Indianapolis, Ind.

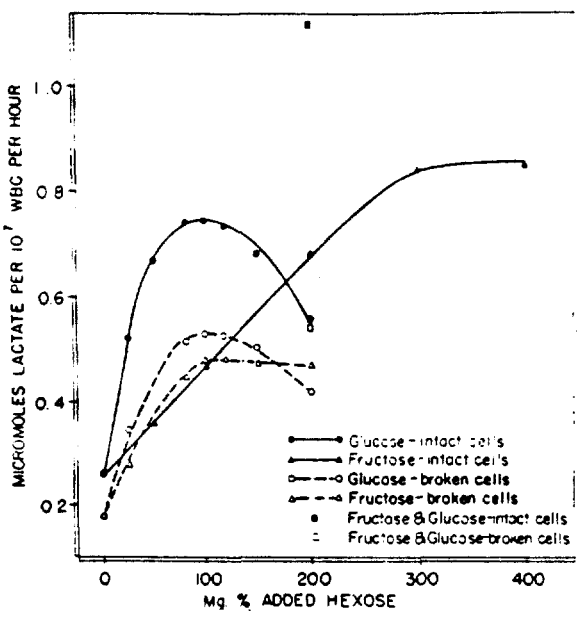

FIG. 1. THE EFFECT OF GLUCOSE AND FRUCTOSE ON THE AEROBIC PRODUCTION OF LACTIC ACID BY LEUKOCYTES FROM HEALTHY SUBJECTS

Curves represent a composite of duplicate determinations on seven subjects.

The utilization of glucose and fructose was measured in the intact leukocyte when the concentration of glucose was 100 mg. per cent, the fructose was 100 mg. per cent, and when the two sugars at this concentration were mixed. When the two sugars were mixed, the production of lactate was a summation of the two alone. The utilization of the hexoses, however, was markedly altered. The utilization of glucose when separate was 0.24 ± 0.09 (SE) micromole per 10 million cells per hour for glucose and when added with fructose was 0.46 ± 0.16. The fructose utilization under similar conditions was 0.16 ± 0.02 micromole per 10 million cells per hour and when glucose was added dropped to 0.04 ± 0.005.

The experiments with intact cells from normal and diabetic subjects with 100 mg. per cent of glucose and fructose, as well as the effect of insulin in the presence of glucose, are presented in Table I. The cells of the diabetic subject have a significantly lower (p 0.01) production of lactic acid with glucose and a significantly higher (p 0.01) production with fructose. This is in contrast to the normal cell. With the addition of insulin, there was a significant increase in the lactate production in the diabetic subject with no response in the control group. Data not included in the table indicated that insulin was effective in concentrations as low as 0.01 u per ml. and did not influence the lactate production in the presence of fructose. Injury or destruction of the cell membrane abolished the insulin response.

The utilization of glucose was significantly lower in the diabetic than in the control group (p 0.02). There was a significant increase (p 0.02) in utilization of glucose in the diabetic group (0.18 ± 0.04 micromole) on the addition of insulin. This brought the value for diabetic leukocytes to the normal range. Insulin did not affect the glucose utilization of the normal leukocyte at 100 mg. per cent level when 0.1 u insulin was used.

DISCUSSION

The data presented would indicate that the lactate production of human leukocytes was proportional to the hexose utilization and may be determined by the ability of the cell to transport the

1110871

TABLE I

The influence of glucose, fructose, and insulin on human leukocytes from normal and diabetic subjects
(The mean and standard error of micromoles of lactate produced or glucose utilized per ten million cells per hour)

Subject	Substrate	Micromoles lactate *10 M WBC/hr.*	Change on addition of insulin	Micromoles glucose *10 M WBC/hr.*
Control 9 subjects	100 mg. % glucose	0.55 ± 0.05	+0.003 ± 0.01 (p 0.10)	0.20 ● 0.02
	100 mg. % fructose	0.38 ± 0.03		
Diabetes mellitus 22 subjects	100 mg. % glucose	0.42 ± 0.03	+0.092 ± 0.03 (p 0.02)	0.12 ± 0.03
	100 mg. % fructose	0.54 ± 0.01		

sugar across the cell membrane. The differences in response of leukocytes in the control group to glucose and fructose could be due to different mechanisms of absorption. This could be through a separate transport mechanism, different affinity for the sugars by the same mechanism, or through variations in cell permeability. Cori and Slein (8) reported a glucokinase and fructokinase in rabbit muscle. Meyerhof and Wilson (9) and Colowick (10) have described different affinities of one hexokinase for glucose and fructose. If this were the mechanism, in the present experiments at 100 mg. per cent, the ratio would be 2 to 1, a figure close to that obtained for rat muscle and brain hexokinase by the above investigators. The work of Cori on absorption of hexoses from the small intestine of rats also showed a similar ratio (11). The increased glucose utilization in the presence of fructose with a drop in fructose utilization cannot be explained. The behavior of the broken cells in these studies closely parallel the studies of Levene and Meyer (12, 13) on exudate leukocytes.

The absence of response to insulin in injured cells and the diminished response to glucose may be related. Previous work with similar preparations of leukocytes had shown that destruction of the cell integrity will also interfere with the action of hydrocortisone, a hormone which diminishes the utilization of glucose and the production of lactate by intact leukocytes (14).

Leukocytes from diabetic subjects produce less lactate in the presence of glucose. The studies of Tolstoi (15) showed no difference in glucose utilization of whole blood, but these were carried out with a different cell population, the red cell being the predominant cell. The erythrocyte and leukocyte might not have the same mechanisms of sugar transport or lactate formation. The diabetic white cell retains the ability to respond to fructose. This is comparable with the *in vivo* effects noted by Root, Stotz, and Carpenter on oral feeding (16) and more recently by Miller and his co-workers after intravenous infusion (17).

The normal leukocyte showed no response to insulin. Eadie, Macleod, and Noble (18) found that there was no response in glycolysis of whole blood of normal animals after the addition of insulin. Macleod (19) reported similar findings in sterile exudate leukocytes of normal animals. The present results indicate that the leukocyte of the diabetic subject responds to insulin by increasing lactate production. There has been a consistent discrepancy between the lactate produced and the glucose utilized. This is greater with the diabetic cells. The source of this lactate may not necessarily be through glycolysis in the complete Meyerhof-Emden System but may be through some other pathway such as that suggested by Cori (20). It may indicate the conversion of 3-phosphoglyceric aldehyde to methyl glyoxal and lactic acid as described *in vitro* by Meyerhof and Lohman (21). Work, currently in progress by McKinney (22), indicates the existence of glyoxylase in leukocytes.

The effect of the metabolic defect noted in the leukocytes from subjects with diabetes mellitus may account for the alterations in susceptibility to infection. Dubos (2) has shown that lactate in the presence of low oxygen tension has a very deleterious effect on the tubercle bacillus. In an area of inflammation, the leukocyte of the diabetic subject might be unable to utilize the available glucose to produce bactericidal quantities of lactic acid.

SUMMARY

Leukocytes from healthy individuals produce more lactic acid from glucose than from fructose,

1110872

and fructose increases the glucose utilization. This difference is abolished by grinding the cells. Leukocytes from diabetic subjects produce more lactic acid from fructose than from glucose. In the healthy subject, insulin had no measured effect on the lactate production of the cell; while in the diabetic, it produced a significant increase in lactate formation with glucose but not with fructose. The utilization of glucose was significantly increased by insulin in the diabetic group but not in the control group. Cell injury blocked the hormone effect.

REFERENCES

1. Cruickshank, A. H., and Payne, T. P. B., Anti-pneumococcal powers of the blood in alloxan diabetes in the rabbit. Bull. Johns Hopkins Hosp., 1949, **84**, 334.
2. Dubos, R. J., Effect of the composition of the gaseous and aqueous environment on the survival of tubercle bacilli in vitro. J. Exper. Med., 1953, **97**, 357.
3. Kempner, W., The nature of leukemic blood cells as determined by their metabolism. J. Clin. Invest., 1939, **18**, 291.
4. McKinney, G. R., Martin, S. P., Rundles, R. W., and Green, R., Respiratory and glycolytic activity of human leukocytes in vitro. J. Appl. Physiol., 1953, **5**, 335.
5. Barker, S. B., and Summerson, W. H., The colorimetric determination of lactic acid in biological material. J. Biol. Chem., 1941, **138**, 535.
6. Somogyi, M., A new reagent for the determination of sugars. J. Biol. Chem., 1945, **160**, 61.
7. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Tissue Metabolism, rev. ed., Minneapolis, Minn., Burgess Publishing Co., 1949, p. 191.
8. Cori, G. T., and Slein, M. W., Gluco- and fructokinase in mammalian tissues. Federation Proc., 1947, **6**, 245.
9. Meyerhof, O., and Wilson, J., Studies on glycolysis of brain preparations. V. Affinity of hexokinase for glucose and fructose. Arch. Biochem., 1948, **19**, 502.
10. Colowick, S. P., Transphosphorylating Enzymes of Fermentation. New York, in the Enzymes, Sumner, J. B., and Myrbäck, K., eds., Academic Press, Inc., 1951, vol. 2, part 1, p. 131.
11. Cori, C. F., The fate of sugars in the animal body. I. The rate of absorption of hexoses and pentoses from the intestinal tract. J. Biol. Chem., 1925, **66**, 691.
12. Levene, P. A., and Meyer, G. M., The action of leukocytes on glucose. J. Biol. Chem., 1912, **11**, 361.
13. Levene, P. A., and Meyer, G. M., On the action of leukocytes on some hexoses and pentoses. Third communication contribution to the mechanism of lactic acid formation from carbohydrates. J. Biol. Chem., 1913, **14**, 149.
14. Martin, S. P., Chaudhuri, S. N., Green, R., and McKinney, G. R., The effect of adrenal steroids on aerobic glycolysis in human leukocytes. In manuscript.
15. Tolstoi, E., Glycolysis in bloods of normal subjects and diabetic patients. J. Biol. Chem., 1924, **60**, 69.
16. Root, H. F., Stotz, E., and Carpenter, T. M., The respiratory quotient and blood pyruvate and lactate responses after oral ingestion of glucose and fructose in diabetes mellitus with and without insulin. Am. J. M. Sc., 1948, n.s., 211, 189.
17. Miller, M., Drucker, W. R., Owens, G. E., Craig, J. W., and Woodward, H., Jr., Metabolism of intravenous fructose and glucose in normal and diabetic subjects. J. Clin. Invest., 1952, **31**, 115.
18. Eadie, G. S., Macleod, J. J. R., and Noble, E. C., Insulin and glucolysis. Am. J. Physiol., 1923, **65**, 462.
19. Macleod, J. J. R., Carbohydrate Metabolism and Insulin, London, Longmans Green and Co. Ltd., 1926.
20. Cori, C. F., Mammalian carbohydrate metabolism. Physiol. Rev., 1931, **11**, 143.
21. Meyerhof, O., and Lohman, K., Über die enzmatische gleichgewichtsreaktion zwischen Hexosediphosphorsäure und Dioxyacetonphosphorsäure. Biochem. Ztschr., 1934, **271**, 89.
22. McKinney, G. R., Unpublished results.

1110873

Reprinted from THE JOURNAL OF CLINICAL INVESTIGATION, Vol. XXXIII, No. 3, pp. 358–360, March, 1954
Printed in U. S. A.

THE EFFECT OF ADRENAL STEROIDS ON AEROBIC LACTIC ACID FORMATION IN HUMAN LEUKOCYTES [1]

BY SAMUEL P. MARTIN,[2] S. N. CHAUDHURI,[3] R. GREEN, AND GORDON R. McKINNEY [4]

(From the Departments of Medicine, Bacteriology, and Pharmacology, Duke University School of Medicine, Durham, N. C.)

(Submitted for publication March 23, 1953; accepted November 12, 1953)

Cortisone has an influence on the course of infectious diseases, as well as on the intensity of the inflammatory response (1–3). One of the important cells in the antibacterial defense and in the inflammatory response is the polymorphonuclear leukocyte. This cell has a very high production of lactic acid in the presence of oxygen and this rate is not altered significantly by the concentration of oxygen (4, 5). The early chemical changes in an area of inflammation such as lowering of pH are in part due to the aerobic lactic acid production by leukocytes.

The present study was undertaken to determine the effect of active and inactive adrenal steroids on this metabolic characteristic of human leukocytes *in vitro*. Compound E and F were found to inhibit aerobic lactic acid production.

MATERIAL AND METHODS

The method for collection and preparation of blood has been previously described (4). Healthy donors in the fasting state were used. The blood was collected from the cubital veins into a non-wetting system. All glassware was siliconed (G.E. Silicone SC 77). Heparin (0.04 mg. per ml.) or a cation exchange resin (Dowex-50) was used to prevent coagulation. The leukocytes were concentrated by the method previously described (4) in which dextran (intrinsic viscosity 0.46) is added to produce a final concentration of 1 per cent. The dextran induced rouleaux formation and rapid sedimentation of red blood cells. After removal of the supernatant plasma containing the leukocytes, the cells were concentrated by centrifugation at 50 x G for 15 minutes. The plasma was decanted and the white cells were resuspended in a sufficient volume of buffered saline (4) with 100 mg. per cent glucose to obtain a concentration of 5,000 to 25,000 cells per cu.mm. Cortisone and hydrocortisone were dissolved in concentrations of 0.01 to 10.0 micrograms per ml. Cholesterol, desoxycorticosterone and Reichstein's compound S [5] in final concentration of 10 to 15 micrograms per ml. were also used. The suspension of leukocytes in buffered saline, with and without the steroids, was distributed into 25 ml. siliconed Erlenmeyer flasks which were suspended in a constant water bath at 37° C. and shaken gently in the horizontal axis at the rate of 10 oscillations per minute. Lactic acid was measured by the technique of Barker and Summerson (6). Measurements of initial lactic acid were made before the cells were placed in the water bath and after four- and six-hour periods of incubation. The values are reported as micromoles of lactate produced by 10,000,000 cells per hour.

In one group of subjects, the *in vitro* effect of cholesterol, cortisone, hydrocortisone, desoxycorticosterone, and Reichstein's compound S on lactic acid production was measured. In another group, the effect of cortisone on the production of lactate and the utilization of glucose, oxygen, and glycogen was measured by techniques previously described (4).

RESULTS

The effect of the various steroids on aerobic production of lactic acid by leukocytes from untreated subjects is presented in Table I. Cortisone and hydrocortisone caused a diminution, while compound S an increase. Cholesterol had no statistically significant effect at four hours. With hydrocortisone, the change was manifest at four hours and was still evident at six hours. With cortisone, the effect at four hours was slight ($P = < 0.05$) and more marked at six hours. Compound S produced a significant increase in lactate at four and six hours, while desoxycorticosterone produced a rise at six hours which was of questionable significance ($P = < 0.05$). This was probably due to the limited number of observations. These experiments were carried out with a concentration of 10 micrograms per ml. of steroid. Experiments on lactate production by

[1] This work was supported in part by a Research Grant from the National Institutes of Health, Public Health Service, the Baxter Laboratory, and the Spanel Foundation.

[2] Markle Scholar of Medicine.

[3] Permanent Address: Indian Institute for Medical Research, Calcutta, India.

[4] Fellow of the American Cancer Society.

[5] Obtained through the courtesy of Dr. Augustus Gibson of Merck and Co., Rahway, New Jersey, and Dr. William Haines, The Upjohn Co., Kalamazoo, Michigan.

1110874

TABLE I

The effect of adrenal steroids on aerobic lactate formation *

	No. of subjects	0–4 hr. change	Change	0–6 hr. change	Change
Control	30	0.59 ± 0.04		0.62 ± 0.05	
Desoxycorticosterone	8	0.64 ± 0.09	+0.05 ± 0.02	0.75 ± 0.05	+0.1 ± 0.02
Cortisone	30	0.53 ± 0.09	−0.06 ± 0.03	0.48 ± 0.04	−0.18 ± 0.02*
Hydrocortisone	16	0.46 ± 0.03	−0.10 ± 0.03†	0.51 ± 0.04	−0.10 ± 0.02*
Compound S	8	0.68 ± 0.05	+0.13 ± 0.05†	0.68 ± 0.04	+0.06 ± 0.02
Cholesterol	8	0.62 ± 0.04	0.02 ± 0.04		

* Values are expressed in micromoles of lactic acid per 10,000,000 cells per hour ± S.E.
† Indicates probability of >0.01.

leukocytes exposed to various concentrations of hydrocortisone are presented in Table II. There was no significant change with 0.1 microgram per ml. This would indicate the minimal effective concentration was between 0.1 and 1.0 microgram per ml. In four trials with broken cells no effect could be noted.

The data on the effect of cortisone on lactate production, and on glucose, glycogen and oxygen utilizations are presented in Table III. There was a diminution of lactate production at four and six hours. The former was of questionable significance and the latter was highly significant. The utilization of glucose was lowered at four and six hours. There was no significant change in oxygen and glycogen utilization. Disruption of the cells by grinding with alumina gel blocked the effect of cortisone and hydrocortisone.

DISCUSSION

These data indicate that some of the adrenal steroids have a direct effect on human leukocytes *in vitro*. The effect of cortisone was diminution in aerobic lactate production and in glucose utilization. The effect was seen only in intact cells which had been handled with care to prevent injury of cell surfaces. The cellular site of action of the steroids in their effect on lactate production is unknown. Preliminary data would indicate that part of the lactate may be produced through an enzymatic mechanism other than lactic dehydrogenase. This interpretation is based on the lack of complete inhibition of lactate production by oxamic acid. Previous work by Hakala, Glaid, and Schwert (7) has shown oxamic acid to be a specific inhibitor of lactic dehydrogenase. With final concentrations as high as 0.02 M there is less than 40 per cent inhibition. Leukocytes show a high glyoxylase activity and this may account for the remainder of the lactic acid. Glutathione in excess (0.005 M) markedly diminishes lactic acid production (8, 9). Since lactic dehydrogenase would seem to account for only a part of the lactate produced by the polymorphonuclear leukocyte, the alteration induced by cortisone could arise from either its effect on pyruvate production or at another site, such as glucose absorption or utilization. Previous work has indicated a close correlation between glucose utilization, glucose concentration, and lactate production by the leukocyte (10). Since the hormones have no effect on broken cells, it would seem that the mode of action is related to the integrative behavior of the cell rather than to the mechanisms of terminal respiration.

The lack of alteration in oxygen utilization despite changes in lactate production cannot be accounted for in the present experiments. Previous studies with human leukocytes have shown

TABLE II

The effect of varying quantities of hydrocortisone on lactate production by human leukocytes in vitro *

Concentration of hydrocortisone *Micrograms/ml.*	5	1	0.1
Change *micromoles/ 10 million cells/hr.*	−0.08 ± 0.03	−0.08 ± 0.02	−.004 ± 0.02

* Studies were carried out at 4 hours. Values are reported as micromoles change over control. Values represent ten subjects in each determination.

1110875

TABLE III

The effect of cortisone on metabolism of human leukocytes expressed as micromoles per 10 million cells per hour

	No. of subjects	Control		Compound E	
		0–4 Hr.	0–6 Hr.	0–4 Hr.	0–6 Hr.
Lactic acid	30	+0.59 ± 0.04	+0.62 ± 0.05	+0.53 ± 0.09	+0.48 ± 0.04*
Glucose	30	−0.205 ± 0.02	−0.106 ± 0.02	−0.150 ± 0.017*	−0.070 ± 0.016*
Oxygen	7	−0.178 ± 0.04	−0.139 ± 0.030	−0.168 ± 0.03	−0.127 ± 0.03
Glycogen	5		−0.007		−0.007

* Indicates probability of >0.01
10 Micrograms of Compound E per ml.

a reciprocal relationship between lactate production and glucose utilization on the addition of glucose or on cell injury. The present experiments may indicate a site of action of the hormone not related to the factors controlling oxygen uptake.

The effective levels in these experiments were found to lie between 1.00 and 0.1 micrograms per ml. Nelson, Sandberg, Palmer, and Tyler (11) found that 200 mg. of compound F given orally produced a blood level of 0.60 micrograms per ml. This level was associated with marked change in polymorphonuclear cell count. This is in the same range as the present experiments.

The effect of compound S is the same as the response noted on injury (4). It may be a pharmacological effect or may be due to some factor present in the material such as pyrogen contamination (12). Selye states that desoxycorticosterone and compound S have a prophlogistic effect (13).

The relation of the antiphlogistic and metabolic effect of cortisone and hydrocortisone is not known. The former has been attributed to the action of these agents on the blood vessels (14). The metabolic effects noted here may also be related to the anti-inflammatory action. This alteration in the leukocyte metabolism would result in a slowing of lactic acid production, less change in pH in the area of inflammation, and possibly longer survival of cells. These factors could be manifested by a slow and abnormal evolution of the inflammatory reaction.

SUMMARY

Cortisone and hydrocortisone have a direct effect on human leukocytes. These hormones cause diminution in production of lactic acid. Oxygen utilization is not altered. It is postulated that these metabolic alterations may play a role in the antiphlogistic effects of adrenal steroids.

REFERENCES

1. Dougherty, T. F., and Schneebeli, G. L., Role of cortisone in regulation of inflammation. Proc. Soc. Exper. Biol. & Med., 1950, 75, 854.
2. Mogabgab, W. J. and Thomas, L., The effects of cortisone on bacterial infection. Group A hemolytic streptococcal infection in rabbits. J. Lab. & Clin. Med., 1952, 39, 271.
3. Michael, M., Jr., and Wharton, C. M., Delay of the early inflammatory response by cortisone. Proc. Soc. Exper. Biol. & Med., 1951, 76, 754.
4. McKinney, G. R., Martin, S. P., Rundles, R. W., and Green, R., The respiratory and glycolytic activity of human leukocytes *in vitro*. J. Appl. Physiol., 1953, 5, 335.
5. Kempner, W., The nature of leukemic blood cells as determined by their metabolism. J. Clin. Invest., 1939, 18, 291.
6. Barker, S. B., and Summerson, W. H., The colorimetric determination of lactic acid in biological material. J. Biol. Chem., 1941, 138, 535.
7. Hakala, M., Glaid, A. J., and Schwert, G. W., Kinetics and specificity of lactic dehydrogenase. Federation Proc., 1953, 12, 213.
8. McKinney, G. R., Glyoxalase activity in human leukocytes. Arch. Biochem., In Press.
9. McKinney, G. R., Martin, S. P., and Green, R., Unpublished observations.
10. Martin, S. P., McKinney, G. R., Green, R., and Becker, C., The influence of glucose, fructose, and insulin on the metabolism of leukocytes of healthy and diabetic subjects. J. Clin. Invest., 1953, 32, 1171.
11. Nelson, D. H., Sandberg, A. A., Palmer, J. G., and Tyler, F. H., Blood levels of 17-hydroxycorticosteroids following the administration of adrenal steroids and their relation to levels of circulating leukocytes. J. Clin. Invest., 1952, 31, 843.
12. Martin, S. P., Chaudhuri, S. N., Green, R., and McKinney, G. R., The effect of bacterial products and hormones on human leukocytes. Clin. Research Proc., 1953, 1, 47.
13. Selye, H., The diseases of adaptation: Introductory remarks. Rec. Prog. Horm. Res., 1953, 8, 117.
14. Ebert, R. H., and Barclay, W. R., Changes in connective tissue reaction induced by cortisone. Ann. Int. Med., 1952, 37, 506.

1110876

OR FORM 368 (JULY 2, 1948)

CONTRACTS AT-40-1-GEN-1081

SUBJECT:

FILE DATE: 1950 Files

TO:

FROM:

SUMMARY:

BROUGHT FORWARD AND FILED WITH— CONTRACTS AT-40-1-GEN-1081

SUBJECT:

FILE DATE: 1951 Files

TO:

FROM:

U. S. ATOMIC ENERGY COMMISSION

CONTINUITY REFERENCE FORM

1110877

September 29, 1952

Dr. Charles L. Dunham
Chief, Medical Branch
Division of Biology and Medicine
United States Atomic Energy Commission
Washington 25, D. C.

Dear Dr. Dunham:

Enclosed you will find our Second Annual Progress Report on the research project Metabolism of the Human Bone Marrow sponsored since January 1, 1951, by the Atomic Energy Commission, and a proposal for extension of the investigation.

We are satisfied with the progress so far and anticipate continuing with the work.

Very sincerely yours,

Wayne Rundles

Wayne Rundles, M. D.

WR:mc

Enclosure

cc: Oak Ridge Operations Office
Atomic Energy Commission
Office of Research and Medicine
Post Office Box E
Oak Ridge, Tennessee

Contract No AT-(40-1)-1081

OCT 8 1952

Plate 1081 c-6052

1110878

PROGRESS REPORT

on

Research Project Sponsored by the Atomic Energy Commission
Metabolism of Human Bone Marrow, R. W. Rundles, M. D.,
Duke University School of Medicine, October 1, 1952

During the past year, our major effort in this project has been expended in perfecting technical procedures required for the study of leukocytes *in vitro*, and in studying certain aspects of the metabolism of normal human leukocytes. The latter has been necessary to provide reference data with which to compare the metabolic activities of immature leukocytes and bone marrow.

Technical Procedures: The method of separating viable leukocytes from normal blood without exposing them to anti-coagulants, wettable surfaces, or excessive gravitational force, as outlined in last year's Progress Report, has proved to be satisfactory. In patients with myelogenous leukemia, an adequate number of leukocytes has been obtained for metabolic studies, in the Warburg apparatus from as little as 50-100 ml. of whole blood. In preparing leukocytes for incubation, the concentrated cells have been resuspended in the donor's plasma, diluted 1/5 with a buffered electrolyte solution containing no divalent ions. It is possible that metabolic effects may be produced by small molecules, unstable proteins, or enzymes in the plasma. This will be studied in coming months by substituting for the plasma in which the leukocytes are suspended a dialyzed solution of albumin and alpha-globulins (SPPS, Cohn).

1110879

The preparation of aspirated human bone marrow tissue for metabolic study involves additional technical problems. The use of Dowex-50 cation exchange resin to prevent coagulation appears to be impractical. The use of the soluble chelating compound, ethylene diamine tetracetate (EDTA) is more promising. Before it can be adopted, however, its effect on the metabolism of leukocytes, if any, will have to be studied.

The separation of immature marrow cells from erythrocytes appears to be feasible if a high density protein solution (SPPS) containing lactose is used. The effect of this medium on the metabolism of leukocytes after they have been exposed to it for varying periods of time awaits study.

Another technical problem involves the determination of the quantity of the marrow tissue being utilized for metabolic measurements. We have found that cell counts are impractical. The possibility of using the nucleic acid content of marrow as a measure of the amount of tissue involved in a given metabolic process is being explored.

<u>Studies on Mature Leukocytes</u>: A number of fundamentally important observations have been made during the last year by Dr. Gordon McKinney and collaborators on the metabolism of mature leukocytes. Three activities in particular have been studied: respiration, glycolysis, and the utilization of carbohydrates. The effects of certain sugars, metabolic intermediates and inhibitors on these processes were also studied.

1110880

Some findings from this study were presented at the Federation meeting in New York City in April, 1952. Reprints of the Federation abstract are enclosed. (McKinney, Gordon R., Rundles, R. Wayne, Martin, Samuel P.: Respiratory activity of human leukocytes *in vitro*. Federation Proceedings. Vol. II, No. 1, March, 1952.) The complete studies have recently been summarized for publication. (McKinney, Gordon R., Martin, Samuel P., Rundles, R. Wayne; Green, Rosalie: Respiratory and glycolytic activities of human leukocytes *in vitro*. Journal of Applied Physiology. To be published.)

The principal conclusions of the latter manuscript are as follows:

(1) The respiration, glycolysis, and the utilization of carbohydrates by mature leukocytes obtained from the blood of humans was studied by methods which avoided exposing the cells to anti-coagulants, excessive gravitational force, or wettable surfaces.

(2) Increasing the glucose concentration from low levels to a physiologic range increased lactate formation proportionately to the amount of glucose added. In the presence of added glucose, oxygen consumption diminished.

(3) Fructose did not increase lactic acid production comparably to glucose and was without effect on respiration. Both fructose and glucose reduced glycogenolysis.

(4) Low oxygen tension did not alter the high rate of aerobic glycolysis of mature leukocytes.

(5) The effect of metabolic inhibitors and intermediates indicated the existance of Krebs cycle activity in the human polymorphonuclear leukocyte.

1110881

During the first year of work on this project, circumstances beyond our control prevented the full utilization of our hematology technician's time on this project. This additional technical assistance enabled us to carry out a study of the effect of triethylene melamine in neoplastic diseases, which has provided an abundance of leads for further study and reference to marrow function. Certain aspects of these studies are being continued at this time. A publication acknowledging the sponsorship of the Atomic Energy Commission in this project has been published. (Rundles, R. Wayne, Barton, W. Bruce: Triethylene melamine in the treatment of neoplastic disease. Blood. Vol. VII, No. 5, 1952.) 493-507

Plans for the Remainder of Coming Year: Technical problems which [illegible] and on which work is being done were outlined above. [illegible] proceeding with studies comparable to those performed on mature human leukocytes utilizing immature granulocytes and lymphocytes obtained from the blood of patients with leukemia, and bone marrow obtained from normal persons and from patients with proliferative [illegible] involving hemopoietic tissues. After these tissues have been studied, we can approach the problem of investigating [illegible] alterations in the cellular metabolism of leukocytes from blood and bone marrow which may be produced by chemotherapeutic agents and by ionizing irradiation.

Wayne Rundles, M.D.
Sept. 19, 1952

1110882

PERSONNEL

Gordon R. McKinney, Ph.D., has worked full time on this project since July 1, 1950. He is supported by an American Cancer Society Fellowship.

Ray Schipke, M. D., continued work on this project as outlined before through February, 1952, when he began the private practice of pediatrics in Hartford, Connecticut.

Tulio Arends, M. D., took over Dr. Schipke's work at first on a part-time basis in January, 1952. He assists us with the clinical supervision of patients whom we use for investigation, with the development of protein solutions in which to study leukocyte metabolism, and with other chemical determinations.

Mrs. Elaine Sullenberger was transferred to this project to work full time, January 1, 1952. She has been our chief technician, performing cell counts, processing blood, doing lactate analyses, etc. She has been helped part time by Miss Bobby J. Callaham and Clinton Talley.

EXPENDITURES: (through August 31, 1952)

Salaries:	Dr. Gordon R. McKinney	$ 0.00
	Dr. Ray Schipke, $100/mo., 6 mos.	600.00
	Dr. Tulio Arends, $100/mo., 6 mos. $275/mo., 2 mos.	600.00 550.00
	Mrs. Elaine Sullenberger, $250/mo.	2,000.00
	Miss B. J. Callaham, $210/mo.	840.00
	Mr. Clinton Talley, $200/mo.	400.00

1110883

Social Security:	$ 17.56
Capital Equipment:	279.86
Expendable Equipment and Supplies:	1,283.31
Communications and Travel:	20.00
University Overhead:	585.97
	$7,176.74

We estimate that the total amount of the grant, $10,800, will be expended by December, 30, 1952.

Summary: The first two years' work on this project has revealed the difficulties involved in this type of work. Progress has been satisfactory and should become more rapid as work continues. Plans have been outlined for another 1-2 years' work in this field.

PUBLICATIONS:

Rundles, R. Wayne, Barton, W. Bruce: Triethylene melamine in the treatment of neoplastic disease. Blood. Vol. VII, 483-507 No. 5, 1952

McKinney, Gordon R., Rundles, R. Wayne, Martin, Samuel P.: Respiratory activity of human leukocytes in vitro. Federations Proceedings. Vol. II, No. 1, p256 March, 1952.

McKinney, Gordon R., Martin, Samuel P., Rundles, R. Wayne, Green, Rosalie: Respiratory and glycolytic activities of human leukocytes in vitro. Journal of Applied Physiology. In press.

Martin, Samuel P., Chaudhuri, S. N., Rosalie, McKinney, Gordon R.: The effect of cortisone on glycolytic rate of human leukocytes in vitro. To be published.

1110884

October 1, 1952

Proposal for Extension of Research Project Supported by the Atomic Energy Commission

1. Title. Metabolism of Human Bone Marrow.

2. Institution. Duke University School of Medicine.

3. Investigators. R. Wayne Rundles, Ph.D., M. D.
Gordon R. McKinney, Ph.D., and others.

4. Scope and Present Status. Summarized in Progress Report dated October 1, 1952.

5. Outline of work to be undertaken. Our plans for the coming months have been outlined in our Progress Report. We plan to extend our studies now to the investigation of the respiration, glycolysis, and the utilization of carbohydrates of immature leukocytes obtained from the blood of patients with leukemia and from the bone marrow.

In addition to the metabolic measurements already being carried out in our laboratory, which pertain to carbohydrate metabolism largely, we shall need to develop methods for studying fatty acid and amino acid utilization. The latter will necessitate the use of isotopes. To attain our goal, a comprehensive study of the metabolism of the human bone marrow in normal people, in disease states, and following chemical and physical injury, will obviously require intensive and continued work for some period of time.

Proposed Budget to be Supplied by the Atomic Energy Commission, January 1-December 30, 1953

Salaries:	Professional (Dr. Tulio Arends)	$3,600.00
	Technical (Mrs. E. Sullenberger)	3,400.00
	Others, part time	1,800

1110885

Capital Equipment:

Manometers, analytic balance, centrifuge accessories	$1,000.00
Expendable Equipment and Supplies:	
Chemicals, reagents, glassware, blood from volunteer donors	1,500.00
Communications and Travel:	200.00
	$11,500.00
Institutional Overhead: 8%	920.00
Total:	$12,420.00

Principal Investigator

R. Wayne Rundles, M.D
Duke Hospital, Durham

Administrative Approval

Duke University

A. D. Brown
Business Manager & Comptroller
Duke University

111088b

In Reply
Refer to: [illegible]

Oak Ridge, Tennessee
February 17, 1954

Dr. Wayne Rundles
Dept. of Medicine
School of Medicine
Duke University
Durham, North Carolina

Subject: FINAL REPORT, CONTRACT NO. AT-(40-1)-1081.

Dear Dr. Rundles:

We acknowledge receipt of seven of the nine reprints comprising the final report under the subject contract, in accordance with your letter of January 21, 1954.

We will be pleased to have reprints of the two final papers when they become available. Your work under the contract is appreciated. We will be glad to hear from you at any time with regard to your progress in further metabolism studies.

Sincerely yours,

[illegible] Shoup
Chief, Biology Branch
Research and Medicine Division

CC: James Haggerty, Wash.

Shoup:ef

CONTRACTS

AT-(40-1)-1081

OFFICE ▶	ORS	ORS				
SURNAME ▶	CEShoup	Roth				
DATE ▶	2/17/54	2/17/54				

Form AEC-318 U. S. GOVERNMENT PRINTING OFFICE 16—62761-2

1110887

Duke University
DURHAM
NORTH CAROLINA

School of Medicine
Department of Medicine
Reply to Undersigned

February 1, 1954

Dr. C. S. Shoup
Chief, Biology Branch
Research and Medicine Division
U.S. Atomic Energy Commission
Oak Ridge, Tennessee

Dear Dr. Shoup:

Enclosed is a list of publications which have resulted from our A.E.C. project, contract No. AT-(40-1)-1081. We are sending additional copies to the Washington office as indicated below. When the last reprints are available, we shall forward them to you and to Washington.

We appreciate very much your interest and help in the undertaking.

Very sincerely yours,

Wayne Rundles

Wayne Rundles, M. D.

WR:mc
Enclosure
cc: Chief, Medical Branch
Division of Biology and Medicine
U.S. Atomic Energy Commission
Washington 25, D. C. with
Enclosed carbon copies for:
Patent Branch, Washington
Technical Library, Washington

OK

1110888

Contract AT-40-1 1081 D-73