HODGKIN'S DISEASE TERMINATING IN ACUTE LYMPHOSARCOMA CELL LEUKEMIA

A Metabolic Study

C. PATRICK BURNS, MD,* RUNE L. STJERNHOLM, PHD,† AND ROBERT W. KELLERMEYER, MD‡

A 40-year-old woman with Hodgkin's disease developed clinical signs of acute leukemia during the fourth year of her illness. She had been treated for Hodgkin's disease with supervoltage radiation therapy and extensive cytotoxic drug therapy. Metabolic studies of her abnormal leukocytes, using ^{14}C-labeled glucose and propionate, confirmed the clinical impression that this was a lymphosarcoma cell leukemia. The development of acute leukemia secondary to radiation and cytotoxic therapy was considered, but the possibility that the lymphosarcoma cell leukemia was a terminal transition of the Hodgkin's disease was also raised. These lymphosarcoma cells also had a more rapid rate of incorporation of thymidine-C^3H_3 into DNA and a higher rate of lipid synthesis from ^{14}C-labeled acetate than those cells recovered from lymphosarcoma cell leukemias studied previously. Leukocytes from this patient and those from another with acute leukemia may exemplify a characteristic metabolic pattern typical of the acute form of lymphosarcoma cell leukemia.

THE ASSOCIATION OF LYMPHOSARCOMA[18] AND reticulum cell sarcoma[33] with leukemia is a well-described clinical phenomenon; however, Hodgkin's disease has rarely been documented as terminating in acute leukemia. In the cases described, it has been difficult to decide from what cell line the blasts were derived, since morphological distinction between the blasts of the major leukocyte lines is uncertain. Most cases reported have been acute myelocytic,[7, 12, 23, 24] myelomonocytic,[16] or monocytic[5, 17] with rare instances of erythroleukemia,[6, 7, 24] histiomonocytic,[9] and Sternberg-Reed cell leukemia.[19] Leukemias of lymphocytic cell origin are even more unusual. Only one substantiated case of acute lymphoblastic leukemia[8] with two additional cases which may fall in that category have been reported.[15, 31] In 4 other cases, the cells of origin were in doubt; they were referred to as stem cell leukemia, or the type was unspecified.[2, 3, 22]

This study summarizes the results of biochemical techniques which differentiate between monocytic, myelocytic, and lymphocytic cells and which were applied to the leukocytes of a patient with Hodgkin's disease who developed acute leukemia. This patient's leukemic cells had the same metabolic behavior as those associated with lymphosarcoma. This association of Hodgkin's disease with acute lymphosarcoma cell leukemia has not been reported previously.

From the Departments of Biochemistry and Medicine, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio.

Supported, in part, by U.S. Public Health Service Grants CA-10706 and No. 5 R01AM-07841-06 from the National Institutes of Health, and Grant T-485 from the American Cancer Society.

* U.S. Public Health Service Trainee in Medicine (Hematology) P01 AM-05420, and recipient of an American Cancer Society Clinical Fellowship (Cuyahoga County Unit, Ohio Division).

† Professor of Biochemistry, Case Western Reserve University.

‡ Associate Professor of Medicine and Pharmacology, and a John and Mary Markle Scholar in Academic Medicine, Case Western Reserve University.

Address for reprints: C. Patrick Burns, MD, 2065 Adelbert Rd., University Hospitals of Cleveland, Cleveland, Ohio 44106.

Received for publication September 17, 1970.

CASE REPORT

J.M., a 34-year-old Caucasian woman, was first admitted to University Hospitals of Cleveland in March 1966, with a non-pitting edema of the left leg of one-month duration. On examination, there were a few palpable small anterior cervical nodes and an adnexal pelvic mass, but no hepatosplenomegaly. The hematocrit was 37%, white blood count (WBC) 8100/mm^3 with 75% segmented neutrophils, 2% bands, 11% monocytes, 8% lym-

phocytes, and 4% eosinophils. Platelets on peripheral smear, urinalysis, uric acid, liver function studies, serum creatinine, chest x-ray, bone survey, and barium enema were normal. Intravenous pyelogram revealed a left hydronephrosis. Lymphangiograms showed enlarged nodes from inguinal to para-aortic area at L_2 level, above which no contrast media passed. During exploratory laparotomy, an external iliac node was excised and histologically revealed Hodgkin's disease (mixed type) with numerous Sternberg-Reed cells (Fig. 1). She was treated initially with 3700 R tumor dose to the abdominal lymph nodes. Subsequently, during the 45 months until her death, she received the following supervoltage therapy: 1700 R to the mandible (July 1966), 1000 R to the left orbit (July 1966), 2700 R to the abdominal lymph nodes (April 1967), 2700 R to the left orbit (April 1967), 2650 R to the left neck (September 1967), 1450 R to the abdominal lymph nodes (August 1969), and 1929 R to each ureter (November 1969). Other therapy included 30 mg of intravenous nitrogen mustard (January 1967), chlorambucil 6–12 mg a day (May-August 1967), 5-fluorouracil 1.5 g intrapleurally (September 1967), vinblastine 10–20 mg weekly (September-November 1967), procarbazine hydrochloride (Natulan, Roche) up to 150 mg daily (April-May 1968), and prednisone 10–30 mg daily (November 1967-November 1969), then 60 mg daily (November 1969 until death one month later). Each form of therapy was continued until no further beneficial response was evident. Complications of her disease included recurrent lymphedema of the legs, pulmonary infiltrates thought to be Hodgkin's disease (January 1967), splenomegaly (August 1967), unilateral exophthalmos (July 1966 and April

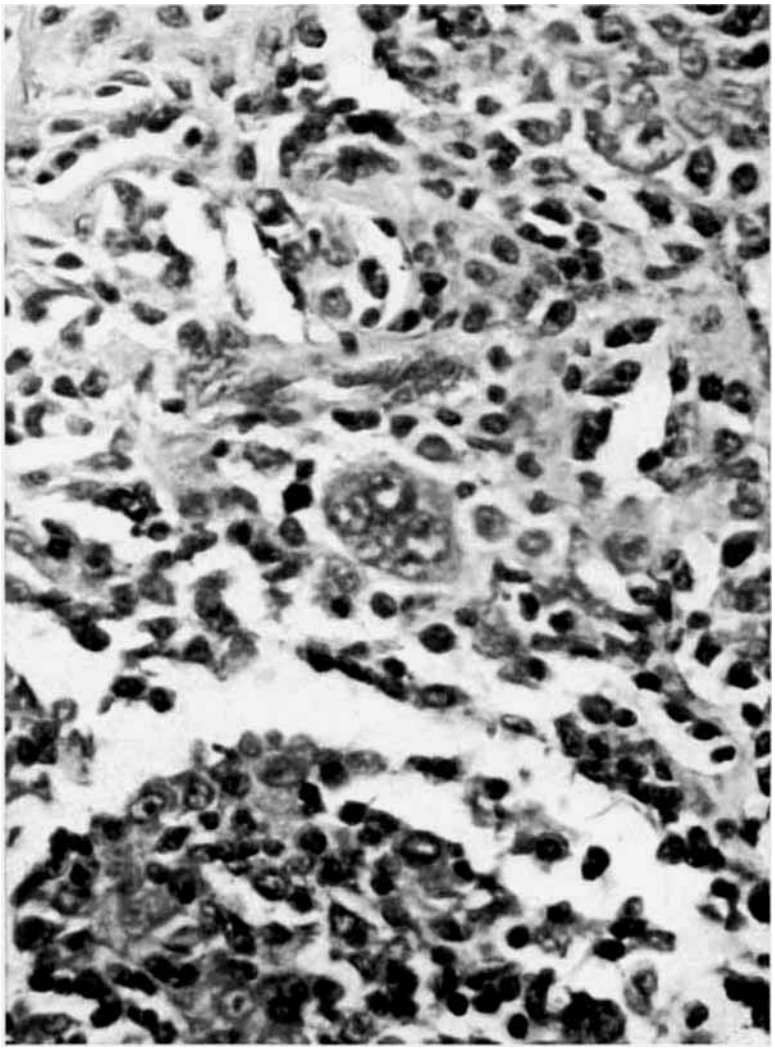

FIG. 1. Lymph node showing Sternberg-Reed cell (H and E, ×430).

1967), pleural effusion containing tumor cells (August 1967), herpes zoster of the trunk (October 1967), pulmonary embolus (November 1967), and anuria due to ureteral compression and invasion (August 1969 and October 1969).

In October 1969, she was admitted with a recurrence of anuria. A left ureteral catheter was inserted after ureterograms showed invasion of the right ureter and extrinsic compression of the left. It was during this admission that the leukemia became evident (Table 1). WBC, which was normal on admission, increased to a peak of 82800/mm^3 on the 30th hospital day (November 24, 1969), at which time 91% of the leukocytes were of an abnormal cell type with sparse cytoplasm, coarsely reticular nuclear chromatin, and notched nucleus. Although a few were blast cells with a single (rarely two) nucleolus rimmed with dense basichromatin, most were thought to be lymphosarcoma cells of intermediate maturity (Fig. 2). Other results included a reticulocyte count of 2.1%, serum creatinine 8.9 mg/100 ml, serum lysozyme 23 μg/ml (spectrophotometric method using cell wall suspension of *Micrococcus lysodeikticus*—normal 5 to 7 μg/ml), uric acid 16.2 mg/100 ml, and a chest x-ray that revealed no infiltrates. A sternal bone marrow aspiration produced only peripheral blood. Subsequent therapy included an increase in the prednisone dose to 60 mg a day and 5 units of packed cells (approximately 200 ml cells per unit). Radiation therapy to the ureteral areas bilaterally and the indwelling left ureteral catheter relieved the azotemia temporarily, but her uremia progressed during the last 2 weeks of life and she died approximately 6 weeks after admission. There was no clinical improvement of the leukemic state with prednisone therapy. No autopsy was performed.

METHODS

On two occasions after the onset of the acute leukemia—10 days and again 1 day before death—15 ml of whole blood were collected in heparinized tubes. The red blood cells were sedimented with 5% dextran. After 40 minutes, the leukocyte-rich supernatant was removed and processed as described previously.[29] Separation of the cells with a column was not necessary. The cells were washed and suspended in 20% serum and Eagle's medium with the final suspension being made up of 95% lymphocytes or lymphosarcoma cells. Cell counts were done with Unopette (Becton, Dickinson and Co.) and a Spencer Hemocytometer.

Radioactive compounds were obtained from New England Nuclear Corporation. Leukocyte incubations with radioactive substrates and isolation of the products by sequential extractions, including ion-exchange and partition chromatography, have been described previously.[26, 30] The cells were incubated with 5 μc of thymidine-C^3H_3 for estimation of DNA turnover, 5 μc of uridine-5-^3H for RNA turnover, 2 μc of mixed tritiated amino acids for protein synthesis, 1 μc glucose-U-^{14}C for glu-

TABLE 1. Hematologic Studies on Patient J.M.

		Oct. 24	Nov. 14	Nov. 19	Nov. 24*	Dec. 2
WBC		6550	7450	36200	82800	42750
Polys		62	34	—	4	0
Bands		24	16	—	2	5
Metamyelocytes		0	3	—	0	1
Myelocytes		0	3	—	1	0
Eosinophils		0	1	—	1	2
Basophils		0	0	—	1	0
Monocytes		7	8	—	0	0
Lymphocytes		7	3	—	0	1
Lymphosarcoma Cells	Mature	0	16	—	61	72
	Intermediate	0	12	—	28	17
	Blasts	0	4	—	2	2
Hematocrit		20	22	20	34†	23
Platelets		—	61000	—	21000	20000

* Differential from Nov. 22.
† After transfusion.

cose utilization and glycogen turnover, 2.5 μc acetate-2-^{14}C for acetate utilization, and 2 μc propionate-3-^{14}C for propionate utilization. The incubation times for glucose, propionate, and acetate were 3 to 5 hours. The phytohemagglutinin (PHA), Difco, for stimulation of RNA turnover was used at a final concentration of 25 μg/ml. All equipment in contact with the intact cells was plastic or silicone-coated glass except the final incubation tubes for DNA, RNA, and amino acids, which were glass. At the time of the first study, the patient was receiving prednisone 15 mg per day; this had been increased to 60 mg per day during the second study.

RESULTS

Glucose utilization by the patient's leukemic cells was 0.52 μmoles per hour per 10^8 cells. The glycogen turnover value was 0.03 μmoles per hour per 10^8 cells. The results of propionate and acetate-labeling experiments are shown in Table 2. Propionate utilization was 17 nmoles per hour per 10^8 cells. Lipid synthesis, reflected by the incorporation of acetate-2-^{14}C, was 8.9 nmoles per hour per 10^8 cells.

The results of nucleic acid and protein-labeling experiments are shown in Table 3. Thymidine incorporation into DNA was 265 picomoles per hour per 10^8 cells. Uridine utilization reflecting RNA synthesis was 11.5 and 0.58 picomoles per hour per 10^8 cells after 4 and 21 hours' incubation, respectively. The uridine incorporation was stimulated only twofold with PHA. RNA turnover and protein synthesis, as determined after a 21-hour incubation period with radioactive precursors, were similar to normal lymphocytes.

Metabolic studies on the abnormal leukocyte population of the patient were repeated one day prior to death, and the results were similar to those given above (Tables 2, 3).

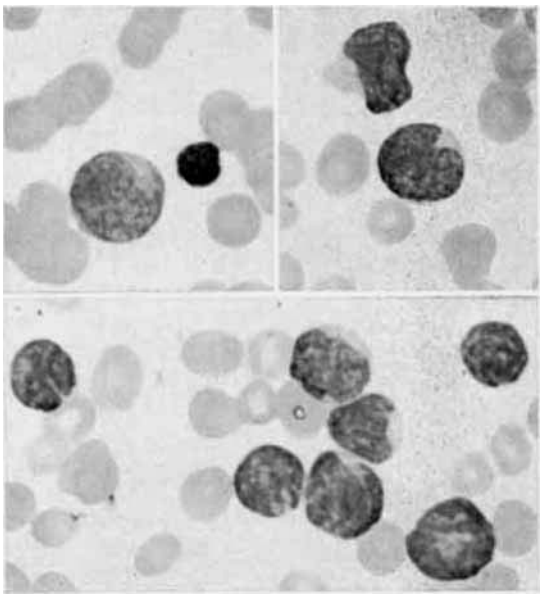

FIG. 2. Peripheral blood smear after development of leukemia. *Upper left*—blast cell with several nucleoli. *Upper right*—lymphosarcoma cell with cleft and nucleolus. Other cell has cytoplasmic tail. *Lower*—clump of lymphosarcoma cells of intermediate maturity (Tetrachrome stain, ×1000).

Results of the metabolic studies of the leukocytes of patient J.M. were similar to those obtained from another patient (J.B.) with acute leukemia (Tables 2, 3). Prior to therapy, J.B. had a leukocyte count of 94,000/mm^3 with 64% of the cells being immature with fine nuclear chromatin and a single large nucleolus. The abnormal leukocytes appeared to be lymphocytic, and some of the more mature-appearing cells had clefts in their nuclei. The abnormal cells in the peripheral blood of this patient were thought to be lymphosarcoma cells. At autopsy, small and large lymphocytes were found invading most of the organs of the body. The morphology of these invasive cells was consistent with lymphosarcoma.

TABLE 2. Results of Propionate and Acetate-labeling Experiments on Cells of J.M. and J.B. Compared to 3 Types of Leukemia

	J.M. 11/22/69	J.M. 12/2/69	J.B.	LSCL*	ALL	CLL
Propionate utilization nmoles/hr/10^8 cells	17	—	29	18–36	2–3	5–6
Lipid synthesis nmoles/hr/10^8 cells	8.9	6.2	12.6	1.2–2.0	0.8–1.7	0.5–0.8

* As previously reported.[27,28]
ALL-acute lymphocytic leukemia; CLL-chronic lymphocytic leukemia.

TABLE 3. Results of Nucleic Acid and Protein-labeling Experiments

	DNA pM/hr/10^8 4 hrs.†	RNA pM/hr/10^8 4 hrs.	RNA pM/hr/10^8 21 hrs.	RNA + PHA pM/hr/10^8 21 hrs.	Amino acids dpm/hr/10^8 21 hrs.
Normal lymphocyte*	8.6±2.9	3.2±2.0	1.6±0.3	10.4±3.6	18200±10000
J.M. 11/22/69	265	11.5	0.58	1.25	20000
J.M. 12/2/69	129	10.0	—	—	—
J.B.	60	—	21	17.8	—

* Mean of 4 normals ±1 S.D.
† Incubation time.
pM = picomoles; dpm = disintegrations per minute.

DISCUSSION

Lymphosarcoma cell leukemia (LSCL) has been identified within the larger group of lymphocytic leukemias.[13, 14, 21] It can be divided into an acute and chronic form. Morphological criteria are used to distinguish these from the corresponding acute and chronic lymphocytic leukemias. Biochemical evidence has substantiated the morphological similarities and differences between LSCL and other lymphocytic leukemias.[27, 28]

The cells from patient J.M. had a low glucose utilization thereby defining their glycolytic rate as that characteristic of a lymphocyte (0.28–0.50 μmoles per hour per 10^8 cells).[27] This low rate which persists even when the lymphocyte is abnormal as in acute lymphocytic leukemia or LSCL is only about 10% of that found in neutrophils, eosinophils, and monocytes.[27] The low glycogen turnover value confirmed the identification of the cell as being of lymphocytic type.[27]

Propionate utilization of the magnitude recorded for the cells of J.M. has been described only for cells from acute monocytic leukemia or LSCL.[28] This result contrasts with the lower values typical of acute and chronic lymphocytic leukemia (Table 2).[28] Together, the rates of glucose and propionate utilization identify the cells studied from J.M. as lymphosarcoma cells.

Serum lysozyme values have been found useful in identifying various forms of leukemia. The lysozyme levels are usually normal or low in the lymphocytic cell leukemias including LSCL.[27] Values greater than 50 μg/ml are characteristic of the monocytic leukemias.[27] Azotemia is a well-documented cause of elevation of lysozyme,[10] and this is the likely cause of the moderate elevation in J.M.

The high thymidine utilization is similar to the values found in acute leukemias.[25] Such high utilization suggests that the cell is undergoing mitosis. This high value supports the presumption that the abnormal cells are immature. The lack of proportional elevation in uridine utilization and protein synthesis has been noted in acute leukemia, and this perhaps reflects the fact that transcription and translation are secondary events during mitosis. PHA added to the incubation mixtures stimulated RNA synthesis by a factor of 2 compared with the five to ten fold increase observed with normal lymphocytes (Table 3). Insensitivity to PHA has been reported to occur in lymphocytes of patients with Hodgkin's disease; the degree of insensitivity seemed to correlate directly with the clinical activity of the disease.[11] Impairment of stimulation is a characteristic of the lymphocytes of chronic lymphocytic leukemia.[20] Lymphocytes obtained during acute viral infection also fail to respond normally to PHA.[32]

Lipid synthesis by the cells from J.M. was higher than that previously reported for LSCL.[27] The fact that J.M.'s cells were dividing more rapidly may account for this increase, since more lipid for cell membrane synthesis would be required. The cells from the patients with LSCL reported earlier had a normal or only slightly elevated DNA turnover.[27]

The other patient with LSCL (J.B.), like J.M., had a markedly elevated DNA turnover and lipid synthesis (Tables 2, 3). From the results obtained with the cells from these two patients, a metabolic pattern may be emerging that is characteristic of LSCL. The high turnover of DNA may correlate with an acute clinical phase of the disease.

The relationship of the terminal leukemia

to Hodgkin's disease in this patient is speculative. When one disease develops in the setting of another, it is difficult to rule out coincidence. However, the appearance of the leukemia at the time of inexorable progression of the Hodgkin's disease suggests a closer relationship. The similarity of Hodgkin's disease and lymphosarcoma before bone marrow involvement occurs and the rarity of reported cases of lymphoproliferative disorders after radiation exposure[1, 4] strengthen the possibility.

Although the relationship of acute leukemia to Hodgkin's disease is as yet uncertain, this study reports an association of the 2 diseases and documents the diagnosis of LSCL with both morphological and metabolic data.

REFERENCES

1. Brill, A. B., Tomonaga, M., and Heyssel, R. M.: Leukemia in man following exposure to ionizing radiation. A summary of the findings in Hiroshima and Nagasaki, and a comparison with other human experience. *Ann. Intern. Med.* 56:590–609, 1962.

2. Cavallero, G., and Bo, G.: Associazione di leucosi acuta indifferenziata o di granulomatosi maligna di Hodgkin in un bambino. *Pathologica* 58:111–120, 1966.

3. Cohen, H., Rubin, S., and Eisemann, G.: Hodgkin's disease (familial) associated with multiple malignant neoplasms. *Cancer* 11:1247–1254, 1958.

4. Court-Brown, W. M., and Doll, R.: Leukaemia and aplastic anemia in patients irradiated for ankylosing spondylitis. Medical Research Council, Special Report Series 295, London, Her Majesty's Stationery Office, 1957.

5. Craver, L. F.: Clinical manifestations and treatment of leukemia. *Amer. J. Cancer* 26:124–136, 1936.

6. Durant, J. R., and Tassoni, E. M.: Coexistent DiGuglielmo's leukemia and Hodgkin's disease. A case report with cytogenetic studies. *Amer. J. Med. Sci.* 254: 824–830, 1967.

7. Ezdinli, E. Z., Sokal, J. E., Aungst, C. W., Kim, U., and Sandberg, A. A.: Myeloid leukemia in Hodgkin's disease: chromosomal abnormalities. *Ann. Intern. Med.* 71:1097–1104, 1969.

8. Gill, A. W., and McCall, A. J.: Lymphoadenoma and leukaemia. *Brit. Med. J.* 1:284–286, 1943.

9. Greenberg, L. H., and Cohen, M.: Histiomonocytic leukemia occurring in patient with Hodgkin's disease. *New York J. Med.* 62:3817–3821, 1962.

10. Hayslett, J. P., Perillie, P. E., and Finch, S. C.: Urinary muramidase and renal disease. Correlation with renal histology and implication for the mechanism of enzymuria. *New Eng. J. Med.* 279:506–512, 1968.

11. Hersh, E. M., and Oppenheimer, J. J.: Impaired in vitro lymphocyte transformation in Hodgkin's disease. *New Eng. J. Med.* 273:1006–1012, 1965.

12. Hollard, D., Morel, P., and Revol., L.: Maladies de Hodgkin terminees en leucose aigue. *Lyon Med.* 215:1373–1377, 1966.

13. Isaacs, R.: Lymphosarcoma cell leukemia. *Ann. Intern. Med.* 11:657–662, 1937.

14. Kundrat: Ueber lympho-sarkomatosis. *Wien. Klin. Wschr.* 6:211–213, 234–239, 1893.

15. Lacher, M. J., and Sussman, L. N.: Leukemia and Hodgkin's disease. *Ann. Intern. Med.* 59:369–378, 1963.

16. Newman, D. R., Maldonado, J. E., Harrison, E. G., Kiely, J. M., and Linman, J. W.: Myelomonocytic leukemia in Hodgkin's disease. *Cancer* 25:128–134, 1970.

17. Razis, D. V., Diamond, H. D., and Craver, L. F.: Hodgkin's disease associated with other malignant tumors and certain non-neoplastic diseases. *Amer. J. Med. Sci.* 238:327–335, 1959.

18. Rosenberg, S. A., Diamond, H. D., Jaslowitz, B., and Craver, L. F.: Lymphosarcoma: a review of 1269 cases. *Medicine* 40:31–84, 1961.

19. Scheerer, P. P., Pierre, R. V., Schwartz, D. L., and Linman, J. W.: Reed-Sternberg-cell leukemia and lactic acidosis: unusual manifestations of Hodgkin's disease. *New Eng. J. Med.* 270:274–278, 1964.

20. Schrek, R., and Rabinowitz, Y.: Effects of phytohemagglutinin on rat and normal and leukemic human blood cells. *Proc. Soc. Exp. Biol. Med.* 113:191–194, 1963.

21. Schwartz, D. L., Pierre, R. V., Scheerer, P. P., Reed, E. C., and Linman, J. W.: Lymphosarcoma cell leukemia. *Amer. J. Med.* 38:778–786, 1965.

22. Shapiro, C. M., Tatar, A., and Rabiner, S. F.: Leukosarcoma in Hodgkin's disease. *Chicago Med. Sch. Quart.* 25:245–249, 1966.

23. Skworzoff, M. A.: Lymphogranulomatose mit akuter myeloider leukämie. *Frankfurt. Z. Path.* 40:81–96, 1930.

24. Steinberg, M. H., Geary, C. G., and Crosby, W. H.: Acute granulocytic leukemia complicating Hodgkin's disease. *Arch. Intern. Med.* 125:496–498, 1970.

25. Stjernholm, R. L.: Unpublished observations.

26. ———: Carbohydrate metabolism in leukocytes. VII. Metabolism of glucose, acetate, and propionate by human plasma cells. *J. Bact.* 93:1657–1661, 1967.

27. Stjernholm, R. L., Dimitrov, N. V., and Zito, S.: Carbohydrate metabolism in leukocytes. XIII. Differentiation by metabolism of leukemic leukocytes into three groups. *J. Reticuloendothel. Soc.* 7:539–550, 1970.

28. Stjernholm, R. L., Noble, E. P., Dimitrov, N. V., Kellermeyer, R. W., and Falor, W. H.: Differentiation of normal and leukemic leukocytes into three groups by propionate metabolism. *J. Reticuloendothel. Soc.* 8:446–457, 1970.

29. Stjernholm, R. L., Noble, E. P., Dimitrov, N. V., Morton, D. J., and Falor, W. H.: Carbohydrate metabolism in leukocytes. XII. Metabolism of the human lymphocyte. *J. Reticuloendothel. Soc.* 6:590–605, 1969.

30. Stjernholm, R. L., Wheelock, E. F., and van den Noort, S.: A lymphotoxic factor in the sera from patients with multiple sclerosis. *J. Reticuloendothel. Soc.* 8:334–341, 1970.

31. Watson, C. J.: Handbook of Hematology, vol. 4, H. Downey, Ed. New York, Paul B. Hoeber Inc., 1938.

32. Willems, F. T. C., Melnich, J. L., and Rawls, W. E.: Viral inhibition of the phytohemagglutinin response of human lymphocytes and application to viral hepatitis. *Proc. Soc. Exp. Biol. Med.* 130:652–661, 1969.

33. Zeffren, J. L., and Ultmann, J. E.: Reticulum cell sarcoma terminating in acute leukemia. *Blood* 15:277–284, 1960.