of infrared spectrophotometry in immunochemistry: (1) It affords a single, rapid physical test, requiring a small sample of the purified material, which permits the identification of the type-specific polysaccharides. (2) By comparison with standard samples, it may be used as a criterion of purity. (3) Comparison of the spectra of different types provides information on chemical structure and relationships.

A more complete report on this work will be published elsewhere.

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The Occurrence of Lymphocytes with Bilobed Nuclei in Cyclotron Personnel[1]

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A preliminary report of observations on the blood of cyclotron workers at the 130″ cyclotron at the University of Rochester called attention to an increased incidence of lymphocytes with bilobed nuclei in cyclotron personnel during the early period of operation of the cyclotron (*1*). It has recently been possible to review the data representing the period extending from 1948, when the initial crew was being formally assembled, through 1951. This review is the subject of the present report. Interest in this subject has been sustained by experimental confirmation of the relationship between an increased number of lymphocytes with bilobed nuclei and exposure to very small amounts of radiation from the cyclotron (*2*).

Hematological monitoring was instituted as a part of the health program for cyclotron personnel in July 1948. The institution of this program prior to the completion of the cyclotron presented a distinct advantage in evaluating the significance of subsequent observations since it permits quantitative comparison of data for the same individuals collected during the control and working periods, as well as comparison of control data for various groups of workers. Throughout the period of cyclotron operation all new employees have had duplicate or triplicate blood studies within 7–10 days after hire, and this has resulted in an ever-expanding body of control data. In most instances, RBC and WBC counts have been done in duplicate, and differential leukocyte counts are based upon examination of 200 leukocytes on a Wright's stained smear. Hemoglobin determinations and platelet counts were done by standard procedures. Throughout the program, particular emphasis has been placed on the cytology of peripheral blood. The basic procedure has been extensive study of good coverslip blood smears of capillary blood. Peroxidase staining was introduced as a routine procedure after the first few cells which appeared to be binucleate lymphocytes were noted. Peroxidase-stained smears are examined from edge to edge, the leukocytes in all good areas counted, and the number and position of all lymphocytes with bilobed nuclei noted. Each cell is subsequently "edited" by the entire group concerned with this study in order to verify the classification. Currently three pairs of peroxidase-stained smears (approximately 8000 leukocytes) are examined for each new employee.

In addition to hematological studies, a complete medical history, as well as the results of physical examinations, is recorded in considerable detail for each individual at the time of hire. A cordial relationship between the cyclotron personnel and the Health Service group facilitated evaluation of the general health of the various individuals during the period covered in this report.

The first evidence of an increased incidence of the abnormal lymphocytes in cyclotron workers was provided by two incidents which occurred during the early days of cyclotron operation. In the first, four physicists were in the cyclotron building when the man at the control panel made certain adjustments that resulted in the possibility that the physicists might have had some slight exposure to the beam.[3] Blood studies were done daily on these four men during the following two weeks and, for the first time in our experience, the presence of several lymphocytes with bilobed nuclei was noted.

Shortly after this, construction of an earth and concrete dike between the cyclotron building and the cyclotron laboratory was begun. This was nearing completion in March 1949, when the second incident occurred. While the cyclotron was running, two machinists started out the back door of the shop in the laboratory building toward the building housing the cyclotron. The men had reached the far end of the dike about 50 feet from the cyclotron building before they realized their mistake and hastily retraced their steps. It was estimated that they were outside the shop only a few minutes.

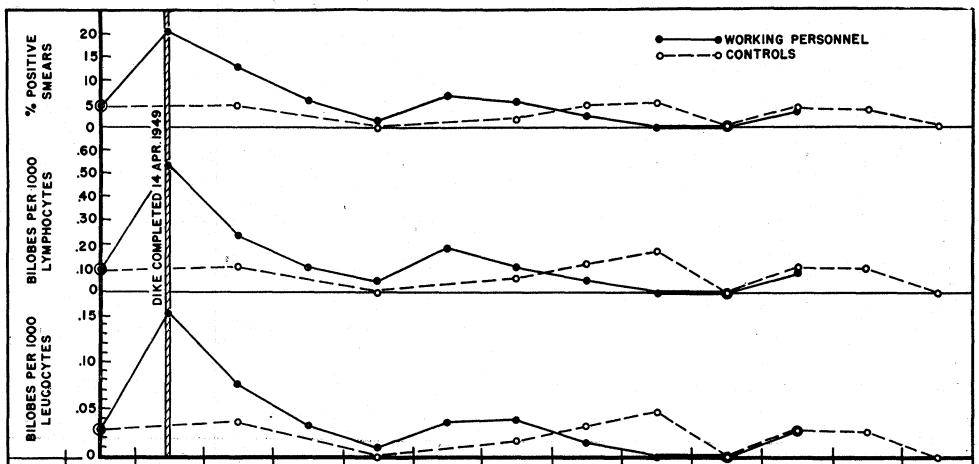
The two men developed slight, transient leukopenia during the week following exposure, and showed a definite increase in lymphocytes with bilobed nuclei for about three weeks after exposure. Seven control

[1] This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.

[2] The authors wish to express their appreciation for the unusually generous cooperation of S. W. Barnes, and the entire cyclotron crew in carrying out the studies on which this report is based.

[3] Both incidents occurred prior to the installation of the various mechanical safety devices which have long since made it virtually impossible for anyone to be in the cyclotron building or the fenced-in cyclotron area while the cyclotron is running.

smears (approx 12,000 leukocytes) from the two men before the incident contained no lymphocytes with bilobed nuclei. By contrast, 11 of 40 smears (28%) pulled during the first 3 post-exposure weeks were positive for binucleate lymphocytes, the incidence in terms of leukocytes being 18/48,000, or roughly 0.4 lymphocytes with bilobed nuclei/1000 leukocytes, or 1.1/1000 lymphocytes (*cf.* Fig. 1). At the time of the incident the men were not wearing badges, a circumstance typical of situations in which biological monitoring is highly desirable. Largely as a result of these two incidents it was decided to review complete blood smears of the entire group, with the idea of obtaining additional information relative to the possible significance of lymphocytes with bilobed nuclei as indicators of radiation exposure.



INTERVAL		*CONTROL	JAN. '49 THRU APR. 14 '49	APR. 15 '49 THRU JULY '49	AUG. 1 '49 THRU OCT. '49	NOV. '49 THRU JAN. '50	FEB. '50 THRU APR. '50	MAY '50 THRU JULY '50	AUG. '50 THRU OCT. '50	NOV. '50 THRU JAN. '51	FEB. '51 THRU APR. '51	MAY '51 THRU JULY '51	AUG. '51 THRU OCT. '51	NOV. '51 THRU JAN. '52
INDIVIDUALS	W	44	44	39	34	18	11	34	26	18	3	34	—	—
	N	44	—	24	—	16	—	12	15	9	11	11	12	9
SMEARS EXAMINED	W	82	160	151	141	49	28	92	62	23	3	50	—	—
	N	82	—	161	—	58	—	42	55	35	28	22	23	23
WBC EXAMINED (thousands)	W	173	857	728	500	203	110	356	249	31	6	72	—	—
	N	173	—	89	—	91	—	58	98	46	38	36	38	34

W - WORKING PERSONNEL N - NEW HIRE (CONTROLS) *W & N IDENTICAL

FIG. 1. Incidence of lymphocytes with bilobed nuclei in cyclotron personnel.

The results of the completed survey are shown in Fig. 1. The incidence of binucleate lymphocytes is presented as (1) the incidence of positive smears (smears which contains one or more of these cells), (2) the number of binucleate lymphocytes/1000 leukocytes examined, and (3) the number of binucleate lymphocytes/1000 lymphocytes examined. Since it was considerably more expedient to keep track of the number of leukocytes rather than the differential count on an entire peroxidase-stained smear, the number of binucleate lymphocytes/1000 lymphocytes is based on an approximation of the number of lymphocytes examined, arrived at by multiplying the number of leukocytes examined during the interval under consideration by the average percentage of lymphocytes found on routine differential counts during that interval. The control data represent the incidence of the unusual lymphocytes in the initial studies of new individuals.

It will be noted that a definite increase of lymphocytes with bilobed nuclei occurred in the group as a whole during the early periods of cyclotron operation. The maximum does not include the incidence in any of the personnel involved in the events mentioned above. Findings in these persons were excluded from the data on the group as a whole for a period of several months after each incident. Since the completion of the protective dike, the number of smears containing one or more lymphocytes with bilobed nuclei has returned to a level about equal to that for the control group. The average leukocyte counts and absolute granulocyte and absolute lymphocyte counts for the group remained relatively stable throughout the period under consideration.

Unusually high numbers of cells with double nuclei have been observed in several tissues following exposure to relatively small doses of ionizing radiation (*3, 4*) and it is not particularly surprising that lymphoid tissue, which is known to be highly radiosensitive, should show a similar response. The findings presented here are directed particularly to the attention of persons concerned with the prevention of radiation

injuries, because (1) the increased occurrence of binucleate lymphocytes appears to be related to exposure to exceptionally small amounts of radiation, and (2) lymphocytes may readily be obtained for examination. Hence examination for the presence of these cells serves as a practicable and sensitive adjunct to physical monitoring when circumstances indicate the desirability of biological monitoring.

Observation of an occasional lymphocyte of this type in irradiated animals and man has been reported by other investigators (*5, 6*). Quantitative estimates of pre- and post-exposure incidence, however, had not been made in these instances; hence it has not been possible to evaluate the significance of radiation exposure as regards the appearance of the cells. Unpublished reports suggest, however, that the cells are observed not uncommonly in radiation workers in other institutions—specifically in a "hazard group" at Los Alamos (*7*) and among luminizers in England (*8*). In both cases, exposure has apparently been kept below the current tolerance level.

It is likely that the increased incidence of lymphocytes with bilobed nuclei in the blood of cyclotron personnel during the early days of cyclotron operation actually indicates exposure to very small amounts of radiation, although physical monitoring at that time did not identify the nature and extent of exposure, and in fact has consistently indicated that exposure is well below tolerance. The supposition that the increased incidence of binucleate lymphocytes represents an effect of irradiation is supported by previously published experimental results (*2*) in which dogs, after a long control period, were exposed three times, 30 min each time, in positions approximating those reached by the two machinists described above. After each exposure there was a marked increase of lymphocytes with bilobed nuclei, followed by a gradual return to approximately normal levels during the period of approximately 2 months between exposures.

Lymphocytes with bilobed nuclei may appear in increased numbers in conditions other than exposure to ionizing radiation, as, for example, in infectious processes and in leukemia. The occurrence of an occasional cell of this type in the controls may reflect the normal incidence of low-grade infections in the group as a whole. It should be mentioned that the general health of the group remained good throughout the period under consideration. In particular, there was no indication of an unusual number of infections to explain the observed increased incidence of lymphocytes with bilobed nuclei during the first few months of cyclotron operation.

Since the occurrence of lymphocytes with bilobed nuclei remained low after completion of the protective dike, the detailed examination of peroxidase-stained smears is currently carried out only for new employees or when there is some definite change in operating procedure (e.g., reversal of the beam) or some question of personnel exposure. In such instances, the growing group of control observations and the individual's own control (new-hire) data are of great value.

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A Quantitative Study of Folic Acid Requirements and Reversal of Aminopterin Inhibition in *Drosophila*[1,2]

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Studies reported by Goldsmith *et al.* (*1*) regarding the response of *Drosophila* to folic acid antagonists made use of a medium for growing the larvae which contained live yeast. This precludes the collection of exact quantitative data. The present report is concerned with results obtained (Table 1) with Drosophila reared on a chemically defined medium (*2*) and under aseptic conditions. With these last-named parameters controlled, the effect on growth of very precise amounts of a test substance can be determined.

Using the Oregon-R wild type strain of *D. melano-*

TABLE 1

Base medium (mg/ml)			(gamma/ml)
L-alanine	1.085	Biotin	0.020
L-arginine	0.794	B_{12}	0.04
L-aspartic acid	1.221	Ca-pantothenate	6.0
L-cystine	0.480	Choline chloride	20.0
L-glutamic acid	4.418	Pyridoxine	3.0
Glycine	2.328	Riboflavin	2.4
L-histidine	0.484	Thiamine	1.5
L-hydroxyproline	0.384	Niacinamide	10.0
L-isoleucine	1.260		
L-leucine	2.345		(mg/ml)
L-lysine	1.337	$MnSO_4 \cdot 4H_2O$	0.246
L-methionine	0.339	$MgSO_4 \cdot 7H_2O$	0.0129
L-phenylalanine	1.008	$FeSO_4$	0.0129
L-proline	1.682	KH_2PO_4	0.606
DL-threonine	1.512	K_2HPO_4	0.606
L-tryptophane	1.745	Thymine	0.004
L-tyrosine	1.240	NaCl	0.0129
L-valine	1.355	$CaCl_2$	0.0129
Sucrose	7.5	Agar	15.0
Cholesterol	0.1	Ribonucleic acid	1.0
Ergosterol	1.0	Inosine	0.25

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[2] G. W. Kidder, of Amherst College, kindly furnished the analogs used.