Technical Papers

Experimental Confirmation of a Previously Reported Unusual Finding in the Blood of Cyclotron Workers[1]

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In an earlier paper (*1*) the authors reported the occurrence of lymphocytes with bilobed or double nuclei in the blood of personnel associated with the 130-inch cyclotron at the University of Rochester. The cells were noted after exposures which were believed to be well below the accepted tolerance levels. The relationship between the increased incidence of lymphocytes with bilobed nuclei and exposure to small amounts of radiation from the cyclotron has now been confirmed experimentally in this laboratory, and considerable additional information has been obtained relative to the occurrence of the cells in cyclotron personnel. Because of the interest which has been expressed in this finding by individuals associated with cyclotrons in other institutions, the experimental results are presented briefly. Analysis of the findings in personnel is being completed and will be the subject of a subsequent paper.

Hematological studies have been made on 3 healthy mongrel dogs repeatedly stationed in relatively protected positions outside the cyclotron building for short periods while the cyclotron was running. These positions, which were roughly 50 ft from the target, were about at the level of the tank and were well outside the neutron beam. Each dog had daily blood studies for approximately 2 months prior to exposure. Several times during the control period the dogs were fastened by a chain in their assigned positions outside the cyclotron building while the cyclotron was not running. No significant changes in the blood picture occurred during the entire control period.

On August 22, October 4, and December 12, 1949, the dogs were fastened in their assigned positions for 30 min while the cyclotron was operating. The dogs' positions were changed with each exposure so that each dog was exposed once in each of the 3 positions. Operating conditions were maintained as nearly constant as possible; however, there were certain inherent variations in the cyclotron over the period of time under consideration, and it is probable that there were definite differences in magnitude of the 3 exposures. Nuclear track plates[2] were taped to the collars of the dogs during the first 2 exposures. They yielded relatively little quantitative information regarding the amount of radiation received, and, in fact, measurement of doses was not possible in any of the exposures. It is felt that exposures were relatively slight, probably below the currently accepted tolerance levels. This assumption is based on (1) earlier studies of the relative intensity of radiation at various points around the cyclotron building, and (2) the fact that the usual hematological signs of overexposure to ionizing radiation were minimal or absent.

Lymphocytes with bilobed nuclei were detected by microscopic examination (oil immersion) of cover-slip blood smears. Good cover-slip smears were selected and stained with peroxidase stain. Each smear was examined from edge to edge, and the number of white blood cells in all good areas was recorded. (As a rule, several thousand white cells are distributed throughout the good areas of a single well-pulled smear of dog blood.) The position of each abnormal cell was recorded in terms of the setting of the graduated mechanical microscope stage, and the classification of each of the cells was subsequently checked by the entire group concerned with the hematological examinations. Photomicrographs were made of many of the cells. The frequency of occurrence of blood smears containing one or more lymphocytes with a bilobed nucleus has arbitrarily been taken as an index of the incidence of these unusual cells.

Blood samples were obtained both morning and afternoon during the first post-exposure week, and once daily thereafter until the experiment tapered off with less frequent counts subsequent to the sixth week following the second exposure and the third week following the last exposure (Table 1). The complete experiment continued over a period of approximately one year. A total of approximately 180 smears was examined during the control period, and a like number during each first post-exposure week. Thereafter approximately 30 smears were examined each week. In all, approximately 3.3×10^6 white blood cells were examined during the experiment.

Examples of lymphocytes with bilobed nuclei are shown in Fig. 1. The cells are slightly larger than typical large lymphocytes, show typical lymphocyte staining with Wright's stain, and are peroxidase-negative. The cytoplasm is clear, light blue, and sometimes appears slightly "clumpy" in peroxidase-stained smears.

The data relative to the occurrence of lymphocytes with bilobed nuclei are presented in Table 1. There was some individual variation in the occurrence of the cells in the 3 dogs; however, these differences do

[1] This paper is based on work performed under contract with the U. S. Atomic Energy Commission and the Office of Naval Research at The University of Rochester Atomic Energy Project and 130-inch Cyclotron Laboratory, Rochester, N. Y.

[2] Kindly made available and interpreted by Herbert Mermagen.

TABLE 1

INCIDENCE OF LYMPHOCYTES WITH BILOBED NUCLEI IN DOGS EXPOSED TO RADIATION FROM 130-INCH CYCLOTRON

	Control period†	Post-exposure period				
		1st week	2nd week	3rd and 4th weeks	5th and 6th weeks	After 6th week
First Exposure						
Percentage of positive smears*	0.6	22.0‡	37.0‡	11.0	2.0	.....
White blood cells examined	372,000	400,000	70,000	194,000	253,000	.....
Smears examined	180	148	30	54	60	.....
Positive smears	1	32	11	6	1	.....
Second Exposure						
Percentage of positive smears		16.0	13.0	6.0	3.0	0
White blood cells examined		430,000	114,000	206,000	208,000	112,000
Smears examined		142	30	62	60	36
Positive smears		22	4	4	2	0
Third Exposure						
Percentage of positive smears		19.0	6.0	11.0	.....	3.0 .....
White blood cells examined		520,000	90,000	18§	.....	96‖ .....
Smears examined		161	30	53,000	.....	288,000 .....
Positive smears		30	2	2	.....	3 .....

* Smears containing one or more lymphocytes with bilobed nuclei are considered "positive."
† Control counts represent a period of approximately 2 months before first exposure.
‡ Associated with a slight depression in the total white blood cell count.
§ Counts were done only 3 days during the third week.
‖ Based on counts done once a week from the fifth through the seventeenth post-exposure weeks.

not significantly alter the interpretation of results. Such differences as did occur appeared to be related to individual variations among the 3 dogs and not to differences in doses at the 3 positions.

The experimental results clearly demonstrate a marked increase in the occurrence of lymphocytes with bilobed nuclei following exposure to radiation from the cyclotron. The incidence was maximal during an approximately 2-week period following exposure.

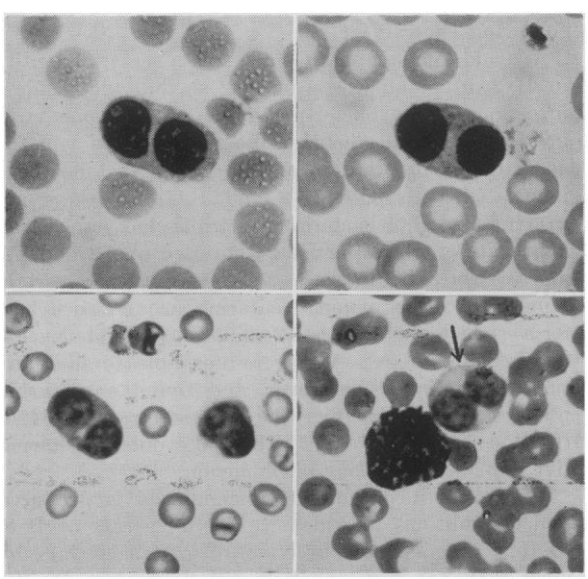

FIG. 1. Left to right: Lymphocyte with bilobed nucleus (peroxidase stain). Other cells are red blood cells. Lymphocyte with bilobed nucleus (Wright's stain. This cell contains several azurophile granules. One lymphocyte with bilobed nucleus, one normal lymphocyte (peroxidase). Normal granulocyte showing characteristic dark peroxidase positive granules and one lymphocyte with bilobed nucleus (arrow).

Thereafter the frequency of positive smears decreased. The highest incidence occurred after the first exposure and was accompanied by a slight depression of the total white blood cell count as determined by routine diluting and counting procedures. The total white blood cell count was not depressed following the second and third exposures. This greater response after the first exposure probably reflects a higher dose. As mentioned above, however, it has not been possible to make quantitative physical estimations of the amount or types of radiation delivered in any of the exposures. It is, of course, also impossible at this time to state which component or components of the radiation are chiefly responsible for producing the "bilobed-nucleus response" in lymphocytes, although studies in this direction are currently being carried out in this laboratory. In our experience, however, the response has been less prominent in persons working with the 26-inch cyclotron and with radioactive isotopes than in personnel associated with the 130-inch cyclotron. R. E. Carter has observed this type of cell in certain personnel at Los Alamos (*2*), and Ethel Browning reports having occasionally seen the cells in the blood of "luminizers and operatives using x-rays and the radium bomb for industrial purposes" (*3*). It should be pointed out, however, that the cells are not an absolute indication of radiation effects. We have observed this type of cell in certain infectious diseases of childhood, as well as in infectious mononucleosis, lymphatic leukemia, etc. As in the case of any hematological finding, interpretation requires careful evaluation of the general condition of the individual.

Perhaps the most significant aspect of the findings reported here relates to the observation that the cells occur following extremely small exposures that do not produce changes detectable by routine hematological

examinations. For this reason studies of the occurrence of lymphocytes with bilobed nuclei appear to be an unusually promising means of identifying potentially harmful operations *before* gross overexposure can occur.

References

1. INGRAM, M., and BARNES, S. W. *Phys. Rev.*, **75**, 1765 (1949).
2. CARTER, R. E. Personal communication.
3. BROWNING, E. Personal communication.

Virus Strains of Identical Phenotype but Different Genotype

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Delbruck and Bailey (*1*) noticed an anomaly in the lysate of bacteria which was obtained by mixedly infecting the B strain of coli with the bacterial viruses T2 and T4. Subsequently, Luria (*2*) found this anomaly to be even more pronounced when he repeated Delbruck's experiment—using, however, virus T2 that had been exposed to ultraviolet irradiation.

When we undertook experiments in an attempt to understand this anomaly, we were led to the following result: If we infect a culture of the B strain of coli mixedly with the bacterial viruses T2 and T4 and incubate to permit lysis of the bacteria, there are present in the lysate 3 easily distinguishable types of bacterial viruses. Two of these, as expected, behave like the original parent strains T2 and T4, i.e., one of them behaves like T2 inasmuch as it is unable to attack the mutant strain B/2 (which is resistant to T2) but is able to grow in the mutant strain B/4 (which is sensitive to T2); the other behaves like T4, being unable to attack B/4 (which is resistant to T4) but is able to grow in B/2 (which is sensitive to T4). The third type of virus present is phenotypically like T4 inasmuch as it is capable of multiplying in the strain B/2 (which is sensitive to T4), but it is genotypically like T2 inasmuch as, after one passage in the strain B/2, it is no longer capable of growing in it but is capable of growing in the strain B/4 (which is sensitive to T2).

The presence of this third type of virus, which may be called "latent T2," can be demonstrated in the following manner: We add to a culture of the B strain of coli viruses T2 and T4 in ratios corresponding to 10 T2 and 10 T4 virus particles per bacterium, incubate to permit lysis of the bacteria, and then filter the lysate.

If we plate a sample of this lysate on agar that is inoculated with the strain B/4 (which is sensitive to T2 but resistant to T4), those virus particles contained in the lysate which have the phenotype T2 will show up as plaques on these plates. T4 virus particles will not give plaques on this plate because B/4 is resistant to T4. The number of plaques is thus a measure of the number of T2 particles in the lysate.

Using a sample of the lysate, we determine in this manner the number of plaques obtained on an agar plate inoculated with the strain B/4. When we repeat this experiment—with the difference that before plating on the B/4 plate we add to the sample of our lysate a certain quantity of the strain B/2, allow 5 min for absorption, dilute with broth, and incubate for 1 hr to permit lysis of the bacteria—then we obtain a ten to twenty-five times larger number of plaques on the B/4 plate.

This phenomenon appears to show that there is present in our lysate a virus (the "latent T2") which is capable of multiplying in B/2 and subsequently forming plaques on B/4. In order to account for our observation, the concentration of the "latent T2" in the lysate would have to be about 10% of the concentration of T2. We were not able to obtain, after one passage in B/2, any appreciable further growth in B/2 of our hypothetical "latent T2." Before drawing the conclusion that the presence of a "latent T2" is in fact responsible for our phenomenon, it is necessary to exclude alternative explanations.

As an alternative explanation of our observation, it appeared a priori conceivable that our lysate contains aggregates of virus particles formed by a T2 and a T4 particle that stick together. Such aggregates might then perhaps be capable of entering into a bacterium of the strain B/2 (by virtue of their T4 component) and, once inside, both virus particles T2 and T4 might then be able to multiply, and thus to produce the observed phenomenon. We were able to rule out this possibility, however, by performing the following experiment.

We add to a sample of our lysate a certain quantity of B/2, using an excess of B/2 so that independent infection of one bacterium by more than one virus particle can be neglected. We then allow 5 min for absorption and plate on an agar plate that has been inoculated with both B/2 and B/4. If there are present any B/2 bacteria into which has entered an aggregate of virus particles composed of T2 and T4, and in which both viruses will grow, then a certain number of clear plaques centering around such bacteria (which yield both T2 and T4) should develop on the agar plate. We were not able to find any such clear plaques, however, and found only turbid plaques (in which either the B/2 is lysed by T4 or the B/4 is lysed by T2). This rules out the alternative explanation of our phenomenon.

We ascertained that our phenomenon is produced under conditions in which we use an excess of B/2, so that independent infection of one bacterium by more than one virus particle can be neglected. We also ascertained that our phenomenon is not produced if, in place of our lysate, we use a mixture of T2 and T4.

We are thus led to conclude that the phenomenon described is due to virus particles that have the phe-