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Deoxyribonucleic Acid Synthesizing Cells in Peripheral Blood of Normal Human Beings

Neoplasia, aplasia, and metaplasia are problems of great importance in medicine today. The mechanism of development of diseases involving these processes is unknown, and treatment is unsatisfactory. Perhaps this is not surprising since the mechanism of control of normal cell destruction and renewal is also obscure. The development of tritium-labeled thymidine, a specific deoxyriboside precursor of deoxyribonucleic acid (DNA), by W. L. Hughes (*1*) provided a powerful tool for the in vivo and in vitro study of cell turnover in man and animals. Individual cell histories can be followed easily using histoautoradiographic techniques (*2*). A review of earlier studies on protection against radiation-induced aplasia of the marrow (*3*) led to the hypothesis that totipotential primitive mesenchymal cells may be circulating under normal conditions. If this were in fact true, one might expect some of these cells to be actively synthesizing DNA preparatory to later mitosis. The in vitro studies reported here were designed to investigate this hypothesis (*4*).

Six healthy males 28 to 45 years of age with peripheral blood counts within normal limits were selected. Nine milliliters of blood from the antecubital vein were withdrawn under sterile conditions and placed immediately into 2 ml of a solution of normal saline containing 5 percent dextran, 2 mg of heparin, and tritiated thymidine. Dextran was used to accelerate erythrocyte sedimentation and to allow easy concentration of white cells (*5*). Tritium-labeled thymidine with a specific activity of 390 mc/mmole was added such that the final dilution was 2 μc/ml. The blood was allowed to incubate for 1 hour at room temperature, during which time the erythrocytes settled. The supernatant was withdrawn and centrifuged at 200*g* for 10 minutes. The cells were then resuspended to a concentration of 100,000/mm^3, and smears were made. The smears were fixed in absolute methyl alcohol, and stripping film was placed over the preparations as described by Pelc (*6*). The film was developed, and the autoradiographs were read after 15 days' exposure. Some smears from each sample were stained with Feulgen's stain prior to application of the photographic film; others were stained through the stripping film with Wright's stain after the film had been developed. Five thousand white cells of each individual were enumerated to determine the percentage of labeled cells. A 500-cell differential was done on each individual.

The results are summarized in Table 1. Labeled cells were found in the blood cell concentrate of all individuals studied. Between 10 and 60 grains were counted above each labeled cell. In terms of the total white cell count of the concentrate, the number of labeled cells amounted to 0.06 percent or less. A typical labeled cell is shown in Fig. 1.

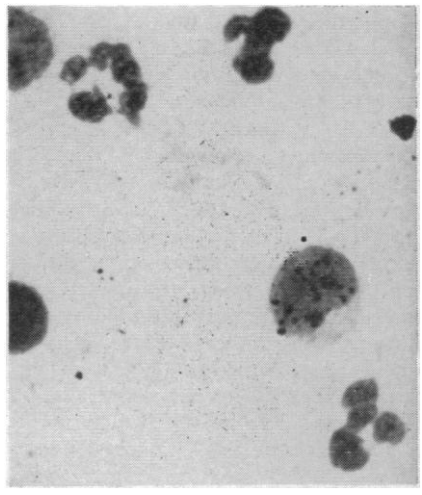

Fig. 1. Large mononuclear cell labeled with tritiated thymidine, found in the peripheral blood of a normal human being.

Table 1. Labeled cells in white cell concentrates from the blood of normal human beings.

Subject No.	Differential counts of cell concentrates (%)				Labeled cells	
	Segmented leukocytes	Lymphocytes		Monocytes	Percentage of total cells	Percentage of large mononuclear cells
		Large	Small			
1	62	7	24	7	0.06	0.4
2	78	3	16	3	0.04	0.7
3	68	11	18	3	0.02	0.1
4	52	25	21	2	0.06	0.2
5	56	13	29	2	0.06	0.4
6	67	14	16	3	0.06	0.4

Labeling was seen only in large mononuclear cells identified as monocytes, or large- and medium-sized lymphocytes. No labeled cells of the myeloid series and no labeled typical small lymphocytes were found.

The existence in the peripheral blood of man of a small percentage (but large absolute number) of circulating leukocytes that have the capacity to synthesize new DNA has been demonstrated. The synthesis of DNA is presumptive but not direct evidence that the cell is destined to divide if given proper conditions for seeding, since present beliefs are that intracellular DNA is synthesized only in preparation for cell division. We consider these cells to be predominantly the classical monocyte. Some resembled large lymphocytes. The monocyte is a phagocytic cell. We, among others, have long considered this cell to be a tissue histiocyte in transit. Does the capacity to synthesize DNA and presumably divide indicate that this cell is in reality a totipotential primitive mesenchymal cell, or does it merely indicate that this cell has the capacity to propagate itself? This fundamentally important question has not yet been answered by direct experiment. The present studies neither support nor refute the contention of Bloom (*7*), Farr (*8*), and Yoffey (*9*) that lymphocytes may be totipotential cells that are in continual transport to the marrow and become transformed into other blood cell lines. Initial in vivo studies with human beings, to whom tritium-labeled thymidine was administered, resulted in the labeling of essentially no small lymphocytes in the peripheral blood, although a small number of labeled large mononuclear cells were found (*10*).

It is clear from current studies of patients with blood dyscrasias that, with

the same techniques reported here, it is possible for large numbers of cells in the peripheral blood to take up tritium-labeled thymidine. With the normal individuals, increase of the incubation time with tritium-labeled thymidine up to 24 hours did not increase the percentage of labeled cells. It thus appears reasonable that in the normal individual only a very small percentage of the cells of the peripheral blood are synthesizing DNA, and these presumably are capable of division. However, the average human being of 60 kg with 7 percent blood volume and 7000 leukocytes/mm^3 has approximately $(0.06 \times 7000 \times 4200 \times 1000)/100$, or approximately 18×10^6 potentially dividing cells in the peripheral blood at one time and presumed to be in transit.

The findings that histiocytes, or specific cells of the reticuloendothelial system, may be multipotential in character, and may be transported to needed sites normally via the blood stream, is pertinent in connection with protection against X-radiation by parabiosis (*11*) and by regionally fractionated exposures (*12*). In the later experiments, exposure of one-half of the body only was followed in a few minutes by exposure of only the other half of the body. Mortality was less than it was in animals that received equivalent single-dose total-body irradiation. Normally circulating multipotential cells would explain these findings. The hypothesis is supported by estimates that only a very few intact cells are required to repopulate a radiation-depleted marrow population (*13*).

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Two Syndromes Caused by Sweetpotato Viruses

Unpublished studies on the nature, host range, and seasonal development of the sweetpotato virus complex have distinguished two distinct virus syndromes, those of feathery mottle and internal cork.

In insect transmission experiments begun in 1954, of which a preliminary report was published (*1*), the aphids *Myzus persicae* (Sulz.) and *Macrosiphum solanifolil* (Ashm.) transmitted a virus from sweetpotato to sweetpotato that was distinguished by chlorotic spots on the foliage, which are typical of the internal cork syndrome. The aphids picked up the virus from two sources: (i) plants of the Porto Rico variety showing the internal cork virosis and (ii) plants of a Russian sweetpotato (plant introduction No. 105945) showing the feathery mottle virosis.

On the basis of the "flush of growth" indexing technique (*2*), which requires about 2 months, there was 100 percent transmission of the chlorotic spot symptom of the internal cork syndrome. Since the syndrome caused by the feathery mottle virus did not appear in plants inoculated with aphids fed on plants of the Russian sweetpotato, it appeared that the aphid was not the vector of the virus.

On the basis of the necrotic root symptom (*1*) of the internal cork syndrome (after storage of roots for 6 months at temperature of 75°F and at 90 percent relative humidity) there was approximately 20-percent transmission, both by insects and by grafting, under greenhouse conditions. The very mild root-symptom expression and the low percentage of incidence must be balanced against the fact that 100 percent of the roots produced some sprouts showing the same virus syndrome. It appears that the development of root symptoms requires a longer incubation period, or multiple inoculations such as undoubtedly occur in the field.

Further studies revealed that there is a definite but limited amount of natural transmission of the feathery mottle virus in the field. Such transmission is revealed only after harvest, in the sprout growth of the bedded, infected roots. The 1957 season was unusual because it stimulated an extended vegetative growth throughout September, after the growth had come to a halt during the summer drought in August, and there was positive evidence of current-season transmission of feathery mottle virus (Fig. 1*B*). Moreover, when the "flush of growth" technique (*2*) was used in the greenhouse with material harvested from the field, there was a much higher incidence of infection than is normally observed. The current-season symptoms, which appeared only on the terminal growth, were similar to those reported by Sheffield (*3*) and could be manifestations of the same disease.

A very important feature of the earlier transmission experiments involving internal cork was the discovery that the original source plant of feathery mottle virus (Russian sweetpotato, P.I. 105945) also contained the internal cork virus, or viruses, and that only the cork virus was transmitted by aphids. This feathery mottle source plant, which descended in a clonal line from plants used by Doolittle and Harter (*4*) in their work on feathery mottle and which was listed by them as No. 029878, also exhibited typical chlorotic spot of the internal cork virus syndrome, and this symptom has been constantly present in this seedling in subsequent studies.

That sweetpotato P.I. 105945 carried both viruses may possibly account for the fact that Webb (*5*) and Webb and Larson (*6*) were able to obtain chlorotic spotting in plants inoculated with virus from their feathery mottle source plants by means of the aphid vector and also by mechanical means. Even though sweetpotato P.I. 105945 has been used in numerous experiments, it has not yet exhibited the necrotic, internal cork root symptom, perhaps because of its immunity from expression of the necrotic root spotting phase of this syndrome.

During a sweetpotato virus disease sur-

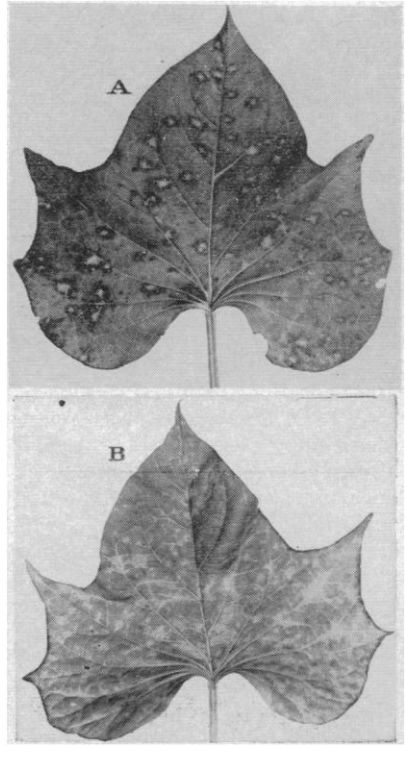

Fig. 1. Current-season symptoms on Porto Rico sweetpotato leaves in two syndromes: (*A*) internal cork; (*B*) feathery mottle.