Histochemical Demonstration of Reducing Activity in Normal and Leukemic Blood and Bone Marrow Cells.* (17664)

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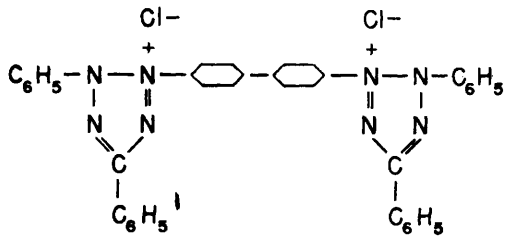
It was found previously that leucocytes from various exudates reduced potassium tellurite and pp′ diphenylene bis 2-(3,5 diphenyl tetrazolium chloride), ("neotetrazolium chloride")(1). The sites of enzymatic activity could be recognized under the microscope either due to the deposition of reduced dark tellurium or the insoluble purple formazan. In the present study, observations were made on normal and abnormal blood and bone marrow cells.

Method: Freshly drawn venous blood was heparinized, thoroughly mixed and centrifuged. The plasma was removed and the upper layer was pipetted into the reagent solution. Aspirated bone marrow or exudate was treated in a similar fashion. Material from lymphnodes was cut into fine pieces in the reagent solution. pp′ diphenylene bis 2-(3,5 diphenyl tetrazolium chloride) was preferred as reagent since purple granules which follow its reduction can be more easily distinguished from preformed refractile granules in blood cells than the dark tellurium deposits. pp′ diphenylene bis 2-(3,5 diphenyl tetrazolium chloride) has the formula:*



The trade name of this substance is "neotetrazolium chloride". It can be obtained from The Pannone Chemical Company, Farmington, Conn. A freshly prepared saturated solution of this substance in M/10 phosphate buffer, pH 7.4 was used. The chemical should be kept in the refrigerator since considerable deterioration was noticed during the hot summer months. The blood or bone marrow suspension was added to the reagent solution at 37°C. A drop of sediment was examined under the microscope after the suspension had been left in the incubator for one hour without further staining. Longer incubation time up to 15 hours did not alter the observed results. The identification of unstained blood cells is admittedly difficult. However, the size and shape of the cell as well as the form of the nucleus and type of cytoplasmic granulation, if present, permits in most cases proper identification. Moreover, by studying leukemic blood in which routine films revealed almost only one type of blood cell, reliable results could be obtained.

Observation. Normal blood: A considerable portion (62-84%) of all white blood cells revealed evidence of reducing activity. Polymorphonuclear leucocytes, lymphocytes and monocytes were seen to contain one to several fine purplish granules. The positive cells were mostly polymorphonuclear leucocytes while only some of the lymphocytes were positive. Eosinophiles contained fine granules which were independent of the coarse eosinophilic granulations.

Leukocytosis. In blood with marked leukocytosis due to various causes, most polymorphonuclear leucocytes contained purplish granules. In some of the cells, these granules were coarser and more numerous than commonly seen in normal leucocytes.

Exudates. Almost all polymorphonuclear leucocytes contained granules that were more numerous and coarser than those seen in normal blood cells (see Fig. 1). Occasionally,

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1. Wachstein, M., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 175.

† The structural formula given in the previous publication(1) shows the chloride ion in the right tetrazine ring erroneously in an asymmetrical position.

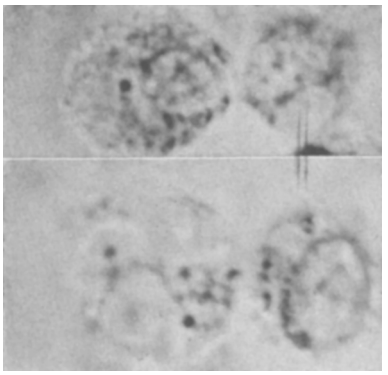

FIG. 1.
Leucocytes from an infected wound showing coarse cytoplasmic granules due to deposition of reduced insoluble pp′ diphenylene bis 2-(3,5 diphenyl tetrazolium chloride). × 1400.

a number of granules were clumped together. In addition, some of the cells contained large vacuoles which stained orange to purplish red.

Acute myeloid leukemia. In the blood of 7 patients with this disease, myeloblasts did not show any staining reaction. Many of the more mature myeloid elements including myelocytes showed purple granules.

Chronic myeloid leukemia. In the blood of 4 patients, a considerable portion of all white blood cells showed reducing activity. As in the acute form, the most immature cells were negative while granules were demonstrable in myelocytes and the more mature forms.

Chronic lymphatic leukemia. Blood from 10 patients with this disease was examined. While in one case none of the lymphocytes were positive, in 5 others the percentage of positive cells varied between 2 and 15. In 3 of the examined cases, 40, 92 and 96% respectively, were positive. In the tenth case, at the first examination the blood contained 137,000 lymphocytes per cu mm of which 19% were positive while three months later it contained 265,000 lymphocytes of which 98% were positive. In the blood of all of these patients, most of the polymorphonuclear leucocytes present showed evidence of reducing activity.

Lymphnodes. Suspensions from tonsils and lymphnodes from surgically removed specimens found to be normal on microscopic examination showed 46-54% of lymphocytes to be positive.

Bone Marrow. In bone marrow suspensions of normal individuals, a considerable portion of all cells revealed reducing activity. The majority of positive cells were found in the myeloid series. Activity could first be identified in myelocytes. Only occasional nucleated red cells showed few purplish granules. Megakaryocytes revealed a varying behavior. In some the cytoplasm was studded with dust-like purple granules while others were entirely negative. In bone marrows from patients suffering from various kinds of anemias, similar findings were obtained. In general only few of the nucleated red cells showed purplish granules. As in the peripheral blood, the myeloblasts in bone marrow suspensions from patients with acute myeloid leukemia were devoid of reducing activity.

Various animals. In the blood of rats, guinea pigs, rabbits and mice, some cells were positive. Apparently the positive cells were of the polymorphonuclear (heterophile) type. In bone marrow suspensions of all of these animals, some of the cells likewise revealed reducing activity.

Comment. Various enzymes are known to occur in white blood cells. In general larger quantities are found in polymorphonuclear leucocytes than in lymphocytes(2). This can be observed under the microscope with several of the enzymes that are demonstrable histochemically *e.g.*, the oxydases and peroxydases and alkaline phosphatase(3). The findings presented here follow the same general pattern since histochemically demonstrable reducing activity is found in normal blood, predominantly in leucocytes. It has been previously reported(1) that in the kidney the deposition of reduced pp′ diphenylene bis 2-(3,5 diphenyl tetrazolium chloride) or tellurium takes place in such a manner as to suggest a localization within the mitochondria. In the blood cells there is, however, apparently an inverse relationship between the occurrence of mitochondria and histochemically demonstrable reducing activity since the mature granulocytes which are almost lacking in these

2. Barnes, J. M., *Brit. J. Exp. Path.*, 1940, v21, 264.

3. Wachstein, M., *J. Lab. Clin. Med.*, 1946, v31, 1.

cellular constituents show most of it. The behavior of reducing activity in normal and leukemic blood cells is paralleled by the findings as obtained by the Warburg technic. The immature cells have a purely oxidative metabolism without any aerobic lactic acid formation. In contrast, the more mature granulocytes show strong lactic acid formation under aerobic conditions(4). No significant changes are present between granulocytes from normal and leukemic blood(5).

Summary. Reducing activity in blood and bone marrow cells can be demonstrated with the aid of pp' diphenylene bis 2-(3,5 diphenyl tetrazolium chloride). Under the microscope, sites of reduction are indicated by small purplish granules due to the formation of insoluble formazan. A positive reaction is seen in the myeloid elements with the exception of the myeloblasts. Lymphocytes and monocytes show a varying degree of activity. Nucleated red cells and megakaryocytes are occasionally positive. In leukemic blood, myeloblasts are consistently negative while the more mature elements show reducing activity.

4. Kempner, W., *J. Clin. Invest.*, 1939, v18, 291.

5. Soffer, L. J., and Wintrobe, M. M., *J. Am. Invest.*, 1932, v11, 661.

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Significance of the Intestinal Flora in Nutrition of the Guinea Pig.* (17665)

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Considerable work has been done to develop a purified ration which will give growth in guinea pigs equivalent to that obtained with commercial rations. In recent reports (1,2) from this laboratory it was shown that the growth was remarkably improved when gum arabic was added to the purified basal ration. Almost normal growth was obtained when in addition to gum arabic potassium acetate and magnesium oxide were used. The reason for the growth response obtained with gum arabic, potassium acetate, and magnesium oxide could not be explained with certainty. Since it seemed possible that this response was not based on a direct effect on the animal but on a beneficial effect on the intestinal flora, we have investigated the intestinal flora of the guinea pig. The chief purpose was to obtain some indication of the numbers of certain representative organisms and to determine whether there are great differences in the absolute and relative numbers when different diets are fed to the animals. Attention was also paid to the excretion of vitamins from animals on different rations.

Experimental. The procedure for growing the animals was similar to that used by Booth *et al.*(1). Two different rations were used. The *first* one, the synthetic basal ration, consisted of: sucrose 60.9%, casein (vitamin-free) 30%, salts IV[3] 4%, fortified soybean oil 4%, sucrose mixture containing B vitamins 0.8%, and choline 0.3%. The fortified soybean oil supplied 1.2 mg of β-carotene, 12 mg of α-tocopherol, 8 μg of calciferol and 0.2 mg of menadione per 100 grams of ration. The vitamin B mixture supplied 1 mg of thiamine hydrochloride, 1.4 mg riboflavin, 1 mg pyridoxine, 3 mg calcium pantothenate, 200 mg inositol, 10 mg niacin, 10 mg p-aminobenzoic acid, 0.04 mg biotin and 0.3 mg folic

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1. Booth, A. N., Elvehjem, C. A., and Hart, E. B., *J. Nutrition*, 1949, v37, 263.

2. Roine, P., Booth, A. N., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 90.

3. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, v138, 459.