Table 2. Phenotypes of acid phosphatase of red cells of 369 individuals from a Brazilian population.

A	B	BA	CA	CB	C
		Observed			
15	220	111	4	19	0
		*Expected**			
14.4	219.9	112.2	4.4	17.7	0.4

* On the basis of Hardy-Weinberg law with allele frequencies: P^a=0.197, P^b=0.772, and P^c=0.031.

titative estimates of enzyme activity of each of the five phenotypes they observed. Exchange of photographs has established that the sample classified as C by Harris and his colleagues (*3*) shows the same pattern as the samples we have classified as C.

The finding of the C phenotype completes the verification of the genetic hypothesis previously offered to explain the genetic data (*1, 2*). This polymorphism promises to be a useful tool for the study of problems ranging from population genetics to the genetic control of enzyme structure.

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5. Supported in part by NIH grants HE 03708, GM 07214, and GM 10424.
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Irradiation of the Blood: Method for Reducing Lymphocytes in Blood and Spleen

Abstract. *Insertion of a beta-emitting source into the right atrium of the heart permits intensive irradiation of the circulating blood, with subsequent depletion of lymphocytes in the peripheral blood and lymphoid organs.*

The lymphocyte, because of its relation to the immunologic response, is the subject of experiments designed to test and define its critical role. McGregor and Gowans (*1*), by creating external thoracic duct fistulas of several days' duration, demonstrated that in the rat such a fistula produces a peripheral lymphopenia and, significantly, a depletion of lymphocytes from the lymph nodes and the spleen. Such animals are altered in their immunologic response to foreign proteins and to skin homografts. Reduction in the peripheral circulating lymphocytes, described by Cronkite *et al.* (*2*), followed extracorporeal irradiation of calf's blood that had been pumped through a spiral of Tygon tubing; after irradiation for 5 hours 90 percent of the blood received more than 1000 rad by random mixing. The lymphocyte population was reduced for several weeks, judging by the decline in lymphocyte counts in the peripheral blood. Moreover, immunologic responses in the calf were altered.

Those studies, attaining much the same end with dissimilar techniques, suggested the advantages of a method capable of modifying the lymphocyte population for more extended periods; an internal radioapplicator appeared to be a logical refinement. Circumferential vascular radioapplicators have been replaced by small cylindrical applicators 20 mm long and 3 mm in diameter containing 300 mc Sr^{90}-Y^{90}, a beta-emitter of 2.18 Mev maximum energy with a half-life of 28 years. The radioisotope is in the form of microspheres and is sealed in the applicator (*3*). The half-value layer of aqueous solutions to this radiation is approximately 1 mm. The stainless steel casing, 0.025 mm thick, results in conversion of less than 1 percent of the radiant energy to bremsstrahlung in the steel. The applicator is suspended in the blood stream within the right atrium where its position depends on the posture of the experimental animal. More often than not it lies touching the walls of the atrium, although the motion of the heart and the concavity of the interior of the heart chamber prevent broad areal contact with the endocardium. The radioapplicator is readily introduced into a branch of the right jugular vein and is advanced into the atrium at the end of a silicone-coated, barium-impregnated, plastic catheter. For protection of the operators, when the applicator is in the operative field, the area of dissection is covered with saline to a depth of several centimeters to absorb the beta rays. Since positioning is not critical, it is determined by dead reckoning; the catheter is advanced in the jugular vein caudad from the angle of the mandible a distance equal to a fixed proportion of the length of the spine,

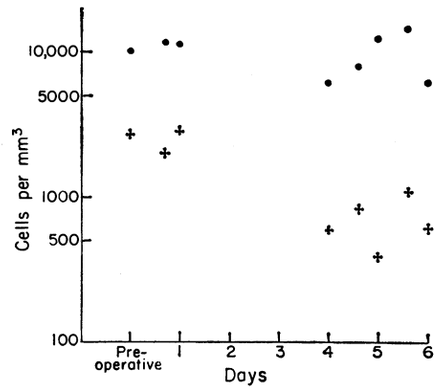

Fig. 1. Leukocyte counts in the peripheral blood of a dog after irradiation of the circulating blood with an intracardiac radioapplicator. Dots = total count; crosses = lymphocytes.

depending on the species. Positioning is verified by x-ray.

The dosimetry, approximate because of the uncontrolled positioning of the applicator relative to the surrounding blood volume, is based on viscosity degradation in vitro and thermal luminescence techniques. Determinations by two independent laboratories were within reasonable agreement. The output of the applicator is expressed as an integral dose-rate in gram-rads per hour; from this datum, the total circulating blood in a dog weighing 10 kg may receive a maximum dose of 300 rads per hour from the intracardiac applicator. The myocardium of the right atrium receives considerable beta-radiation, but this tissue is known to be resistant to larger doses of radia-

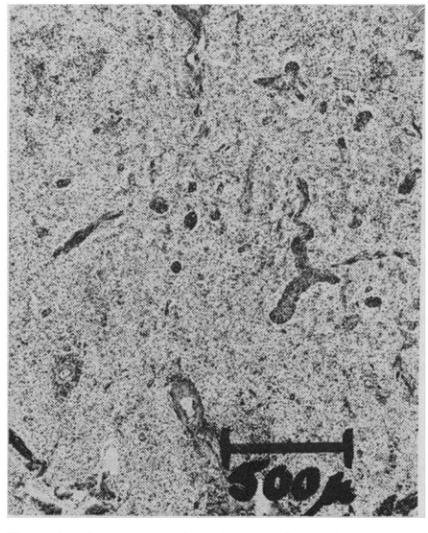

Fig. 2. Section of spleen after 6 days of irradiation of the circulating blood, showing very marked reduction of the lymphoid elements in the white pulp.

tion than are used in these studies, except for the portion of heart muscle immediately adjacent to the applicator. If radiation to the myocardium proves to be a problem, the applicator may be modified with bumpers to prevent its making direct contact with the endocardium.

A pronounced effect on the peripheral lymphocyte count in the circulating blood is seen in dogs weighing about 10 kg. Polymorphonuclear leukocyte and erythrocyte counts do not change significantly during the 1st week of irradiation. Figure 1 shows a typical series of total white blood cell counts and lymphocyte counts per cubic millimeter of periphal blood plotted against duration of the radiation. In this particular experiment the spleen was excised and studied histologically after 6 days (Fig. 2). Lymph nodes removed at the same time were atrophic grossly; microscopic sections revealed that they share lymphocyte depletion with the spleen, but to a lesser degree. Control studies with a dummy applicator were negative. The lymphocyte count is restored after removal of the radioapplicator.

If current knowledge of the radiosensitivity of lymphocytes, as determined under different circumstances, may be applied here (*4*), the intensity of the radiation has been sufficient in these studies to render nonviable all circulating lymphocytes remaining within the vascular compartment after a few hours. The cell count will be reduced promptly if the nonviable cells are removed or are obviously altered in their staining properties on routine smear. However, our repeated observation that the lymphocyte count *gradually* declines from the level preceding irradiation is consistent with continuous replacement of nonviable circulating lymphocytes by "fresh" cells mobilized from such centers as the white pulp of the spleen, lymph nodes, and thymus. The importance of the reentry into circulation of slightly irradiated cells after a period of convalescence while sequestered in the spleen, thymus, or a lymph node is yet to be determined. There is evidence that the total doses of radiation, when fractionated, are less lethal to cells (*5*). Thus the established movement of the lymphocyte in and out of the vascular compartment may contribute to a greater tolerance to radiation. Whatever ultimately proves to be the anatomical route of the individual lymphocyte, in aggregate they are reduced in number by this technique of irradiation, with major depletion of cells located within the white pulp of the spleen.

Extensions of this method for altering the lymphocyte population, alone and in combination with regional x-ray treatment, have obvious implications for the study of immunologic responses to standard antigens, of homotransplantation rejection, of cell populations in the thymus, and of the life cycle of the normal and pathologic lymphocyte.

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Infrared Absorption of Hydroxyl Groups in Kaolinite

Abstract. *Effects of sample orientation on the hydroxyl absorption of kaolinite can be interpreted in terms of the structure indicated by x-ray investigations, in which the surface hydroxyl groups of each layer are directed toward the oxygen atoms on the surface of the next layer.*

The hydroxyl groups of kaolinite give four sharp infrared absorption bands, one of which, at 3697 cm^{-1}, arises from a dipole oscillation which is perpendicular, or nearly so, to the kaolinite sheets; the other three, at 3670, 3652, and 3620 cm^{-1}, arise from dipole oscillations nearly parallel with the sheets. In addition, there are indications of diffuse absorption, with a dipole component perpendicular to the sheets underlying the bands at 3670 and 3652 cm^{-1} (*1*). Most workers (*1–6*) have assumed that each sharp absorption band arises from a distinct type of hydroxyl group, and on this assumption it has proved difficult to reconcile the infrared spectrum with the structure of the kaolin minerals indicated by x-ray diffraction studies (*3*). Although the structure established by x-ray investigation does not determine the exact position of the protons, it strongly suggests that the hydroxyl groups in the surface sheet of each kaolin layer are nearly equivalent and are directed, at small angles to the normal to the sheet, toward the oxygen atoms of the next kaolin layer (Fig. 1). This structure agrees with results of nuclear magnetic resonance experiments (*5*). In contrast, on the basis of infrared studies, it has been suggested that one of the three surface hydroxyl groups of each primitive lattice cell lies nearly in the plane of the sheet, directed toward the empty octahedral site (*1, 4, 6*).

In the structure indicated by x-ray diffraction (Fig. 1), the three surface hydroxyl groups of each primitive cell are approximately related by a threefold axis of symmetry passing through the aluminum ions to which they are attached. Farmer and Russell (*7*) pointed out that, with an exact threefold axis, coupling between these three hydroxyl groups would give rise to one symmetrical in-phase vibration, with a dipole oscillation along the threefold axis—that is, perpendicular to the sheets—and two mutually degenerate, out-of-phase, vibrations with dipole oscillations in the plane of the sheets. The hydroxyl absorption of kaolinite can then be readily interpreted by ascribing the strong 3697 cm^{-1} band to the symmetrical in-phase vibration, and the weaker bands at 3670 cm^{-1} and 3652 cm^{-1} to the out-of-phase vibrations, their degeneracy being lifted by the fact that the kaolinite structure as a whole does not have a threefold axis of symmetry. The 3620 cm^{-1} band can then be reasonably assigned to the fourth hydroxyl group, which lies within the kaolinite layers (*7*).

Ledoux and White (*6, 8*) have now shown, elegantly and conclusively, that the three higher frequency absorption bands do in fact arise from the surface sheet of hydroxyl groups, and the 3620 cm^{-1} from the inner hydroxyl group. This was achieved by introducing potassium acetate and hydrazine between the kaolinite layers, followed by washing with D_2O, when only the protons in the surface sheets of hydroxyl groups were replaced by deuterium.