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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References and Notes

1. D. D. McGregor and J. L. Gowans, *J. Exptl. Med.* **117**, 303 (1963).
2. E. P. Cronkite, C. R. Jansen, G. C. Mather, N. O. Nielsen, E. A. Usenick, E. R. Adamik, C. R. Sipe, *Blood* **20**, 203 (1962); E. P. Cronkite, personal communication.
3. By the Minnesota Mining and Manufacturing Co., St. Paul, Minn.
4. H. B. Hewitt and C. W. Wilson, *Brit. J. Cancer* **13**, 69 (1959); R. Schrek, *Ann. N.Y. Acad. Sci.* **95**, 839 (1961); L. H. Smith and O. Vos, *Radiation Res.* **19**, 485 (1963).
5. R. J. Berry and J. R. Andrews, *Ann. N.Y. Acad. Sci.* **95**, 1001 (1961).
6. Supported by a grant from the Burroughs Wellcome Co. (U.S.A.), Inc.

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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.

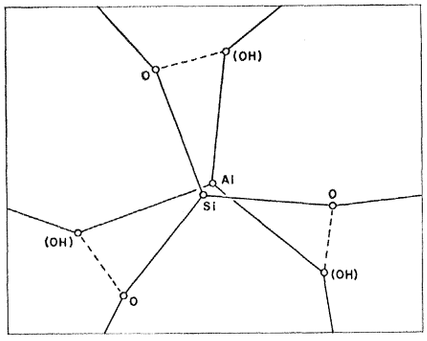

Fig. 1. Relation of the surface hydroxyl groups of one layer to the surface oxygens of an adjacent layer in dickite. A similar relation exists in kaolinite (*3*).

Their results show two features which require further discussion. First, weak sharp bands persist at 3695, 3670, and 3650 cm^{-1} in the deuterium-substituted kaolinite. These can reasonably be ascribed to a small proportion of kaolinite layers which were not expanded by hydrazine treatment, and so were not available for deuterium exchange. Although x-ray diffraction measurements on hydrazine-treated kaolinite showed only a weak 7.1 Å line (*6*), it must be remembered that an x-ray reflection requires a number of adjacent 7.1 Å spacings; a small number of isolated 7.1 Å spacings would not be detectable. Second, although the 3670 cm^{-1} band of well-crystallized kaolinite is the weakest of the four and has its dipole change nearly parallel to the sheets (*1*, *7*), the residual absorption in the deuterium-exchanged kaolinite is as strong as that at 3695 cm^{-1} and has a component perpendicular to the kaolinite layers (*8*). This can be ascribed to residual hydroxyl groups which have deuteroxyl groups as neighbors. The sharp absorption bands of well-crystallized kaolinite arise from dipole-dipole coupling between the hydroxyl groups in the surface layer and probably require a well-ordered region of hydroxyl groups covering several primitive cells in any one sheet. In the deuterium-substituted kaolinite, some residual hydroxyl groups would be entirely surrounded by deuteroxyl groups; others would have one, two, or more hydroxyl groups as first and second neighbors, giving a range of coupling forces, which would be expected to give rise to broad hydroxyl absorption in the 3640 to 3700 cm^{-1} region, showing a marked degree of polarization perpendicular to the kaolinite sheets. The spectra of Ledoux and White (*8*) do in fact show this feature, of which the maximum appears to be near 3670 cm^{-1}. Similar arguments apply to the deuteroxyl absorption at 2698 cm^{-1} (*8*). The effects of disorder are also evident in halloysite, which, because of its poorer crystallinity, does not show the fine details of the kaolinite spectrum (*8*). The diffuse absorption of kaolinite in the 3650 to 3690 cm^{-1} region (*1*), which has a component perpendicular to the kaolinite layers, probably arises from less highly crystalline regions such as crystal surfaces and slip planes within the crystals.

It is noteworthy that the position at which the inner hydroxyl group absorbs, 3620 cm^{-1}, is unaffected by replacing the surface sheet of hydroxyl groups in kaolinite by deuteroxyl groups. This indicates that there is little coupling between the inner hydroxyl group and the surface groups, presumably because they are oriented nearly perpendicular to each other.

Although the argument presented here shows that the results of infrared studies need not be taken to conflict with those of x-ray investigations on kaolinite, it must be noted that the related minerals, dickite and nacrite (*7*), give hydroxyl absorption patterns surprisingly different from those of kaolinite. Application of the selective deuteration technique of Ledoux and White (*8*) should prove valuable in interpreting these differences.

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References and Notes

1. J. M. Serratosa, A. Hidalgo, J. M. Vinas, *Nature* **195**, 486 (1962); *Proceedings of the International Clay Conference, Stockholm* (Pergamon, New York, 1963), vol. 1, p. 17.
2. R. J. P. Lyon and W. M. Tuddenham, *Nature* **185**, 835 (1960); H. W. van der Marel and J. H. L. Zwiers, *Silicates Ind.* **24**, 359 (1959).
3. R. E. Newnham, *Mineral. Mag.* **32**, 683 (1961).
4. R. G. Wolff, *Am. Mineralogist* **48**, 390 (1963).
5. J. J. Fripiat and F. Toussaint, *J. Phys. Chem.* **67**, 30 (1963).
6. R. L. Ledoux and J. L. White, *Science* **143**, 244 (1964).
7. V. C. Farmer and J. D. Russell, *Spectrochim. Acta* **20**, 1149 (1964).
8. R. L. Ledoux and J. L. White, *Science* **145**, 47 (1964). These authors kindly showed their manuscript before publication.

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Adenovirus-like Particles from Cancers Induced by Adenovirus-12 but Free of Infectious Virus

Abstract. *Particles resembling adenovirus were recovered from tumors induced by adenovirus-12. The particles were similar to incomplete adenovirus in size, subunit morphology, and density. The presence of such incomplete viral units helps to explain why attempts to isolate infectious virus from adenovirus-induced tumors have failed despite the serological evidence that viral elements are present.*

There are three human adenoviruses (types 12, 18, and 7) that induce malignant tumors in laboratory animals (*1*). Such tumors appear to be free of infectious virus, although they may contain high concentrations of viral complement-fixing antigens (*2*). These tumor antigens found by Heubner *et al.* (*2*) are similar to the type-specific antigens produced by adenoviruses during their normal growth in tissue culture cells. In the present study adenovirus-induced tumors were extracted, and the extracts were examined by quantitative electron microscopy (*3*) to determine whether they contained organized viral structures.

Hamsters bearing tumors induced by adenovirus-12 (*4*) were killed by decapitation and exsanguinated as completely as possible. Tumors were removed aseptically, cut into small pieces, and rinsed three times in Eagle's serum-free medium. Small weighed pieces of individual tumors were ground into a thick paste with a mortar and pestle, and measured volumes of Eagle's medium were added slowly during grinding until the concentration of tumor (in suspension) was 20 percent. These homogenates were then frozen and stored at −20°C. When needed they were thawed and allowed to stand for a few minutes until large debris had settled. The turbid supernatant fluids were filtered through thin layers of Celite held by filter paper in Buchner funnels. The resulting filtrates, which showed no visible turbidity, were used for electron microscopy and density-gradient fractionation studies.