sult. Thus the molecular or ion clusters or pseudo-embryos that agglomerate to produce active condensation nuclei seem to be always present in pollution-free air. Since turpentine and iodine are used commercially to polymerize resins, it is likely that their vapors serve in a similar manner to produce condensation nuclei.

I first encountered this phenomenon while studying the reaction of iodine (*3*) with lead particles in the exhaust of internal combustion engines, such as the automobile, when powered with leaded gasoline. After catching a sample of air at our field station in Arizona and checking it for lead content in a supercooled cloud at −20°C by reacting it with iodine vapor, I found that it had only about one ice nucleus per cubic centimeter (which was one to two orders of magnitude lower than usual); I then noticed that a dense supercooled cloud had formed. This was very unusual since, when the concentration of lead particles is even as high as 100 per cubic centimeter, such as is commonly found in eastern New York, the concentration of cloud-forming nuclei is rarely more than 1000 or 2000 per cubic centimeter. With the effect observed in Arizona I found the cloud nucleus concentration to be in excess of 30,000.

Since this discovery, I have checked this effect hundreds of times and find it to be an extremely common reaction. In fact, only in heavily polluted air or in extremely pure air is it absent. It is likely that in polluted air the clusters or pseudo-embryos that cause this reaction have become adsorbed on the Aitken nuclei, which in polluted air generally have concentrations of 50,000 per cubic centimeter or more.

I have attempted to duplicate the effect observed by injecting various types of pure gases into filtered air. Such gases as CO_2, H_2S, NO, N_2O, NH_3, SO_2, CO and Cl_2 have been tried without success. The only gas that seems to have an effect is NO_2. Because of the current interest in gas-to-particle conversion reactions and ion reactions, I believe that this interesting phenomenon should be brought to the attention of the scientific community without further delay (*4*). It might be directly related to a basic and worldwide source of Aitken nuclei.

Thus far, I have not had adequate opportunity to determine if this phenomenon can have an important effect in the free atmosphere. Experiments to date indicate the necessity of exposing the air sample for a few seconds to saturated vapor of iodine or turpentine with a temperature of +10° to +20°C.

There is a good chance that this reaction may be a useful way to measure the degree of cleanliness of the air (as many as 90,000 cloud nuclei per cubic centimeter have been found in very clean air on a mountaintop). Conversely, absence of the effect may denote a particularly potent type of air pollution. A better understanding of this phenomenon may help to elucidate some of the puzzling features of gas-to-particle conversion reactions recently summarized by Mohnen and Lodge (*5*).

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1. Gardner small-particle detector (George Gardner Associates, Schenectady, New York); Rich condensation nuclei detector (Environment-One Corporation, Schenectady, New York).
2. The measurement was made in an upwelling air stream emerging from Government Cave 15 miles northwest of Flagstaff, Arizona. Air apparently passes through an old lava flow and apparently is completely free of all particulates. Air also is slightly supersaturated with respect to water and has a temperature of 37.5°F (3.1°C).
3. V. J. Schaefer, *Science* **154**, 1555 (1966).
4. I made a brief announcement of the "iodine effect" at the Seventh International Conference on Condensation and Ice Nuclei held in Prague, Sept. 1969, but have not published any data.
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6. Research studies conducted under grant No. GA-1112, Atmospheric Sciences Section, National Science Foundation.

20 July 1970

Crystal Structure of Serotonin Picrate, a Donor-Acceptor Complex

Abstract. *The crystal structure of the red picric acid salt of serotonin was determined by x-ray diffraction methods. The structure consists of parallel hydroxyindole and picrate planes which are intimately stacked with an interplanar separation of 3.3 to 3.4 angstroms. The stacking interaction appears to be of the donor-acceptor (charge-transfer) type, involving specific contacts between picrate nitro groups and atoms of the hydroxyindole moieties. Similar interactions might mediate biological processes involving serotonin.*

It has been suggested that many of the physiological properties of serotonin (*1*) might be related to the exceptional electron donor capabilities of the hydroxyindole moiety and the resultant propensity to form donor-acceptor (charge-transfer) complexes with biological electron acceptors (*2*). In support of this possibility, it has been found that, in vitro, serotonin forms donor-acceptor complexes with various electron acceptors, including flavin and nicotinamide derivatives (*3*). We report here the crystal structure of serotonin picrate monohydrate (Fig. 1), which provides structural information about the specific molecular interactions that lead to the formation of a serotonin donor-acceptor complex.

Red crystals of serotonin picrate monohydrate were obtained by slowly cooling a hot aqueous solution containing approximately equimolar amounts of picric acid and serotonin creatinine sulfate. The crystal structure of the complex was determined by single crystal x-ray diffraction methods (*4*).

An outstanding feature of the crystal structure is the vertical stacking association of the approximately parallel picrate and hydroxyindole planes. These planes are stacked in an alternating pattern, forming continuous columns running parallel to the *b* crystallographic axis. Within these columns adjacent picrate and hydroxyindole planes form a dihedral angle of about 6° and are separated by an average interplanar spacing of 3.3 to 3.4 Å. As verified by the determination of hydrogen atom positions, serotonin picrate consists of picrate anions and serotonin cations, with the formal positive charge confined to the ethylamino group of serotonin; thus the stacking interactions involve association of negatively charged picrate ions and uncharged hydroxyindole moieties. Figure 2 shows the two types of stacking patterns in the crystal structure, along with the shortest interatomic distances between adjacent planes. Although no distances are significantly shorter than the sums of the van der Waals radii of the atoms involved, it is noteworthy that several intimate contacts are formed.

In addition to the hydroxyindole-picrate stacking interactions, the crystal structure is stabilized by a hydrogen-bonding scheme which utilizes all of the hydrogen atoms covalently bonded to nitrogen or oxygen atoms. One hy-

drogen bond (from the protonated ethylamino group of serotonin to a nitro oxygen atom of the picrate ion) occurs between adjacent serotonin and picrate ions within the stacked columns. The remaining hydrogen bonds connect the columns and join the water molecules to the picrate and serotonin ions.

The hydroxyindole-picrate stacking interactions (Fig. 2) appear to be of the donor-acceptor type (*5–7*). A donor-acceptor complex receives contributions from a resonance structure arising from the transfer of an electron from the donor group (the hydroxyindole moiety) to the acceptor group (the picrate anion) (*6*). Since serotonin is a good electron donor (*3*) and picric acid a good electron acceptor (*8*), it is not surprising that these compounds would combine to form a donor-acceptor complex. The red color of the serotonin picrate crystals is characteristic of donor-acceptor complexes which picric acid forms with various aromatic electron donors (*8*), and appears to be attributable to charge-transfer absorption bands. The arrangement of picrate and hydroxyindole planes in stacked arrays is similar to the pattern of interaction between donor and acceptor molecules found in the crystal structures of other aromatic donor-acceptor complexes (*7*, *9*).

There are no accurate determinations of serotonin structures with which to compare our results, but the bond lengths and angles within the picrate ion agree with those found in the crystal structures of the ammonium and potassium salts of picric acid (*10*). Therefore, since charge-transfer is expected to affect the bond lengths and angles within the picrate anion [chapter 5 in (*6*)], it is possible that the charge-transfer resonance form makes only minor contributions to the ground state structure. This possibility is supported by several studies which indicate that the ground state stabilities of aromatic donor-acceptor complexes may be due largely to factors other than the contributions of the charge-transfer state (*6*, p. 301; *7*, *9*, *11*). However, regardless of the factors governing the hydroxyindole-picrate stacking interactions, it is significant that the observed geometry is such that charge-transfer electronic transitions apparently can occur and impart color to the crystals.

Similar to the ring-ring interactions found in other crystalline donor-acceptor complexes (*9*), the stacking of hydroxyindole and picrate moieties is

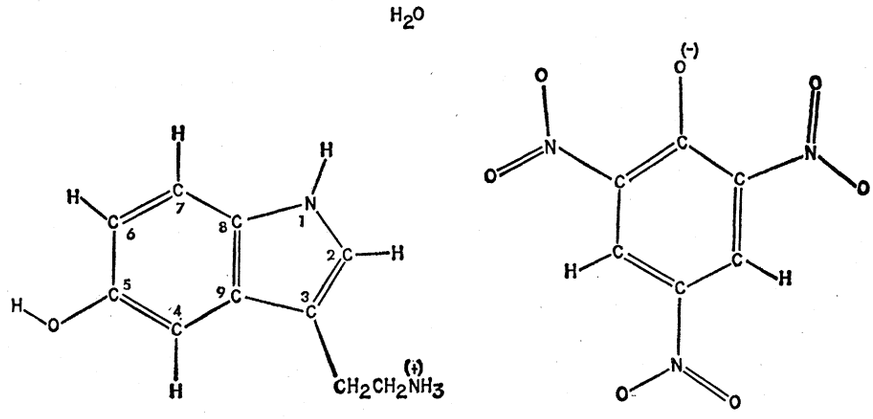

Fig. 1. Structural formula of serotonin picrate monohydrate.

accompanied by short, vertical contacts between specific pairs of atoms. The stacking pattern does not involve extensive ring overlap, but does permit interactions of the picrate ions with several sites of the hydroxyindole moieties (Fig. 2). In particular, the picrate nitro groups participate in the majority of the close contacts shown in Fig. 2. The crystal structure of serotonin picrate is thus consistent with the hypothesis that aromatic donor-acceptor complexes of picric acid are stabilized by specific association of the polar nitro groups with the polarizable pi electron systems of the donor molecules (*7*, *8*).

Several experimental studies have indicated that donor-acceptor complexes of indoles involve interactions of the acceptor molecules with specific sites in the indole ring, rather than with the pi electron system as a whole (*12*); theoretical considerations have implicated atoms C-2 and C-3 of the indole ring as the sites which are most likely to be involved in the formation of donor-acceptor complexes (*13*). A nitro group of the picrate ion does interact with this region of the indole ring; as seen in Fig. 2, a nitro group is sandwiched between two indole rings with the nitro oxygen atoms forming close contacts with atoms C-2 and C-4 of one ring (Fig. 2a), and the nitrogen atom forming a close contact with atom C-3 of the other ring (Fig. 2b). A similar situation occurs in the crystal structures of skatole and indole donor-acceptor complexes with trinitrobenzene, where a nitro group is sandwiched between two indole rings with close contacts between the nitrogen atom of the nitro group and the C-3 carbon atom of the indole rings (*14*).

The crystal structure of serotonin picrate provides additional evidence

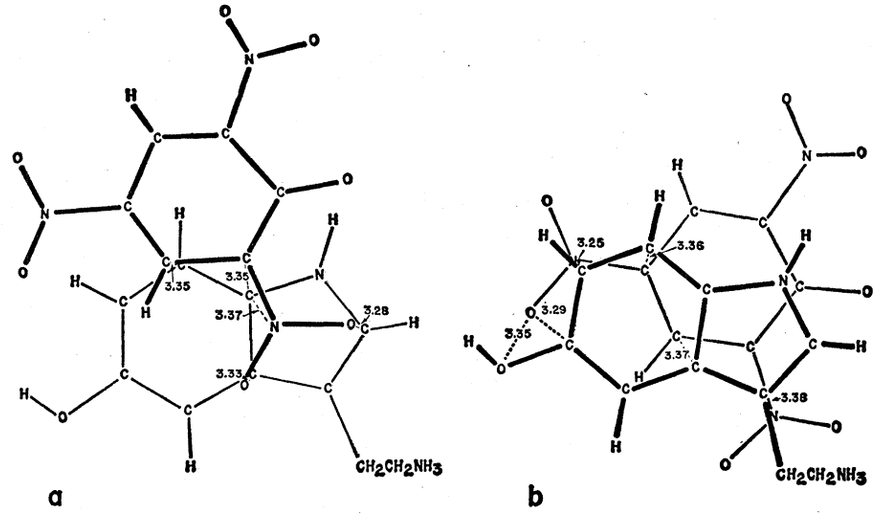

Fig. 2. The two types of stacking interactions as viewed perpendicular to the hydroxyindole plane (the numbers represent interatomic distances, in angstroms, between stacked planes). (a) Picrate anion above hydroxyindole moiety; (b) hydroxyindole moiety above picrate anion.

that the hydroxyindole moiety of serotonin can associate strongly with aromatic systems, and that such association is accompanied by specific interactions of the donor-acceptor type. Considering the evidence that serotonin also forms donor-acceptor complexes with aromatic compounds in solution (*3*), it would not be surprising if interactions similar to those found here occur in biological systems. Associations of this type could conceivably mediate a number of biological processes. Of potential importance are the contributions that such interactions might make to processes involving charge movement. It is also likely that related interactions between serotonin and biological aromatic compounds might lead to the formation of stable complexes, like those which serotonin apparently forms with adenosine triphosphate (*15*) and with certain aromatic coenzymes (*3*).

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4. Serotonin picrate monohydrate crystallizes in the monoclinic space group $P2_1/c$ with four formula units in a cell of dimensions $a = 14.172(3)$ Å, $b = 6.908(2)$ Å, $c = 18.749(3)$ Å, and $\beta = 101.65(2)°$ (measured at 25°C). The crystal density measured by flotation is 1.55 g/cm³; the calculated density is 1.56 g/cm³. With the use of nickel-filtered copper radiation and a scintillation detector, three-dimensional intensity data were collected on a Picker FACS-1 automated diffractometer; measurements were made for all independent reflections in the range $4° \leq 2\theta \leq 128°$. A suitable trial structure was obtained by the symbolic addition procedure [I. L. Karle and J. Karle, *Acta Crystallogr.* **16**, 969 (1963)], and the structure was refined by block-diagonal least-squares methods. The hydrogen atoms were located in a difference Fourier map calculated during the latter stages of refinement. All atomic positional parameters, along with anisotropic temperature parameters for the nonhydrogen atoms and isotropic temperature parameters for the hydrogen atoms, were refined to a final R index $(\Sigma||F_o| - |F_c||/\Sigma|F_o|)$ of 0.073. The final estimated errors in the positional parameters are 0.002 to 0.003 Å for the nonhydrogen atoms and 0.02 to 0.04 Å for the hydrogen atoms.
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20 July 1970; revised 8 September 1970 ■

Transfer of Interferon-Producing Macrophages: New Approach to Viral Chemotherapy

Abstract. *Mice were protected from infection with Semliki Forest virus and encephalomyocarditis virus by the transfer of peritoneal macrophages that were stimulated to produce interferon in vitro by exposure to a nonreplicating virus. This method of therapy was also utilized in animals infected with encephalomyocarditis virus after onset of clinical signs. Of these animals 40 percent recovered, but only 9 percent of the control group recovered.*

The production of interferon is one characteristic of the host response to infection with an intracellular parasite (*1*). Baron (*2*) has reviewed the evidence suggesting that this circulating interferon may be one factor in the control of virus replication in the infected host. Phagocytic cells of the reticuloendothelial system (*3*) and peripheral leukocytes (*4*) may be sources of the interferon in the serum. This concept is supported by evidence that interferon production by macrophages may protect a population of susceptible cells from a viral infection in vitro (*5*) and that when macrophages that have been infected with a nonreplicating virus in vitro have been transferred to mice circulating interferon can be detected in the recipients (*6*). One interpretation of these data is that macrophages have the capacity to produce interferon in vivo and are a source of circulating interferon found in many viral infections.

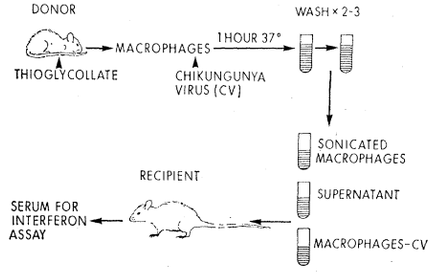

Fig. 1. The macrophage transfer experiment.

Interferon has not yet fulfilled its promise as a practical antiviral substance; and in almost all experiments reported up to now, interferon treatment has required the initiation of therapy prior to, simultaneously with, or immediately after inoculation of the virus. I now report my experiments on the transfer of interferon-producing cells as an approach to therapy of two viral infections (Fig. 1).

Macrophages were harvested from donor C3H mice 5 to 7 days after inoculation with thioglycolate broth, exposed to Chikungunya virus (CV) in vitro for 1 hour, washed to remove unadsorbed virus, divided into equal portions, and inoculated into recipient animals. The control groups for these