cent 3-methylcholanthrene (MCA) in paraffin (*1*) were placed in the subcutaneous tissue of the animals. Two pellets were located anteriorly, one on either side, on the lateral aspect of the thorax. The other two pellets were similarly implanted on either flank posteriorly. The first tumors arose approximately 3 months after the pellet implants. Whenever a tumor arose, the tumor bearer was immediately paired with an animal that had not yet developed a tumor. The tumor and pellet and the corresponding pellet in the control animal were then excised. (Tumors were approximately 5 mm in average diameter at the time of excision.) The pair was then observed for development of subsequent tumors at the remaining pellet sites. Two series of such experiments were done.

In addition, the same type of experiment was repeated twice, but a variety of MCA-induced tumors, transplanted at a pellet site shortly after pellet implantation, was substituted for the primary tumors of the previous experiments. Each tumor implant was by trocar and was adjacent to the right anterior pellet.

The first two experiments (Table 1) show that an animal in which a primary tumor had arisen earlier was an animal of significantly increased susceptibility, that is, the average such animal developed a tumor adjacent to one of the three remaining MCA pellets before the paired control. In contrast, animals in which a tumor transplant had been excised were not significantly more susceptible to induced tumor formation than were the control animals not previously exposed to tumor. The difference between the first and second pairs of experiments approached statistical significance as judged by the Mann-Whitney U test ($P = .07$). This suggests that at least a part of the increased susceptibility to tumor formation in the mice that had developed an early primary tumor was probably not a result of tumor growth per se.

Regardless of the statistical probabilities, this conclusion cannot be reached without considerable reservation. The physiological effects of the growth of a tumor transplant on oncogenesis, especially early in the course of tumor formation, might differ from the effects of the later growth of a primary tumor. Furthermore, differences in induced immunity probably exist between an autochthonous, untransplanted tumor and a syngeneic implant. However, these immunological differences are unlikely to have affected the results because independently induced MCA tumors produce, after their excision, an immunity that is not cross reactive (*2, 3*).

Although the possible role of the first tumor in altering the susceptibility of the animal to oncogenesis remains uncertain, the data do suggest that the animals had varied susceptibility prior to the initial tumor formation.

This variability was presumably of nongenetic origin since the F_1 mice were derived from highly inbred strains. Inbred animals are different in a variety of epigenetic ways, such as litter seriation, size of litter, location of the fetus in the uterus, age of parents, weight, and so on. Any one (or more) of these might correlate, for unknown reasons, with tumor susceptibility. Furthermore, and perhaps in relation to some of these epigenetic sources of variation, mice of an inbred strain can vary in their immune responses to certain antigens. It may be that the variability in tumor susceptibility was related to an underlying variability in the immune system; this is a reasonable hypothesis that can be examined experimentally.

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Deoxycorticosterone-Adenine Interactions in a Crystalline Complex

Abstract. *Deoxycorticosterone-adenine monohydrate is the first complex involving a steroid and a component of DNA to be successfully crystallized and studied by single crystal x-ray analysis. Hydrogen bonds between O(20) and N(6) as well as O(21) and N(1) connect the corticoid side chain to an adenine molecule. The molecules are also packed such that a second adenine moiety is situated over the Δ^4-3-one region of the steroid. These observations of the solid state suggest ways in which steroids and nucleic acids may interact in vivo.*

The biological effects of steroid hormones result from modification of the rate of protein synthesis in target tissues. After entering the target cell, the steroid binds to a cytoplasmic receptor protein. The hormone-receptor complex then moves to the nucleus, where it binds to a specific acceptor site on the genome and induces the appearance of RNA species absent from the unactivated cell. The mechanism of this binding is unknown, and the role of the steroid hormone may simply be the induction of a conformational change in the receptor protein which allows the protein to bind to chromatin. The steroid-induced RNA is transported to the cytoplasm, where it directs the synthesis of the proteins that are responsible for the characteristic changes associated with hormone administration (*1*). This sequence of events has been demonstrated to be very similar for the estrogens (*2*), androgens (*3*), progesterone (*4*), and the corticoids (*5*).

Although there is no evidence that direct interaction of DNA with steroid molecules precedes the appearance of steroid-induced species of RNA, steroids have been shown to bind to purified native and denatured DNA and to protect the DNA secondary structure from thermal denaturation (*6*).

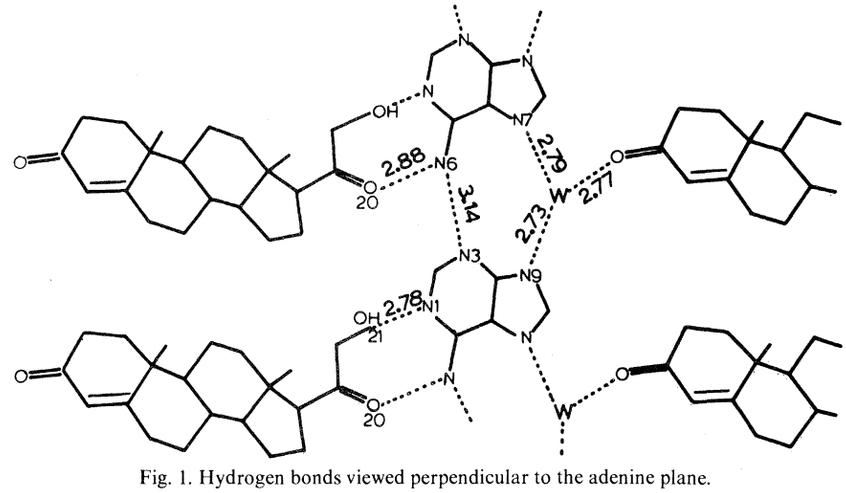

Fig. 1. Hydrogen bonds viewed perpendicular to the adenine plane.

Hydrogen bonds and hydrophobic forces have both been implicated in these steroid–nucleic acid interactions. We report here the results of single crystal x-ray analysis of deoxycorticosterone (DOC)-adenine monohydrate. The steroid-adenine interactions observed in this structure may provide models for the way steroids bind to nucleic acids.

Deoxycorticosterone-adenine (1 : 1) monohydrate crystallizes in the orthorhombic space group $P2_12_12_1$ with four steroid and four adenine molecules in a unit cell having the dimensions $a = 17.147_3$ Å, $b = 20.311_4$ Å, and $c = 7.170_1$ Å. The crystals were grown by slow evaporation of an aqueous (50 percent) pyridine solution. The intensities for 2836 reflections having $2\theta < 145°$ were measured using a GE XRD-5 diffractometer with Cu$K\alpha$ radiation monochromatized by balanced nickel and cobalt filters. The structure was solved by direct methods (*7*) and refined by full-matrix least squares. The final reliability index was 12.3 percent for all data.

The hydrogen bonding network is illustrated in Fig. 1. Pairs of DOC and adenine molecules are formed by hydrogen bonds between atoms O(20) and O(21) in the 17β–side chain of the steroid and atoms N(6) and N(1) in the adenine moiety. Additional hydrogen bonds involving water molecules link steroid-adenine pairs in adjacent unit cells to form chains parallel to the *b*-axis and layers perpendicular to the *a*-axis. A weak hydrogen bond between N(6) and N(3) also occurs in these layers.

Crystallographic studies of almost two dozen corticosteroids (*8*) have shown that the orientation of the 17β–side chain is remarkably constant despite wide variation in the number, orientation, and strength of hydrogen bonds involving the side chain oxygens. The usual orientation is observed in the DOC-adenine complex. As shown in Fig. 2, the side chain is oriented over the D-ring with the C(16)-C(17) bond nearly eclipsing the C(20)-O(20) bond. The C(16)-C(17)-C(20)-O(20) torsion angle of –10° agrees closely with the value of –11° observed in the uncomplexed structure of DOC (*9*). Atoms O(20) and O(21) are *cis* coplanar in all corticoid structures which have been examined, and the O(20)-C(20)-C(21)-O(21) torsion angle is 10° in the adenine complex. A least-squares plane through the four nonhydrogen atoms in the side chain is inclined at 37° with respect to the plane of the adenine moiety to which it is connected by hydrogen bonds.

In most nucleoside and nucleotide crystals, the bases form stacks (*10*) in which the interplanar distances between the bases range from 3.25 to 3.45 Å. In the DOC-adenine complex, the adenine molecules are not stacked. Instead, each adenine is

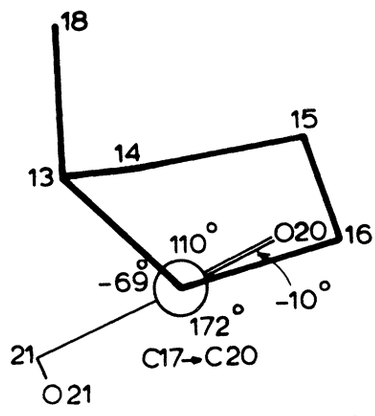

Fig. 2 (left). Newman projection with torsion angles about the C(17-C(20) bond.

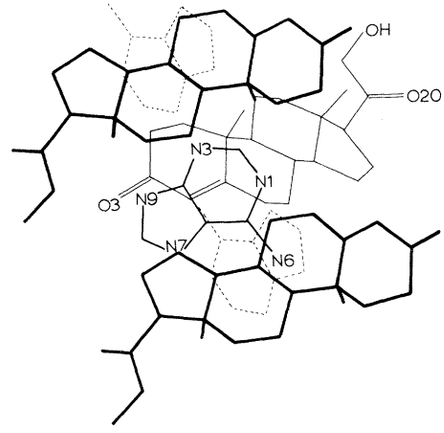

Fig. 3 (right). Stacking of adenine and the Δ^4-3-one region of DOC.

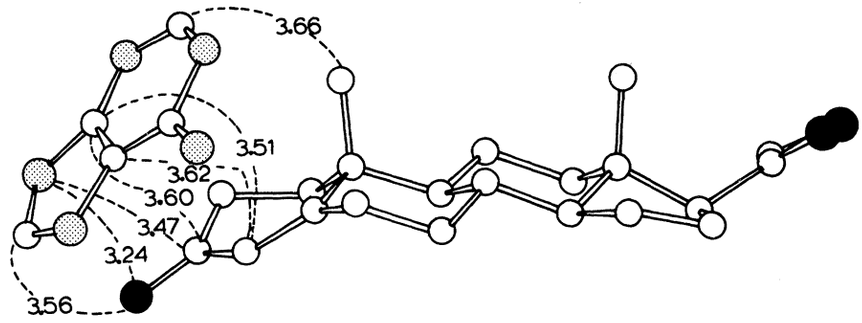

Fig. 4. Intermolecular contacts < 3.7 Å between stacked steroid and adenine molecules.

situated above the unsaturated A-ring of a steroid molecule, as illustrated in Fig. 3. Figure 3 also shows that the adjacent steroid and adenine molecules in the stack are displaced so that there is minimal overlap with the stacked DOC-adenine pair. The average distance of the atoms C(3), C(4), C(5), and O(3) comprising the conjugated system from the plane of the adjacent adenine is 3.47 Å, and the plane of these four atoms is inclined at 16° with respect to the plane of the adenine. Intermolecular contacts less than 3.7 Å in the steroid-adenine stacks are labeled in Fig. 4.

In conclusion, two kinds of interaction, hydrogen bonds and steroid-base stacking, have been observed in DOC-adenine monohydrate. Although the nature of the interactions between the hormone-receptor complex and the DNA molecule is unclear, the mere fact that a stable DOC-adenine crystalline complex can be formed is interesting and possibly significant. It seems unlikely that the hydrogen bond pairing between the corticoid side chain and the adenine observed here could be a biologically significant initial reaction between a corticosteroid and DNA since the adenine atoms involved in these hydrogen bonds normally participate in Watson-Crick base pairing. However, this corticoid-base pairing could stabilize a DNA conformation in which a break has already been made. On the other hand, the fact that adenine stacks over the unsaturated DOC A-ring, in preference to forming stacks of adenine molecules alone, indicates that it may not be unreasonable to regard the Δ^4-3-one A-rings of progesterone and the corticoids as potential intercalators.

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