greatly enhances *early* significant labeling (five or more grains per cell). This effect can be further improved by emulsion exposure at low temperatures (–85°C was optimal in our system) (Table 1). Previously, such experiments have required prolonged exposure times (for example, 6 to 8 months); with our technique results can be obtained in 14 days or less. There are additional potential applications in thin-layer chromatography, paper chromatography, and electron microscopy (*10, 12*).

Because HSARG allows careful analysis of mixed cell populations it has an advantage over other methods of rapid cell cycle analysis (*13*). With Giemsa staining, labeling can be related to morphology, and DNA synthesis rates can also be assessed (*14*). (An example of labeling in a mixed cell population is shown in Fig. 1. Note extremely low background.)

Thus, HSARG provides a rapid, reliable technique which should find wide clinical and research application.

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6. The combination of PPO, dimethyl-POPOP, and dioxane was selected after several other combinations were tested. Scintillator could not be premixed with emulsion because this resulted in an opaque gel. Addition of naphthalene (10 g/liter) to the scintillator resulted in significant improvement in the rate of grain development. However, a dense precipitate formed on the emulsion. This precipitate could be removed during processing by dipping the slides into methanol or toluene after emulsion development. If energy emission is very low, this can be of use. Tritiated thymidine was obtained from New England Nuclear Corp., Boston, Mass., NET-027Z; specific activity, 40 to 60 c/mmole. Shandon cytocentrifuge, Shandon Southern Instruments, Sewickley, Pa. $NTB_3$ nuclear track emulsion (obtained from Eastman Kodak Co., Rochester, N.Y.) is mixed with an equal volume of distilled water and 1.0 ml of Tween 80. PPO and dimethyl-POPOP were obtained from Amersham/Searle, Arlington Heights, Ill. Dioxane (stabilized $C_4H_8O_2$) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Kodak D-19 developer, catalog No. 146-4593; Kodak fixer, catalog No. 197-1746 (Eastman Kodak Co.).
7. The Giemsa stain must be carefully filtered and the *p*H of the citrate buffers checked. We use *p*H 5.75 for 30 seconds followed by *p*H 5.40 for 30 seconds. Giemsa blood stain was obtained from Fisher Scientific Co., G-146; 734319.
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## Nongenetic Variability in Susceptibility to Oncogenesis

Abstract. *Genetically homogeneous mice varied in susceptibility to tumor induction by 3-methylcholanthrene. The early appearance of an induced tumor identified an animal of relatively great susceptibility to tumor induction as compared to other animals of the same genotype.*

Although inbreeding leads to genetic uniformity, it may sometimes result in phenotypic diversity. For example, among inbred multiparous BALB/c mice that carry the mammary tumor virus, approximately half of the animals exhibit the mammary tumor character; the other half do not. Most tumor systems show a similar, though often not so striking, degree of diversity. When a carcinogen is given to inbred mice, especially at near-threshold dosage, there is usually great variation among the animals in how fast they develop tumor and even in whether or not they develop tumor at all. The usual explanation of such diversity in genetically uniform animals is that the genotype is such that the character in question is close to the threshold of penetration. Uncontrollable, minor environmental variables can then determine the presence or absence of the phenotypic character.

In the case of the character, tumor, some of the diversity that is found among genetically uniform, inbred animals might be due to phenotypic variations that affect their susceptibility to tumor induction. Alternatively, tumors are often considered to result from random somatic mutations. Furthermore, there are of necessity small, uncontrolled variations in carcinogen application and the like. Thus, the presence or absence of tumor among genetically identical animals might reflect chance events associated with transformation rather than a predisposing phenotypic variation. The two explanations are not mutually exclusive.

I have attempted to answer the question of whether or not, in an induced tumor system, genetically uniform animals are equally at risk or, alternatively, whether some are more susceptible to tumor induction than others. The relative susceptibility of such animals can be tested, in principle, by determining whether or not multiple primary tumors are more frequent than would occur by chance. However, if multiple tumors are more frequent, the increased susceptibility might be the result rather than the cause of the first tumor. In other words, it would not be clear whether a relative hypersusceptibility was present prior to the appearance of the first tumor or had been caused in some way by that tumor. It is well known that tumor growth can alter mammalian physiology and perhaps influence future oncogenesis. In my work, the possible physiological alterations that might be caused by tumor growth per se were controlled by the use of transplanted tumors.

The mice used were (C57BL/6JNIcr × BALB/cAnNIcr) $F_1$ hybrid females approximately 2 months old. The animals were produced in the animal production facility of the Institute for Cancer Research (Fox Chase, Philadelphia); the parental strains are maintained by vigorous inbreeding and are routinely monitored for homozygosity by syngeneic skin grafting. Four pellets per mouse of 0.5 per-

Table 1. Number of pairs in which a tumor arose first at a remaining pellet in an experimental (tumor and pellet excision) or a control (pellet excision) animal.

| Experiment | Origin of first tumor | Tumor and pellet excision animal | Pellet excision animal | *P* value (sign test) |
|---|---|---|---|---|
| 1 | Pellet induced | 24 | 7 | .002 |
| 2 | Pellet induced | 19 | 7 | .014 |
| Average latent period* | | 23.6 ± 12.7 | 34 ± 23.2 | |
| 3 | Trocar implantation | 18 | 9 | .061 |
| 4 | Trocar implantation | 8 | 10 | |
| Average latent period* | | 88.8 ± 19.9 | 90.26 ± 18.1 | |

*Latent period is the interval in days between excision of the first tumor and of the pellet and the subsequent appearance of a tumor at one of the remaining pellets.

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 $\Delta^4$-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*).

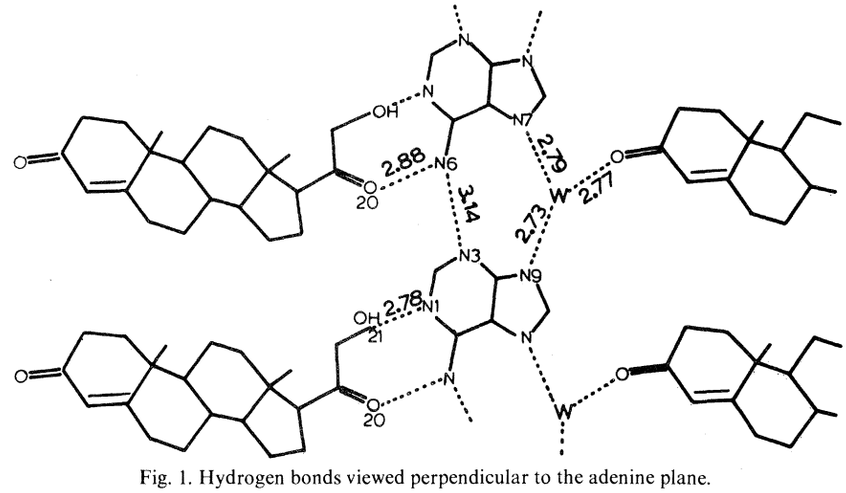


Fig. 1. Hydrogen bonds viewed perpendicular to the adenine plane.