That such cycle responses are probably genetic and not derived from cycled conditioning during ontogeny is established by the noncyclic nature of the temperature of the hatchery water, which comes from a depth of 27.5 m in Lake Whatcom. It is of further interest that adults of this variety live in the more stenothermal, less cyclic marine environments, while only the young are normally obligated to the cyclic stream temperatures (*15*).

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Inhibition by 5-Iododeoxyuridine of the Oncogenic Effects of Adenovirus Type 12 in Hamsters

Abstract. *Subcutaneous tumors induced in newborn hamsters by type 12 adenovirus were suppressed when 0.5 mg of 5-iodo-2′-deoxyuridine (IUdR) was given at the same subcutaneous site as the virus. Although one injection of IUdR given immediately after the virus was effective, additional injections on subsequent days reduced further the number of hamsters developing tumors. These effects of IUdR are especially interesting since replication of infectious adenovirus 12 cannot be demonstrated in the hamsters at any time before or after tumor development.*

The suppressive effects of 5-halogenated pyrimidine deoxyribonucleosides on replication of DNA viruses (*1–4*) suggested that they might also influence induction of neoplasia by certain oncogenic DNA viruses, such as adenovirus types 12 and 18 (*5–8*). The activity of 5-iodo-2′-deoxyuridine on herpes simplex and vaccinia viruses both in vivo (*1, 2*) and in vitro (*2, 3*), and the effect on adenovirus multiplication in vitro (*4*) made this drug a logical choice for the studies reported herein in which a strain of type 12 adenovirus was used that leads to the induction of tumors in newborn hamsters with greater regularity than does type 18 (*6, 7, 9*). The tumors produced by adenoviruses in hamsters are of particular interest, since no infectious virus, but only incomplete replication of virus, can be demonstrated in those hamsters that develop neoplasms (*8*).

We used a pool of human adenovirus type 12 which contained approximately 10^7 $TCID_{50}$'s (tissue culture infectious doses 50 percent effective, per 0.1 ml). This was derived from the prototype strain of adenovirus 12 supplied by us to the Viral and Rickettsial Registry of the American Type Culture Collection. Virus (0.04 ml, undiluted) was inoculated intraperitoneally or subcutaneously, as described previously (*8*). Hamsters in the last stages of pregnancy were supplied from the colony of the National Institutes of Health, and were held in isolated quarters. Two or three litters, containing approximately eight young each, were used routinely for each test group; they were injected with virus within 24 hours after birth (*6*). All hamsters were inspected daily for tumors, illnesses, and deaths. Only those hamsters surviving at the time of first occurrence of tumors in the experiment (aproximately 30 days) were included for tabulation of results.

The 5-iodo-2′-deoxyuridine (IUdR) was dissolved in twice-distilled sterile water at a concentration of 5.0 mg per 1.0 ml; after passing it through a Millipore filter it was then maintained at room temperature until use. Each dose that was inoculated consisted of 0.1 ml of solution (0.5 mg of IUdR).

We conducted two separate experiments. In the initial experiment, five groups of hamsters (two litters per group) were subjected to different regimens of virus and IUdR inoculations. Two groups were given virus subcutaneously and three, intraperitoneally. In the second experiment, four test groups (two litters each) were given virus subcutaneously and one group, intraperitoneally. In each experiment we included control hamsters which received only the virus.

No definite evidence of toxicity attributable to IUdR was noted in the newborn hamsters, except possibly when multiple injections of the drug were given intraperitoneally. In one experiment, 13 out of 16 hamsters given a single injection of virus and repeated doses of IUdR by this route on alternate days died before the onset of grossly observable tumors; however, in another experiment done in the same way, the majority survived this period of therapy. The overall 30-day survival rates of the groups given IUdR subcutaneously compared favorably with untreated, but virus-injected, groups, and with our general experience with uninjected controls. However, in the one litter which was given virus subcutaneously and multiple subcutaneous injections of IUdR, in the second experiment, there was high mortality during the pre-tumor period.

Tumors produced by intraperitoneal injections were difficult to evaluate for a number of reasons. The presence of multiple small tumors, or even single tumors with a diameter as great as

Table 1. The effect of 5-iodo-2-deoxyuridine (IUdR) on the induction of subcutaneous tumors in hamsters.

Injections of IUdR	No. in test	No. with tumors *	No. dead with tumors *
Virus (SC)† plus IUdR (SC)			
Immediately, plus 9 or 10 additional doses	37	8	5
One dose immediately, or 2 hours later	34	14	10
24 hours later, plus 9 additional doses	11	6	5
Control			
None	28	26	15

* 90-day period of observation. †Subcutaneous.

1.0 cm, could not be determined except at autopsy; furthermore, the more friable intraperitoneal tumors were subject to massive hemorrhages that resulted in early death, an event frequently followed by cannibalism by the mother or cage mates (*6*). The possibly greater toxicity of IUdR given intraperitoneally also complicated the evaluation of data. Consequently, accurate objective evidence of tumor inhibition by IUdR was not obtainable with the groups injected intraperitoneally.

More objective and definitive effects were obtained in those groups given both virus and IUdR subcutaneously. Here, the survival rates were much better than those observed with intraperitoneal injections of virus, and the time of onset of tumors could be determined with considerably greater precision.

In the first experiment (Fig. 1), all 13 of the untreated control hamsters developed tumors within 90 days; of these animals, seven died with large tumors. This rate of tumor induction was somewhat higher than that ordinarily produced by the virus pool used in these experiments. The overall occurrence of subcutaneous tumors with this pool during the same period of observations was approximately 85 percent.

Figure 1 shows the results of two test groups given virus and multiple doses of 0.5 mg of IUdR subcutaneously, when the drug was given immediately after the virus and on alternate days (ten times) during the following 20 days. In one group the drug was given subcutaneously at the same site as the virus, while in the other group, the subcutaneous site was on the opposite side of the hamster. The incidence of tumors was reduced to 2 out of 15 (13.3 percent), and to 4 out of 14 (28.6 percent), respectively; in both test groups, only 4 of the 29 hamsters died, all of them after 60 days.

A similar test group was included in the second experiment (Fig. 2). Of eight hamsters given IUdR at the same site immediately after virus, and nine additional doses during the following 15 days, only two developed tumors; both tumors were first observed after 60 days, and only one animal died within the 90-day period. In the control group, 13 out of 15 hamsters developed tumors, 11 of them within 60 days. Eight of the hamsters with tumors died within 90 days, and two

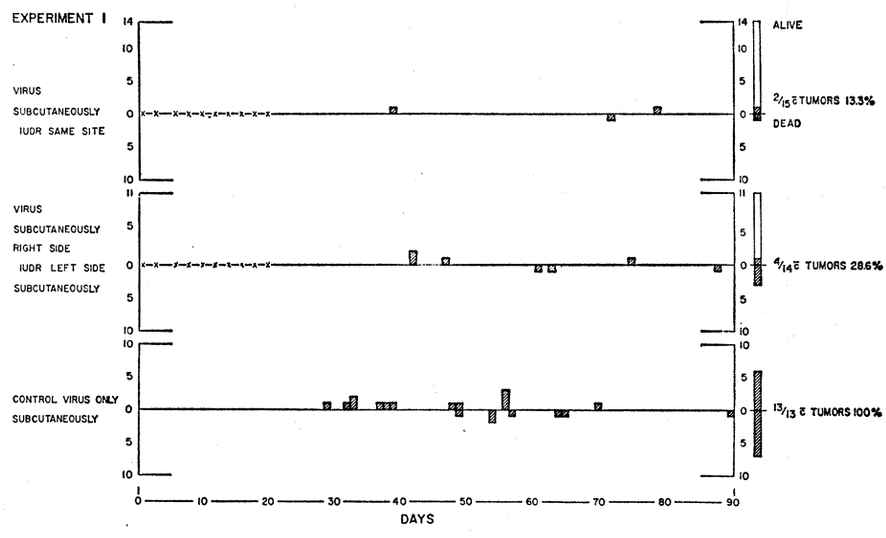

Fig. 1. Tumor development in hamsters given adenovirus type 12 subcutaneously: the effect of subcutaneous injections of IUdR given immediately after the virus and on 10 alternate days during the following 20 days. [0.4 ml of prototype adenovirus 12 suspension (containing approximately 10^7 $TCID_{50}$'s per 0.1 ml) was injected subcutaneously on day-zero]. All hamsters were checked daily for tumors; bars represent onset of palpable tumor. The IUdR (0.5 mg in 0.1 ml) was given subcutaneously as specified (x = days on which IUdR was administered after initial inoculation).

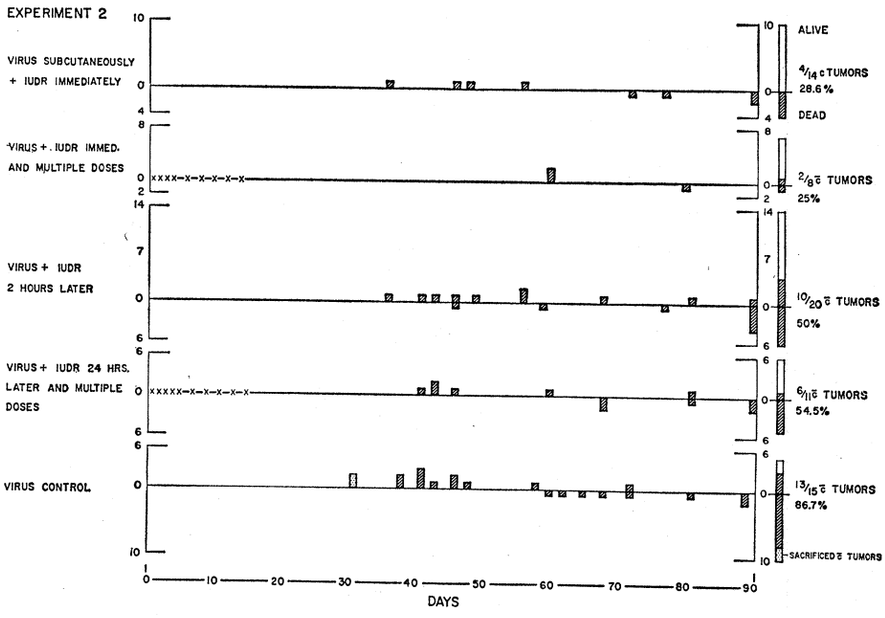

Fig. 2. Tumor development in hamsters given adenovirus type 12: the effect of IUdR when given immediately after the virus or at varying intervals during the following days. Further details are described in the legend of Fig. 1.

of the hamsters with large tumors were killed and subjected to autopsy at 40 days.

Three other groups were given different regimens of treatment with IUdR; all of them were given the virus and drug subcutaneously at the same site. Fourteen hamsters were given only one dose (0.5 mg) of IUdR immediately after virus; of these, only four developed tumors. Of 20 hamsters given a single dose of IUdR 2 hours after virus, ten developed tumors; and of 11 hamsters given the first dose of IUdR 24 hours after virus, plus nine subsequent doses, six developed tumors.

Table 1 summarizes the data in Figs. 1 and 2. It is apparent that suppression of the induction of tumors was achieved when IUdR was given at the same site immediately after the injection of virus,

especially when nine or ten succeeding doses of the drug were given on alternate days. Even a single dose of IUdR, however, when given at the same site either immediately after the virus or 2 hours later, provided definite, though possibly somewhat less, protection. The effect of multiple injections of IUdR, when initiated 24 hours after the injection of virus, was difficult to evaluate because of the small number of hamsters that survived for 30 days in this group.

In both experiments, virtually every hamsters which developed tumors, despite treatment with IUdR, also developed complement-fixing antibodies to adenovirus type 12 viral antigen, which were similar in reactivity and titer to those described previously (*7, 8*). Those which did not develop tumors did not develop complement-fixing antibodies.

In previous reports (*7, 8*), we concluded that the specific complement-fixing "viral" antigens in adenovirus 12 induced hamster tumors, and the total absence of infectious virus could best be explained by the incorporation of genetic material from the viral genome into the genetic apparatus of tumor cells (*8*).

In the hamsters which developed tumors despite administration of IUdR, we therefore conclude that IUdR did not interfere with the transfer of viral genetic material, with its oncogenic activity or with its expression in the form of specific adenovirus antigens.

On the other hand, in those hamsters in which tumors were suppressed, it is possible to suppose that either IUdR prevented effective incorporation of viral DNA into the genomes of the cells which, in the absence of drug, would have been transformed, or that following incorporation into the cell genome, the oncogenic effect of the viral DNA was somehow rendered impotent.

Possible action of the drug on the tumor cells induced by virus cannot be ruled out; however, the preventive effect of a single dose of the drug given simultaneously or 2 hours after injection of virus makes this an unlikely mode of action, a conclusion supported by the studies already cited (*2–4*) concerning the mode of action by IUdR and related compounds on the replication of DNA viruses.

It is clear that IUdR can suppress the formation of tumors by adenovirus type 12 tumors in the newborn hamster, provided the drug is injected within 2 hours after the virus and in either the same or another subcutaneous site. One experiment in which the initial injection of IUdR was delayed until 24 hours after the injection of virus gave equivocal evidence of the inhibition of tumor development. It was also clear, however, that despite multiple injections of the drug, some of the tumors grew to sizes large enough within the 90-day observation period to kill the hamsters, and that hamsters with tumors developed typical complement-fixing antibodies to adenovirus 12 antigens.

These preliminary studies provide only qualitative data concerning the inhibitory action of IUdR on the development of these virus-induced neoplasms. Since replication of infectious adenovirus 12 has not been demonstrated in hamsters with or without tumors (*10, 11*), any effects of IUdR on this phenomenon could not be measured. Additional studies of the suppression by IUdR of the development of tumors initiated by adenovirus 12 may provide opportunities for gaining insight into the mechanism of adenoviral oncogenesis; at the same time a new means of evaluating the action of 5-halogenated pyrimidine deoxyribonucleosides on the differing host cell relationships exhibited by DNA viruses may be afforded.

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Abnormal Myoglobin Ultraviolet Spectrum in Duchenne Type of Progressive Muscular Dystrophy

Abstract. *Met-myoglobin isolated from gluteal muscle of cases with Duchenne type of progressive muscular dystrophy showed an abnormal ultraviolet spectrum. The maximum of the spectrum at pH 7.0 was at 275 mμ in contrast to that at 281 mμ in normal met-myoglobin. Such an abnormality was not found in the limb-girdle type of dystrophy and in progressive spinal muscular atrophy. The results indicate the presence of an abnormal myoglobin in the Duchenne type of progressive muscular dystrophy.*

In 1962 Whorton *et al.* (*1*) reported that the myoglobin from cases of progressive muscular dystrophy (PMD), differed from normal controls in the visible absorption spectrum. Their results did not seem indisputable, however, because the optical density of their sample was too low (*2*). Perkoff *et al.* (*3*) analyzed the myoglobin from skeletal muscle by chromatography on a column containing diethylaminoethyl cellulose and said that the F_3, one of the three major components of normal myoglobin, preponderated in muscle samples obtained from two cases of childhood dystrophy.

We have been studying the etiology of PMD and have assumed that it might be a "myoglobinopathy" similar to a hemoglobinopathy. We have found that the ultraviolet spectrum of some types of PMD myoglobin definitely differs from that of normal myoglobin.

Ten cases of PMD, five cases each of the Duchenne and limb-girdle types, were examined. As controls we used muscle obtained at autopsy from cadavers showing no signs of muscular disease, and muscle obtained by biopsy from one patient with progressive spinal muscular atrophy. The met-myoglobin was isolated from the gluteal muscle of these subjects according to the procedure of Theorell and de Duve, modified by Singer *et al.* (*4*).

In a paper electrophoresis at *p*H 8.6, no obvious differences were found in the mobility of met-myoglobin from patients with PMD and that from controls.

Spectrophotometry in the visible region also revealed no recognizable differences in the absorption curves between the met-myoglobin from patients