also consistent with our hypothesis. X-rays or γ-rays, delivered at high dose rates in total doses above 600 rad, produce a "therapeutic" effect on the incidence of lymphomas in LAf_1 mice (*4*, 6), the incidence falling considerably below the 20- to 30-percent incidence in unirradiated controls. Therefore, with total doses of 618 to 1335 rad, lymphomas were more frequent after γ-irradiation at low dose rates than after x-irradiation at high dose rates, despite the fact that, as with the liver, chromosome aberrations in the hematopoietic tissues were more frequent after x-irradiation at the high dose rate (*1*, *11*).

Even if such an explanation of the carcinogenic effects of irradiating mice at low dose rates is accepted, there remains the question of why only hepatomas and ovarian adenomas were increased in frequency in this study; gastrointestinal tumors, renal tumors, and miscellaneous malignancies, though uncommon in both groups, appeared to be even less frequent than in earlier experiments in which high dose rates were used (*2–5*). The fact that induction of ovarian adenomas by radiation involves an indirect mechanism, hormonal imbalance (*12*), may be significant. Since the incidence of hepatomas in unirradiated mice is markedly influenced by hypophysectomy (*13*) and by castration in males (*14*), it is conceivable that hormonal imbalance was also a factor in development of the hepatomas in this study. Perhaps irradiation at low dose rates is more effective in producing such imbalance than are comparable total doses delivered at high dose rates; this possibility remains to be investigated.

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Coxsackie A9 Virus: Mutation from Drug Dependence to Drug Independence

Abstract. *During the multiplication of Coxsackie A9 virus dependent on 2 - (α - hydroxybenzyl) - benzimidazole there arise some drug-independent virus particles, which are either drug-resistant or drug-sensitive. We studied the properties of such revertant particles under conditions which did not favor the selection of resistant over sensitive virus particles. These particles exhibited a wide range of responses to 2-(α-hydroxybenzyl)-benzimidazole, from high sensitivity to high resistance. Subclones showed the same degree of sensitivity or resistance as the respective parent clones.*

Mutants of Coxsackie A9 virus which require 2-(α-hydroxybenzyl)-benzimidazole (HBB) for multiplication give rise among their progeny to some HBB-resistant or HBB-sensitive virus particles (*1*). We have now determined the proportions of the drug-resistant and drug-sensitive revertant particles and their degree of resistance or sensitivity. Previous work provided only an estimate of the relative proportions of resistant and sensitive revertant particles, and HBB may have selectively favored the multiplication of drug-resistant particles by inhibiting the multiplication of drug-sensitive revertants. This difficulty can be overcome since unsubstituted benzimidazole permits optimal multiplication of HBB-dependent Coxsackie A9 virus at concentrations which do not inhibit the reproduction of sensitive particles (*1*).

The HBB-dependent Coxsackie A9 virus was plated in the presence of 1 m*M* unsubstituted benzimidazole in the overlay medium (Fig. 1) on monolayer cultures of monkey kidney cells (*2*). The infectivity titer was 2.6×10^8 plaque-forming units (PFU) per milliliter as compared to a titer of 1.7×10^8 PFU per milliliter with 0.1 m*M* HBB in the overlay. Three plaques that developed in the presence of unsubstituted benzimidazole and at a high dilution of virus were picked and suspended individually in 1 ml of phosphate-buffered saline (*p*H 7.2). The plaques contained 0.9×10^6, 1.3×10^6, and 1.1×10^6 PFU of HBB-dependent virus and 2.8×10^3, 2.9×10^3, and 5.7×10^3 PFU of HBB-independent virus, respectively, as determined by titration in the presence or absence of 0.1 m*M* HBB. These results indicate a high probability that each plaque had been initiated by a single HBB-dependent virus particle. To isolate HBB-independent revertants, virus from plaques 1, 2, and 3 was plated in the absence of the compound. The developing plaques, now initiated by drug-independent virus particles, were picked at random and subjected to one passage in monkey kindey tube cultures in the absence of the compound to eliminate residual HBB-dependent virus which might have contaminated the drug-independent clones. A total of 79 clones of drug-independent virus, derived from the HBB-dependent virus contained in plaques 1, 2, and 3, were thus prepared.

The HBB sensitivity of the drug-independent clones was determined by plaque titrations without and with 0.1 m*M* HBB in the agar overlay. Sensitivity to HBB was expressed as the ratio of the number of plaques formed in the absence of the compound to the number formed in its presence. Under the experimental conditions, clones of HBB-sensitive Coxsackie A9 stock virus gave ratios in the range from 1×10^3 to 8×10^3. The virus resistant to 0.1 m*M* HBB gives a ratio of 1. The procedure, though adequate, has limitations in that only plaque number, and not plaque size, is taken into account, and testing is confined to one concentration of HBB.

Tests of the 79 drug-independent clones revealed many degrees of sensitivity to HBB. At one extreme, the most sensitive clone showed a ratio as high as 1.7×10^5, whereas at the other extreme, some clones were completely resistant to 0.1 m*M* HBB.

Table 1. Sensitivity to 2-(α-hydroxybenzyl)-benzimidazole (HBB) of HBB-independent mutant clones derived from three HBB-dependent parent clones.

Parent clones (HBB-dependent)	No. of mutant clones* (HBB-independent)		
	Sensitive	Intermediate	Resistant
Plaque 1	4	7	9
Plaque 2	11	9	12
Plaque 3	10	10	7
Total	25	26	28

* HBB sensitivity is expressed as the ratio of the number of plaque-forming units (PFU) obtained without HBB in the overlay to the number of PFU with 0.1 m*M* HBB in the overlay. Sensitive clones, ratios $\geq .4 \times 10^{3}$; intermediate clones, ratios from 3.9×10^{3} to 2; resistant clones, ratios from 1.9 to 1.

Table 1 lists the distribution of the HBB sensitivities of the HBB-independent clones, designated as "sensitive," "intermediate," or "resistant." The grouping is arbitrary; each group is composed of clones exhibiting many sensitivities, so that there is a more or less continuous series of variants, each with characteristic sensitivity. Similarly, the resistant group comprises clones with differing degrees of resistance.

The genetic stability of the revertant virus particles was also investigated. Five clones of HBB-independent particles, grouped either as intermediate or resistant, were cultured in the absence of the compound to obtain 10 to 18 subclones of each group, and these subclones were then tested for sensitivity to the drug. Without exception all subclones exhibited the same HBB sensitivity as the respective parent clones. This indicates that HBB-independent revertant particles are not highly unstable genetically. Thus, the majority of the HBB-independent clones is, in all likelihood, derived directly from HBB-dependent virus.

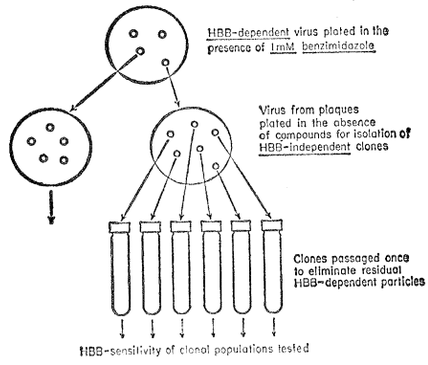

Fig. 1. Isolation of HBB-independent virus particles from HBB-dependent Coxsackie A9 virus in a nonselective system.

Although it is not possible on the basis of the present data to determine precisely the mutation frequency from HBB dependence to HBB independence, the mutation indices for plaques 1, 2, and 3 given above nevertheless indicate a frequency of the order of 10^{-4} mutations per replication, a remarkably high rate compared with other genetic systems.

Passage of HBB-sensitive wild-type virus in the presence of HBB permits ready isolation of HBB-resistant mutants of varying degrees of resistance (*3*). The simplest explanation of this finding is to assume that, during replication of HBB-sensitive virus, mutants of varying degrees of resistance to HBB arise which are selected during passage of sensitive virus in the presence of HBB.

The origin of HBB-dependent mutants has not yet been studied in detail, mainly because of the lack of suitable selective procedures. Yet, since HBB-dependent virus does give rise to both HBB-sensitive and HBB-resistant virus, it is not unreasonable to assume that, conversely, the drug-dependent virus may be derived from either HBB-sensitive or HBB-resistant virus particles.

Results similar to those with Coxsackie A9 virus and HBB have been obtained with poliovirus and guanidine. Guanidine, like HBB, is a selective inhibitor of picornavirus reproduction, and guanidine-resistant and guanidine-dependent mutants have been isolated (*4, 5*). A nonselective system for the study of guanidine-independent virus particles arising from guanidine-dependent poliovirus has not yet been devised. However, when we determined the guanidine sensitivity (1 m*M* guanidine) of 13 guanidine independent clones obtained from a guanidine-dependent virus population, strain Ledinko (*5*), three were sensitive, three intermediate, and seven resistant. Thus, mutation to guanidine-independence may occur according to a pattern similar to that observed with HBB-dependent Coxsackie A9 virus. When drug-sensitive poliovirus is subjected to passage in the presence of guanidine, guanidine-resistant mutants of varying degrees of resistance are obtained (*5*). The origin of guanidine-dependent poliovirus mutants, however, is not known.

On the basis of all of the available results we propose that picornaviruses, occurring in any state of drug sensitivity, drug resistance, or drug dependence, may mutate directly to any other state.

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Leukemia in Husbands and Wives

Abstract. *Study of the death certificates of 876 spouses of widows and widowers who died of leukemia revealed that seven of the spouses also died of leukemia. However, five cases of leukemia were identified among matched controls of the spouses. The incidence of leukemia in husbands and wives of individuals who die of leukemia is not significantly greater than that of a control group. This is consistent with the hypothesis that adult leukemia is not contagious in the usual sense.*

With the possible exception of the demonstration of small "time-space" clusters of acute childhood leukemia (*1*), contagion has not been demonstrated in human leukemia. A recent analysis (*2*) of childhood leukemia cases in Connecticut revealed no tendency for the cases to cluster. In rare

Table 1. Statistical analysis of leukemia in matched spouse-control pairs (*7*).

Leukemia in		Number of matched pairs
Spouses	Controls	
−	−	864
−	+	5
+	−	7
+	+	0

$$\chi_1^2 = \frac{(7-5-1)^2}{(7+5)} = 0.083$$

$$p\{\chi_1^2 \geq .083\} > 75 \text{ percent}$$