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.

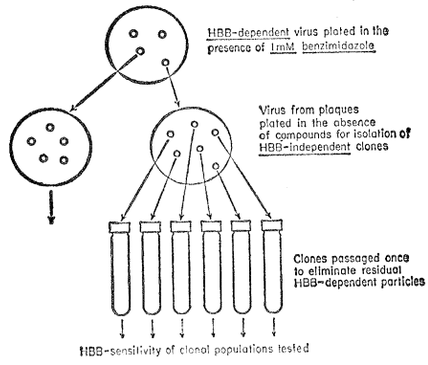

Fig. 1. Isolation of HBB-independent virus particles from HBB-dependent Coxsackie A9 virus in a nonselective system.

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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25 January 1965

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

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

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

instances, striking familial aggregation of leukemia has been reported (*3*), but in such aggregates the relative roles of heredity and environment cannot be separated. In order to investigate the occurrence of leukemia in individuals intimately associated with, but not genetically related to, persons with leukemia, I have studied the disease in married couples. This approach has been used previously in studies of cancer (*4*) and other diseases (*5*), but not in studies of large series of leukemia cases.

Cancer has been a reportable disease in New York state since 1940, when a cancer reporting system was established at the New York State Department of Health. A study in 1945 revealed that the completeness of cancer reporting at that time was between 84 and 96 percent, depending on the sites of the tumors. In addition to the reports filed by physicians and laboratories, cases of cancer reported on death certificates are routinely added to the reporting system.

From a deck of punch cards containing information from death certificates (*6*), 1241 index cases were selected on the following criteria: (i) cause of death: the causes being leukemia and aleukemia, as designated by the International Classification of Diseases code number 204; (ii) marital status: widow or widower; (iii) death during the years 1951 to 1961; (iv) residence in the state of New York, excluding New York City.

Selection of index cases among widows and widowers is a device which ensures that a mate has predeceased each index case and that, presumably, a death certificate and a stated cause of death will be available for both marital partners.

The death certificate of each index case was examined for information which would identify the spouse of each. Of the 1241 death certificates of index cases, all but ten named a mate. A search was then made of the death indexes of the New York State Department of Health for the 1231 death certificates of the respective mates of each index case. A death certificate was located for 876 of the mates of the 1231 index cases. The 355 index cases whose mates' death certificates could not be located were studied further, since a significant bias would be introduced if their mates had a leukemia incidence different from that of the mates of index cases whose death certificates were located.

Table 2. Marital pairs concordant for leukemia; the index cases died during the years 1951 to 1961 in New York state excluding New York City.

Couple No.		Age at death (years)	Date of death	Stated cause of death
1	Husband	65	Oct. 1945	Aleukemic leukemia
	Wife*	65	Oct. 1951	Chronic lymphatic leukemia
2	Husband	77	Jan. 1949	Lymphatic leukemia
	Wife*	77	July 1959	Blast cell leukemia
3	Husband*	75	Feb. 1958	Chronic lymphatic leukemia
	Wife	36	Feb. 1941	Subacute myelogenous leukemia
4	Husband*	82	June 1954	Leukemia
	Wife	80	Dec. 1952	Lymphatic leukemia
5	Husband	62	Aug. 1932	Lymphatic leukemia
	Wife*	77	Nov. 1951	Chronic lymphatic leukemia
6	Husband	56	Jan. 1923	Lymphatic leukemia
	Wife*	82	Aug. 1954	Acute myelogenous leukemia
7	Husband*	64	July 1952	Myelogenous leukemia
	Wife	62	Oct. 1951	Acute myelogenous leukemia

* Index case.

A 10-percent sample of the 355 incompleted marital pairs was studied by contacting the respondent who had provided personal information for completion of the death certificate of the respective index case. Usually, the respondent was a close relative or friend who could provide information on the untraced spouse of the index case. Twenty-four of 35 such respondents were successfully contacted, and in 20 of 24 cases it was determined that the untraced spouse had died outside the state of New York. None of the 20 spouses so traced died of leukemia. Death out of the reporting jurisdiction of the study was, therefore, the primary reason for inability to locate a death record for the spouses of these index cases. Misspelling of name, miscoding of marital status, and clerical errors in searching for death certificates were other reasons for failure to locate the spouse's death record.

Table 3. Number of deaths among spouses of individuals who died of leukemia and their matched controls, listed by cause of death.

Causes of death*	Code numbers*	Number of deaths among	
		Spouses of leukemia cases	Matched controls of spouses of leukemia cases
Infective and parasitic diseases	001 to 138	28	22
Neoplasms, except leukemia	140 to 203; 205 to 239	145	155
Leukemia	204	7	5
Allergic, endocrine system, metabolic, and nutritional diseases	240 to 289	53	42
Diseases of the blood and blood-forming organs	290 to 299	4	0
Mental, psychoneurotic, and personality disorders	300 to 326	2	4
Diseases of the nervous system and sense organs	330 to 398	94	102
Diseases of the circulatory system	400 to 468	364	351
Diseases of the respiratory system	470 to 527	23	33
Diseases of the digestive system	530 to 587	38	42
Diseases of the genito-urinary system	590 to 637	31	35
Deliveries and complications of pregnancy, childbirth, and the puerperium	640 to 689	2	2
Diseases of the bones and organs of movement	720 to 749	2	3
Congenital malformations	750 to 759	1	0
Symptoms, senility, and ill-defined conditions	780 to 795	43	40
Accidents, poisonings, and violence	800 to 999	39	40

* As designated in the International Classification of Diseases.

Seven cases of leukemia were discovered among the spouses of the 876 index cases while five cases of leukemia were found among matched controls of the spouses. This difference is not statistically significant (Table 1; 7).

For the controls of the spouses, death certificates were matched on the following criteria: age within 5 years; sex; race; marital status; township of residence; date of death within 4 weeks. Since the death certificates used were bound in books of 500 by county of residence and month of death, selection of a control death certificate from the same or adjacent death book as that of the spouse automatically matched for county of residence and month of death. Conscious matching for marital status, age, sex, and race was done by first examining the death certificate of the spouse to be matched and looking at adjacent certificates alternately toward the front and back of the death book until a certificate which fulfilled the matching criteria was located.

Table 2 shows information obtained from death certificates of the seven couples, both members of which died of leukemia. No trend is evident for the interval between death, and in three of the seven couples, death was attributed to different types of leukemia.

Table 3 shows that the distribution of stated causes of death in mates of index cases is not significantly different from that of matched controls of the mates.

In summary, husbands and wives of individuals who die of leukemia do not have a demonstrably increased risk of dying of leukemia. This is consistent with the hypothesis that adult leukemia is not contagious in the usual sense.

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7. See W. G. Cochran, *Biometrika* **37**, 256 (1950) for details of this test.
8. This study would have been impossible without the dedicated death certificate searching of Mrs. Mary Postmayer and Miss Madeleine Igoe.

24 December 1964

Cyclic Structure of Adenovirus DNA

Abstract. *The deoxyribonucleic acids of adenovirus types 2, 7, and 12 were extracted and examined in the electron microscope. Cyclic forms of the molecules were found to exist in each viral type. The lengths of cyclic strands extracted from the most dense fraction of purified particles of adenovirus type 2 were highly variable, averaging about 2.5 microns. This corresponds to a molecular weight of about 5 million.*

Fiers and Sinsheimer (*1*) showed that the DNA molecule of ϕ174 bacteriophage is circular, and Dulbecco (*2*) obtained similar evidence for polyoma virus DNA. Kleinschmidt and Zahn devised a method for preparing nucleic acid molecules so that they can be studied in the electron microscope (*3*). With this technique, Weil and Vinograd confirmed that polyoma virus DNA exists in a cyclic form (*4*). We used a different preparative method in which DNA molecules were recovered from the surface of agar and made electron opaque by reaction with uranyl acetate. The details of this technique and others applied in our study have been described (*5*). The droplet rather than the centrifugation method was used for placing samples on agar.

In the course of studying artificially disrupted adenovirus particles, long, circular strands of small diameter were obtained from adenoviruses types 2, 7, and 12 after treatment with sodium lauryl sulfate (SLS). In this report we present some of the characteristics of these strands and the evidence for the conclusion that they are viral DNA molecules.

In order to compare the results obtained by Kleinschmidt and Zahn's method with those obtained in this study, polyoma virus purified by cesium chloride density gradient was disrupted with SLS, and the degradation products were prepared for microscopy by the agar method. Large numbers of circular strands were produced after treatment with SLS. Electron micrographs were made of typical fields and the lengths of the strands were determined with a map-measuring device. Magnification of the electron micrographs was checked against a carbon replica of a standard grating having an average dimension of 0.883 μ between lines. The lengths of the circular strands of polyoma virus ranged from 1.22 μ to 1.90 μ, and averaged 1.64 μ. This average is in agreement with the value of 1.58 μ previously reported by Weil and Vinograd for circular polyoma virus DNA (*4*) and thus confirms their determination by a different method. Noncyclic (linear) strands were also seen and were about the same length. Treatment of viral suspensions containing more than $10^{9.5}$ particles per milliliter produced masses of tangled strands which were difficult to interpret and measure.

Efforts were made to use very mild conditions for disruption of the particles. Experiments were first designed to determine the lowest concentration of SLS which would disrupt a given number of particles. Serial dilutions were made from a 1-percent stock solution of SLS in 0.15*M* saline, then constant amounts (about $10^{9.5}$ particles per milliliter) of purified adenovirus type 2 were added in equal volumes to each dilution of SLS. The purified virus suspensions used in our first experiments were pools containing particles of density 1.30 to 1.35 g/cm^3; they were obtained from cesium chloride density gradients. Mixtures were allowed to stand at room temperature (23°C) for 5 to 60 minutes. Samples were prepared at intervals and examined in the electron microscope. Disruption of at least 99 percent of the particles was achieved within 5 minutes after addition of SLS (final concentration, 0.12 mg/ml).

Suspensions of disrupted particles contained many circular strands and other viral components readily observable in the electron microscope. Figure 1 shows a group of purified particles of adenovirus type 2 before disruption. The DNA cores are intensely stained with uranyl acetate, whereas the protein coats are relatively unstained. Figure 2*A* shows an electron micrograph (low magnification) of a typical circular strand obtained by disruption of purified adenovirus type 2. Figure 2*B* shows a micrograph (higher magnification) of a portion of this same circular strand. Aggregates of viral protein subunits (capsomeres) can be seen in the background (see arrows); some of these capsomeres remained loosely attached to the circular strand. Circular strands were of various lengths, measured about 30 Å in diameter (although this small dimension was difficult to measure), and stained more intensely with uranyl acetate than did the protein capsomeres. A micrograph