vated when stem disks and scales were excised. Experiment 4 was performed in October, experiment 3 in January; this explains the differential effect of the 13°C treatment. It appears that the length of the 13° treatment required to obtain a threshold condition in the bulbs is strongly dependent upon the length of the preceding storage period at 25.5°C.

2) Excised stem disks were placed on a medium on which a scale fragment had previously been incubated. As shown in Table 1, experiments 5 and 6, some of these stem disks became reproductive, while the controls did not.

On the basis of these results attempts were made to extract and identify the active material. It was assumed that this material would be more highly concentrated in the apexes and the primordial leaves than in the scales. Cold methanolic extracts of the buds of bulbs that had been stored at 13°C for varying periods of time were prepared by the method of Harada and Nitsch (*4*). *Bud* in this connection signifies that part of the bulb that remains after removal of the scales. The extract was evaporated to dryness at 40°C under reduced pressure. The residue was dissolved in water, sterilized by filtration, and incorporated into the nutrient medium. The first tests were made with extracts of buds of bulbs that had been stored at 13°C for 3 and 4 weeks. After such storage the shoot apexes of these bulbs are still vegetative in appearance, although physiologically the transition to the reproductive state may already be in progress. This time the explants were more severely trimmed, weighing only about 30 mg. They were cultured in small tubes containing 5 ml of nutrient agar. The ratio of the number of extracted buds to the number of explants was 1 to 1. As shown in Table 1 (experiments 7 and 8), after 7 weeks a greater percentage of apexes had become reproductive on the medium containing bud extract than on the control medium.

The next step was to chromatograph the crude methanolic extracts in 80-percent isopropanol on paper strips, according to the method of Harada and Nitsch (*4*). The Avena first-leaf-section test was applied to detect eventual gibberellin-like substances. On the strength of these determinations it appears that the buds of iris bulbs contain several gibberellin-like substances. During the first 4 weeks of the 13°C treatment the main growth substances are those having R_F values of 0.2 to 0.4 and 0.6 to 0.8, respectively, in 80-percent isopropanol. Gibberellic acid (*5*), which has an R_F of 0.45, did not seem to be present in the extracts, although in earlier experiments (*6*) this growth substance had been shown to exert a strong flower-inducing effect.

In extracts of buds of bulbs that had been stored at 13°C for 7 weeks and thus were definitely reproductive, the fraction with R_F of 0.2 to 0.4 was extremely active in the Avena first-leaf-section test, whereas no activity was found between R_F 0.6 and 0.8. In preliminary experiments the capacity of these fractions to induce flower development was tested. The extract was chromatographed, and the regions of the strips corresponding to R_F values 0.2 to 0.4 and 0.6 to 0.8, respectively, were eluted. The eluates were sterilized by filtration and incorporated into the nutrient agar. On these media small explants were incubated, as in experiments 7 and 8. Each tube, carrying one explant, contained an amount of extract originating from one bud. The results seem to indicate that flower initiation is promoted by the fraction having an R_F value of 0.2 to 0.4 in 80-percent isopropanol.

These findings suggest that in the bulbs of Wedgwood iris, flower induction is associated with changes in endogenous gibberellin-like substances. By systematically screening extracts prepared at several different times in the course of the flower-inducing 13°C treatment, both by means of a test specific for gibberellins and with the technique of aseptic culturing of apexes, it should be possible to draw more specific conclusions about the mode of action of the gibberellin-like substances in floral initiation.

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3 August 1961

Leukemogenesis by Urethan in C57Bl Mice Bearing Isologous Tissues from X-Irradiated Mice

Abstract. A single exposure of mice of a low-leukemic strain (C57Bl/6) to 400 r of total-body x-irradiation caused the early appearance of a factor which, though not itself capable of inducing leukemia in adult C57Bl recipients, did cause its appearance when the recipients also received urethan by injection. Positive results were obtained with seven different tissues from irradiated animals, whether urethan was injected 1 day or 30 days after the irradiation. No such effect was obtained in either series in the absence of urethan treatment. When urethan was injected into normal C57Bl mice, or into mice previously implanted with tissues from nonirradiated mice, only marginal leukemogenic activity was evinced. The possible involvement of a precursor virus is discussed.

Experimental leukemogensis by x-irradiation differs from other forms of carcinogenesis with respect to the involvement of several complicating factors. (i) Radiation leukemogenesis does not operate in thymectomized mice but becomes again effective when normal thymus is reimplanted after the irradiation (*1*). Thus, the eventual target organ (the thymus) is required for leukemogenesis, though it need not be acted upon by the inciting agent (x-irradiation). (ii) Radiation leukemogenesis is interfered with by the presence of normal bone marrow, as evidenced by the ineffectiveness of irradiation when one limb is shielded (*2*), and by the marked depression of leukemogenesis by total-body irradiation when normal, isologous bone marrow is injected subsequent to the irradiation (*3*). (iii) While the presence of a leukemia virus, demonstrable in mice of a high-leukemic strain (*4*), is not demonstrable in untreated mice of a low-leukemic strain (see *5*), it becomes demonstrable in mice of a low-leukemic strain after irradiation, either after (*6*) or shortly before (*7*) the disease appears.

Radiation leukemogenesis can be augmented by urethan treatment when this is given either simultaneously with the irradiation (*8, 9*) or 2 weeks later, but not when it is given 2 weeks prior to the irradiation (*9*). The possibility that the urethan effect might be due to a further depression of the bone marrow has been excluded (*10*). It would seem (*9, 10*) that urethan operates as a "promoting" factor in leukemogensis, with x-irradiation (in the low-dose ranges used) acting as "initiating" factor, in

Table 1. Incidence of leukemia* in strain C57Bl/6 mice injected with tissues from irradiated and nonirradiated donors, and subsequently with urethan, and in mice injected with tissues but not with urethan. The figures in parentheses are percentages. Comparable totals for mice injected with urethan only and for untreated controls are 2/110 (2 percent) and 0/141 (0 percent), respectively.

Tissues injected	Injected 1 day after tissue irradiation (No.)		Injected 30 days after tissue irradiation (No.)		Injected with tissues from nonirradiated animals and subsequently with urethan (No.)
	Subsequent urethan injections	No urethan injections	Subsequent urethan injections	No urethan injections	
Bone marrow	8/40 (20)	1/47	4/45 (9)	0/51	1/44 (2)
Lymph nodes	5/41 (12)	0/48	6/39 (15)	0/46	1/41 (2)
Spleen	5/39 (13)	0/48	4/39 (10)	0/48	1/49 (2)
Thymus	8/35 (23)	0/49	2/24 (8)	1/48	3/40 (7.5)
Lung	5/40 (12.5)	0/45	6/36 (17)	0/50	1/47 (2)
Brain	7/37 (19)	0/48	3/40 (7.5)	0/52	2/41 (5)
Skin	4/30 (13)	0/49	4/46 (9)	0/53	2/43 (5)
Total	42/262 (16)	1/334 (0.3)	29/269 (11)	1/348 (0.3)	11/305 (4)

* Incidences are based on the number of survivors at the time of the first leukemia (24 weeks after the injection of tissue).

terms of the "two-stage mechanism" demonstrable in skin carcinogenesis (*11*).

The long latency (5 to 10 months or more) in radiation leukemogenesis and the fact that a leukemia-inducing virus becomes demonstrable (in a normally low-leukemic strain) late in the leukemogenic process raises the intriguing possibility (*7*) that there is an intervening precursor-virus stage. If we assume that the promoting phase of leukemogenesis is concerned with the postulated conversion of the precursor virus into a virus, the question arises whether the urethan effect may be implicated in the process. The experiment reported here is the first of a series designed to test this hypothesis.

The mice used were of the C57Bl/6 strain, having a low spontaneous incidence of leukemia and normally lacking a demonstrable leukemia virus, yet sensitive to radiation leukemogenesis. The x-irradiation consisted of a single exposure of 400 r. The tissues used for transplantation were minced and injected intraperitoneally, in the following amounts: a whole organ per recipient in the case of spleen, thymus, lung, and brain; the contents of four bones in the case of bone marrow; several lymph nodes; and about 12 cm^2 of skin. The experiment was carried out in parallel series, one with transplants 1 day after irradiation and the other with transplants 30 days after irradiation. Controls included the following: mice that received similar transplants without urethan treatment; mice that received transplants from nonirradiated mice, followed by urethan treatment; mice not previously injected with tissues that received urethan treatment; and untreated mice. The urethan treatment consisted of 15 weekly intraperitoneal injections of 0.2 ml of a 10-percent solution—that is, 20 mg per injection—totaling 300 mg per animal. The interval between tissue transplantation and first urethan injection was 1 week.

The experiment has now been in progress for 14 months, and many of the mice, still alive, are being kept under further observation till they die. The results reported here are, therefore, of a preliminary nature.

The results (see Table 1) were as follows. (i) No leukemias developed in the untreated controls. (ii) Urethan alone, or urethan given to mice previously injected with nonirradiated tissues, appeared to be slightly leukemogenic (two leukemias in 110 mice [2 percent]; and 11 in 305 mice [4 percent], respectively. (iii) Injections of tissues from irradiated animals, without subsequent urethan treatment to the recipients, failed essentially to induce leukemia (one leukemia in 334 and in 348 mice, respectively). (iv) Injections of tissues from irradiated animals, followed by urethan treatment to the recipients, yielded significantly higher incidences of leukemia, both in the 1-day series (42 leukemias in 262 mice [16 percent]) and in the 30-day series (29 in 269 mice [11 percent]).

After the demonstration by Gross (*4, 5*) of a specific virus in spontaneous mouse leukemia in the high-leukemic AK strain, attempts were made to find evidence of such a virus in irradiated mice of low-leukemia strains (in which the virus is normally not demonstrable). Gross (*6*) succeeded in transmitting the disease, using cell-free extracts from leukemic tissues of x-irradiated adult C3H mice and injecting them in newborn C3H mice; Lieberman and Kaplan (*7*) obtained similar results with cell-free extracts of tissues from irradiated C57Bl mice shortly before the actual appearance of the induced leukemia. The two common factors in these studies are (i) that in irradiated mice of low-leukemic strains the virus appears only at, or shortly before, the onset of the induced leukemia, and (ii) that newborn mice are required as responsive recipients for testing for the presence of the active virus. The results make consideration of the role of viruses in induced leukemogenesis imperative.

The results of the experiment under discussion indicate that total-body irradiation of mice of a low-leukemic strain (C57Bl/6) causes the immediate appearance of a "transmissible" factor which, though not itself capable of inducing leukemia, renders *adult* mice into which it is injected subject to leukemia when they are subsequently injected with urethan.

The nature of this "transmissible" factor cannot, of course, be discerned from this experiment. It is evidently not an active leukemia virus, since in the absence of urethan treatment of the recipients no leukemia develops, and since the factor appears so soon after the irradiation. Nor is there proof that the factor is a precursor virus, though the results are compatible with such an interpretation. An experiment is now in progress to determine this point, by injecting cell-free extracts of the tissues into normal mice, which are then injected with urethan.

The fact that the choice of tissue for transplantation did not seem to affect the results was disappointing, since it was hoped that observed differences might have indicated the primary target organ, on which the irradiation was acting (the thymus is presumably the secondary target organ, on which the eventual agent acts).

Two apparent discrepancies call for comment—the fact that the incidence of leukemia was somewhat higher in the control series that received nonirradiated tissues followed by injection of urethan than in the control series given only urethan (4 as against 2 percent), and the fact that the incidence of leukemia with injection of irradiated thymus seemed rather low in the 30-day series (though not in the 1-day series). It is not possible to say, until the experiment is completed, whether these are significant discrepancies (*12*).

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Effect of Ultrahigh Vacuum on Viability of Microorganisms

Abstract. Three species of resistant microorganisms were exposed for 5 days to an ultrahigh vacuum approaching that of interplanetary space. Since no lethal effect was observed, there is no indication that the vacuum of outer space would prevent transport of viable microorganisms on unsterilized space vehicles.

The effect of ultrahigh vacuum on microorganisms is of current practical interest in connection with the concern that interplanetary vehicles be sterile. Although ordinary laboratory vacuum (10^{-2} to 10^{-4} mm-Hg) is used to preserve microorganisms, Phillips and Hoffman (*1*) pointed out that the effect of the extreme vacuum of outer space [estimated as low as 10^{-10} mm-Hg (*2*)] on microorganisms is unknown. Prince (*3*) reported that microorganisms withstood pressures from 10^{-5} to 5×10^{-4} mm-Hg for 32 days. However, data on the effect of higher vacuums are needed, especially since odd surface effects have been noted (*4*) on some materials in ultrahigh vacuum (defined as pressures lower than 10^{-8} mm-Hg). The experiment reported here was performed in an 85-liter chamber (*5*) at the National Research Corporation (*6*), where pressures as low as 2×10^{-10} mm-Hg have been reached. It was hoped that if the vacuum of outer space is lethal to microorganisms, evidence of it would be obtained at this level of pressure.

Three of the more resistant types of saprophytic microorganisms were exposed to ultrahigh vacuum for 5 days, a period chosen arbitrarily as a little longer than the time required for transit from the earth to the moon. Exposure was at ambient temperature which corresponds to the usual temperature maintained within space vehicles for the proper functioning of instruments. The chamber chosen for these tests operates at room temperatures, not at elevated temperatures as many high-vacuum sources do.

Each of a number of ashless filter paper patches (½ inch in diameter) was contaminated with one of the three test microorganisms, *Bacillus subtilis* var. *niger* spores, *Aspergillus fumigatus* spores, or *Mycobacterium smegmatis* cells suspended in water, 0.1-percent Tween 20, and Dubos broth base, respectively. After drying over calcium sulfate, three patches of each type were assayed for viable microorganisms to serve as a baseline control.

At the same time that the baseline control patches were assayed, nine test patches, three with each microorganism, were placed in the ultrahigh vacuum chamber. For comparison, similar patches, kept within desiccators in the same room, were maintained under five other environments. These environments were air, atmospheres lacking oxygen or water vapor or both, and low vacuum. At the end of a 5-day exposure period, each patch was removed from its exposure chamber, placed in distilled water, and shaken until the paper patch disintegrated. Samples from serial decimal dilutions were then plated by the spread plate technique, with a trypticase soy agar as the culture medium. The plates were incubated at 37°C, and colonies were counted after the optimum growth period which was 24 hours for *Bacillus subtilis* var. *niger*, 48 hours for *Aspergillus fumigatus*, and 72 hours for *Mycobacterium smegmatis*.

The results shown in Table 1 indicate that, in general, ultrahigh vacuum, ordinary laboratory vacuum, and storage in a nitrogen atmosphere were the environments most conducive for the preservation of microbial viability. Statistically, the recoveries of *Bacillus subtilis* var. *niger* spores or *Aspergillus fumigatus* spores obtained after exposure to these three conditions were not significantly different from each other or from the baseline control. With the vegetative cells of *Mycobacterium smegmatis*, the recoveries obtained after exposure to these three conditions were also not significantly different from each other, but were significantly lower than the baseline control assayed 5 days before.

Table 1. Microorganisms recovered after 5 days of exposure to ultrahigh vacuum and other test conditions. Each entry is an average count for three patch samples. Temperature during test ranged from 23° to 24°C.

Test condition	*B. subtilis* ($\times 10^4$)	*A. fumigatus* ($\times 10^4$)	*M. smegmatis* ($\times 10^2$)
Baseline control	102.2	31.8	469.1
Vacuum, ultrahigh*	118.2	21.9	156.0
Vacuum, lab†	121.9	27.4	106.6
Nitrogen + $CaSO_4$	96.3	6.4	10.8
Nitrogen only	96.4	23.2	140.9
Air + $CaSO_4$	37.6	0.1	0.8
Air only	132.1	1.1	3.3

* At 6 hours, pressure was 5.0×10^{-9} mm-Hg; at 24 hours, pressure was 7.2×10^{-10} mm-Hg; and at 5 days, pressure was 3.6×10^{-10} mm-Hg. † Pressure was 4×10^{-2} mm-Hg, produced by a Welch high-vacuum air pump.

These data furnish no evidence to indicate that the vacuum of outer space would kill microorganisms and thus prevent their conveyance in a viable state on interplanetary vehicles. At the vacuum reached, the effect upon the test organisms was less adverse than exposure to normal atmosphere (*7*, *8*).

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6. The apparatus was operated by the personnel of the National Research Corp.

7. This work was sponsored by the National Aeronautics and Space Administration through an interagency agreement with the U.S. Army Chemical Corps.

8. Since this report was prepared our attention was called to a Hughes Aircraft Company technical memorandum, by E. E. Brueschke, R. H. Suess, and M. Willard (*Planetary and Space Science*, in press), where an opposite conclusion was reached. These authors report that microorganisms survived a pressure of 8×10^{-9} mm-Hg for 10 days but failed to survive 1.2×10^{-8} mm-Hg for 30 days. No control data are given.

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