continuous radiation and 3 minutes for ultrafractionated radiation were used.

Immediately after irradiation, a drop of cell suspension was placed on each counting area of a hemocytometer slide. A drop of 0.5-percent aqueous eosin B (*8*) was mixed with each. The slide with cover slip was allowed to stand in subdued light for 10 minutes. Two hundred cells were then counted from each area. Stained and unstained cells were tabulated, and the percentage of stained cells was calculated. Maximum variation between the two 200-cell samples from the slide was 3 percent. The percentage of stained cells for 1 minute of continuous radiation served as a basis for testing the reproducibility of the method. The staining method was selected to indicate the early effects of, or damage by the radiation, or both.

The results of five experiments, covering single exposure times from 0.1516 sec to 0.0012 sec, are shown in Fig. 1. Starting at a single exposure time of 0.1107 sec, there was a decrease in the percentage of damage with decreasing single exposure time. A minimum in percentage of damage, averaging 36.7 percent of the continuous irradiation damage, was found at an average single exposure time of 0.024 sec. When single exposure times were further shortened, the percentage of damage approached the continuous irradiation value. A second minimum of less magnitude, averaging 77 percent of the continuous irradiation damage, occurred at an average single exposure time of 0.0025 sec. For further decrease in the single exposure time, the percentage of damage again approached the continuous irradiation damage. The straight line at 100 percent indicates the damage with continuous irradiation—that is an average of 65.2 percent of the total cells counted.

Similar to the findings of Witte (*1, 2*), Witte and Sigmund (*3*), and Hofmann *et al.* (*4, 5*) for ionizing radiation, ultrafractionated ultraviolet radiation also shows in its biologic effect a dependency on the frequency (single exposure time) of the incident radiation. Attempts have been made to interpret these findings in terms of the space and time distribution of the radiation-produced ions (*1*), as well as in terms of reciprocal actions between the radiation impulse and the restitutional pause (*4*). A theoretical approach to the problem by Krohn and Michie (*9*) proposed a radiation-produced chemical substance, the mean level of which is a function of the single exposure time. A possible explanation could be based on the "time-existence" of the radiation-induced chemical reactions. Photochemical studies by Allmand and Style (*10*) and radiochemical studies by Hummel *et al.* (*11*) have shown the usefulness of pulsed-radiation techniques in studying these reactions. These conclusions for chemical systems can be extended to biological systems with the assumption that a biological system behaves as a complex chemical system. Therefore, it is suggested that the minima observed in these studies on ultrafractionated ultraviolet irradiation of yeast cells are related to the "time-existence" of specific radiation-induced reactions.

J. G. KEREIAKES
R. H. HODGSON
A. T. KREBS
Radiobiology Department, Army Medical Research Laboratory, Fort Knox, Kentucky

References and Notes

1. E. Witte, *Strahlentherapie* **82**, 209 (1950).
2. ——— *ibid.* **89**, 586 (1953).
3. ——— and R. Sigmund, *ibid.* **88**, 384 (1952).
4. D. Hofmann and K. Muller, *ibid.* **96**, 403 (1955); **97**, 64 (1955).
5. D. Hofmann and R. K. Kepp, *ibid.* **97**, 64 (1955).
6. Listed as No. 4125 in the *American Type Culture Catalog.*
7. J. G. Kereiakes, R. H. Hodgson, A. T. Krebs, *Army Med. Research Laboratory Rept. No. 195* (1955).
8. C. G. Fraser, *J. Phys. Chem.* **24**, 741 (1920); D. W. King *et al., Cellular Research,* in press.
9. R. W. Michie, and L. H. Krohn, *Army Medical Research Laboratory Rept. No. 202* (1955).
10. A. J. Allmand and D. W. G. Style, *J. Chem. Soc.* **1930**, 596 (1930).
11. R. W. Hummel *et al., Science* **119**, 159 (1954).

7 May 1956

Agronomic Use of an X-ray-Induced Mutant

In 1941, Genter and Brown (*1*) reported on the effects of varying x-ray dosages applied to the seed of *Phaseolus vulgaris* L. variety Michelite, a vine type of Navy or pea bean. Many different types of mutants were produced, and 90 percent of these were distinguishable within 5 weeks after emergence, 67 percent being chlorophyll abnormalities. No mention was made of any agronomic value being attached to any observed mutants. In 1941, progenies from this material without known mutants were planted in the regular breeding plots to insure against overlooking any valuable material. A mutant bush or nonvining type of bean that was smaller than Michelite and that matured about 12 days earlier than Michelite was found. Two years of testing proved the progeny of this mutant to be breeding true.

An attempt (*2*) was made to transfer the earliness of this mutant to the vine type by crossing and backcrossing to Michelite, followed by selection. The vine type of Michelite was found to be dominant to the bush and lateness was dominant to earliness. In this particular mutant, earliness and bush type were closely linked, so that no early vine type was obtained. However, during the process of selection, the size of the bush was increased considerably.

In 1948, the bean-breeding program of the Michigan Agricultural Experiment Station was made cooperative with the U.S. Department of Agriculture. At that time, a survey was made of the bean industry of Michigan to determine the priorities in bean improvement. It appeared desirable to develop a variety which might lend itself to harvesting by combine without pulling. It also appeared necessary that resistance to either bean anthracnose, which is caused by the fungus *Colletotrichum lindemuthianum* (Saec. and Magn.) Scrib., or to common bean mosaic, which is caused by *Phaseolus* virus 1 and its variant strain, be incorporated into any new variety which might be developed and released. The ultimate purpose of this would be to combine the resistance to these diseases in one commercial variety.

Several of the bush types that were recovered from the irradiated material were crossed to different anthracnose-resistant strains of beans belonging to a collection received from Cornell University. Four generations of back-crossing followed by five generations of selection resulted in a large number of anthracnose-resistant bush strains that were first tested in 1953. Twenty acres of one of these strains were grown in 1955. This strain has been named Sanilac and will be grown by foundation growers in 1956 for certification in 1957.

Table 1 compares the variety Sanilac with Michelite, the present variety which constitutes 95 percent of the 400,000 acres planted to Navy beans in Michigan.

The advantages of the new bush type, Sanilac, over the vine type, Michelite, can be enumerated as follows. (i) Sanilac is resistant to the alpha strain of bean anthracnose fungus, which attacks the Michelite and all other pea beans in Michigan. (ii) Because of better air movement resulting from the bush habit of growth, Sanilac has been found to be considerably less liable to injury by *Sclerotinia sclerotiorum* (Lib.) D By., the cause of sclerotinia wilt, or white mold disease, of beans. This is indicated by the differences in yield between the two varieties in 1953. (iii) The new variety is 6 days earlier than Michelite, indi-

Table 1. Yield comparisons and development rates of Sanilac versus Michelite (1953–55).

Variety	Yield (bu/acre)			Days to bloom	Days to harvest
	1953	1954	1955		
Sanilac	39.4	22.4	28.0	47	86
Michelite	22.0	24.6	23.1	53	92

cating that the linkage between earliness and bush type has been partially broken. (iv) Because of the upright type of growth of Sanilac, the ripe beans withstand the periods of wet and humid weather which frequently occur in Michigan during the harvest period, resulting in a lower percentage of discolored beans than is usual with the vine type.

The new variety is similar to Michelite in seed type, canning quality, and resistance to common bean mosaic. It is adapted to the better bean soils of Michigan and produces large upright plants, thus making up for loss of vines. More data are needed to determine whether Sanilac can be harvested by combine without pulling.

This agronomic use of an x-ray produced mutant may be added to those reported by Gustafsson and Tedin (*3*), who state "as far as we know, there are now three x-ray varieties released into the market, the Primex white mustard of Svalof, the 'Strålärt' of Weibullsholm, and, according to Knapp, the 'Schäfers Universal' in *Phaseolus*."

E. E. Down
Department of Farm Crops, Michigan State University, East Lansing
Axel L. Andersen
Horticultural Crops Research Branch, U.S. Agricultural Research Service, and *Botany and Plant Pathology Department, Michigan State University*

References and Notes

1. C. F. Genter and H. M. Brown, *J. Heredity* 32, 39 (1941).
2. Journal paper No. 1908 of the Michigan State University Agricultural Experiment Station.
3. A. Gustafsson and O. Tedin, *Acta Agr. Scand.* 4, 636 (1954).

30 April 1956

Isolation of Dengue Virus from a Human Being in Trinidad

Epidemics of dengue fever have not been reported from Trinidad, British West Indies, in the past. Although some of the physicians on the island have made the clinical diagnosis of dengue fever, the presence of the disease has not been generally accepted. This brief report (*1*) describes the isolation from a human being in Trinidad of a virus that has been found to be related to previously isolated strains of dengue virus.

The patient (TRVL case No. 18) was an 18-year-old, white, unmarried, female resident of Port-of-Spain. She became ill on 16 Aug. 1953. The onset of illness was abrupt, with fever, muscle and joint pains, malaise, and headache as the chief complaints. A rash appeared on the following day, spreading rapidly over the face, trunk, and extremities of the patient. She did not visit a physician, however, until 20 Aug. 1953. At the time of the visit, her complaints were the same as those present at the onset of the illness and were not significantly changed in intensity. Her temperature was 100°F. A rash covered most of the body surface; it was a light red color and discrete and maculopapular in character. The rash faded on pressure. No other physical signs were found on examination. Her physician diagnosed dengue and informed the Trinidad Regional Virus Laboratory. A blood sample was obtained at this time and was inoculated intracerebrally into a group of 4-day-old mice and a group of adult mice. The clinical picture was so typical of dengue that no treatments other than salicylates were advised. The disease subsided during the next few days and the patient made an uneventful recovery, except for a somewhat prolonged period of convalescence, during which she complained of weakness and of becoming easily tired on exertion. A sample of serum was obtained during the patient's convalescence on 11 Sept. 1953.

Table 1. Results of cross-neutralization tests between dengue-1, dengue-2, and the Trinidad-1751 strains of virus and serums from immune rhesus monkeys.

Serum		Virus		
Monkey No.	Immunity status	Trinidad 1751	Dengue 1	Dengue 2
5007	Normal	4.6*	3.8	4.4
5007	Trinidad-1751 immune	< 1.0	3.4	2.3
5026	Normal	6.5	6.4	5.9
4975	Dengue-1 immune	4.2	2.6	5.3
4961	Dengue-2 immune	3.4	5.1	3.4
4963	Dengue-2 immune	2.6	4.2	3.0
5007	Trinidad-1751 immune	< 2.0	4.8	2.2
4975	Normal	4.6	Not tested	Not tested
4975	Dengue-1 immune	< 1.0	Not tested	Not tested
4961	Normal	> 5.4	Not tested	Not tested
4961	Dengue-2 immune	2.3	Not tested	Not tested

* Reciprocal of the logarithm of the 50-percent mortality end-point.

Definite signs of illness were first noted in the infant mice 12 days after inoculation. A brain suspension prepared from the sick mice was passaged into a group of adult and a group of 1-day-old mice. An agent that is lethal for young mice was isolated. No evidence of illness was observed in the adult mice. In the early passages, the incubation period in 1- to 2-day-old mice was from 7 to 10 days or more. The onset of the disease was characterized by irritability and marked ataxia. The disease developed rather slowly, and the mice gradually became prostrated and died. Occasionally a mouse was found with one or more legs paralyzed. On continued passage, the disease in the mice became more virulent, judging by the shortening of the incubation period and the duration of the disease. The incubation period became finally fixed at from 5 to 6 days. Following the inoculation of large doses of the virus, the disease lasted but a day or two from onset until death. It was found that the agent passed easily through bacteria-tight Seitz-EK pads. Suspensions that would produce the typical illness in mice failed to grow in ordinary broth or thioglycolate medium. The strain of virus is identified in this laboratory as TRVL specimen No. 1751.

The neutralizing capacities of serum samples obtained during the acute and convalescent stages of the patient's illness were compared with the neutralizing capacity of the homologous virus. It was found that the sample obtained during convalescence would neutralize about 1000 times more virus than the sample obtained during the acute stage. The serums were inactivated at 56°C for 30 minutes prior to being mixed with the virus suspension.

The virus has been adapted to adult mice following a series of passages in mice of gradually increasing ages. Although the incubation period and the duration of the disease in the adult mice are essentially the same as they are in the 2-day-old mice, the titers of virus in the adult-mouse brains have been lower, generally 10^{-5} to 10^{-6}. Titers of 10^{-7} and greater have been obtained with lower passage material when 2-day-old mice were used.

The clinical illness of the patient, together with the behavior of the virus in mice, strongly suggested that the agent might be a strain of dengue virus. Accordingly, studies were undertaken in this laboratory and by Max Theiler in the New York Virus Laboratories of the Rockefeller Foundation to test this hypothesis.

Normal rhesus-monkey serum and serums of rhesus monkeys that were immune to each of the known dengue strains, dengue 1 (Hawaiian) and dengue 2 (New Guinea "B"), and to the Trinidad strain (1751), were compared for their capacity to neutralize each of the corresponding virus strains (*2*). In the tests, tenfold dilutions of virus were mixed with equal volumes of serum. Following incubation, the serum-virus mixtures were inoculated into groups of six adult white mice, intracerebrally. In several of the