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*."

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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).

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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

tests, the preimmunization serum from the monkey was available for comparison with the postimmunization serum. In other instances, a known normal serum was employed in the control titration. All the serums were inactivated at 56°C for 30 minutes prior to being mixed with the virus suspensions.

The significant data pertaining to the cross-neutralization tests are summarized in Table 1. The virus titers obtained in the presence of the various serums have been expressed as the reciprocal of the logarithm of the 50-percent mortality endpoint, which was calculated by the method of Reed and Muench (*3*). The results indicate an immunological relationship between the Trinidad strain and each of the two type strains with which the Trinidad strain was compared. Taking the results as a whole, it would seem that the Trinidad strain is somewhat more closely related to the dengue-2 strain than to the dengue-1 strain. However, the data are not sufficiently clear-cut to permit a final decision in the matter.

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References and Notes

1. The studies and observations on which this paper is based were conducted by the Trinidad Regional Virus Laboratory with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme, and the Rockefeller Foundation.
2. A. B. Sabin and R. W. Schlesinger, *Science* 101, 640 (1945); R. W. Schlesinger and J. W. Frankel, *Am. J. Trop. Med. Hyg.* 1, 66 (1952).
3. L. J. Reed and H. Muench, *Am. J. Hyg.* 27, 493 (1938).

16 April 1956

Characteristic Electrophoretic Patterns of Plasma Proteins of Orders of Amphibia and Reptilia

Immunological methods have been used for many years to study the phylogenetic relationships of animals (*1*). Electrophoresis would seem to offer another useful technique for such studies, since the electrophoretic patterns of the plasma proteins of an animal are characteristic, and distinct differences are found between patterns of closely related forms (*2*).

Paper electrophoretic patterns of the plasma proteins of amphibians and reptiles were determined under uniform conditions as part of a study of the comparative biochemistry of these forms. In each electrophoretic run, a parallel sample of human plasma served as a standard reference. All protein separations were carried out on an LKB paper-electrophoresis unit. A sodium barbital buffer of *p*H 8.6 with an ionic strength 0.05, containing 15 percent glycerol by volume, was used. A linear rate of movement of protein fractions was achieved by overcoming evaporation with the aid of the glycerol (*3*). Six 1-in. strips of Munktell No. 20 S filter paper were dipped into the buffer. Excess buffer was expressed from the paper with a rubber film roller. Seven hundred milliliters of buffer were added to each electrode vessel, and the two buffer surfaces were leveled with a siphon. The ends of the cassette holding the wetted strips were placed in the vessels. Following a 3-hour preliminary equilibration a potential of 170 v was imposed across the strips for an additional 3 hours. Two hundredths of a milliliter of fresh plasma was then spotted across each strip and fractionated for 17 hours at room temperature (26 to 28°C), 170 v, and 4 ma. Each strip was subsequently dried and stained with bromphenol blue (*4*). The optical density at 2-mm. intervals along strips made translucent with mineral oil was measured with a Beckman DU spectrophotometer at 590 mμ.

To date, we have examined about 1200 plasma patterns from more than 800 specimens of more than 100 kinds of amphibians and reptiles. Since genetic and physiological factors are known to influence plasma-protein composition, we have attempted to recognize such effects. Differences in the patterns due to sex, age, starvation, season, and geographic variation have been found (*5*).

The patterns of the various vertebrate orders that were studied seem to possess certain general diagnostic characteristics, provided that allowance is made for variations cited. In Fig. 1, a typical pattern from one species of each amphibian and reptilian order is shown adjacent to a "tentative key." The key is also in agreement with patterns obtained by

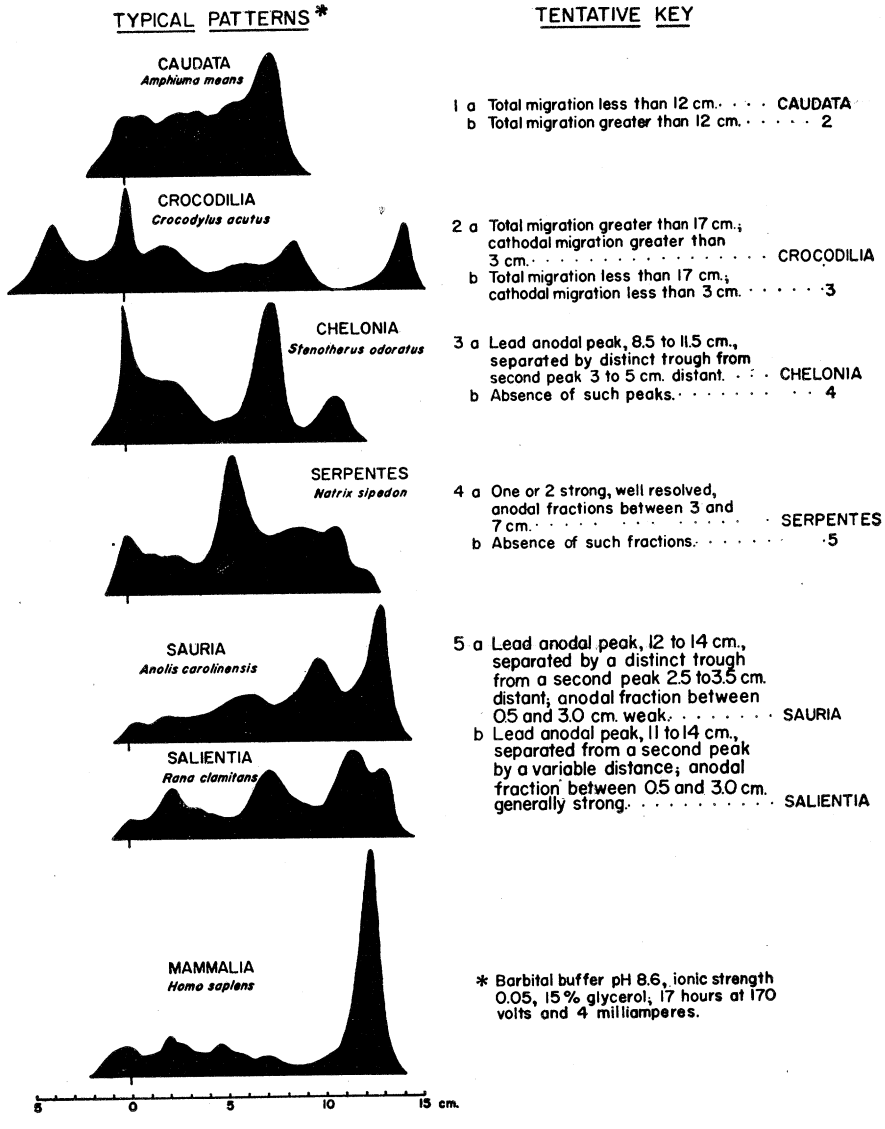

Fig. 1. Characteristic plasma protein patterns of the orders of Amphibia and Reptilia. Plasma samples were applied at the points indicated by the short line below each pattern. The anode is to the right of this mark.