of the second instar which, according to the curve, reaches a final width, on molting, of 5.1 mm and it, in turn, again becomes a new initial width. Continuing in this manner, it has been possible to estimate the number of postlarval molts and the width of each instar, of which

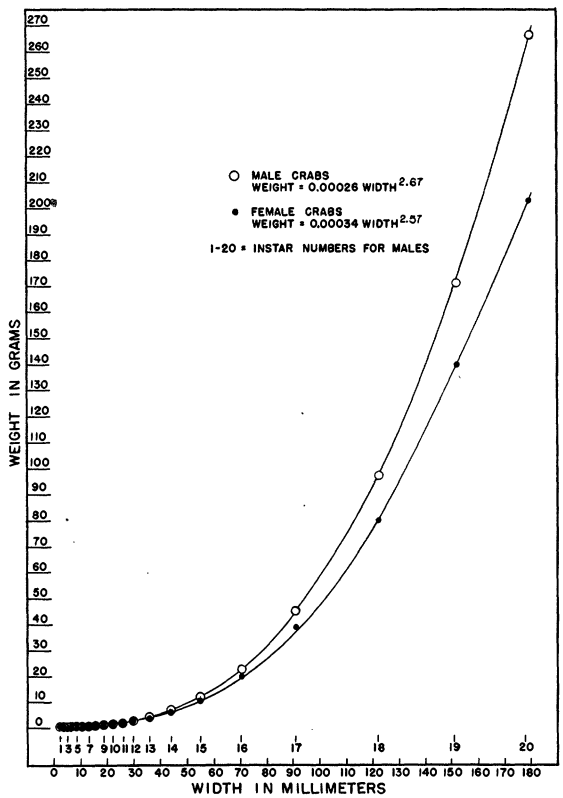

FIG. 2. Showing growth trends in *Callinectes sapidus* Rathbun, based upon growth curve of Fig. 1.

there are believed to be 19 or 20 in males and 18, or possibly 19, in female blue crabs (*7*). A mathematical equation was fitted to the molting curve for males and females so that from any initial width one can calculate directly the final width after molting. Hence, knowing the width of the first postlarval instar, it was possible to calculate the widths of the remaining instars.

To complete the story, it was necessary to establish the relationship between the width dimension, selected for a basic index of size, and other linear and also weight measurements. The allometry equation $Y = aX^b$, was found to constitute a satisfactory expression of the different linear and also weight relationships. Consequently, from a width measurement, any of the corresponding linear and weight dimensions can be readily ascertained. Since the width of each instar has been determined, it is now possible to calculate the remaining linear dimensions and the weights of each instar (Fig. 2).

The information provided by this procedure makes it possible to analyze certain characteristics of the growth of body parts in relation to one another and to the whole body. Also, a basis is provided for interpreting the effect of environmental conditions and mechanical injury on the normal growth curve as established by the preceding technics. The method has potential use for comparing differential growth rates in geographic varieties as well as closely related species.

References

1. CALVERT, PHILLIP P. *Proc. Amer. philos. Soc.*, 1929, **68** (3), 227–274.
2. GRAY, ELLEN H., and NEWCOMBE, CURTIS L. *Growth*, 1938, **2** (3), 235–46.
3. GRAY, ELLEN H., and NEWCOMBE, CURTIS L. *Growth*, 1938, **2** (4), 285–96.
4. MACKAY, DONALD C. G., and WEYMOUTH, FRANK W. *J. Biol. Bd. Canad.* 1935, **1** (3), 191–212.
5. MUSCONI, L. *Boll. Lab. Zool. gew. agrar. R. Scuola Sup. Agric.*, 1925, **18**, 95–118.
6. NEWCOMBE, CURTIS L., ECKSTINE, ALLEN M, and CAMPBELL, FRANK. *Southern Fisherman*, in press.
7. NEWCOMBE, CURTIS L., SANDOZ, MILDRED D., and ROGERS-TALBERT, ROSALIE. *J. exp. Med.*, in press.
8. ROBERTSON, ROY L. Masters Degree Thesis, University of Maryland, 1938, 1–30.
9. SHAFER, G. D. *Stan. Univ. Publ. biol. Sci.*, 1923, **3**, 307–338.
10. WESENBERG-LUND, C. *Inter. Rev. gesam. Hydrobiol. Hydrogr.*, 1913, **6**, 4–5, 373–422.

Cultivation of the Lansing Strain of Poliomyelitis Virus in Cultures of Various Human Embryonic Tissues[1]

John F. Enders, Thomas H. Weller,[2] and Frederick C. Robbins[3]

Research Division of Infectious Diseases, Children's Hospital,* and *Departments of Bacteriology, Comparative Pathology,* and *Pediatrics, Harvard Medical School, Boston

An extraneural site for the multiplication of the virus of poliomyelitis has been considered by a number of investigators (*2, 5*). The evidence that this may occur is almost entirely indirect, although recent data indicate that Theiler's mouse encephalomyelitis virus as well as various mouse pathogenic poliomyelitis-like viruses of uncertain origin may multiply in nonnervous tissue (*1, 3*). Direct attempts by Sabin and Olitsky (*4*) to demonstrate *in vitro* multiplication of a monkey-adapted strain of poliomyelitis virus (MV strain) in cultures composed of certain nonnervous tissues failed. They obtained, however, an increase in the agent in fragments of human embryonic brain.

The general recognition that the virus may be present in the intestinal tract of patients with poliomyelitis and of persons in contact with them emphasizes the desirability of further investigation of the possibility of extraneural multiplication. Accordingly, experiments with tissue cultures were undertaken to determine whether the Lansing strain of poliomyelitis virus could be propagated

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[2] Post doctorate Fellow of the U. S. Public Health Service.

[3] Senior Fellow in Virus Diseases of the National Research Council.

in three types of human embryonic tissues. The results are summarized here in a preliminary manner.

The technique was essentially the same as that recently described for the cultivation of mumps virus (*6*). The cultures consisted of tissue fragments suspended in 3 cc of a mixture of balanced salt solution (3 parts) and ox serum ultrafiltrate (1 part). Tissues from embryos of 2½ to 4½ months as well as from a premature infant of 7 months' gestation were used. These were: the tissues of the arms and legs (without the large bones), the intestine, and the brain. Each set of cultures included 4 or more inoculated with virus, and usually a similar number of uninoculated controls. The primary inoculum consisted of 0.1 cc of a suspension of mouse brain infected with the Lansing strain of poliomyelitis virus.[4] The identity of the virus was verified by (a) the character of the disease it produced in white mice following intracerebral inoculation; and (b) its neutralization by specific antiserum.[5] Subcultures were inoculated with 0.1 cc of pooled *centrifuged* supernatant fluids removed from the previous set of cultures.

The procedure of cultivation differed from that usually followed by other workers in that the nutrient fluid was removed as completely as possible and replaced at periods ranging from 4 to 7 days. Subcultures to fresh tissue were prepared at relatively infrequent intervals, ranging from 8 to 20 days.

Two experiments have been carried out employing cultures composed chiefly of skin, muscle and connective tissue from the arms and legs. The findings in each have been essentially the same. In the first, a series of cultures has now been maintained for 67 days. During this interval, in addition to the original set, three successive subcultures have been made to fresh tissue and the fluids have been removed and replaced 10 times (Table 1). Assuming that at each change of fluid a dilution of approximately 1: 15 was effected and that at the initiation of each set of cultures the inoculum was diluted 30 times, it has been calculated that the 10% suspension of infected mouse brain used as the primary inoculum had been diluted approximately 10^{17} times in the fluids removed from the third subculture on the 16th day of cultivation. These fluids, however, on inoculation into mice and monkeys, produced typical paralysis. Accordingly, since the mouse LD_{50} of the original inoculum was 10^{-2}, it would appear that the increase in virus during the course of the experiment was at least of the order of 10^{15} times. During the 67-day period of cultivation a progressive decrease in mouse infectivity was recorded (Table 1). On the other hand, in the second experiment, mentioned above, the calculated increase in virus during a 52-day period is now of the order of 10^{16} times and no decrease in mouse infectivity has so far been observed.

The agent propagated in the first experiment continued to exhibit the principal characteristics of the Lansing strain during the period of cultivation, as indicated by the following observations: (a) fluids from each set of cultures produced paralysis and death in mice after intracerebral inoculation; (b) the agent present in the fluids of the second set of subcultures was neutralized by antiserum specific for the Lansing strain; (c) following intracerebral inoculation, the fluids from the third set of subcultures produced flaccid paralysis within 7 and 10 days, respectively, in two rhesus monkeys. Microscopic examination of the spinal cords of these animals revealed lesions characteristic of poliomyelitis.

TABLE 1

MULTIPLICATION OF LANSING POLIOMYELITIS VIRUS IN TISSUES OBTAINED FROM THE EXTREMITIES OF HUMAN EMBRYOS

Culture set	No. of nutrient fluid changes prior to subculture		Day of incubation subculture done		Mouse LD_{50} of pooled fluids used to inoculate subcultures		Calculated dilution of original inoculum at time of subculture	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Original*	3	4	20th	20th	$10^{-2.0}$	$10^{-1.57}$	10^{-5}	$10^{-6.2}$
1st subculture	2	2	19th	12th	$10^{-1.57}$	$10^{-1.71}$	$10^{-8.8}$	$10^{-10.0}$
2nd subculture	2	4	12th	20th	$10^{-0.63}$	$10^{-1.34}$	$10^{-12.6}$	$10^{-16.2}$
3rd subculture	3	..	16th	....	$10^{-0.16}$	.....	$10^{-17.7}$	.....

* The LD_{50} of the suspension of mouse brain used as the inoculum in the first experiment was 10^{-2}; that of the suspension employed in the second experiment was $10^{-1.01}$.

Cultures of intestinal tissue were prepared with fragments from the entire intestine of human embryos, except in one experiment in which jejunum of a premature infant was used. In the latter, the bacteria were eliminated in the majority of cultures by thorough washing of the tissue and by the inclusion in the original nutrient fluid of 100 units/cc of penicillin and of streptomycin.

In one experiment with embryonic intestine, which included two subcultures and 7 changes of nutrient fluid, the calculated dilution of the original inoculum was of the order of $10^{13.7}$ times. On the basis of the mouse LD_{50} of the original inoculum and that of the last supernatant fluid, it was calculated that the virus had increased about $10^{12.7}$ times. The identity of the agent thus cultivated in intestinal tissue has not yet been confirmed by neutralization tests or monkey inoculation, but it elicits a response in the mouse typical of the Lansing virus.

The cultures prepared with intestine of the premature infant have, so far, been maintained 17 days with 3

[4] This strain was isolated by Dr. Charles Armstrong and obtained through the courtesy of Dr. S. D. Kramer.

[5] This serum was obtained through the courtesy of Dr. Isabel Morgan. It was prepared by hyperimmunization of rhesus monkeys with her strain of the Lansing virus.

changes of nutrient fluid. Virus has been demonstrated, by mouse inoculation, in the fluids removed during the course of the experiment, including that of the 17th day. The calculated multiplication of the virus was approximately 10^3 times. This finding suggests that multiplication occurred in this tissue which, from the embryologic point of view, is more mature.

To compare the increase of virus in nervous tissue with that in tissue of the intestine and the extremities, cultures of embryonic brain were prepared. The multiplication of the virus in this medium has been comparable to that in the other types. Thus in one experiment carried out contemporaneously with the series of embryonic intestinal cultures mentioned above, the calculated multiplication of virus was of the order of 10^{12} times.

No evidence was obtained which indicated that an agent other than the Lansing strain of virus was propagated in any of the three types of tissue. Mouse infectivity tests for the presence of virus in the supernatant fluids of uninoculated control cultures were negative. Aerobic and anaerobic cultures of supernatant fluids yielded no growth of bacteria.

On microscopic examination of fragments of the three types of tissue, removed after about 30 days of cultivation, differences have been observed in cell morphology between those derived from inoculated and uninoculated cultures. Many of the fragments from uninoculated cultures contained cells which appeared to be viable at the time of fixation, as indicated by the normal staining properties of the nuclei and cytoplasm. In contrast, the nuclei of the majority of the cells in fragments from inoculated cultures showed marked loss of staining properties. Since the amount of material which has been studied is as yet relatively small, one cannot conclude that the changes observed in the inoculated cultures were caused by the virus.[6]

It would seem, from the experiments described above, that the multiplication of the Lansing strain of poliomyelitis virus in the tissues derived from arm or leg, since these do not contain intact neurons, has occurred either in peripheral nerve processes or in cells not of nervous origin.

References

1. Evans, C. A., and Chambers, V. C. *Proc. Soc. exp. Biol. Med.*, 1948, **68**, 436.
2. Howe, H. A., and Bodian, D. *Neural mechanisms in poliomyelitis.* New York: Commonwealth Fund, 1942. P. 89.
3. Rustigian, R., and Pappenheimer, A. M. *J. exp. Med.*, 1949. 89, 69.
4. Sabin, A. B., and Olitsky, P. K. *Proc. Soc. exp. Biol. Med.*, 1936, **34**, 357.
5. Van Rooyen, C. E., and Rhodes, A. J. *Virus diseases of man.* (2nd ed.) New York: Nelson, 1948. P. 939.
6. Weller, T. H., and Enders, J. F. *Proc. Soc. exp. Biol. Med.*, 1948, **69**, 124.

[6] We are indebted to Dr. Duncan Reid and members of the staff of the Boston Lying-in Hospital for providing the human embryonic tissues and to Dr. Alwin M. Pappenheimer for the preparation and examination of sections of tissue culture material.

Insect Vectors of Phony Peach Disease[1]

William F. Turner

United States Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine

Phony peach is a virus disease of economic importance in the southeastern United States (*1*). While the disease attacks other species of the genus *Prunus*, its most serious effects result from heavy infections in commercial peach orchards, particularly in the South Atlantic and Gulf Coast States from South Carolina to Louisiana. The disease has caused the loss of more than 1,500,000 peach trees during the period from 1929 to 1947, inclusive.

Since 1936 the Bureau of Entomology and Plant Quarantine, in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, has been conducting research in an effort to find the insect vector, or vectors, of phony peach. Following an extensive survey of insects associated with peach orchards in areas where the annual disease incidence was high, in other areas where few new cases occurred each year, and finally, in areas where there appeared to be no local spread, a test program utilizing the most likely insect suspects was undertaken.

Results of the survey, together with data derived from experimental transmission of the disease, suggested the probability that phony peach was being spread by one or more stem-feeding Cicadellidae (leaf hoppers) belonging to the subfamily Tettigellinae. Although 53 species of insects, including members of several different families of Homoptera and a number of species of Cydnidae (Hemiptera), have been used in transmission tests, most attention for the last four years was given to four species of leaf hoppers.

From 1939 through 1944 the work was conducted at Chattanooga with field-grown peach trees that were unprotected from possible natural infection. During the course of these tests three of the experimental trees developed symptoms of the disease. One had been subjected to inoculation by *Cuerna costalis* (F.), one by *Graphocephala versuta* (Say), and one by *Homalodisca triquetra* (F.),[2] all members of our selected group of suspects. One case of phony peach occurred in one of the many check trees that were maintained.

In 1945 the work was transferred to Fort Valley, Georgia, where it was necessary to grow the experimental trees in a large screened cage because of the certainty of considerable natural spread in unprotected trees. Insects

[1] The diagnoses on which this report is based were made by Lee M. Hutchins and L. C. Cochran, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, and by L. D. Christenson and William F. Turner, Bureau of Entomology and Plant Quarantine, of the United States Department of Agriculture.

[2] The leaf hoppers actually used in each of these and all other tests were identified by P. W. Oman and J. S. Caldwell, Division of Insect Identification, Bureau of Entomology and Plant Quarantine.