on 9 volunteers indicated that the mouse-adapted dengue virus had undergone a very extensive modification in its pathogenicity for human beings. The intracutaneous or subcutaneous injection of 0.2 cc of a 5 per cent. centrifuged brain and cord suspension gave rise either to no systemic symptoms or after an incubation period of 8 to 10 days to reactions not exceeding in severity those following typhoid vaccination, *i.e.*, fever with or without headache and malaise for 24 hours or less. A marked and extensive maculo-papular eruption and terminally also petechiae on the feet and ankles were the rule. However, when this dose of mouse-adapted dengue virus was given simultaneously, or mixed, with the regular U. S. Army dose of yellow fever vaccine, the rash was reduced to a small number of faint macules and the petechiae were entirely suppressed. This combination was tried because one of us (A.B.S.) had previously observed an interference phenomenon between yellow fever vaccine and the regular, unmodified dengue virus in human beings.[4] It has, furthermore, been demonstrated that the virus present in the blood at the onset of the rash in the volunteers inoculated with 7th passage virus was of the modified type, and that *Aëdes aegypti* feeding during that period became infected with difficulty since large numbers of mosquitoes and an extrinsic incubation of more than 3 weeks were required to transmit the virus. However, the virus that these mosquitoes transmitted was also of the modified type, since rash without fever or significant symptoms and the leukocyte changes seen in dengue were the only reactions observed in the bitten individual. The 9 volunteers who were inoculated with the 7th, 9th or 10th mouse-passage dengue virus, either alone or in combination with yellow fever vaccine, and 1 volunteer who was bitten by mosquitoes carrying the modified virus, were all found to be immune when they were exposed to the bites of *Aëdes aegypti* mosquitoes of proved infectivity at intervals of 12 days (2 men), 21 days (5 men), 24 days (1 man) and 28 days (2 men) after inoculation. Four volunteers, who served as controls for these tests and were bitten by mosquitoes from the same lots, developed typically severe unmodified dengue. The volunteers, who received the combination of dengue and yellow fever vaccine, also developed neutralizing antibodies for the yellow fever virus.[8]

SUMMARY

It has been demonstrated that dengue virus can be propagated by intracerebral inoculation in mice. Although initial adaptation to the mouse is a tedious and difficult process, 16 consecutive passages have been achieved already in one series and further passages are in progress. The virus propagated in mice produced dengue in human volunters, but was not pathogenic for cotton rats, hamsters, guinea pigs or rabbits. Although it was evident that even after 2 serial passages in mice the virus produced a modified type of disease in human beings, tests with the 7th, 9th and 10th passage material indicated that the modification had become so marked that it could be used as a vaccine for the production of immunity against dengue.

LT. COL. ALBERT B. SABIN, M.C., AUS
CAPT. R. WALTER SCHLESINGER, M.C., AUS
ARMY EPIDEMIOLOGICAL BOARD,
THE CHILDREN'S HOSPITAL
RESEARCH FOUNDATION,
CINCINNATI 29, OHIO

[8] We are indebted to Dr. Max Theiler, of the International Health Division of The Rockefeller Foundation, for carrying out the yellow fever neutralization tests on these sera.

BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF 2,4-DICHLOROPHENOXYACETIC ACID

THE specificity of 2,4-dichlorophenoxyacetic acid in bringing about the death of certain weeds[1, 2] and the well-known bacteriostatic properties of some other aromatic compounds suggest that 2,4-dichlorophenoxyacetic acid might affect the growth of some lower organisms such as fungi and bacteria.

It was observed that open test-tubes of potato-dextrose agar[3] containing 0.1 per cent. 2,4-dichlorophenoxyacetic acid dissolved in 0.5 per cent. Carbowax 1500[4] remained sterile for 30 days, while similar tubes containing no 2,4-dichlorophenoxyacetic acid became contaminated with bacteria and fungi.

In a subsequent experiment two lots of potato-dextrose agar were prepared, one containing 0.1 per cent. of 2,4-dichlorophenoxyacetic acid and 0.5 per cent. Carbowax 1500 and the other containing 0.5 per cent. Carbowax only. The reaction was adjusted with N/1 NaOH to give pH values of 5, 6, 7 and 8, respectively, in aliquots of each of the two series. All manipulations involving the media were made in the open laboratory. The media and utensils used were not sterilized and no attempt was made to avoid contamination. Five Petri dishes were used for each medium, 15 ml being used for each dish. After the media had solidified, spores of *Penicillium* sp. were dusted over the surface of all plates. The plates were then closed and incubated at 28° C. Within two days colonies of

[1] J. W. Mitchell, F. F. Davis and P. C. Marth, *Golfdom*, October, 1944.

[2] P. C. Marth and J. W. Mitchell, "2,4-Dichlorophenoxyacetic Acid as a Differential Herbicide," *Botanical Gazette*, 106: 224–232, 1944.

[3] 200 gms peeled potatoes, 20 gms dextrose, 15 gms agar, 1,000 ml water.

[4] J. W. Mitchell and C. L. Hamner, *Bot. Gaz.*, 105: 474–483, 1944.

Bacillus subtilis[5] and the *Penicillium* sp. were observed on all the control plates from pH 5 through pH 8. No colonies of bacteria were observed on the 2,4-dichlorophenoxyacetic acid media at pH 5, 6 or 7, but some very small apparently static colonies about 3 mm in diameter appeared on the treated agar at pH 8. These colonies were not visible a few days after their appearance. The bacteria overran the control plates at pH 7 and 8 so rapidly that the fungus barely made pin-point spots before the surfaces of the media were covered with bacteria, thus checking the growth of the fungus. The *Penicillium* sp. grew vigorously on all media treated with 2,4-dichlorophenoxyacetic acid except that at pH 8 in which case its growth was somewhat limited probably because of the relatively high alkalinity. There was no apparent antagonism between the fungus and the bacteria. The relative appearance of these plates remained unchanged for 15 days, even though they were opened on several occasions after 3 days' incubation.

For a further test of this bacteriostatic action of the Carbowax-acid mixture, two lots of potato-dextrose agar were prepared; one contained 0.1 per cent. of the acid and 0.5 per cent. of Carbowax; the other 0.5 per cent. of Carbowax. The acid-treated medium was diluted with the Carbowax medium to give concentrations of 0.02, 0.04, 0.06 and 0.08 per cent. of the acid, one lot was maintained at 0.1 per cent. and one lot was of Carbowax control agar. N/1 NaOH was added so that media at each concentration of 2,4-dichlorophenoxyacetic acid were represented at pH 6 and pH 7. The Carbowax control was also adjusted to pH 6 and pH 7. These media were then autoclaved 15 minutes at 15 pounds steam pressure and 15 ml poured in each Petri dish. After cooling, 5 plates of each medium were then inoculated on the left side with mycelial transfers of a *Fusarium* sp., streaked on the right side with *Aerobacter cloacae* isolated from bean plants, and streaked down the center of the plate with *Bacillus subtilis,* which had appeared in the control plates of the previous test. The plates were incubated at 28° C. After two days' incubation the fungus was growing vigorously in all plates. However, there was a definite bacteriostatic effect on both bacteria at all concentrations. The growth of *B. subtilis* was retarded more than that of *A. cloacae* and at 0.08 per cent. concentration the former was completely inhibited, while the latter appeared to be definitely retarded. Very slight growth of both bacteria took place at the 0.1 per cent. concentration of 2,4-dichlorophenoxyacetic acid. After one week the inhibiting effect was more pronounced and transfers were made to potato-dextrose agar from the margins and from the centers of the streaks of *A. cloacae* which had grown in the medium containing 0.5 per cent. Carbowax and 0.08 per cent. of the acid. There was no growth from the marginal transfers, or from 4 out of 5 of the central transfers, and it was concluded that most of the bacteria had been unable to maintain growth in the 0.08 per cent. concentration. The *Fusarium* sp. grew well at all concentrations.

In order to determine what role the Carbowax might play in the action of the acid, the sodium salt, which is soluble in water, was prepared by adding N/1 NaOH to 2,4-dichlorophenoxyacetic acid. The media used were: (1) potato-dextrose agar + 0.02 per cent. of the Na salt + 0.5 per cent. Carbowax; (2) potato-dextrose agar + 0.08 per cent. of the Na salt + 0.5 per cent. Carbowax; (3) potato-dextrose agar + 0.02 per cent. of the Na salt, and (4) potato-dextrose agar + 0.08 per cent. of the Na salt. Control media consisted of one lot of plain potato-dextrose agar and one lot of potato-dextrose agar containing 0.5 per cent. Carbowax. These media were adjusted to pH 7 with N/1 NaOH, sterilized at 15 pounds steam pressure for 20 minutes and 15 ml poured into 9 cm Petri dishes. After cooling, ten plates of each medium were streaked with three bacteria, *Bacillus subtilis, Staphylococcus aureus* (Food and Drug Administration strain 209) and *Phytomonas tumefaciens* (strain 671 Plant Industry Station, Beltsville, Md.)

At 0.02 per cent. concentration of the sodium salt of 2,4-dichlorophenoxyacetic acid there was a decided retarding effect on the growth of all three bacteria. Eight hundredths per cent. concentration of the salt completely inhibited *S. aureus* and *P. tumefaciens,* and with *B. subtilis* there was just a faint cloudiness visible along the streak.

The presence or absence of the Carbowax 1500 had little effect on the action of the sodium salt of 2,4-dichlorophenoxyacetic acid, although there were some indications that the salt alone at 0.02 per cent. had a greater retarding effect than the salt in combination with Carbowax 1500.

In a subsequent test it was found that 0.08 per cent. of the sodium salt of 2,4-dichlorophenoxyacetic acid in potato-dextrose agar completely inhibited *B. subtilis* but had no apparent effect on the growth of *Penicillium notatum* (strain 1249–B21 Northern Regional Research Laboratory) when the two were grown in the same Petri dish.

The salient points observed in these limited tests are that the addition of 0.02 per cent. 2,4-dichlorophenoxyacetic acid or its sodium salt with or without 0.5 per cent. Carbowax into potato-dextrose agar had a decided retarding effect on the growth of *Bacillus*

[5] Identified by N. R. Smith, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agriculture Research Administration, U. S. Department of Agriculture, Beltsville, Md.

subtilis, Aerobacter cloacae, Staphylococcus aureus and *Phytomonas tumefaciens,* while it had no apparent effect on the growth of *Fusarium* sp. and *Penicillium* sp. A concentration of 0.08 per cent. of 2,4-dichlorophenoxyacetic acid or its sodium salt in potato-dextrose agar with or without 0.5 per cent. Carbowax prevented the growth of *B. subtilis, S. aureus* and *P. tumefaciens* and retarded the growth of *A. cloacae,* being toxic to this organism in 4 out of 5 cases after a period of one week. The growth of *Fusarium* sp. and *Penicillium* sp. was not affected noticeably by the 0.08 per cent. concentration of the acid or its salt nor was the growth of *P. notatum* visibly affected by the salt.

Further studies are in progress using common test organisms and a number of plant pathogens.

E. C. STEVENSON,
Associate Pathologist
JOHN W. MITCHELL,
Physiologist
BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING,
U. S. DEPARTMENT OF AGRICULTURE,
BELTSVILLE, MD.

THE PRODUCTION OF CARCINOMA AND SARCOMA IN TRANSPLANTED EMBRYONIC TISSUES[1]

SEVERAL years ago it was found that embryonic mammalian tissues could be grown in adult alien species and that such transfer was as readily effected as homologous embryonic transplantation.[2] At the same time an attempt was made to induce tumor formation in heterologous embryonic transplants with chemical carcinogens; for control parallel experiments with homologous embryonic tissues were performed. Cancer resulted in a number of heterologous transplants, including several of human origin; but an exploration of the diverse lines of research suggested by the results is still in progress and demands a more detailed report than is possible at present. The results of the control studies entailing the production of cancer in homologous embryonic transplants appear of sufficient immediate interest to warrant an independent report at this time. The intent of the present note is to draw attention to the method and its general results. Precise details and other pertinent observations will be discussed in a later paper.

The technique employed is comparable to that used in routine tumor or tissue transfer. The embryonic organ or tissue is placed in the mouth of a trocar and impregnated with crystals of methylcholanthrene. The trocar is inserted through an incision and the tissue fragment expelled in the desired region of the new host. The anterior chamber of the eye, testicle, peritoneal cavity, muscle and subcutaneous regions have been utilized as inoculation sites but, in general, subcutaneous transfers have proved most satisfactory. Mouse embryos have been used most extensively in homologous transfers, but in several experiments guinea-pig embryos were utilized, and it is significant that despite the resistance of adult animals of this species, the embryonic tissues proved as susceptible to the carcinogenic action of methylcholanthrene as did mouse material.

In experiments utilizing mice, treated embryonic tissue was transferred to adult animals of the same and of different strains without apparent variation in the incidence of takes or in the ultimate fate of the transplant. The organs from embryos of C_3H extraction grew as readily in C57 black or Bagg albino mice as in the parent strain, and present data reflect no influence of the genetic constitution of the new host on the action of the carcinogenic chemical.

A variety of embryonic organs and tissues, including lung, stomach, intestine, skin, muscle and cartilage, have been employed with comparable results. Successful transfer is followed by rapid growth which reaches a peak toward the end of the second week. A short interval of apparent quiescence ensues, but early in the fourth week renewed growth becomes evident. The transplants in animals killed between the thirtieth and thirty-fifth days measure 1 to 1.5 cm in diameter and show all the cellular and structural changes characteristic of cancer. It should be emphasized that in all these experiments, the diagnosis of cancer is based on biological behavior as well as on morphology and such diagnosis is not made unless the tissue in question possesses the ability to grow and to duplicate its structure in alien species. At the present point in this series of experiments, approximately 60 per cent. of the transplants examined between the thirtieth and thirty-fifth days fulfil both morphological and biological requirements. It appears significant that embryonic tissues undergo such modifications within 35 days, whereas from 90 to 200 or more days are required before comparable changes appear in adult tissues.

In view of present efforts directed toward the production of cancer of the alimentary canal with chemical carcinogens, it is noteworthy that carcinoma of the glandular portion of the stomach and of the intestine may be induced by the application of the chemicals to embryonic transplants. A pronounced hyperplasia precedes the appearance of malignant changes and, in the intestine, results in glandular

[1] From the Departments of Pathology and Surgery, Yale University School of Medicine, New Haven, Conn. This investigation was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research and the International Cancer Research Foundation.

[2] H. S. N. Greene, *Cancer Research,* 3: 809, 1943.