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.

masses simulating polyps. Gastric mucosa produces an abundance of mucin in early stages and may terminate either as a colloid carcinoma or a typical fundic adenocarcinoma.

Involvement of host tissues is apparent by the forty-fifth day and is characterized by the active invasion of tissues as well as by the passive infiltration of tissue spaces. Metastasis has occurred, but the majority of animals have been killed for early study and significant data are not yet available.

The carcinomas arising in subcutaneous regions are obviously derived from the transplanted embryonic organ, but some question obtains with reference to the origin of the sarcomas, for their appearance is often identical with that of growths obtained in adult animals. However, their early inception together with their position in relation to embryonic remnants is suggestive. Moreover, in one instance in which the primary tumor consisted solely of sarcomatous fibroblasts, a splenic metastasis contained areas of newly formed cartilage, thus identifying the growth as a chondrosarcoma and rendering an origin from the subcutaneous connective tissue of the adult host highly improbable.

These experiments were instituted on the assumption that the reserve stores of stem or partially differentiated cells of the body formed the source of neoplastic cells in adult animals and that embryonic tissues might prove a more favorable medium for the experimental production of tumors than the corresponding tissues of adult animals. The results obtained support this assumption. The method described offers a means of producing carcinomas in a variety of internal organs in a relatively short time. Moreover, the ability to transplant treated tissues heterologously and to test the susceptibility of embryonic organs of resistant species after transfer to susceptible hosts and vice versa offers a new approach to a study of the nature and mode of action of carcinogenic chemicals.

HARRY S. N. GREENE

ACETYL CHOLINE AND THE ACTIVATION OF MARINE EGGS

RECENT studies have made it appear probable that acetyl choline is of fundamental importance in the excitation process in nerves, muscles and electric organs.[1, 2] The activation of marine eggs has certain features in common with excitation of nerve,[3] and it seemed worth while to examine the effects of acetyl choline on such activation.

The eggs of the echiuroid worm *Urechis caupo* Fisher and MacGinitie, and of the sea urchin *Strongylocentrotus purpuratus* (Stimpson) were used. *Strongylocentrotus* eggs were activated by (*a*) very dilute sperm suspensions or (*b*) treatment with distilled water for ½ to 2 minutes. *Urechis* eggs were activated by (*a*) dilute sperm suspensions, (*b*) brief treatment with hypotonic sea water or distilled water, (*c*) treatment with isotonic calcium chloride solution

[1] J. F. Fulton and D. Nachmansohn, SCIENCE, 97: 569, 1943.

[2] D. Nachmansohn, R. T. Cox, C. W. Coates and A. L. Machado, *Jour. Neurophysiol.*, 5: 499, 1942, and 6: 383, 1943.

[3] R. S. Lillie, "Protoplasmic Action and Nervous Action," Chicago, 1932 (2nd ed.).

TABLE 1

INHIBITION OF ACTIVATION IN MARINE EGGS BY ACETYL CHOLINE AND PHYSOSTIGMINE

Eggs of:	Activating agent:	Per cent. of eggs activated, controls	Difference from control, and standard error of the difference, with	
			acetyl choline	physostigmine
	Dilute sperm	93	−93 ± 2	
		64	−64 ± 2	
		87		−67 ± 3
		72		−16 ± 3.5
		58		−27 ± 7
		24		+63 ± 8
Urechis caupo	Hypotonic sea water	35	−29 ± 3	−26 ± 3
	Isotonic $CaCl_2$	97	−15 ± 2.4	− 7 ± 1.7
		22	−20 ± 3	−22 ± 3
		17	−15 ± 3	−16 ± 3
	Ca-free artificial sea water	87	−87 ± 2	
		9	− 8 ± 1	
		4	− 4 ± 1	
		97		−96 ± 1
		68		−41 ± 7
	Ultraviolet	93	−92 ± 2	−80 ± 3
		42	−41 ± 4	−40 ± 5
		22	−21 ± 3	−21 ± 3
Strongylocentrotus purpuratus	Dilute sperm	64	−60 ± 3	+12 ± 3.5
		19		+16 ± 5
		5.8	−5.6 ± 1.1	+0.9 ± 1.6
	Distilled water	40	−39 ± 2	−27 ± 3
		37	−37 ± 2	−25 ± 3
		30	−29 ± 2	−11 ± 3