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

for 10–20 minutes, (*d*) treatment with artificial sea water lacking calcium ion for 1 to 4 hours, or (*e*) irradiation with ultra-violet light of wave-length about 2537 Å for ½ to 3 minutes. Membrane elevation was used as a criterion of activation in *Strongylocentrotus* eggs, dissolution of the germinal vesicle in the eggs of *Urechis*. A small and variable percentage of the eggs of *Urechis* cleaved when activated by any of the artificial treatments. Artificial activation of *Strongylocentrotus* eggs did not result in cleavage.

The addition of 1 mg of acetyl choline bromide to 10 ml of the activating solution before the eggs were placed in the solution resulted, in almost every case, in a marked decrease in the number of eggs activated by the particular treatment. Typical results are presented in Table 1. The addition of 1 mg of physostigmine salicylate to 10 ml of activating solution had effects wholly comparable to those of acetyl choline, except when the eggs were activated with sperm. With the eggs of *Strongylocentrotus* activated by sperm, the only effect noted was a small increase in activation. With the eggs of *Urechis* activated by sperm, a marked decrease similar to that produced by acetyl choline was evident in most cases, but in one experiment an equally marked increase was apparent.

Further analysis of these effects is in progress, and a more detailed account will be published later.

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THE OXYDASE REACTION IN CHICK EMBRYOS AND BROTH MEDIA CONTAINING NEISSERIA[1, 2]

THE value of the oxydase reaction described by Gordon and McLeod[3] for the preliminary identification of meningococci and gonococci has been well established. This reaction is ordinarily elicited by spraying or flooding a petri dish culture with a fresh, one per cent. aqueous solution of either dimethyl or tetramethyl paraphenylene diamine hydrochloride. Colonies of members of the genus Neisseria will then undergo a series of characteristic color changes: pink to maroon to black with the former reagent, and lavender to purple with the latter.

In the course of certain experiments concerned with meningococcic and gonococcic[4, 5] infections of chick embryos, it was found that typical oxydase reactions could be produced upon the infected chorio-allantoic membranes. Forty-eight hours after the inoculation[6] of the chorio-allantoic membranes of eleven-day-old embryos a portion of the shell was removed, the membrane cultured, and a drop of either the dimethyl or tetramethyl paraphenylene diamine hydrochloride solutions deposited thereon. In all instances the Neisseria-infected chorio-allantoic membranes gave positive reactions. Control embryos, either uninfected or infected with organisms other than the Neisseria, yielded negative reactions. Parallel passages on appropriate solid media gave similar findings.

Since the allantoic fluids of Neisseria-infected embryos contain large numbers of organisms, they were subjected to similar tests. This was achieved by overlaying one milliliter of allantoic fluid in a narrow tube with one or two drops of oxydase reagent. The typical sequences of color changes were noted at the interphase. Similar reactions were obtained in broth cultures, including those in the Neisseria fermentation broth previously described.[7]

The specificity of the reaction in both infected allantoic fluid and broth media suggested the possibility that the reacting enzyme was of a soluble char-

TABLE 1

SUMMARY OF OXYDASE REACTIONS OBTAINED IN CHICK EMBRYOS INFECTED WITH VARIOUS AGENTS

Infecting agent	Total number strains	Total number embryos	Chorio-allantoic membrane		Allantoic fluid*		Supernate†	
			Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
N. intracellularis	15	41	18	0	23	0	22‡	1
N. gonorrheae	3	13	12	0	1	0	N.D.§	N.D.
N. catarrhalis	1	2	2	0	N.D.	N.D.	N.D.	N.D.
Staph. aureus	2	2	0	2	N.D.	N.D.	N.D.	N.D.
Sh. alkalescens	1	2	0	2	N.D.	N.D.	N.D.	N.D.
Sh. paradys. Boyd 88	1	2	0	2	N.D.	N.D.	N.D.	N.D.
Paracolon	2	4	0	2	0	2	0	2
P. gallinaceum	1	1	0	1	0	1	N.D.	N.D.
Influenza A virus	1	2	0	2	0	2	0	2
Normal embryos	..	8	0	8	0	1	0	1

* Uncentrifuged.
† Centrifuged, bacteria-free.
‡ The allantoic fluid whose cell-free supernate gave a negative reaction was also negative on culture but morphologically and tinctorially positive for N. intracellularis on smear examination.
§ N.D. = Not done.

[1] With the technical assistance of Frances S. Friedman.
[2] The valuable aid of First Lieutenant (then Sgt.) Barclay R. McGhee, Sn. C., A. U. S., during the early phases of this study is gratefully acknowledged.
[3] J. Gordon and J. W. McLeod, *Jour. Path. and Bact.*, 31: 185–190, 1928.
[4] G. J. Buddingh and A. D. Polk, SCIENCE, 86: 20–21, 1937.
[5] G. Morrow and G. P. Berry, *Jour. Bact.*, 36: 280, 1938.
[6] The surface of a chocolate agar culture which had been incubated for twenty-four hours was scraped and the growth suspended in tryptose-phosphate broth. One drop of this heavy suspension served as the inoculation dose.
[7] D. M. Kuhns and H. A. Feldman, *Am. Jour. Pub. Health*, 33: 1461–65, 1943.