

and of 100 children receiving Toxoid 7 B (0.75 per cent formalin) 36 per cent were negative.

CONCLUSION.

Diphtheria toxoid (anatoxin) is an ideal preparation for active immunization against diphtheria. This is especially true for children of school age and of pre-school age who are relatively free of protein sensitiveness and in whom the toxoid produces very slight local or constitutional reactions.

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Studies upon the biological reactions of growing tissues to radiant energy. I. Effect of radiumized media upon tissue cultures *in vitro*.

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The *in vitro* cultivation of embryonic tissues seemed to us the most promising method of gaining important information of the biological reaction of growing tissues to radiant energy. Up to the present, experimentation along physical lines has claimed major interest, and very scant attention has been paid to the biological importance of radiant energy. Consequently very little is known concerning the biological phenomena accompanying radiation.

It was highly desirable therefore to study the immediate and latent behavior of tissues growing *in vitro*, when placed in immediate contact with radium salts and radium emanations.

The experiments to be reported were conducted upon chick embryo hearts, embryo spleens, and upon Flexner-Jobling rat carcinoma. Along with these, numerous experiments were made upon the effects of radium salts, radium emanations, as well as X-rays, upon different media, such as plasma, serum, embryonic tissue extracts, etc.

In the present studies chick embryo hearts from 7 to 11 day embryos, and spleen from 18 to 20 day embryos were used for *in vitro* studies. Small fragments were planted in a medium of chicken plasma and embryonic tissue juice. In the cultures where

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spleen tissue was used, the embryonic tissue extract was omitted. Fine glass seeds about 0.5 centimeter in length, and 1 millimeter in thickness, containing 0.1, 0.25, 0.35, 0.5 millicuries to 1.0 millicurie of radium emanations, were placed in direct contact with the tissue, or, 1 and 2 millimeters distant.

A series of 12 experiments were conducted comprising 100 cultures, without radium or with empty glass seeds. Growth was charted by using the camera lucida, and the area of new growth measured with the planimeter one hour, 24 hours, and 48 hours after incubation. After this period subculturing was continued.

In the 133 controls a normal growth was observed in 124 cultures, 9 cultures showed either no growth or a very scanty one.

In the 100 cultures treated with radium emanation 49 showed no growth whatsoever, 30 were markedly inhibited, especially on the side toward the radium seed, and in 21 growth was fair or normal. It was of especial interest to observe that cultures with 0.1 millicurie of radium emanations were effected in practically the same proportion and manner as those with the larger amounts.

Aside from these experiments with radium emanation, other cultures were treated by applying needles containing 5, 10, 25, and 50 milligrams of Radium Bromide to the mica cover slip over the small fragment of tissue, for varying lengths of time. In the majority of these cultures growth occurred, but the outgrowth was studded with fine vacuoles indicating early degeneration, and upon subculture, showed complete degeneration within 8 to 10 days.

No. Exp.	Experimental					Control		
	No. Cult.	Me. Rad.	No. Growth	Inhi-bited	Fair	No. Growth	No. Growth	Normal
18	8	0.1	0	8	0	8	0	8
22	6	0.13	5	1	0	6	0	6
38	10	0.24	3	4	3	10	0	10
50	4	1.05	4	0	0	19	0	19
54	10	0.25	7	3	0	19	5	14
64	11	0.12	5	5	1	35	2	33
70	6	0.5	0	3	3	9	0	9
72	18	0.25	12	0	6	9	0	9
74	12	0.25	5	6	1	10	1	9
80	5	0.35	4	0	1	4	0	4
92	5	1.02	0	0	5	5	1	4
93	5	0.21	4	0	1	4	0	4
Totals	100		49	30	21	138	9	129

In connection with this work I wish to express my appreciation to Dr. Muir, who supplied the Radium Emanation seeds.

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A biochemical reaction associated with sex in Cladocera.

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A biochemical test for sexual differences reported by Manoilov² and used as the Station for Experimental Evolution by Miss Sophia Satina and others with various plants and animals, has been applied to three races of Cladocera. This is probably the first time that such a biochemical difference between the sexes has been demonstrated in a crustacean.³

The first test with Cladocera was with small samples (0.02 gram) of females and males of *Moina macrocopa*. The female sample gave a faint violet coloration. The male sample gave no color. These samples, though each involving 200 or 300 individuals of this minute species, were too small to give the striking reaction obtained with more material.

The other Cladocera used were obtained from the wilds near Cold Spring Harbor. One form, a distinct type closely related to *Daphnia pulex*, was in an actively sexual condition. Every female was producing sexual eggs, and adult males were in the population in great numbers. A sample in duplicate of the females consisting of nearly 200 individuals was taken. A second sample was of males exclusively, about 350 individuals. A third sample was of a different type of *Daphnia pulex*, but obtained at the same time and from the same pond. The third sample was of a stock which was exclusively in parthenogenetic reproduction,

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² Manoilov, E. O., *Bul. Appl. Bot. and Plantbreed*, 1922-1923, xiii (2), 503.

³ A statement of the method used is appearing in *Science*, "Manoilov's Reaction for Identification of the Sexes" by Sophia Satina and M. Demerec.