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Tissue Culture Growth Stimulants from Ground Frozen-Dried Chick Embryos.

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It has been demonstrated[1] that frozen-dried plasma and similarly treated embryo juice used in combination, after solution in distilled water, form an adequate and satisfactory medium for the growth of tissue cultures. Further experimentation with such preparations has brought forth evidence that a much more potent embryo juice could be secured if the embryos themselves were ground and frozen-dried before extraction.

Eleven-day chick embryos were removed from their shells and membranes and reduced to a gray, grumous mass with sea sand in a Ten Broeck grinder. The material was pipetted off and allowed to stand in a container for a short while to permit the larger particles of grit to settle. Later the ground substance was introduced into pyrex ampoules, frozen-dried and sealed *in vacuo* by means of a Lyophile[2] apparatus. All manipulations were carried out aseptically.

The dried matter at first was crusty but could be reduced to a powder by forcibly shaking or by tapping the containers. The ampoules were stored at room temperature for 14 months before any tests were instituted. A preliminary series of cultures was made with embryo juice prepared by steeping a portion of the dried powder in Ringer-Tyrode's solution in an ice box prior to centrifuging. The cultures grew so exuberantly that it was decided to test statistically the growth-promoting effect of this extract on cultures of embryo chick heart.

By calculation from fresh embryos dried to constant weight in an oven, 70 mg of dry powder were equivalent to one gram of fresh embryo. This figure was only approximately accurate since there was no simple way of determining how much sand and glass dust might have been retained in the powder. However, 20% embryo juice was made by adding 70 mg of powder for each 5 cc of Ringer-Tyrode's solution. The mixture was agitated gently, steeped for

1 Hetherington, Duncan C., and Craig, Jane Stanley, PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 831.

2 Flossdorf, Earl W., and Mudd, Stuart, *J. Immunol.*, 1935, **29**, 389.

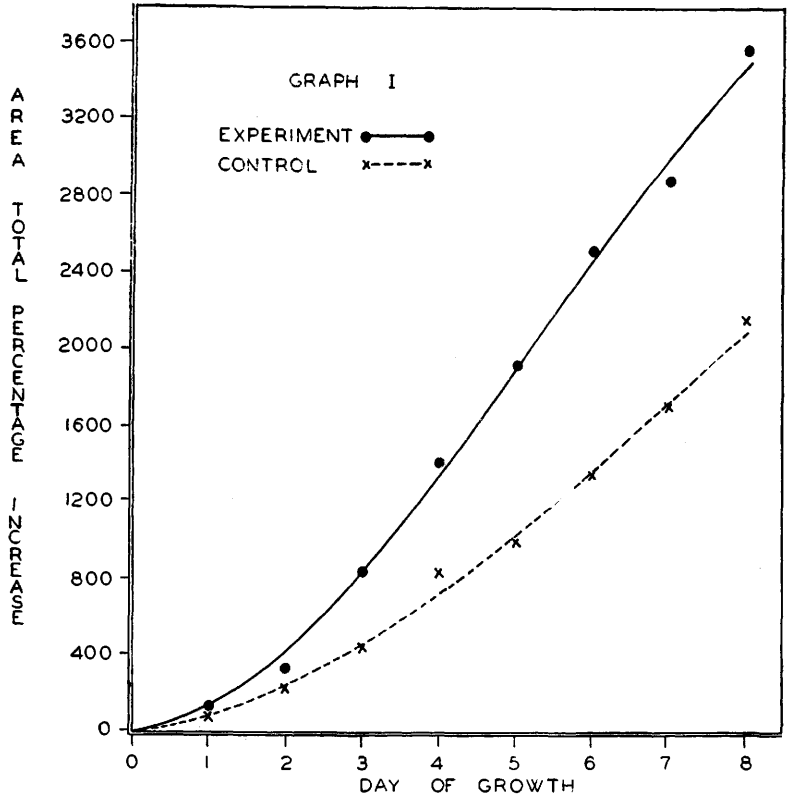
TABLE I.

Days of growth	Control Series: Mean total area in mm²	Control Series: Probable error of mean	Control Series: σ	Control Series: Total growth in %	D_M	σ_D	$3\sigma_D$	Significant difference	Experimental Series: Mean total area in mm²	Experimental Series: Probable error of mean	Experimental Series: σ	Experimental Series: Total growth in %
0	0.77	±0.02	0.27	0	0.13	0.04	0.12	+	0.64	±0.02	0.31	0
1	1.45	±0.05	0.65	88	0.04	0.11	0.33	0	1.49	±0.05	0.81	133
2	2.51	±0.07	1.12	225	0.32	0.19	0.57	0	2.83	±0.10	1.46	342
3	4.33	±0.17	2.42	462	1.75	0.46	1.38	+	6.08	±0.28	3.92	850
4	7.28	±0.22	3.17	845	2.48	0.71	2.13	+	9.76	±0.38	5.47	1425
5	8.61	±0.32	4.53	1018	4.36	0.77	2.31	+	12.97	±0.40	5.74	1926
6	11.26	±0.38	5.33	1362	5.56	0.85	2.55	+	16.82	±0.43	6.08	2528
7	14.06	±0.39	5.59	1726	5.02	0.91	2.73	+	19.08	±0.47	6.53	2881
8	17.54	±0.40	5.66	2178	5.84	0.88	2.64	+	23.38	±0.43	6.14	3553

D_M, difference of the means; σ_D, standard error of the difference of the means; significant difference $= D_M > 3\sigma_D$.

24 hours in an ice-box and centrifuged. The resultant slightly pink, opalescent fluid was removed and delivered in 1 cc quantities into ampoules and frozen-dried. Again aseptic technic was employed throughout. After 3 months storage this frozen-dried embryo juice made from the frozen-dried embryos was tested for growth-promoting properties. Two series of tissue cultures were planted from 11-day chick embryo hearts. At any one planting the same heart was used for both the experimental and the control cultures. The former were planted in equal parts of the new type embryo juice and frozen-dried plasma (stored for 6 months); while the latter were similarly treated except that 20% juice from fresh 11-day embryos was employed.

Delineascope and planimeter records were kept of the areas of the original explants; thereafter for 8 days, the total area of each culture was measured at 24-hour intervals. The results obtained from 90 cultures in each series of the experiment appear in Table I and Graph 1. From these it will be seen that there was a significant



growth increase in the experimental series, indicating rather conclusively the greater potency of the embryo-juice. Furthermore, careful microscopic examination of the living cells indicated that those in the test series did not develop fat droplets as readily as the controls and at all times appeared in better condition.

Fowler[3] in a series of titrations of embryo juices upon chick heart fibroblasts concluded that best growth was obtained from utilizing extracts from 11- to 14-day whole embryos since during that growth period greater morphological and physiological changes took place within the developing animal—attributed possibly to endocrine production.

The potency of embryo juice prepared by the present method may be ascribed to increased accessibility of the cell contents to extraction and solution by the Ringer-Tyrode's saline. The preliminary grinding reduced the tissues to smaller particles than could be accomplished by mere mincing and the subsequent freezing* and drying disrupted most of the cells. In consequence many more substances from all parts of the embryo went into solution, probably in an undenatured condition.[2, 4]

The experiment reported has shown that a very excellent embryo juice may be prepared from ground frozen-dried chick embryos. The dried powder retained its potency for 14 months and frozen-dried embryo juice derived from it was extremely active after 3 months' additional storage. There seems hence to be no reason why such products as frozen-dried embryo powder and frozen-dried plasma could not be used to considerable advantage in tissue culture laboratories.

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[3] Fowler, Ona M., *J. Exp. Zool.*, 1937, **76**, 235.

* Neutral red spreads made from tissues of chick embryos frozen to —70°C and then thawed rapidly at 37°C showed that very few of the cells remained intact. The nuclei alone appeared to be unbroken.

[4] Elser, William J., Thomas, Ruth A., and Steffin, Gustav I., *J. Immunol.*, 1935, **28**, 433.