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Effect of Frozen-Dried Plasma and Frozen-Dried Embryo Juice on Tissue Cultures.

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In view of the remarkable results obtained in keeping bacterial antigens, sera and other products without alteration for long periods of time, following freezing and drying *in vacuo*, it was undertaken to determine whether plasma and embryo juice for tissue culture could also be treated similarly. The following report describes briefly the procedure and the results obtained.

Young chickens were deprived of food for 24 hours and then bled aseptically by cardiac puncture. Not more than 20 cc of blood was removed at a time. It was prevented from clotting by being drawn into syringes moistened with a solution of heparin. The plasma was collected after centrifuging the blood on ice, and was pooled before distribution in one cc quantities in small glass ampoules prior to freezing and drying with a Lyophile* apparatus. The ampoules sealed *in vacuo* were labeled and stored in an icebox.

Twenty percent embryo juice was prepared in the usual manner,† using Ringer-Tyrode solution (glucose 0.2%) and chick embryos of 11 days' incubation. This material in one cc quantities was likewise frozen, dried, sealed *in vacuo* and stored. To determine whether these 2 products would serve as adequate media for tissue cultures, the following experiment was performed.

The ampoules of dried plasma and embryo juice which had been kept in storage for 3 months were filed, sterilized in alcohol, and wrapped in sterile gauze before breaking the necks to open them. The dried powders were dissolved and diluted to original volume with sterile, triply distilled water. The plasma always dissolved without residue but a few flecks of insoluble material remained in the embryo

* The apparatus and method of freezing and drying the plasma and embryo juice were those described by Flossdorf, Earl W., and Stuart Mudd, *J. Immunol.*, 1935, **29**, 389.

† Twenty per cent embryo juice was made by weighing out 10-day embryos in a sterile dish and adding for every gram, wet weight, 4 cc of Ringer-Tyrode solution. The embryos were minced with sharp scissors and the mixture centrifuged at high speed. The supernatant fluid constituted the juice which was then frozen and dried *in vacuo*.

TABLE I.

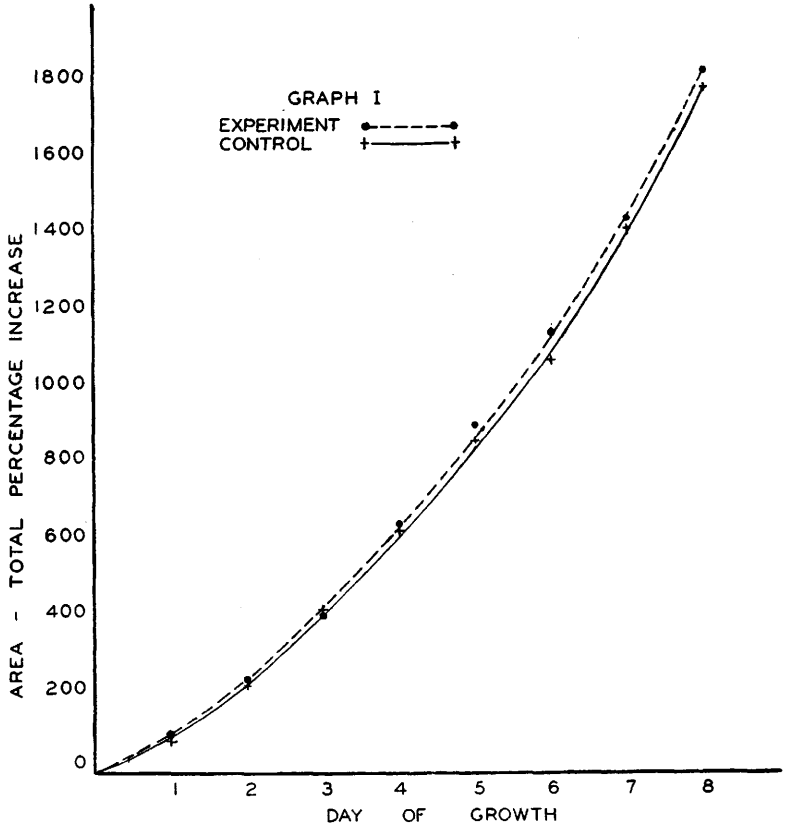
Days of growth	Control Series: Mean total area, mm²	Probable error of mean	Standard deviation	Total growth, %	D_M*	σ_D	$3\sigma_D$	Significant difference	Experimental Series: Mean total area, mm²	Probable error of mean	Standard deviation	Total growth, %
0	0.63	±.01	0.20	0	0.12	0.03	0.09	+	0.75	±.02	0.24	0
1	1.19	±.04	0.48	84	0.32	0.09	0.27	+	1.51	±.05	0.66	101
2	2.12	±.06	0.83	236	0.48	0.15	0.45	+	2.60	±.08	1.10	246
3	3.32	±.10	1.39	427	0.52	0.26	0.78	0	3.84	±.14	1.99	412
4	4.63	±.14	1.97	634	1.01	0.35	1.05	0	5.64	±.19	2.63	652
5	6.11	±.18	2.55	869	1.51	0.46	1.38	+	7.62	±.25	3.48	916
6	7.44	±.24	3.30	1080	1.90	0.58	1.74	+	9.34	±.31	4.28	1145
7	9.60	±.32	4.36	1423	2.01	0.73	2.19	0	11.61	±.38	5.16	1448
8	11.88	±.38	5.21	1785	2.60	0.87	2.61	0	14.48	±.45	6.12	1830

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

juice. Cultures of 11-day chick embryo hearts were planted in equal parts of the experimental plasma and embryo juice. From portions of the same hearts, control cultures in equal numbers were planted in freshly-drawn plasma and freshly prepared 20% embryo juice. The hanging-drop, cover-slip method was used and all cultures were incubated at 37.5°C.

Delineascope and planimeter were used to measure the areas of the original explants. During the succeeding 8 days the total area of each culture was measured at 24-hour intervals. The results were treated statistically and appear in Table I. In each series there were about 110 cultures, and from each of these groups the records of the first 85 cultures in full growth at the end of 8 days were tabulated.

It is apparent from Table I and Graph 1 that the growth of the experimental cultures and of the controls was almost identical. Furthermore, careful microscopical study of the living cells revealed no discernible difference between those growing in the new media as compared with those in the standard media.



The simplicity of planting cultures using frozen-dried plasma and embryo-juice is particularly attractive when technical help is limited, since much routine of preparation can be eliminated. The only disadvantage so far experienced with the plasma is that clotting time is slightly delayed.

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Mechanism of the Therapeutic Effect of Metrazol and Insulin Convulsions.*

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Anoxemia has been regarded as a common factor in the effects of insulin and metrazol convulsions, in that hypoglycemia diminishes the oxygen utilization of brain tissue (Holmes,[1] Wortis[2]), while the metrazol convulsions interfere with the respiratory movements (Himwich[3] and coworkers). Anoxemia may act by stimulating the sympathetic system (Gellhorn[4]), it may, however, affect the brain cells directly by increasing the permeability of the cellular surface films (Spiegel and Spiegel-Adolf[5]). The question may, therefore, be raised whether insulin and metrazol convulsions change the permeability of the cells of the central nervous system.

In 10 guinea pigs metrazol convulsions were produced (2 cc metrazol† intraperitoneally), in 10 others insulin convulsions (20-40 units of insulin). The brains of 3 guinea pigs were studied after the animals had received insulin, but before the onset of convulsions, while the measurements on 10 further animals served as normal controls. Part of the animals were killed by decapitation during the

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1 Holmes, E. G., *Biochem. J.*, 1930, **24**, 914; 1932, **26**, 2010.

2 Wortis, S. B., *New York State J. of Med.*, 1938, **38**, 1015.

3 Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F., *J. Am. Med. Assn.*, 1939, **112**, 1572.

4 Gellhorn, E., *Arch. Neur. and Psych.*, 1938, **40**, 125.

5 Spiegel, E., and Spiegel-Adolf, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 799; *J. Nerv. Ment. Dis.*, 1939, **90**, 188.

† The metrazol was kindly supplied by the Bilhuber-Knoll Corp.