been reported by Kiyono and associates[2] to exhibit an increased oxygen uptake as measured in the Warburg respirometer.

It is possible that the presence of the dye in some way stimulates the oxygen uptake and thus induces an increased growth rate of the cells. This hypothesis is only a tentative one since increased metabolic rate is not necessarily a sign of growth.

*Summary.* Cardiac explants from 10-day chick embryos planted in a medium of lyophiled chicken plasma and embryo juice containing 1/4000 trypan blue, grew significantly better than control cultures from the same hearts grown in an identical medium without the dye.

## 14752

## Frozen-Dried Serum as a Medium Constituent for Tissue Cultures.

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It has been demonstrated that frozen-dried chicken plasma and also embryo juice sealed *in vacuo* and stored for variable lengths of time, will, when redissolved to volume with sterile distilled water, form adequate media for growth of tissue cultures from chick embryos.[1] Furthermore a very potent embryo juice was obtained from previously ground, frozen-dried chick embryos. Juice prepared from this dried material by extraction with Ringer-Tyrode's solution can be used at once or can be frozen dried and stored for future use.[2]

Plasma and embryo juice constitute an adequate medium, among others, for cultivation of chick embryo tissues by the cover-glass method where experiments demand maintenance of the cultures for only 7-14 days. Cultivation of mammalian tissue requires a fuller medium met best by the addition to the plasma and embryo juice of serum,[3,4] either homologous or heterologous.

Since lyophiled embryo juice and plasma proved so satisfactory for cultivating chick tissues it was decided to determine what effect frozen-dried serum as an added constituent of the medium, might have upon the growth of cardiac explants from young mice.

Human cord serum was collected on occasions by the method outlined by the Geys.[4] At other times by cooperation of the Department of Obstetrics and Gynecology, when routine cord blood Wassermann samples were taken at the time of delivery, an additional tube was set aside in an ice-box for our use.

The serum, after retraction of the clots, was removed aseptically and pooled. Highly colored, cloudy or hemoglobin tinted serum was discarded. When a sufficient quantity was collected and tested for sterility by bouillon cultures, it was delivered into sterile 1 cc ampoules, lyophiled, and sealed *in vacuo*.

In the following experiments during any one planting of cultures upon cover-slips 3 series were set up. The heart of a 4-weeks-old white mouse was removed aseptically under ether anesthesia and placed in Ringer-Tyrode where it was carefully cut into uniformly small pieces by cataract knives. Cultures of Control A series were planted in a mixture of fresh embryo juice (20% strength prepared from 11-day chick embryos), fresh chicken plasma, and fresh human cord serum. Control B series cultures were set up in fresh serum, and frozen-dried embryo juice (20% made from previously ground frozen-dried 11-day chick embryos) and frozen-dried chicken plasma. In the Experimental series of cultures

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

2 Hetherington, Duncan C., and Craig, Jane Stanley, PROC. SOC. EXP. BIOL. AND MED. 1940, **44**, 282.

3 Lewis, W. H., *Contrib. to Embryology*, No. 150, Carnegie Inst. of Washington, 1935, **25**, 163.

4 Gey, George O., and Gey, Margaret K., *Am. J. Cancer*, 1936, **27**, 45.

TABLE I.
Statistical Results of Cultures Grown in All Lyophiled Media as Compared with Growth of Control Cultures.

| Days of growth | Control A Series: Mean total area in mm² | Control A Series: Probable error of mean | Control A Series: σ | Control A Series: Total growth in % | Control B Series: Mean total area in mm² | Control B Series: Probable error of mean | Control B Series: σ | Control B Series: Total growth in % | Experimental Series: Mean total area in mm² | Experimental Series: Probable error of mean | Experimental Series: σ | Experimental Series: Total growth in % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.85 | ±.01 | 0.23 | 0 | 0.87 | ±.02 | 0.31 | 0 | 0.85 | ±.02 | 0.26 | 0 |
| 1 | 1.15 | ±.02 | 0.30 | 35 | 1.21 | ±.03 | 0.37 | 39 | 1.23 | ±.03 | 0.35 | 44 |
| 2 | 1.50 | ±.03 | 0.37 | 76 | 1.69 | ±.04 | 0.49 | 94 | 1.80 | ±.04 | 0.53 | 112 |
| 3 | 1.97 | ±.04 | 0.56 | 132 | 2.38 | ±.05 | 0.74 | 173 | 2.56 | ±.07 | 0.86 | 201 |
| 4 | 2.67 | ±.05 | 0.58 | 214 | 3.11 | ±.06 | 0.95 | 257 | 3.64 | ±.10 | 1.31 | 328 |
| 5 | 3.75 | ±.08 | 1.04 | 341 | 4.38 | ±.10 | 1.35 | 403 | 5.72 | ±.11 | 1.37 | 573 |
| 6 | 4.99 | ±.11 | 1.45 | 487 | 5.86 | ±.11 | 1.43 | 573 | 7.91 | ±.19 | 2.39 | 831 |
| 7 | 6.35 | ±.16 | 1.91 | 647 | 7.44 | ±.12 | 1.53 | 755 | 10.07 | ±.20 | 2.62 | 1085 |
| 8 | 8.32 | ±.16 | 1.95 | 879 | 9.39 | ±.15 | 1.92 | 979 | 12.14 | ±.18 | 2.25 | 1328 |

Significant Difference

| Days of growth | Control A vs. Control B: $D_M$ | $\sigma_D$ | $3\sigma_D$ | Sig. D | Control A vs. Experiment: $D_M$ | $\sigma_D$ | $3\sigma_D$ | Sig. D | Control B vs. Experiment: $D_M$ | $\sigma_D$ | $3\sigma_D$ | Sig. D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.02 | .04 | .12 | — | .00 | .04 | .12 | — | .02 | .04 | .12 | — |
| 1 | 0.06 | .05 | .15 | — | .08 | .06 | .18 | — | .02 | .06 | .18 | — |
| 2 | 0.19 | .07 | .21 | — | .30 | .08 | .24 | + | .11 | .08 | .24 | — |
| 3 | 0.41 | .11 | .33 | + | .59 | .12 | .36 | + | .18 | .13 | .39 | — |
| 4 | 0.44 | .13 | .39 | + | .97 | .17 | .51 | + | .53 | .19 | .57 | — |
| 5 | 0.63 | .20 | .60 | + | 1.97 | .20 | .60 | + | 1.34 | .23 | .69 | + |
| 6 | 0.87 | .25 | .75 | + | 2.92 | .33 | .99 | + | 2.05 | .34 | 1.02 | + |
| 7 | 1.09 | .32 | .96 | + | 3.72 | .39 | 1.17 | + | 2.63 | .39 | 1.17 | + |
| 8 | 1.07 | .35 | 1.05 | + | 3.82 | .35 | 1.05 | + | 2.75 | .38 | 1.14 | + |

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

all of the constituents of the medium were frozen-dried. The lyophiled serum was 2 months old, while the embryo juice and plasma had been kept *in vacuo* for 18 months. In all cultures equal portions of serum, plasma, and embryo juice were used. Cultures were incubated at 37.5°C.

Records were kept by means of delineascope and planimeter of the areas of the original explants; thereafter for 8 days, the total area of each culture was measured at 24-hour intervals. The statistical results of 69 cultures in each series appear in Table I. From these it will be seen that in both the Control B and Experimental series growth was significantly better than the growth in Control A series where all components of the medium were fresh. Significant difference between the Control B and Experimental series appeared only after the first 4 days of growth. It would seem therefore that growth is enhanced by using frozen-dried serum in addition to the lyophiled plasma and embryo juice. The explanation is not clear since freezing-drying is not believed to denature biological material such as antigens, etc.[5] If this be true, fresh plasma and serum may contain some substance or substances acting as mild growth depressants, which during lyophiling are removed by volatilization.

The practical advantage of lyophiled media in tissue culture lies in the fact that with an adequate supply of material on hand much routine labor can be avoided. Furthermore for any given series of comparative experiments a constant medium can be maintained.

*Summary.* Lyophiled serum, embryo juice and plasma when used together form excellent media for the growth of cardiac explants from white mice. They have the added advantage of forming a standardized medium for comparative experiments.

5 Elser, William J., Thomas, Ruth A., and Steffin, Gustav L., *J. Immunol.*, 1935, **28**, 433.