days after alloxan injection. From examination of the tissues in this series it might be suspected that the majority of the cells present are degenerate forms of the original beta cells. There is little difference in the appearance of islets between the third and seventh days. In several alloxan-treated animals (30A, 6A, 26A), the pancreatic islets retain a normal appearance. In each instance where this occurred the animals failed to develop diabetes.

Almost all the sections of kidney in this series of animals with alloxan diabetes showed varying grades of parenchymatous degeneration most notable in epithelium of the convoluted tubules. In several cases there was marked swelling and hydropic degeneration of individual and small groups of epithelial cells. Two animals (29A, 10A) showed marked degeneration of epithelium of both limbs of loops of Henle. In these cases the changes appeared most marked in the ascending limbs where much of the epithelium was necrotic and the lumina were often filled with blue-stained granular debris.

Liver sections revealed frequent widespread parenchymatous degeneration and early stages of hydropic degeneration. No consistent changes were observed in the myocardium.

Summary. Glycogen determinations were made on the heart, liver, and skeletal muscle of rats with alloxan-produced diabetes mellitus and on the same tissues of untreated controls. The alloxan-treated animals showed a statistically significant increase in glycogen content of heart muscle and a statistically significant decrease in liver and skeletal muscle glycogen.

Rats which developed diabetes mellitus had a high blood sugar and high blood ketones at the time of autopsy.

On histological examination of the tissues, the islet cells of alloxan-treated rats which developed diabetes showed a marked reduction in cytoplasm, pycnosis and decrease in size of the nuclei with resultant collapse of islet structure. Almost all kidney and liver sections showed some degenerative changes.

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Effect of Trypan Blue upon Cardiac Explants in Tissue Culture.

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During some previous work with trypan blue in tissue cultures it was observed that the growth of explants in the dye-containing medium appeared to be more luxurious than in the control cultures. When buffy coat[1] of chicken blood was cultivated in a trypan blue medium, monocytes markedly increased in numbers as they became transformed into macrophages. Attempts were made to estimate this increase in numbers of phagocytic cells by making surface counts of macrophages in so many contiguous microscopic fields and comparing them with similar counts made upon control cultures stained by neutral red at the time of counting. There was a noticeable difference, but since cultures of buffy coat did not make a compact or membranous growth on the under surface of the cover-slip, comparative areal measurements could not be made and the matter was dropped.

The following experiment was designed to test the presence or absence of a stimulating effect upon growth of tissue cultures in a medium containing trypan blue.

At any one planting, control and experimental cultures were made on cover-slips from fragments of the same heart removed from a 10-day chick embryo. Lyophiled embryo juice (20%) and plasma diluted to volume with

* The author wishes to express his thanks to Jane Stanley Craig, who assisted in these experiments.

1 Hetherington, Duncan C., *Arch. f. exp. Zellforsch.*, 1931, **11**, 520.

TABLE I.
Showing Statistical Results of Cardiac Explants Grown with Trypan Blue Versus Growth of Controls.

	Control series				Significant difference				Trypan series			
Days of growth	Mean total area in mm²	Probable error of mean	σ	Total growth in %	D_M	σ_D	$3\sigma_D$	Sig. D	Mean total area in mm²	Probable error of mean	σ	Total growth in %
0	0.71	±.01	0.20	0	0.02	0.10	0.30	—	0.73	±.06	0.77	0
1	1.75	±.04	0.53	145	0.19	0.10	0.30	—	1.94	±.05	0.70	165
2	3.26	±.06	0.83	359	0.43	0.19	0.57	—	3.69	±.13	1.39	405
3	5.25	±.13	1.83	639	1.12	0.37	1.11	+	6.37	±.20	2.53	772
4	8.01	±.20	3.28	1028	3.28	0.64	1.92	+	11.29	±.33	4.25	1460
5	11.39	±.34	4.31	1504	7.24	0.86	2.58	+	18.63	±.40	5.76	2452
6	14.22	±.40	5.07	1902	8.49	0.95	2.85	+	22.71	±.47	6.16	3010
7	17.22	±.47	5.72	2325	10.30	1.01	3.03	+	27.52	±.47	6.23	3669
8	21.15	±.53	6.22	2878	10.62	1.10	3.30	+	31.77	±.54	6.78	4252

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

distilled water, were mixed in equal proportions and constituted the medium for growth of all control cultures. The experimental cultures were planted in the same manner except that to the embryo juice trypan blue was added beforehand in the ratio of 1/2000, the final dilution in the cultures becoming 1/4000. Sterile precautions were used throughout and all cultures were incubated at 37.5°C.

Delineascope and planimeter records were made of the original explants and thereafter at 24-hour intervals for 8 days. The results involving the records of 70 cultures in each series were treated statistically and appear in Table I.

The cultures were composed of a mixed population of cells, cardiac muscle, endothelium, mesothelium, a moderate number of macrophages, and predominating fibroblasts. As is common with such mixed groups of cells, the fibroblasts constituted the bulk of the radial outgrowth almost immediately. As far as could be determined microscopically the cell types in the control and trypan blue cultures were comparable except for the differential reaction of the cells in acquiring trypan blue inclusions and a more noticeable number of macrophages in the experimental series. These phagocytic cells did not migrate fast enough to keep up with the outgrowth of fibroblasts so that at no time were they a confusing factor in making the delineascope tracings and therefore did not complicate the marked areal increase noted in the dye cultures.

Only occasionally and usually late in the life of the culture, did cardiac muscle acquire a very few dye inclusions. These could be seen to be moved about in the cytoplasm of the contracting fibers. Mesothelial and endothelial cells which were never very numerous, accumulated only after a time a small number of very fine dye inclusions, arranged usually close to the nucleus. Fibroblasts also took up trypan blue and segregated it into vacuoles of various sizes, but without any patterned orientation within the cytoplasm. Many of these cells contained no dye. The most obviously loaded cells were the macrophages some of which underwent epithelioid transformations toward the end of the culture period. Record keeping was terminated on the eighth day of growth because the dye cultures became patchy rather suddenly and began to die off after this period. This behavior was attributed to the exhaustion of the small amount of food available in an undisturbed hanging drop culture.

It is apparent from Table I that the growth of cardiac explants in trypan blue medium was significantly more copious than in the control cultures. The explanation for this trend is not clear. Some tissues, notably liver, kidney, and spleen, excised from animals which had previously been treated with trypan blue have

[2] Kiyono, K., Sugiyama, S., and Amano, S., *Die Lehre von der Vitalfärbung*, Kyoto, II Hauptteil, 1937, p. 282.

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.

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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

* The author wishes to express his thanks to Jane Stanley Craig, who assisted in this work.

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.