the animal. With medium intensity the first signs of cytolysis appear in one minute and in three to four minutes disintegration is far advanced. Disintegration ceases after cessation of the vibration, intact parts remain alive and may reconstitute whole animals.

Tubifex tubifex, subjected to supersonic vibration, is very active showing intense stimulation. High intensity results in almost instantaneous death and disintegration, but with lower intensity the posterior growing segments disintegrate first, and disintegration progresses anteriorly. Simultaneously, a short gradient appears in the anterior region of the body.

Observations on hydra show that the tentacles disintegrate basipetally, but the body contracts so strongly that it is difficult to determine the course of disintegration there.

These supersonic disintegration gradients are the same as those observed with other agents and, so far as data are available, the same as the respiratory gradients and gradients in reduction of vital dyes. Evidence of alteration of reconstitution and of head frequency in *Euplanaria* by supersonics has already been obtained and work is being continued.

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ARTIFICIAL MEDIA FOR THE CULTIVATION OF FIBROBLASTS, EPITHELIAL CELLS AND MONOCYTES

THE object of the present communication is to describe two synthetic media that have been developed for the cultivation of cells outside the body. One was designed to promote the rapid proliferation of fibroblasts and epithelial cells; the other, that of monocytes. These media contain practically the same ingredients; they differ in the concentration of these ingredients.

Two synthetic media for fibroblasts, less complete than the one to be described here, have already been devised. The first of these contained a peptic digest of casein, glycocoll, nucleic acid, glutathione, hemoglobin, glucose and some ash of liver. A pure strain of fibroblasts from Crocker 10 sarcoma proliferated in this medium for 40 days as rapidly as did control tissues that were cultivated in embryo juice.[1] This medium did not suffice, however, for the cultivation of normal fibroblasts. The second medium, one devised by Vogelaar and Erlichman,[2] has given better results with the normal cells. It contained Witte's peptone, hemin, cysteine, insulin, thyroxine, glucose and the usual salts. Fibroblasts emanating from human thyroid tissue that was embedded in irradiated cow plasma proliferated in this medium for three months. The medium failed, however, to nourish chicken heart fibroblasts that were embedded in coagula made of horse plasma.[3]

The new medium to be described here contains all the substances used by Vogelaar and Erlichman, and in addition a number of others that greatly enhance its power to promote growth and maintain cell life. Its composition is as follows:

	Per 100 cc
Witte's peptone	675.00 mg
Cysteine hydrochloride	9.00 "
Hemin	0.0036 "
Insulin	0.09 units
Thyroxine	0.0009 mg
Glucose	100.00 "
Serum homologous to the tissue	10.00 cc
Vitamin A[4]	900.00 to 1800.00 units
Vitamin D[4]	about 15.00 to 30.00 "
Vitamin C (crystalline ascorbic acid)	0.25 mg
Glutathione	1.00 "
Phenol red	5.00 "
Sodium chloride	720.00 "
Potassium chloride	18.00 "
Calcium chloride, anhydrous	18.00 "
Magnesium chloride, 6 H_2O	9.00 "
Sodium dihydrogen phosphate	4.50 "
Sodium bicarbonate, anhydrous	100.00 "

When the solution is used for the cultivation of organs, the glucose is increased to 300 mg per cent., and the sodium chloride reduced sufficiently to keep the solution isotonic. A small amount of iodine is added when the medium is used for the cultivation of the thyroid gland.

This new medium promotes more rapid and more prolonged growth of fibroblasts than does any artificial medium previously devised. Chicken heart fibroblasts that were embedded in horse plasma proliferated in this medium two or three times as rapidly as did control tissues that were cultivated in the feeding solution of Vogelaar and Erlichman. A pure strain of these cells multiplied actively for six weeks without showing any deterioration or decrease in growth rate. Control fibroblasts, cultivated under the same conditions in the Vogelaar feeding solution, underwent fatty degeneration and died in 12 or 14 days. The new medium causes fibroblasts to proliferate for a time fully as rapidly as they do in embryo juice. In one experiment, two tiny fragments of heart fibroblast that were in their sixth passage *in vitro* increased in size so fast that they completely covered the coagulum in a D-3 flask in 11 days. This rapid growth does not continue indefinitely, of course, for the medium is still incomplete.

[1] L. E. Baker, *Jour. Exp. Med.*, 49: 163, 1929.
[2] J. R. M. Vogelaar and E. Erlichman, *Am. Jour. Cancer*, 18: 28, 1933.
[3] Unpublished experiments of the author's.
[4] Vitamins A and D were supplied together by using a concentrate prepared from halibut liver oil.

In addition to promoting proliferation of fibroblasts, this medium causes growth of muscle cells and epithelial cells. Fragments of fresh heart produced a considerable mass of actively pulsating tissue when they were cultivated in this medium. Iris epithelium from a chick embryo proliferated in the medium for 65 days.

When the medium was used in the Lindbergh[5] apparatus for the cultivation of whole adult organs,[6] the following interesting results were obtained. Ovaries of the adult cat tripled their weight in 5 days. Thyroids doubled their weight in 3 or 4 days. The epithelial cells proliferated within and also outside the follicles. The follicles also increased in number to a marked extent. The glucose consumption of the organs was considerably greater than that of organs cultivated in 40 per cent. serum. Thus, cat thyroids cultivated in the artificial medium metabolized 17 mg of glucose daily, whereas those cultivated in serum metabolized only 7 mg daily. After the thyroids had been cultivated for some time in this medium, they were sectioned, and fragments from them were cultivated according to the usual techniques. These fragments grew as actively as do fragments of embryonic thyroid.

The medium designed for the cultivation of monocytes has the following composition:

	For cells cultivated in a fluid medium per 100 cc		*For cells cultivated in a coagulum per 100 cc*	
Serum	25.00	cc	25.00	cc
Witte's peptone*	85.00	mg	170.00	mg
Vitamin A[4]	50.00 to 100.00	units	100.00 to 200.000	units
Vitamin D[4]	1.00 to 2.00	"	2.00 to 4.00	"
Vitamin B_1[7]	0.0053	"	0.0106	"
Vitamin B_2[7]	0.0001	"	0.0002	"
Vitamin C (crystalline ascorbic acid)	0.085	mg	0.17	mg
Glutathione	0.34	"	0.68	"
Cysteine hydrochloride	1.125	"	2.25	"
Hemin	0.00045	"	0.0009	"
Insulin	0.012	units	0.024	units
Thyroxine	0.000113	mg	0.000225	mg
Phenol red	5.00	"	5.00	"
Glucose	200.00	"	200.00	"
Sodium chloride	581.00	"	581.00	"
Potassium chloride	15.00	"	15.00	"
Calcium chloride, anhydrous	15.00	"	15.00	"
Magnesium chloride, 6 H_2O	7.50	"	7.50	"
Sodium dihydrogen phosphate	3.75	"	3.75	"
Sodium bicarbonate	75.00	"	75.00	"

* Monocytes proliferate more rapidly in tryptic digests of fibrin than they do in Witte's peptone.[8] Such digests may be substituted for the peptone. The peptone has been used here because of the greater ease with which the medium can be reproduced.

[5] C. A. Lindbergh, *Jour. Exp. Med.*, 62: 409, 1935.

[6] A. Carrel and C. A. Lindbergh, SCIENCE, 81: 621, 1935.

[7] Obtained from a concentrate prepared by Burroughs Wellcome and Company.

[8] L. E. Baker, *Jour. Exp. Med.*, 57: 689, 1933.

When the medium is used for organ cultivation, the glucose is increased to 300 mg per cent., and the sodium chloride reduced sufficiently to keep the solution isotonic.

Chicken monocytes have been cultivated in this medium for 80 days. At first, they proliferated so actively as to cover the entire area of the flask in four days. Half of the cells were then removed. In another three days, the flask was again covered with cells. In order to prevent overcrowding, a portion of the cells was removed every four or five days for at least a month. After that, proliferation was not so rapid. It continued, however, throughout the entire 80 days of cultivation. Control cells that were cultivated in 25 per cent. serum without any of the other constituents of the medium proliferated very slowly. It was not necessary to remove any cells to prevent overcrowding during their entire time of cultivation.[9]

The quantity of serum used in the medium may be varied to a considerable extent, according to the results desired. When it is increased to 50 per cent. proliferation is still more rapid. When it is reduced to 15 or to 10 per cent., the cells proliferate less rapidly. It can not be eliminated altogether. With as low a concentration as 10 per cent. serum, the medium sustained the proliferation of monocytes for 62 days. Control cells cultivated in 10 per cent. serum and Tyrode solution, without the other ingredients of the medium, died in 12 days. It is evident, therefore, that the constituents used with the serum have a true nutritive value, and can replace serum to a considerable extent. This medium is now being used with success for the cultivation of whole adult spleens in the Lindbergh apparatus.

To summarize: Artificial media have been developed that cause fibroblasts, epithelial cells and monocytes to proliferate rapidly. Although still incomplete, and needing serum as one constituent, they allow the cultivation of cells for considerable periods of time. For the present, they are the most efficient artificial media as yet devised for the cultivation of tissues and entire organs.

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[9] The controls were kept for only a month.

BOOKS RECEIVED

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