

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 Lindbergh⁵ 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 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 B ¹ | 1.00 to 2.00 | " | 2.00 to 4.00 | " |
| Vitamin B ² | 0.0053 | " | 0.0106 | " |
| Vitamin B ⁶ | 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 ₂ O .. | 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

- CLARK, J. G. D. *The Mesolithic Settlement of Northern Europe; A Study of the Food-Gathering Peoples of Northern Europe during the Early Post-Glacial Period.* Pp. xvi+284. 74 figures. 8 plates. Cambridge University Press, Macmillan. \$9.00.
- HULL, GORDON F. *An Elementary Survey of Modern Physics.* Pp. xxiv+457. Illustrated. Macmillan. \$4.50.
- PENNELL, VERNON. *A Handbook of Urology for Students and Practitioners.* Pp. viii+224. 34 figures. Cambridge University Press, Macmillan. \$2.75.