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A New Technique for the Study of Tissues in a State of Latent Life in vitro.

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We know that active proliferation prevents the normal functions of tissue cells *in vitro*.[1] For this reason we have found it necessary to modify the present technique of tissue cultivation in order to study more carefully the innate properties of the cells and their relationship one to another. The methods developed by Carrel and his collaborators have provided an excellent means for the study of growth phenomena in general. In the hands of the physiologists, cultures of growing tissues have been mainly used as reagents for the study of the rate of cell proliferation under all possible conditions. But morphologists and embryologists, who expected much from the method, have been rather disappointed. The characteristic structure of the tissue cultivated was very early lost when it was attempted to retain it permanently *in vitro*. All cells soon came to look alike despite the complexity of the original tissue. In view of these difficulties, many morphologists developed a technique of their own. But in most cases such methods have allowed merely a survival of the cells in various media until death finally occurred.

The method to be reported here is an attempt to create a simple technique for the cultivation of pure strains of tissue cells which will more nearly imitate the conditions found in the adult organism. It is an attempt to discover new properties in the cells, properties which may never be detected under conditions of excessive multiplication.

Into a Carrel flask, of the 3½ cm. D-type, is introduced 0.5 cc. plasma and 1.0 cc. Tyrode solution. Coagulation of the plasma is initiated by the addition of one or 2 drops of embryonic tissue juice. The fragment of tissue is placed in the medium as soon as the tissue juice has been added and before coagulation has occurred. The flask is then closed and placed in the incubator. After 2 days the flask is reopened and 2 cc. of Tyrode solution is poured over the surface of the clot. The flask is then returned to the incubator for ½ to 1 hour. At the end of this time the Tyrode solution is aspirated and replaced by 0.5 cc. plasma containing 1% of a 10%

[1] Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 583.

solution of heparin. The flask is again returned to the incubator for 2 or 3 hours. The concentration of the heparin should be just sufficient to keep the plasma in a fluid state over this period. The plasma is next removed and the flask left with a dry clot for another 2 days, when the process is repeated as before. This procedure allows a washing out of accumulated waste products with the Tyrode solution, and a replacement of food materials with the fresh plasma. And if this procedure be carried out regularly, the cells are provided with food materials not very different in quality and quantity from those supplied in the organism.

We have found the rate of proliferation, under these conditions, to be very low. However, cultures which have been so treated, and allowed to remain in the same clot for a month, have, upon histological examination, proved to be in just as healthy a condition as those which were allowed to grow vigorously in embryonic tissue juice according to the usual method. Under these conditions, we have a more ideal relationship between the individual cells of the colony. The cells become dependent upon each other. They adapt themselves to the poorer conditions in such a manner that their metabolism is reduced to a minimum and the rate of proliferation becomes so adjusted that a condition of equilibrium is reached between the supply of nourishment and the elimination of metabolic substances. And finally, it is believed that a condition is ultimately reached where cell death may induce new cell divisions, thus reestablishing the equilibrium.

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The Oestrous Hormone in the Urine of Pregnant Cows.*

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Through the kindness of Dr. L. J. Cole of the Genetics Department a number of pregnant cows were placed at our disposal to make a quantitative study of hormones in the urine. One of the points of interest found in this study was the great amount of the oestrous hormone present. A quantitative determination of the oestrous hormone in the urine of pregnant women has been recently

* Assisted by grants from the National Research Council, Committee on Problems of Sex.