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## STUDIES ON THE PHYSIOLOGICAL CONDITIONS PREVAILING IN TISSUE CULTURES

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An analysis of some of the physiological factors active in Maitland tissue cultures has been presented in the hope that it may be of some value in clarifying the principles underlying tissue cultures in general.

It has been found that the empirically determined necessity of using relatively small amounts of tissue in such cultures is dependent upon the fact that excessive tissue leads to a rapid change of reaction toward the acid side. Whereas tissue may remain viable in an environment as alkaline as pH 9 and over, viability is rapidly destroyed when the reaction approaches pH 6.

Evidence is presented to indicate that the changes in electrode potentials which take place in Maitland cultures are not, as has been suggested, the determining factors upon which virus multiplication depends, although they may, of course, be incidentally important.

It has been shown that there are fundamental differences between those conditions in Maitland cultures which favor the multiplication of a typical virus and those upon which the growth of the *Rickettsiae* of typhus fever depends.

The virus which we have studied (equine encephalitis virus, western type) multiplies during the period of active tissue metabolism. The maximum virus titrations are obtained at about the time at which metabolism has come to a standstill. Thereafter the virus not only ceases to increase but rapidly deteriorates. The period of viability of the tissue cells themselves is shortened by several days in the presence of virus multiplication. There is some evidence that a temporary acceleration of oxygen uptake takes place during the time of active virus multiplication. Technical difficulties in controlling such experiments prevent certainty in regard to this point.

In contrast with the conditions determining the growth of a virus agent in the Maitland cultures the multiplication of *Rickettsiae* does not begin to any determinable extent until after active cell metabolism has either become stabilized or has ceased. The *Rickettsiae* continue to grow at a time when the cells are no longer viable. It appears likely that these organisms find the most favorable conditions for growth in cells which are no longer metabolically active but in which some delicately heat-susceptible elements have not yet been disturbed. As a consequence of these observations, frozen and preserved embryonic tissues have been successfully used for *Rickettsia* cultivation. A report on these experiments will be made in a separate communication.

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In the tissue engineering (TE) paradigm, engineering and life sciences tools are combined to develop bioartificial substitutes for organs and tissues, which can in turn be applied in regenerative medicine, pharmaceutical, diagnostic, and basic research to elucidate fundamental aspects of cell functions in vivo or to identify mechanisms involved in aging processes and disease onset and progression. The design of 3D engineered tissue models is currently in its development stage, showing high potential in overcoming the limitations of already available models. Plant tissue culture plays an important role in the production of agricultural and ornamental plants and in the manipulation of plants for improved agronomic performance. Abebe et al. [43] get a reduction of 37% of callus growth in saline stress conditions at 100 mM NaCl. Salinity is regarded as being a major factor limiting development of plants and crops production potential. In vitro tissue culture could be an important means of improving crop tolerance and yield through genetic transformation as well as by induced somaclonal variation. Tissue culture is the growth of tissues or cells in an artificial medium separate from the parent organism. This technique is also called micropropagation. This is typically facilitated via use of a liquid, semi-solid, or solid growth medium, such as broth or agar. Tissue culture commonly refers to the culture of animal cells and tissues, with the more specific term plant tissue culture being used for plants. The term "tissue culture" was coined by American pathologist Montrose Thomas Burrows. Suspension culture under controlled conditions may be used to solve many physiological or biochemical problems and also provides a system for the production of important plant products, such as, plant alkaloids. From cell and organ culture under controlled environmental conditions nutritional and metabolic processes can be studied. Some mutant cells cannot grow in a medium which does not contain a special nutrient. The behaviour of substances, which can prevent virus attack has been studied on virus infected cells. In tissue culture the behaviour of normal and cancer cells can be studied. It has been noted that some viruses and carcinogenic chemicals can produce cancer. Effect of radiation and chemicals on normal and cancer cells has been studied. Tissue culture is an excellent example of such a technical revolution. An editorial published in 1910 in the Journal of the American Medical Association commented that "it lays bare practically a whole new field for experi Only four years later, a review discussed the applications of tissue culture in studies on cell morphology and differentiation, cancer, bacteriology, virology, immunology, radiobiology, and toxicology.2. But even such a revolutionary technique as tissue culture has a long history in which the principles of the technique were recognized and various partially successful attempts were made.