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**Observations on Mitochondrial Growth in Artificial Culture Media.**

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In former publications,<sup>1</sup> it was shown that when rabbit liver from new-born rabbits is planted in rabbit liver infusion media containing urea (0.5%), the mitochondria of the planted livers will grow independently in the culture medium. Two types of growth develop in the media: surface growth, which is similar to ordinary bacterial surface growth, and a deep growth which produces a clouding in the medium round about the planted liver tissue. It was shown that the clouded growth in some instances could be subcultured to produce a surface growth.

In recent experiments it has been again very evident that the deep clouded growth is a true bacterial growth. This is shown by the fact that when surface growths have developed on the media there has been no, or very little, deep growth. So also, when the deep clouded growth developed there has been no surface growth. In a few instances where neither surface nor deep growth developed in the usual time, it was found that surface growth may ultimately appear; in one case, 21 days after the tissue was planted. Thus, it may be stated that independent mitochondrial growth occurs in practically 100% of cases when liver of new-born rabbits is planted in the media.

Considerable time has been spent in attempting to determine the factors responsible for surface growth in some instances and the deep clouded growth in other cases. So far, I have been able to recognize certain general conditions only. The significance of the various ingredients of the culture media have been studied, but any pronounced significant factors have not been recognized. Water used in the media was distilled in various ways and obtained from different sources. There appeared to be some variation in the results from the different waters used, but the results were not constant. Another ingredient of the media, namely peptone, is difficult to evaluate. Witte's peptone has been used in the preparation of all media. To what extent the heat in sterilization produces changes in this complex ingredient is not known, but it appears probable that heat produces changes in the peptone and that these changes will not be the same in the preparation of different batches of media.

While the chemical constitution of the medium appears to be the

prime factor in the successful culture medium, the physical condition of the medium also appears to play an important rôle. The consistency of a medium may vary from a very firm jelly to one that is barely solid depending on the amount of water present. In many instances it appears that a particular consistency is favorable to the production of surface growth.

It has become evident that the mitochondria vary in their physiology in different new-born rabbit livers, and that the physiological state is an important factor in determining the character of growth in the culture media. A variation in the response of mitochondria is shown in stained smear preparations of liver from different new-born rabbits. In some cases the mitochondria are well preserved and numerous, in other cases they are few in number. The gross characteristics of the liver also vary in the new-born rabbits. The color varies from a yellowish brown to a deep maroon. So also, the physical nature of the livers vary; some are stringy and tough, while others are friable and easy to cut into pieces.

Mitochondria appear to be extremely delicate in their responses. With variable factors in the culture media on the one hand and variable physiology of the mitochondria on the other, it appears nigh impossible to devise a culture medium that will constantly produce surface growth of mitochondria.

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<sup>1</sup> Wallin, Ivan E., *Am. J. Anat.*, xxxiii, 1; xxxv, 3; xxxvi, 1.

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**Experimental Typhoid Fever Induced in Guinea Pig With In Vivo Prepared B. Typhosus Toxic Product.\***

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Since typhoid fever represents a syndrome manifestly accompanied by toxemia, many and varied attempts have been made to obtain the specific toxic moiety from the typhoid bacillus. While certain of these procedures have yielded toxic materials which when injected into animals produce tissue reactions, they are not, as far as ascertained, analogous to the pathological changes occurring in typhoid fever of man.

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