CULTIVATION IN VITRO OF THE THYROID GLAND.*

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Plates LV-LVII.

In a previous paper\textsuperscript{1} we have described in full the method employed by us in cultivating tissues and organs of cold- and warm-blooded animals \textit{in vitro} and the general results thus far secured with the method. In this paper we wish to present in greater detail the experiments performed with the thyroid gland.

We cultivated the thyroid gland for the first time on September 22, 1910. Since then we have cultivated this gland, taken from mammals, many times. Small fragments of the gland were extirpated from living and anesthetized dogs, cats, and guinea pigs, and cultivated in the plasma obtained from the same animal or an animal of the same species. These cultures are distinguished as autogenic and homogenic. In all, we have now made fifteen series of cultivation experiments with the gland, each of the series being composed of from four to thirty separate cultures.

\textbf{PRIMARY, SECONDARY, AND TERTIARY CULTURES, AND SECOND GENERATION OF THYROID CELLS.}

A primary culture consists of the growth in plasma of a fragment of the gland obtained directly from an animal. A secondary culture is secured by extirpating a fragment of the gland growing in the primary culture and transplanting it to a new plasmatic medium. When a living fragment of thyroid is removed from a primary culture and transferred to a fresh medium, growth often begins anew. A fragment which has produced chiefly or only connective tissue in a primary culture may, on being transferred, produce a continuous layer or tubular formations of epithelial cells

\textsuperscript{*}Received for publication, February 7, 1911.
\textsuperscript{1}\textit{Jour. Exper. Med.}, 1911, xiii, 387.
in the secondary culture (figure 1). In the course of this process of growth, the original fragment becomes more and more translucent, apparently from the out-wandering of the constituent cells. A culture, therefore, contains emigrated as well as proliferated cells. The secondary cultures are best obtainable from primary cultures that are still growing actively. From the third to the eighth day the period is favorable; later it is unfavorable. The second generation of cells may be abundant or sparse or fail altogether to appear. In some instances the plasmatic medium may be rapidly and generally invaded with cells that present no distinct outlines and are detachable by reason of the bright and refractive granules that they contain. A tertiary culture is made in the same way from a secondary culture. In a few instances a fresh plasmatic medium was inoculated with cells from a thyroid fragment grown outside the body. The attempt rarely succeeds because it is very difficult to prepare a suitable section of the old clot, and the cells contained in it are therefore injured or killed by the act of transfer.

Another and more successful procedure for obtaining a second generation of growing cells is to extirpate the original fragment of tissue from the middle of the clot and to fill the resulting space with fresh plasma. The cells contained in the old plasma multiply and invade the new. The cultivation of cells in series was attempted in a few instances only, and in most of these a second generation could be secured. Thus far we have not secured a third generation of the thyroid cells. We do not, however, believe this to be impossible, as the number of experiments performed by us is too few to be conclusive. The technique of cultivation of tissue cells in series is far from being worked out. Indeed, the results obtained in primary cultivations are subject to wide fluctuations depending on the exactness of the technique. The positive results increase in direct ratio to the precision with which the experiments are conducted. In January, 1911, we secured eighty per cent. of positive results, while in September and October, 1910, when the technique was less exact, the percentage was less than fifty.

Failure of the thyroid tissue to grow may be due to one or more causes. When the medium has coagulated properly about the fragment and growth does not take place, it is probable that undue
crushing or drying of the tissue before transplantation has occurred, or that a trace of antiseptic may have entered the tissue or plasma. When sudden cessation of the active growth takes place during the first few days, the plasma has generally suffered drying or bacterial infection, or the temperature of the oven has changed markedly. Bacterial infection is usually attended with liquefaction of the medium, but liquefaction may arise from other causes.

**THE THREE PERIODS OF A CULTURE.**

Almost all our observations have been upon primary cultures of the thyroid gland, the phases of which can be divided artificially into three periods; namely, latency, growth, and death.

1. The latent period covers the time from the inoculation of the fragment in the plasmatic medium until the appearance of the first cells. Note must first be taken of the appearance of the culture immediately after its preparation: the thyroid fragment appears as an opaque body with more or less sharply defined edges, lying within a clear medium. Within the fragment it is possible to distinguish, under the microscope, between the glandular and the supporting tissues. The period of latency endures from twelve to seventy-two hours, according to the age of the animal supplying the fragment, and some other conditions. In the case of a six day old kitten, growth began in twelve hours; in that of a young dog, it began after twenty-four to forty-eight hours. In the case of adult animals, two to three years old, the period extended to forty-eight or seventy-two hours.

2. The period of growth varies considerably. It may extend to eighteen days and is determined, doubtless, by many factors, of which few or none have been worked out up to the present. We shall confine ourselves to a description of the new cells produced in the culture.

The earliest cells produced are fusiform or polygonal and wander freely from the fragment of tissue into the medium. Some of the later cells tend to form continuous layers that spread from the fragment into the plasma (figure 2). We do not possess at present any criteria for determining absolutely the nature of the proliferated cells, but we believe that they consist of connective tissue
and epithelial cells. The earlier cultures of the thyroid of the dog and chicken yielded exclusively a connective tissue growth. The cells were isolated and elongated or irregular, possessed a large clear nucleus with one or two nucleoli and protoplasm containing many granules which were grouped about the nucleus or filled the cytoplasm. These cells invaded the medium either as isolated single cells or in rows or chains of cells (figure 3). Ultimately their processes united to form an open network. They did not give rise to continuous layers, as we have observed the cells of the epidermis to do. On the other hand, their multiplication goes on rapidly so that they come in a few days to occupy several planes of the plasma. In a few instances they surrounded the fragment concentrically and produced a kind of dense network or capsule.

The cells of another type were polygonal in form and appeared somewhat later than the fusiform cells. They presented less distinct outlines and a finely granular protoplasm surrounding a large clear round nucleus which in turn contained one or two opaque nucleoli (figure 4). These cells differ from the others in remaining in a community and not wandering separately into the medium, and in producing sometimes tubular formations (figure 5) and sometimes continuous layers (figure 2). Moreover, these cells grow from the edges of the fragment as far as the upper surface, and in a single plane. In one instance, the tubular proliferation was traced to the circumference of a thyroid vesicle which formed its base. In some instances the growth was cup-shaped, and later budding occurred so that ramifying tubules were produced. Hence we think that there is good reason to regard these cells as of epithelial origin.

The foregoing descriptions refer to the fresh, living cells. The cultures can be fixed at will and stained in the usual manner. Hematoxylin and eosin stained preparations showed that the layers and tubules of cells consisted of a faintly stained cytoplasm containing obvious nuclei but showing only slightly the demarcation between the cells (figure 6). Now and again the limits defining the cells could be made out and were similar to those of the epidermis.

The period of active growth of a thyroid culture is from six to eight days. When it exceeds this, the proliferation of cells goes
on very slowly. In a culture that remained alive for eighteen days, there was slight multiplication of cells after the tenth day. During the proliferation of cells, the original fragment becomes progressively clearer and the alveoli are easily visible, an effect produced probably by the wandering of many cells into the medium. As respects rate of growth, the thyroid equals that of the ovary, testicle, and kidney, and exceeds that of the peritoneum and cartilage. The medium becomes progressively darker as growth proceeds, and the fibrin network more apparent. The plasmatic clot tends to rarefy at the edges of the tissue, and at times it retracts so that a clear area of fluid surrounds the fragment. This occurrence prevents further growth.

3. The death of the culture takes place after ten to eighteen days, that is, after a complete cessation of the multiplication of cells. Coincidently, the cytoplasmic granules coalesce and increase in number. The outlines of the cells grow faint and finally disintegration of the cells takes place. The death of the culture is bound up with changes induced in the plasma by the growing cells. This is shown by the fact that on secondary transplantation the cells continue to multiply. It may be caused by exhaustion of the nutriment or by accumulation of metabolic products or by both of these factors together.

CONCLUSION.

The thyroid gland of mammals can be cultivated outside the body. The proliferated elements consist of connective tissue and epithelial cells, the former predominating. The cells survive in cultures for two weeks or longer, which period can be increased by secondary and sometimes by tertiary cultivations. It is to be noted that the method of growing in vitro organs such as the thyroid gland may come to be used with advantage in the study of the substances concerned with the internal secretion of certain glands.
Fig. 3.

Fig. 4.
EXPLANATION OF PLATES.

PLATE LV.

Fig. 1. Tubular growth of a twelve day old secondary culture from the thyroid gland of a cat. Stain hematoxylin.

Fig. 2. A continuous layer of thyroid cells from a ninety-six hour culture, and two tubes located near the border of this layer and opening on its surface. Stain hematoxylin.

PLATE LVI.

Fig. 3. The connective tissue cells shown in a portion of Fig. 2.

Fig. 4. Cellular growth in a living culture, seventy-two hours old, of the thyroid gland of a dog.

PLATE LVII.

Fig. 5. Tubular formation from a living culture, ninety-six hours old, of the thyroid gland of a dog.

Fig. 6. The culture shown in Fig. 5, after it was fixed and stained with hematoxylin.