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Studies on cancer.
I. The effect of circulation on the functional activity, migration and growth of tissue cells.

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In a previous article I had shown that rhythmical muscular contraction as it occurs in fragments or the whole hearts of embryos in simple hanging drop cultures is regular only for a few hours. After this it becomes irregular; there are periods of activity followed by periods of rest. These active periods are ushered in by rapid strong contractions which gradually become less and less forcible and rapid until they finally cease. After a shorter or longer rest period of several minutes, the contractions begin again, as before.

These irregularities I had long ago concluded were due to the accumulation of waste products about the contracting cells. In the simple hanging drop cultures the only means of escape of such substances is by diffusion into the outer portion of the hanging drop of medium. I prepared at that time a culture[1] which allows one to wash continuously a greater part of such hanging drops of plasma with a stream of serum. The serum in this apparatus is dropped at the rate desired on the upper end of a wick which passes to the hanging drop and then into a receiving chamber. In the hanging drop on the surface of a cover glass the fibres of the wick are teased apart so that they transverse all parts of the hanging drop. The fragment of tissue or cells to be tested is placed in the center of the hanging drop. Plasma is used as the medium for the hanging drop. It is allowed to clot before the stream of serum is started.

In such cultures I found that as the serum is passing the contractions of the heart become regular and forcible in those cells which are sufficiently separated to be well washed by the serum. In the more compact part of the fragments the contractions are

[1] Burrows, M. T., *Anat. Rec.*, 1912, vi, 141.

irregular as before. Again it is interesting to note that these rhythmical contractions of the muscle cells can be prolonged by this method. One group of cells in one of the fragments continued to contract for as long as 28 days. In the hanging drop cultures all activity ceases after 8 or 9 days as a rule. A few have contracted, however, at intervals up to the 18th day.

In a later study I investigated the migration of the embryonic heart muscle, connective tissue and epithelial cells by the same method. About carefully cut compact fragments the migration of the cells is little disturbed over that seen in the simple hanging drop cultures. The migration begins after a given latent period, continues active for a time and then ceases. The area covered by new cells is the same as if the serum had not been passed through the culture medium. This had led me to believe in my earlier studies that the passing serum had no effect on, or possibly accelerated the migration of these cells.[1] [2]

About the fragments which were teased apart, however, this is not true. I had noted at an earlier time that teasing itself always leads to a marked decrease in the extent of migration. Washing further decreases the migration of the mesenchyme, muscle and connective tissue cells. In the more compact epithelial membranes, however, this effect of washing is less marked. Breaking up these epithelial membranes lessens the movement. The single cells will not migrate. Washing small groups of these cells has little further effect upon this activity.

What is true for the fixed tissues is not true, however, for the lymphoid cells migrating from the spleen. The migration of these cells is decidedly affected by the flowing serum. In several instances the entire fragment of spleen disappears. All the cells migrate out as lymphoid cells. They accumulated at tangle portions of the wick. In the simple hanging drop culture such a complete dissipation of the cells never occurs from these fragments. When the fragments are transplanted repeatedly, a few may so dissipate. Many of the fragments of spleen cease, however, after a few transplants to new plasma to give forth lymphoid cell; connective tissue-like cells migrate from them. In many of the cultures which were washed with serum all the cells of the spleen became transformed into lymphoid cells and migrated out.[2] In a few of the cultures washed with serum I

[2] Burrows, M. T., to appear in *Sci. Bull., U. of Kans.*, 1923.

noticed also connective tissue-like cells migrating later from these splenic fragments. Whether this complete transformation of all the cells of the fragment into lymphoid cells in one case and as part lymphoid and part connective tissue-like cells in others is dependent alone on the serum is yet to be established. In all of these cultures, as it must be noted here, however, the migration of the wandering type of cell from the spleen is increased by the flowing serum. This fact places these cells in definite contrast to the fixed tissue cells.

Having established these facts it became of interest to study the effect of this flowing serum upon the growth and division of embryonic heart muscle cells and mesenchyme cells in these cultures. The lymphoid cells have never been seen to grow and divide in these cultures. The cells from fragments of young embryonic heart muscle fragment and fragments of body wall of chick embryos migrate with great activity after a short latent period, grow and divide by mitosis and later degenerate. The flowing serum not only delays slightly the migration of the cells from the compact fragment but inhibits decidedly the growth and division and delays greatly the degeneration of the cells in the outer medium. How long it may delay this degeneration has not been determined. The complications of preparing and handling the serum leads generally to infection sooner or later. Stopping the flow of serum allowed growth and the degenerative changes to proceed again.

While it is impossible by such a device to duplicate completely the circulation as it exists in the body there seemed little doubt that, quite the reverse of function, the growth and the migration of fixed tissue cells is inhibited by an active circulation. Growth fails in all parts of these cultures where the cells are well washed by the serum.