have been no summation with the subminimum central effect of the ether.

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Note on the production of acid by tissues growing in vitro.

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Connective-tissue cells of the chicken, growing *in vitro* in chicken plasma to which a little blue litmus has been added, produce rapidly a focal, pink coloration of the medium. If a number of small fragments of one tissue (heart muscle or the aorta of young chicks, or chicken sarcoma) be plated out with the plasma medium in a petrie dish, it will be found that all the tissue bits are at first stained blue, but that those from which growth occurs become pink, while the growing tissue itself is unstained. The fragments remaining permanently inert keep the blue color.

Often a pink coloration of tissue bits can be observed at a time when growth is found, microscopically, to have barely started. The acid change is in general sharply localized to the neighborhood of the growing tissue. When growth is checked by placing the preparation in the ice-box, neutralization in the acid foci is often incomplete at the end of forty-eight hours, and this even when the bulk of alkaline plasma is relatively large and its plasma network thinned by dilution. Diffusion in the plasma medium as thus indicated is very slow. Under the ordinary circumstance of *in vitro* life without artificial provision for a circulation of fluid, tissue proliferation must take place almost from its beginning, in an acid medium. This constitutes a serious fault in the method of cultivation.

The nature of the acids produced by the growing tissue has not been determined. Carbonic and lactic acids are presumably present in greatest quantity. That the amount of acid formed may be very considerable has been shown by titrating out the as yet unclotted blue plasma to the tint acquired by the tissue cultures. The acid does not affect methyl orange, but very occasionally it changes congo red toward violet, a change best seen

in the interior of degenerating cells which have taken up the indicator. The violet change is not due to free carbon dioxide, for bubbling the gas through unclotted plasma containing congo red fails to bring about an alteration in color.

The observations show that tissue cells can withstand much more considerable changes in the reaction of the medium round about than has been supposed; and that their growth *in vitro* may be very active in an acid medium.

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The location of the optic anlage in Amblystoma and the interpretation of certain eye defects.

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The early embryos of *Amblystoma* were operated upon so as to remove definite areas from the open medullary plate in order to determine the position of the materials which would give origin to the future eyes.

Preliminary experiments of sticking and disturbing the cells in the anterior end of the medullary plate without actually removing these cells did not prevent the subsequent development of the optic vesicles in an apparently normal manner. Cutting out rectangular pieces of the anterior part of the medullary plate, reversing the pieces and transplanting them merely caused the eyes to develop in misplaced positions. These two experiments demonstrate the fact that unless the future eye material is well removed by the operation the optic vesicles may form. This fact is important in considering the results of the following experiments.

A general statement of the results of the experiments may be expressed as follows: Thirty embryos studied after various operations in which lateral portions of the medullary plate were removed at slightly different developmental stages showed in twenty-four individuals, or eighty per cent. of the cases, subsequent development of both eyes, while only six specimens, or twenty per cent.