formation of the typical tongue pseudopodia, but also that of the balloon pseudopodia and the circus movements which were produced in mixtures of urea and potassium chloride.

The fact that other agencies which inhibit these processes (KCl, NH_4Cl for instance) likewise exert a certain preserving effect on the blood cells seems to support this interpretation. However, further experiments must decide whether in addition other factors may not contribute to this preserving effect of alkali.

125 (2357)

The effect of acid on the amebocyte tissue of Limulus in tissue cultures.

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In previous publications[1] we have shown, (1) that a small amount of acid added to an isotonic sodium chloride solution exerts a certain preserving effect on the amebocytes in a drop of *Limulus* blood surrounded by an excess of the solution. (2) That in tissue cultures of amebocyte tissue there occurs not only an active migration of the amebocytes into a sodium chloride solution, which has been made slightly acid, but that under certain conditions the addition of acid may increase the duration and intensity of this migration, which is followed by, or associated with, a formation of tissue-like structures not unlike those produced by the mesenchyme of vertebrates.[2] (3) That this effect of acid is due to its action on the cells, which consists in an increase in contraction and consistency of the protoplasm. (4) Inasmuch as we had previously shown that there exists a parallelism between the effect of various agencies on the state of con-

1 Loeb, L., *Folia Haematologica*, 1907, iv, 313. *Pflüger's Arch.*, 1910, cxxxi, 465. *Washington Univ. Studies*, 1920, v, 8. *Scient. Series* 3. *Am. J. Physiol.*, 1921, lvi, 140. *Science*, 1922, lvi, 237. Loeb, L., and Blanchard, K. C., *Am. J. Physiol.*, 1922, lx, 277.

2 We use the term "outgrowth" to designate these effects.

traction and consistency of the amebocytes on the one hand, and on the other hand, on the character of ameboid movement, the shape of pseudopods, the mode of movement of the granuloplasm, the power of resistance to the stereotropic tendency on the part of these cells, we found in accordance with these observations under the influence of acid a sharpening and lengthening of the pseudopods and a tightening of the granuloplasm and a greater state of contraction of the cells. (5) We found that it is possible, through grading the amount of acid added, to grade correspondingly the effects of the acid on the consistency of the cells in such a way that all gradations could be obtained from the extreme condition of round contracted cells, too hard to carry out ameboid movements, and contracted cells sending out very long, often multiple thread pseudopodia, to a medium state in which the plasticity of the cell is greater and the ordinary tongue pseudopods are formed, and finally to the other extreme condition of cells which are very soft, which spread out readily and soon degenerate (Loeb & Blanchard).

In continuing these investigations during the summer of 1922 and 1923, at Woods Hole, we found (1) that, if we add small amounts of hydrochloric acid to solutions of NaCl, a steady decrease in the hydrogen ion concentration takes place in the fluid surrounding the tissue. This diminution in the hydrogen ion concentration begins shortly after adding the tissue, increases in the next few hours and continues for from one to two days or longer. The intensity of the loss varies in different cases in accordance with the character of the tissue, with the size of the piece of tissue used, and probably also with the amount of outgrowth that has taken place. (2) Outgrowth could be obtained when the pieces of tissue were placed in solutions of HCl varying in strength from M/1000 to M/8000 HCl. The optimal concentration varied in different cases according to the variable factors stated above.[3]

[3] The method of colorimetric determination of hydrogen ion determinations used by us is similar to the one described by Felton, L. D., *J. Biol. Chem.*, 1921, xlvi, 299. However, in this paper, of which we learned only recently, several improvements are suggested which we intend to use next summer. Since our first observations on the effect of acid on the movements of cells in tissue culture were published (1920), the following papers which are of interest in this connection appeared: Chambers, R., PROC. EXP. BIOL. AND MED., 1921, xix, 37; Lewis, M. R., and Felton, L. D., *Science*, 1921, liv, 636; Lewis, M. R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 373; Fenn, W. O., *J. Gen. Physiol.*, 1922, v, 169; Loeb, J., *J. Gen. Physiol.*, 1922, v, 231.

(3) The beneficial effect of acid on the outgrowth of amebocyte tissue may be observed in hypertonic as well as in hypotonic solutions of sodium chloride. In certain respects the effects of acid and of hypertonicity are additive, while those of acid and hypotonicity are antagonistic. However, acid merely diminishes to some extent the injurious effects of hypotonicity without being able to prevent them. A concentration of acid which still permits outgrowth in hypotonic solutions may be too strong for this purpose, if added to hypertonic solutions.

(4) We have found previously that while fresh *Limulus* serum is usually a favorable medium for the movement of amebocyte tissue, *Limulus* serum, from which the proteins have been removed through heat, has lost to a great extent its favorable character. This loss can partly be made good if we add acid to the protein-free filtrate of *Limulus* serum. The filtrate then regains some of the effects of fresh serum; however, it is necessary to add much larger quantities of acid to filtrate than to sodium chloride solutions on account of the buffer substances present in this filtrate of *Limulus* serum.

(5) Acid acts primarily on the amebocyte tissue by increasing the consistency and state of contraction of the cells. This effect has two consequences: (1) the cells are made less plastic and their ameboid activity is thereby decreased; (2) the cells are to a certain extent protected against injurious effects of the surrounding fluid and particularly the secondary injurious effects of the stereotropic reaction are delayed.

The first one of these two effects tends to make the outgrowth in acid solutions smaller than in corresponding neutral solutions, while the second one tends to preserve the cells for some time and thus to enable the tissue in certain cases to be still active at a time when in the neutral control solutions degenerative changes have cut short further active outgrowth of the tissue. Thus the outgrowth in an acid medium can under certain conditions surpass that of the control tissue in a neutral medium.

The maximum outgrowth is usually obtained when the tissue is sufficiently soft to allow a limited number of cells to spread out and hyalinize, and when at the same time a preserving effect of the acid is still noticeable. With the gradual decrease in the acidity of the medium and with the tendency of tissue even in neutral solution to become soft and to spread out in the course of time, the optimum condition for outgrowth is obtained in later

periods of the experiment, in preparations in which the concentration of acid was at first too great to allow an active migration of the cells; at a still later period the optimum of consistency is passed in this solution, the cells become still softer and degenerative processes predominate. Thus the optimum passes in the course of time from preparations in which the concentration of acid was relatively low to preparations which had originally a higher concentration of acid.

Whether in a specific case the outgrowth in acid surpasses in duration and ultimately also in intensity that in the control solutions, depends upon a number of variable factors, especially important among which is the original power of resistance of the tissue to injurious factors.

126 (2358)

Presence of toxic and insulin-like substances in oranges, grape fruit and lemons.

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During the past few months Winter and Smith,[1] Best and Scott,[2] Funk and Corbitt,[3] Collip,[4] Perry,[5] and Dubin and Corbitt[6] have reported the presence of a substance in yeast and in plants and vegetables, which causes a fall in blood sugar upon injection into normal rabbits. In view of these results the possible presence of this substance in various fruits suggested itself to us.

Various methods of extraction were used with oranges, grape fruit and lemons with the following results:

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[1] Winter, L. B., and Smith, W. J., *J. Physiol.*, 1922, lvii, 100.

[2] Best, C. H., and Scott, D. A., *J. Metabol. Research*, 1923, iii, 177.

[3] Funk, C., and Corbitt, H. B., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 422.

[4] Collip, J. B., *J. Biol. Chem.*, 1923, lvi, 513; 1923, lvii, 65.

[5] Thalhimer, W., and Perry, M. C., *J. Am. Med. Assoc.*, 1923, lxxx, 1614.

[6] Dubin, H. E., and Corbitt, H. B., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 16.