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**Effect of ion combinations on protoplasm, amoeboid movement, tissue formation in experimental amoebocyte tissue.**

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In former investigations we have shown that the consistency and state of contraction of the protoplasm determines various functions and transformations in amoebocytes and in the experimental amoebocyte-tissue, such as: (1) The character of the amoeboid movements and pseudopods. The greater the consistency, the more rounded are the cells, the sharper are the pseudopods, the slower is the movement of the granuloplasm. At certain stages the movement of the granuloplasm is entirely or almost entirely prevented, while the ectoplasm of the pseudopods may still actively move. Then the cell *in toto* may move in a snail-like manner, the whole cell being pulled along by the extension and contraction of the pseudopods. However, snail movement may also be produced under other conditions. The other extreme is represented by the circus movement and *paripassu* movement. In the former, the pseudopod has the shape of a drop or balloon, and moves along the periphery of the cell; the granuloplasm pours into it like a liquid mass. Subsequently the cell as a whole rounds off, and the same cycle begins again after a period of latency. In the latter form of movement, the protoplasm is so soft that the pseudopod is rounded off, and the granuloplasm moves into the pseudopod as soon as it develops, so that the pseudopod is from the beginning filled with granuloplasm.

(2) The consistency of the protoplasm influences the extension of the cell. The greater the consistency, the less is the cell liable to extend in contact with solid surfaces; conversely, the softer the cell, the more liable it is to extend. Associated with

the extension are usually solution processes in the cell, in particular, solution of the cell granules. However, other factors in addition to extension, influence such solution processes; thus it may occur that in extended cells the granules remain preserved for some time at least; and, conversely, in contracted cells the granules may be lost. The chemical constitution of the fluid surrounding the cells largely determines these conditions.

(3) The agglutination of the cells depends upon the consistency of the protoplasm. A very great consistency diminishes the agglutination of the amœbocytes to one another as well as to the glass by making the surface of the cells hard. Stickiness of a gelatinous material depends upon a limited taking up of fluid on the part of the gelatinous material.

(4) Agglutination, extensive migration of the cells, and the character of the pseudopods and cell outlines, together with solution of granules determine the production from amœbocytes of the tissue-like structures, most often resembling mesenchymatous tissue, but also, under certain conditions, epithelial and other kinds of tissues. The consistency of the protoplasm which is largely influenced by the environment, and also by differences in the cells of various individuals, determines the character of the tissue-like structure resulting from these transformations.

(5) The character of the degenerative changes taking place in amœbocyte tissue depends largely upon the consistency of the cells. In very consistent tissue hyalinization of the contracted cells may occur. With intermediate stages of consistency, vacuolization and the transformation of the tissue into fibrillar structures of various kinds may take place; or individual cells may disintegrate into a mass of cell granules. If the consistency is very low, the whole tissue may disintegrate into an opaque, structureless mass.

The consistency and state of contraction of the protoplasm depends: (1) On inner factors which vary in the case of different individuals. Amœbocytes derived from *Limuli* which are in a bad condition of health, tend especially to be very soft and to show little resistance. (2) On physical condition of the environment. A low temperature tends to increase the consistency and state of contraction, while, within a certain range, a higher temperature favors softness and extension. (3) On the chemical constitution of the fluid which surrounds the tissue.

If we consider the action of various ions on the consistency of the protoplasm of amoebocytes, and on the correlated phenomena mentioned above, each ion has a characteristic effect. If we take serum as a standard, sodium tends to cause contraction of the cell and to lead to hyalinization. Potassium in small amounts causes a softening, leading to circus movements, especially in combination with hypotonicity; in stronger concentration it causes fixation and death. It tends to preserve the granules. It alters the normal plasticity of the cell, either in the direction of too great a liquidity and mobility, or in the direction of too great a stiffness, depending upon the concentration of the potassium and associated factors, such as osmotic pressure, temperature, simultaneous action of other ions, and character of the individual amoebocyte tissue. The effect of calcium and rubidium is intermediate between that of sodium and potassium. Ammonium does not permit normal movements of the cells. It leads to the formation of multiple drop-pseudopodia and mulberry cells. Ca leads to solution processes, hyalinization and cytolysis and complete disintegration of the amoebocytes. The anions we can arrange in the following order: (1)  $\text{NO}_3$ , (2) Cl, (3)  $\text{SO}_4$ .  $\text{NO}_3$  tends to cause softening;  $\text{SO}_4$  hardening of the cells; and Cl exerts an intermediate effect. H-ions in the form of HCl or other acids tend to increase the consistency of the amoebocytes. OH-ions tend to preserve the cells, temporarily, if used in a higher concentration. Gradually the solution processes predominate. If weaker concentrations are used the softening affects predominate from the beginning. In former investigations we influenced the consistency of the cells and the associated changes through combinations of various ions. Thus through combination of NaCl solutions and acid we could increase the consistency of the cells, improve the preservation and increase in certain cases the outgrowth of the tissue.

During the summer of 1923, with Miss Elizabeth Gilman, we determined the action of various ion combinations on the consistency of amoebocytes and the associated phenomena mentioned above. We repeated and extended these experiments during the summer of 1924 with Mr. H. Beerman and Miss Ida P. Genther. We found that the effect of various combinations of ions on the consistency, amoeboid movement, agglutination, tissue formation and degeneration of amoebocytes can be pre-

dicted on the basis of the action of the individual ions, provided we take into consideration also the concentration of the various ions, the osmotic pressure, the temperature of the solutions, and the character of the tissue used. Various ions may mutually antagonize or intensify their separate actions according to the character of the combinations. This rule applies in cases in which a kation and an anion are united in the same salt, as well as in cases in which several salts are combined in the solution. Thus  $\text{SO}_4$  counteracts the softening effect of low concentration of K more effectively than does Cl; while  $\text{NO}_3$  under certain conditions intensifies softening and toxic effects. Therefore  $\text{KNO}_3$  will neutralize the hardening effects of  $\text{Na}_2\text{SO}_4$  more completely than KCl. A combination of NaCl and KCl can be as effective as a combination of  $\text{Na}_2\text{SO}_4$  and  $\text{KNO}_3$ . The results also depend on the character of the tissue. A naturally soft tissue is comparable to a more resistant harder tissue to which a small amount of KCl or a still smaller amount of  $\text{KNO}_3$  has been added. A harder, more resistant tissue resembles a softer tissue to which an adequate amount of acid has been added. If, on the basis of such and other similar considerations, the consistency of the cells can be predicted, we can therefrom also predict the action of the various ion combinations on amœboid movement, character of pseudopods, agglutination, extension of the cells, tissue formation, rapidity of outgrowth and secondary degeneration in the amœbocyte tissue.

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**Decolorization by acids and alkalis of amoebocytes and of filter paper stained by neutral red.**

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We have previously found<sup>1</sup> that amoebocytes of *Limulus* stained with neutral red are almost instantaneously decolorized

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<sup>1</sup> Leo Loeb and Elizabeth Gilman, *Am. J. Physiol.*, 1924, lxii, 526; Leo Loeb and K. C. Blanchard, *Biol. Bulletin*, 1924, xlvi, 284.