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The reaction of the protoplasm of the living amoeba to injected salts.

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The salts, NaCl, KCl, $CaCl_2$, and $MgCl_2$, in various concentrations both separately and combined, were injected into the living Amœba by means of the micromanipulation apparatus.[1] The bore of the micropipettes averaged between one and two micra in diameter, and the volume of fluid injected could be controlled to amounts varying from about half the volume of the nucleus to that of the entire Amœba. Amœba proteus is ideal for this micro-operative work, not only because of the ease with which it can be injected, but also because of its characteristic reactions to the fluids injected. All the salts introduced produce a momentary dilution, as indicated by a scattering of the cytoplasmic granules in the injected area.

When NaCl or KCl is introduced, the dilution is accompanied by a decided liquefaction of the injected region which becomes quiescent. The surrounding protoplasm then pours into this area where movement ceases. During this quiescent phase the larger and presumably heavier crystalline granules fall to the bottom of this region and there tend to clump against the lower surface of the Amœba. Recovery takes place by a reappearance of currents in and around the quiescent area, and a gradually increasing flow, back and forth, until the Amœba resumes its normal state. The size of the region involved is conditioned by the strength of the salt solution used, and by the amount injected. The Amœba quickly recovers from an injection of a 2M solution of NaCl equal in amount to the volume of its nucleus. If more than this be injected the entire Amœba is converted into a quiescent globule of dead, liquid protoplasm surrounded by a delicate pellicle which readily disrupts when torn with the micro-needle. The granular contents then scatter in the surrounding

[1] Chambers, Robert, *Anat. Record*, 1922, xxiv, 1.

medium. Injections of M/4 to M/8 NaCl produce only a momentary, localized quiescence, and the Amœba rapidly recovers, even from very large doses, by a rushing back and forth of the cytoplasm between the injected and uninjected regions. Solutions in concentrations weaker than M/8 produce effects in the Amœba which approach those occurring when water alone is injected.[2] KCl, except for being more toxic, closely resembles NaCl in its effect on the Amœba.

$CaCl_2$, on the other hand, produces an effect quite different from either that of NaCl or of KCl. The injection of $CaCl_2$ is immediately followed by a contraction and a solidification of the injected region. Strengths of $CaCl_2$ varying from 2M to M/2 when introduced in volumes from ⅓ to ½ of the Amœba, immediately set the entire Amœba into an irreversible solidified mass with protruded pseudopodia. Small amounts of 1M, M/2 and large amounts of M/4 to M/104 $CaCl_2$ produce only a localized solidification. Sometimes, especially with the stronger of these solutions, there is an immediate flow of protoplasm to the injected area with a consequent increase in volume of the involved region. The Amœba then subsequently reacts by a flow of its healthy protoplasm away from the involved region, which is thus left behind as an inert, solidified mass. By an active "pinching-off" process this mass is rejected by the Amœba. A surprising feature of the $CaCl_2$ injection is the rapid "pinching-off" reaction on the part of the Amœba. The area affected tends in this way to be eliminated, and leaves the rest of the Amœba apparently unaffected and normal. When $MgCl_2$ is injected, the protoplasm solidifies in much the same way as with $CaCl_2$. However, no "pinching-off" reaction takes place, and the solidifying process, instead of being limited, gradually spreads throughout the Amœba.

In brief, NaCl and KCl produce a liquefaction of the injected area, whereas $CaCl_2$ and $MgCl_2$ cause a solidification.[3]

Combinations of these salts in various proportions were also injected. It was found that NaCl and $CaCl_2$ in certain very definite proportions, *viz.*, 1M NaCl with M/52 $CaCl_2$, M/2 NaCl with M/104 $CaCl_2$, and M/4 NaCl with M/208 $CaCl_2$, antago-

[2] Chambers, Robert, Sect. V, General Cytology, Univ. of Chicago Press, 1924.

[3] Chambers, Robert, 1924 Meeting of the Pathological Society, Federation of American Societies for Experimental Biology.

nize one another in such a manner as to neutralize the solidifying effect of the $CaCl_2$ and the liquefying action of NaCl. When combinations of KCl and $CaCl_2$ were injected it was found that these two salts neutralize one another when combined in the proportions of 1M KCl with M/26 $CaCl_2$, M/2 KCl with M/104 $CaCl_2$, and M/4 KCl with M/208 $CaCl_2$.

It may, therefore, be inferred that at least one of the features of the antagonistic action of NaCl or KCl to $CaCl_2$ is the maintenance in protoplasm of a definite balance between its liquid and solid phases. This phenomenon possibly depends upon the formation of a balanced proportion of Na and Ca or of K and Ca protein salts. It may also be due to the formation of Na or K and Ca soaps.

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The lactic acid content of blood and spinal fluid.

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As a preliminary to the study of variations of the concentration of lactic acid in blood and spinal fluid in pathological conditions, it was found essential to establish the normal limits for human blood and spinal fluid. Clausen's method has been adopted as the most satisfactory procedure. This method comprises the following steps: The precipitation of the blood proteins by tungstic acid by the Folin-Wu procedure, and the removal of glucose from this filtrate by means of copper sulphate and calcium hydroxide; the extraction of the lactic acid from this glucose-free filtrate with ether; the oxidation of the lactic acid to acetaldehyde with potassium permanganate; the distillation of the acetaldehyde into sodium bisulfite solution, and the titration of the excess and combined bisulfite, with iodine. With this method, Clausen states the provisional figures for normal human blood are from 15 to 32 mg. per 100 cc. This indicates a variation of more than 100 percent. The object of the present