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Combinations of Various Metals with Urease of Amoebocytes of Limulus or with its Coenzyme.

LEO LOEB AND I. LORBERBLATT.

From the Department of Pathology, Washington University School of Medicine, St. Louis, and the Marine Biological Laboratory, Woods Hole, Mass.

In former papers Loeb and Bodansky[1] have shown that if amoebocyte tissue of Limulus is extracted with various salts, the activity of urease in the extract differs very much in accordance with the character of the metal present in the salt. Furthermore, Loeb, Lorberblatt and Field[2] found that after extraction with NaCl a subsequent addition of $CaCl_2$ is without benefit, while after extraction with $MgCl_2$ subsequent addition of $CaCl_2$ increases markedly the efficiency of the extract.

In continuing these investigations we found that after extraction with certain salts in which the character of the anion is the same but which differ in respect to the kation, the efficiency coefficients of the various urease preparations differ very much from each other; we found that by adding a second salt to the extract made with a first salt the urease preparation can, under certain conditions, be made to assume the efficiency coefficient characteristic of the second salt and the readiness with which this transformation can be accomplished may be represented in definite curves, the abscissa of which indicates the time at which the second salt was added and the ordinate the amount of urease transformed into the new urease preparation. These curves are specific for each combination used. Thus, if we add NaCl to $CaCl_2$ extract one hour after the beginning of the extraction, almost one-half the $CaCl_2$ effect can be replaced by the NaCl effect; but during the next 4 hours the $CaCl_2$ effect gradually increases in strength and at last has become so strong that it remains unaffected by addition of NaCl. In the urease preparation obtained by extraction with NaCl, only within the first 30 minutes after the beginning of extraction can the $CaCl_2$ effect replace to a slight extent the NaCl effect. After completion of extraction (2 hours) the effect of addition of Ca has become nil. Thus each preparation has a specific time curve. These facts can best be explained if we assume that definite metal-urease or metal-coenzyme combinations form, which in some curves are

[1] Loeb, L., and Bodansky, O., *J. Biol. Chem.*, 1926, lxvii, 79; *J. Biol. Chem.*, 1927, lxxii, 415.

[2] Loeb, L., Lorberblatt, I., and Field, M. E., *J. Biol. Chem.*, 1928, lxxviii, 417.

at first of a relatively loose character, but which become more firm in the course of the next few hours; the rapidity with which this change takes place varies in the case of different metals. In this connection we have not only to consider the primary metal combination but also the second metal, which is meant to replace the first one. Thus the Na combination becomes a very firm one soon after the beginning of extraction with a NaCl solution, while the Ca combination is at first relatively loose and the Ca in this combination can much more readily be replaced by Na, but, as stated, after having been in contact with the urease for 3 hours after completion of extraction, it can no longer be replaced by Na. The Mg combination behaves approximately like the Ca combination. The Mn combination, on the other hand, becomes at once so firm that from the very beginning the Mn cannot be dissociated from the urease (or coenzyme) through the addition of other salts as far as we have tested the latter in our experiments. Conversely Mn can readily replace Ca and Mg.

The fact that these metal-urease or coenzyme combinations became fixed after a certain time, which varies considerably in the case of different metals, makes it seem improbable that the metals are combined with the urease or coenzyme in the form of readily dissociable ion combinations; it rather appears as if combinations of a different nature develop under these conditions.

Whether the metals in these combinations are bound to the urease or to a hypothetical coenzyme cannot be answered definitely at the present time, but the fact that Ca extracts, in which the urease efficiency has been weakened through heating, do not show an activating effect on NaCl extracts or on urease in blood serum, as well as certain other facts, make it more probable that we have to deal with metal-urease combinations. However, we intend to study this problem further especially by means of diffusion experiments.

Previously we have found that urease occurs in various kinds of cells and also in blood serum of Limulus, but apparently not in other arthropods. There is every reason for assuming that the urease present for instance in the blood serum and the blood cells of Limulus is of the same kind. It was of interest to determine whether the urease in all tissues of Limulus shows the same specific relations to salts which we observed in the case of urease extracted from the amoebocyte tissue. We found this not to be the case; neither does lipase extracted from amoebocyte tissue of Limulus show comparable reactions. We must then conclude that the metal urease combinations, which we observed, are specific for the par-

ticular constellation in which urease occurs in amoebocyte tissue of Limulus. These conditions can be altered, if we extract with certain salts, one constituent of which undergoes at once a very firm combination with urease (coenzyme), and they are also modified if we extract with blood serum which contains protein. It might be suggested therefore that, under usual circumstances, enzymes are in stable union, either with constituents of salts or with proteins, which make it impossible for them to form readily other combinations comparable to those into which the urease of amoebocyte tissue of Limulus or its enzyme can enter.

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Fate of Skin Bacteria after Autogenous and Heterogenous Transplantation of Skin into Subcutaneous Tissue.*

L. ERMATINGER, F. B. QUEEN and R. F. PARKER
(Introduced by Leo Loeb)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

In comparing auto-, homoio- and heterotransplants of various tissues Loeb observed, in general, a greater number of polymorphonuclear leucocytes around or in the transplanted tissues in the last type of transplantation than in the 2 former. The question arose whether the appearance of these leucocytes was due to the presence of bacteria in heterotransplants or whether the leucocytes were attracted directly by the heterotoxins. These observations suggested the following investigations in which we wished to determine (1) what the fate is of bacteria normally present on the skin after transplantation into the subcutaneous tissue, (2) whether in different species the fate of the bacteria after subcutaneous transplantation differs, and (3) whether there is a difference in the fate of bacteria adherent to the skin in case of autogenous and heterogenous transplantation.

We transplanted pieces of ear skin of approximately the same size into pockets of the subcutaneous tissue. Before transplantation the hair of the skin to be used was clipped, but otherwise the skin was left unchanged. In different experiments the pieces were

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