rabbits in which the vagus has been cut, insufflation of air into the bronchi prevented edema of the lung, and who attributed this effect to the diminution in negative pressure during expiration. These authors believe that negative pressure is an important factor in the development of pulmonary edema. We conclude that also the opening of the chest prevents or diminishes edema of the lung by this same mechanism.

[1] Johnson, Scott, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 181.
[2] Graham, E. A., *J. Am. Med. Assn.*, 1921, lxxvi, 784.
[3] Auer, John, and Gates, F. L., *J. Exp. Med.*, 1917, xxvi, 201.

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The Specific Action of Salts in Preparation of Urease from Amoebocyte Tissue of Limulus.

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In previous investigations Loeb and Bodansky[1] found that different salts have a specific action in the extraction of urease from amoebocyte tissue of Limulus, in accordance with the character of the kations. The salts of alkaline earths are by far the most favorable; the salts of alkali metals are very unfavorable, and the salts of Mg and in decreasing order Mn are intermediate in effectiveness. While extracts prepared with salts of heavy metals are apparently of similar strength to those prepared with salts of alkali metals, the addition of the salts of heavy metals to active extracts is very much more injurious than the addition of salts of alkali metals.

We recently analyzed the effect of mixtures of salts in the preparation of the urease, and the effect of addition of salts after the extraction with certain salts had been completed. If mixtures of salts are used in the preparation of extracts, the activity of the extracts thus prepared is approximately intermediate between the activity that is characteristic of each component salt; this applies to various combinations of NaCl, $MgCl_2$ and $CaCl_2$. If on the other hand, the extraction is first completed and then another salt is added, the results vary in accordance with the salt used for extraction. If we extract with NaCl and add a more favorable salt to the extract the addition of the latter salt is ineffective. On the other hand, if the extract has been prepared with $MgCl_2$, the subsequent addition of $CaCl_2$ leads to a condition in which the activity coefficient is inter-

mediate between the activity coefficient of $MgCl_2$ extract and the activity coefficient of $CaCl_2$ extract.

While extraction with Limulus serum in which the preformed urease has been previously inactivated through heating to 80° for 30 minutes, is more favorable than extraction with 0.5 m. NaCl solution, only a slight improvement in the activity of this extract can be produced through addition of $CaCl_2$ at the beginning of the extraction, and the effect is injurious if $CaCl_2$ is added to the extract. On the other hand, if we use unheated Limulus serum as extractive, the extract is much more potent than that prepared with heated serum, and in this case the activity coefficient of the preparation is increased through addition of $CaCl_2$ previous to extraction as well as after completed extraction. If we substitute for Limulus (or Lobster) serum indifferent colloids, like gum arabic, no effect is produced. Addition of salts to the preformed urease, as present in fresh Limulus serum, is injurious.

The lowering of the pH which we observe after addition of $CaCl_2$ to the extractive or to the extract depends probably largely on the formation of $CaCO_3$ as the result of the interaction between $CaCl_2$ and $(NH_4)_2CO_3$; in addition there takes place in all probability an interaction between a buffer substance in the $CaCl_2$ extract and the alkali which is produced in the course of the urease action.

Tentatively we may assume that urease or a substance associated with the enzyme, or a substance which exerts a strong influence on the activity of the enzyme, combines with various kations and that the activity coefficients of the urease in these combinations differ greatly. While the Mg combination is not so potent as the combinations with Ca, Ba or Sr, the former preserves the enzyme in such a condition that Ca can take the place of Mg in such a combination. Therefore in a mixture of Ca and Mg salts, both Mg and Ca combinations seem to form side by side and the activity coefficients are, therefore, intermediate. On the other hand, the combinations with alkali metals are apparently of such a nature that the enzyme is changed irreversibly and that subsequently no effective combinations with Mg or Ca can be produced. It is, therefore, necessary to add the salt of the alkali earth to the salt of the alkali metal in the beginning of extraction, before the alkali metal has had a chance to enter into a combination with the enzyme or with associated substances, if we wish to increase the activity coefficient above that of the NaCl extract.

We may conclude that the specific effect of the salts in these experiments does not depend upon their specificity in the process of extraction as such, but upon their interaction with the enzyme or

with a specific associated substance which takes place as soon as the latter has been made accessible to the salt.

Furthermore, it seems that we can distinguish at least 3 different effects of salts in their interaction with the urease: (1) Specific combinations of kations with the enzyme or with associated substances. In these combinations alkali earths are by far the most favorable. (2) Injurious salt effects of a non-specific character. Salts of heavy metals are more injurious than salts of alkali earths and the latter are more injurious than salts of alkali metals. (3) In addition osmotic effects of salts play in all probability a certain rôle. Increased osmotic pressure of salts or of non-electrolyses, at least within the range we have examined so far, acts favorably on the enzyme. Furthermore, we have found specific actions of proteins which may modify the effect of salts on urease.

[1] Loeb, Leo, and Bodansky, O., *J. Biol. Chem.*, 1927, lxxii, 415.

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Sex Characteristics in Monkeys.

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Female monkeys during sexually mature life show the sex characteristics of reddening and swelling of the skin about the external genital organs, on the buttocks and medial surfaces of the thighs. These phenomena disappear after double ovariectomy and can again be induced in spayed animals by injections of ovarian and placental extracts.[1] Three male monkeys in our colony had never shown this cutaneous reddening. (It has, however, been reported that male monkeys may show this reddening during the breeding season.) During the late summer and fall the 'sexual skin' of a large 7-year-old male began to redden. During November and December the regions affected covered an area 15x13 cm. in extent. The reddening reached a moderate intensity but never approached the maximum 'sexual skin' color of the mature female. From our experiments with ovarian hormone in inducing this condition in spayed animals, it seemed logical to infer that this condition in the male might be due to the male sex hormone.

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