

TABLE II.

No.	Units per 100 cc.		Date.		Remarks.
	1st assay	2nd assay	1st assay	2nd assay	
					All exposed in light from north windows.
159	66	57	Feb., 1924	Aug., 1926	Ether solution of liquor folliculi extract, green glass bottle.
6A32	333	200	May, 1925	Aug., 1926	Ether solution of placental and ovarian extract in clear flint glass.
R.R.	>500	<100	May, 1925	Aug., 1926	Petroleum ether solution of placental extract in amber bottle part(?) of time.
125	116	80	Dec., 1923	Aug., 1926	Alcoholic solution of a liquor folliculi extract in clear flint glass.

The method of preparation already referred to, but carried out by the illumination from a photographer's red light, gives almost total recovery of the hormone in a purified condition (0.01 to 0.02 mg. per rat unit).

¹ Ralls, J. O., Jordan, C. N., and Doisy, E. A., *J. Biol. Chem.*, 1926, lxi, 357.

² Allen, E., and Ellis, M. M., *J. Am. Med. Assn.*, 1925, lxxxv, 94.

3304

Specific Effect of Salts in Extraction of Urease from Amoebocyte Tissue of *Limulus*.

LEO LOEB AND OSCAR BODANSKY.

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

In a former communication¹ we have shown the presence of urease in the amoebocyte tissue, blood serum, muscle tissue and even in the unfertilized eggs of *Limulus*. In experiments carried out during the past summer we investigated means of extraction of this enzyme from experimental amoebocyte tissue, and we observed very striking differences in the extracting power of different salts.

Distilled water with or without the addition of urea is entirely or almost entirely ineffective. The Woods Hole sea water, which

is alkaline, or sea water previously neutralized, heated blood serum of *Limulus*, or, serum from which the proteins have been removed through heating, show a very moderate degree of efficiency. Solutions of NaCl or KCl, approximately isotonic with sea water, behave in a similar manner to sea water, or are even slightly less effective than the latter. On the other hand, extracts prepared with isotonic solution of CaCl_2 , BaCl_2 or SrCl_2 have a very high degree of activity. Solutions of MgCl_2 and also of MnCl_2 have an intermediate position, but approach more closely the former solutions than they do CaCl_2 , SrCl_2 or BaCl_2 . Solutions of CuCl_2 or ZnCl_2 are entirely or almost entirely without effect. Extracts made with isotonic solutions of CaCl_2 , BaCl_2 or SrCl_2 are perhaps 40 to 100 times more active than extracts made with $M/2$ NaCl or $M/2$ KCl.

The anions tested thus far did not seem to possess a much greater extracting power than the corresponding chlorides. However, through increase in the concentration of various salts, we can increase their effectiveness. Thus the optimal concentration of NaCl seems to lie between 1.25 and 2.5 m, while in the case of CaCl_2 and also of MgCl_2 it seems to be approximately between 0.38 and 0.75 m. However, further investigations in this respect are needed. If, instead of comparing the effectiveness of isotonic solutions of various salts, we compare that of solutions representing the optimal concentrations of various salts, the advantage of CaCl_2 , BaCl_2 , and SrCl_2 over NaCl, although less pronounced, is still quite marked, the former being about 8 times more effective than the latter.

If we add CaCl_2 merely to the finished extracts made with solutions of NaCl, no noticeable improvement in the activity is observed. It is therefore the process of extraction as such which is improved through CaCl_2 and related salts rather than the action of the enzyme on urea.

Extracts made with CaCl_2 , SrCl_2 or BaCl_2 differ still in another respect from all other extracts so far tested: in mixtures of urea solutions and extract they prevent the rise of the pH from 7.0 to 9.0, which normally takes place as the result of the conversion of urea into ammonium carbonate. This neutralizing power of CaCl_2 extracts seems to run parallel to their concentration in urease. It is therefore possible that it is a substance (protein?) specifically extracted by CaCl_2 and perhaps associated with the enzyme which is responsible for this neutralizing power. On the other hand, it can be shown that the neutralization as such is not the cause of the great effectiveness of CaCl_2 and similar salt solutions in the preparation of the urease. Variations in the pH of NaCl solutions may

increase or diminish their effectiveness as extracting agents of urease, but the greatest activity thus attained remains considerably below that attainable through the use of salts with more favorable kations.

Our experiments prove thus that urease is extracted from amoebocyte tissue in a quantitatively graded way by certain kations and that among these Ca, Ba and Sr are by far the most effective.

¹ Loeb, Leo, and Bodansky, Oscar, *J. Biol. Chem.*, 1925, lxxvii, 79.

3305

The Presence of a Polysaccharide-Like Substance in Blood.

MICHAEL SOMOGYI AND ETHEL RONZONI. (Introduced by P. A. Shaffer.)

From Departments of Biological Chemistry and Internal Medicine, Washington University School of Medicine, St. Louis, Mo.

In 1909 and the following years Lépine and Boulud¹ asserted that besides the usual form of sugar, blood contains a latent form of glucose which does not reduce alkaline copper solutions, but after being set free by acid hydrolysis behaves as ordinary glucose, reduces copper, and is fermented by yeast. This "sucre virtuel" plays an important part in carbohydrate metabolism. They believed that glucose and "sucre virtuel" were readily converted into one another, and that free sugar before utilization had to be incorporated into the larger complex molecule. In the diabetic this elastic shift from one form to the other was impaired. Sugar disappearing during glycolysis they claim to have recovered as "sucre virtuel." Lépine characterizes this sugar as glucose in combination with blood proteins in a glucoside linkage.

Lépine's analytical method consisted of precipitation of the blood proteins by coagulation with Na_2SO_4 and heat. The coagulum was separated by filtration, washed and hydrolyzed by means of hydrofluoric acid in lead crucibles for from 22 to 30 hours. Each determination required 10 cc. portions of blood and the life of a rabbit. Few have tried to repeat Lépine's work, and those who have failed to confirm his observations and his theories have been entirely discredited.

During the early part of last year we observed that the blood of animals in extreme insulin hypoglycemia yielded, on heating in a