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, lxvii, 79.

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The Presence of a Polysaccharide-Like Substance in Blood.

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In 1909 and the following years Lépine and Boulud[1] 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.

Lêpine'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

water bath for 2 hours with N/2 HCl, a substance giving typical reduction of alkaline copper solutions, and which disappeared completely if incubated 24 hours with yeast.

This observation agreed so completely with those of Lépine that we believed we were dealing with the same substance. After working out a simple technique for its determination (consisting in hydrolysis, for 2 hours, with N/2 HCl, in a boiling water bath, with subsequent precipitation of protein by Hg $(NO_3)_2$), we found this sugar present in the blood of all animals examined: man, ox, sheep, dog, rabbit, and guinea pig, in varying concentration in different species and in different members of the same species. Further, investigation into its relation to carbohydrate metabolism was undertaken, and observations were made of its behaviour during changes produced in the blood sugar by the following procedures: (1) Ingestion of glucose, (2) injection of insulin, (3) glycolysis *in vitro,* (4) fermentation.

During sugar tolerance tests the latent sugar remains fairly constant and uninfluenced by the concentration of the blood sugar. Occasionally, there is contrary to Lépine's findings a slight fall when the glucose of the blood is at its highest.

The injection of insulin left the latent sugar near the usual level, in guinea pigs, in insulin convulsions. An experiment on rabbits also shows the failure of insulin to influence the level of the latent sugar. In one experiment in a diabetic patient, entering the hospital in coma, treatment with insulin caused a drop in blood sugar from 360 mgs. to 59 mgs., at which point there were symptoms of insulin reaction. The latent sugar showed relatively slight changes. It is evident that changes in combined sugar are not related to changes in ordinary blood glucose.

Our test tube experiments show quite conclusively that there is no change in the latent sugar during glycolysis of a duration long enough to remove completely the glucose from the blood. In fact, if the blood remains sterile, incubation for a period of 18 hours failed to influence the latent sugar.

Fermentation tests carefully controlled showed that our earlier observations when 24 hours incubation was used were worthless. In non-sterile blood, frequently without the addition of yeast, all reducing substances, including non-sugar, disappears during 24 hours incubation. With yeast, 3 to 4 hours incubation at 37.5° C is sufficient to remove the glucose from the blood, together with added glucose, or pure glucose from its solution. When hydrolized blood is fermented under these conditions the liberated latent sugar

remains untouched. If the same sample of hydrolized blood is allowed to stand over night one finds but slight reducing properties remaining.

The non-fermentability of our reducing substance gave rise to the question of whether or not we were actually dealing with a sugar. The fact that the copper reduction is typical cannot decide this. We obtained typical osazone crystals, but only in sufficient quantity for an approximate determination of the melting point, which was about 180°C. The small amounts of latent sugar, which we were able to obtain free from glucose, we found to be dextrorotatory after hydrolysis, the rotation in relation to its reducing power being considerably less than that of glucose.

Because of the fact that this sugar is not contained in the protein free filtrate, regardless of the method of removing protein, we concluded that it is chemically combined with blood proteins. It remained to decide whether it was the monosaccharide of Lépine in glucoside linkage with the protein or Pavy's[2] amylose carbohydrate described in 1893. Following Pavy's technique we submitted the blood proteins to alkaline hydrolysis, as for glycogen determinations, subsequently precipitating with 10 volumes of alcohol. The precipitate was then separated and hydrolyzed by acid, giving a reducing substance qualitatively and quantitatively identical with that found in the same blood by direct acid hydrolysis. This shows that the combined carbohydrate of the blood is a polysaccharide.

Since this combined carbohydrate is largely contained in the plasma, we determined its distribution between globulin and albumin fractions. About 70 per cent is in the globulin fraction, the remainder being recovered from the albumin.

We have then, in blood, a substance combined with the protein which can withstand alkaline hydrolysis, which is susceptible to acid hydrolysis, as a result of which it yields a substance which gives a typical sugar reduction of alkaline copper solutions, which forms an osazone, which is dextrorotatory, with a rotation less than that of glucose, if based on its reduction value. It is not fermented by yeast after hydrolysis, nor is it susceptible to glycolysis. Its concentration varies in different individuals of the same species, and its level is independent of the amount of glucose in the blood and is unaffected by insulin injection.

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[1] Lépine, R., and Boulud, *J. de Physiol. et de Pathol. Général*, 1909, xi, 557; 1911, xiii, 178. *Compt. R. Soc. Biol.*, 1912, lxxiii, 589; 1913, lxxiv, 76.

[2] Pavy, F. W., "The Physiology of the Carbohydrates," J. & A. Churchill, 1894, London.