But the specific gravity of the chick's blood is not known for the incubation period, so that glucose should be expressed in milligrams per so many grams of blood pending such investigation.

This technique avoids the use of anticoagulants as the blood does not clot in the cotton, and it also makes unnecessary the use of oil to prevent evaporation. These advantages permit the drawing of the smallest possible samples of blood.

By means of such technique it is proposed to determine the normal blood sugar curve for the developing chick.

TABLE 1.

Hyperglycemia in chick embryo resulting from injection of glucose into air-sac. Blood sugar in mg. per 1.120 grams of blood × 100.

Age in days	Amount of glucose injected	Volume of fluid injected	Time in hours	Blood sugar	Remarks
15	400 mg.	2 cc.	0	202	Normal.
			1	441	Absorption incomplete.
			2		Death from loss of blood.
16	200 mg.	1 cc.	0	202	Normal.
			1	347	Absorption incomplete.
			2		Death from loss of blood.
16	100 mg.	½ cc.	0	221	Normal.
			1	859	Complete absorption.
			2	804	
			3	801	
			4	226	Death resulted.

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The nature of insoluble urease.

By JAMES B. SUMNER and VIOLA A. GRAHAM.

[*From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca, N. Y.*]

Soluble urease is prepared by the authors from the jack bean by the use of two principles: (1) repeated precipitation from slightly acid 30 per cent alcohol by cooling and centrifuging; (2) removal of the two globulins, concanavalin A and B, by al-

lowing them to crystallize out from the solution of urease in dilute aqueous neutral phosphate solution. Urease prepared in this manner has an activity of nearly 30,000 units per gram of dry material. A unit is defined by the authors as the amount of urease capable of producing 1 mg. of ammonia nitrogen from a urea phosphate solution in 5 minutes at 20° C.

Contrary to the previous belief of the authors, it is not possible to obtain urease entirely free from carbohydrate by the above mentioned procedure. A large amount of a pentose gum can be washed out during the precipitation by cooling, but small amounts of the gum appear to be firmly bound to some of the protein present. It is possible also that soluble urease is contaminated with traces of the third jack bean globulin, canavalin, although canavalin is insoluble in acid 30 per cent alcohol, the solvent used for extracting urease.

The authors have been able to free urease from the last traces of pentose gum, and also from any canavalin that might be present, by means of a third new principle, the conversion of urease into an insoluble, though still active form. This is effected by adding small amounts of sodium chloride to neutral 30 per cent alcohol urease, and allowing the solution to stand in a cool place for one or two days. The alcohol and sodium chloride are not added until after the crystallizable globulins have been removed and the material has been filtered. The precipitate of insoluble urease is centrifuged off and washed several times with aqueous neutral phosphate solution.

Insoluble urease is not as active, weight for weight, as soluble urease, but the authors believe it to be purer than the latter. When digested by trypsin its activity is considerably increased, and in a few instances has been observed to be more than doubled. Further action of trypsin leads rapidly to inactivation.

In the table below are given the percentages of cystine, tyro-

	Canavalin	Concanavalin A	Concanavalin B	Soluble Urease	Insoluble Urease
Ash per cent	0.13	0.67	0.22	trace	4.3
Percentages on ash-free basis					
Total "N"	15.97	15.74	15.79	14.18	15.33
Cystine	1.0	0.4	3.23	+	1.2
Tyrosin	5.5	5.17	9.44	4.4	4.94
Tryptophan	0.24	2.25	2.34	0.9	1.46
Urease units per gram				28,000	20,000

sin and tryptophan in the three jack bean globulins, and in preparations of soluble and insoluble urease. The methods of Folin and Looney[1] have been used because they permit the use of very small quantities of material. The value for cystine in soluble urease could not be obtained on account of the interfering action of the pentose gum present in this preparation. It is interesting to note that insoluble urease contains a slightly greater percentage of total nitrogen and of tyrosin than soluble urease, and that both of these extremely active enzyme preparations appear to be protein. We are unable to say why the percentage of tryptophan is so much larger in insoluble than in soluble urease. This may have been due to a loss of tryptophan caused by condensation with the pentose gum present in the soluble urease during the hydrolysis with barium hydroxide.

The amino acid percentages of the three jack bean globulins indicate, although they do not prove the matter conclusively, that both soluble and insoluble urease consist of a protein which is not identical with canavalin, concanavalin A, or concanavalin B. The three jack bean globulins differ from the urease preparations also with respect to such physical properties as solubility, crystalline form and color.

[1] Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, li, 421-434.