XLVIII. A NEW METHOD FOR THE PREPARATION OF THE PLANT GLOBULINS.

By GEORGE REEVES.

*From the Biochemical Laboratories, Department of Plant Physiology
and Pathology, Imperial College of Science.*

(Received November 9th, 1915.)

The plant globulins have been hitherto prepared by the extraction of the material with warm saline solutions. For this purpose sodium chloride has been most frequently employed, and relatively large volumes of solution have been necessary to obtain a satisfactory yield. In the ordinary process, furthermore, several tedious filtrations must be performed, the technique of which has been described in detail by Osborne [see, especially, Osborne, 1909].

It has been shown by Schryver [1910] that the globulins are much more soluble in solutions of those salts which lower the surface tension of water than they are in sodium chloride solution, and it was thought that by the use of these the bulk of material employed in the extraction of the globulins might be very considerably reduced and the troublesome filtration processes to a large extent avoided. At the suggestion of Dr Schryver, I have endeavoured to take advantage of the properties of certain salt solutions already referred to, and have succeeded in elaborating a process by means of which relatively large amounts of plant globulins can be prepared by processes, the chief of which can be completed within the course of a single day. The method is illustrated by the following examples.

Preparation of edestin from hemp-seed.

The hemp-seed is first ground and extracted by light petroleum. It is then filtered off on to a large Buchner-funnel, washed on the funnel with more petroleum and air-dried. The husks are then removed by sifting through coarse muslin.

500 g. of the material prepared in this way are then mixed with 500 cc. of semi-normal solution of sodium benzoate (about 7 % solution). After standing for about half an hour, so much torn blotting paper is pressed into the mass as to make it appear almost dry. The whole is then wrapped round with filter-paper and placed in a Buchner press and submitted to high pressure. About 400 cc. of liquid can be expressed. The residual mass is extracted again with about 500 cc. of the benzoate solution, and after standing for a short time, more blotting paper is added (relatively, only a small amount is necessary) and the whole is again pressed in the Buchner press, a further quantity of extract amounting to about 500 cc. being thereby obtained. The amount of edestin remaining in the residue is so small that a third extraction is almost superfluous.

The combined extracts are then thrown into about 10 litres of cold water and the whole is allowed to stand over night in a cool place. The edestin separates at the bottom of the vessel; the clear liquid is decanted off, and the creamy paste at the bottom is centrifuged. The edestin separates as a compact powder, and is then washed on the centrifuge once or twice with water, once with 95 % alcohol, once with absolute alcohol, and twice with dry ether. It is then rapidly transferred to a vacuum desiccator. A product is thereby obtained which is almost completely soluble in 10 % sodium chloride solution from which it can be obtained in a crystalline form in the usual manner. Any small impurity which is insoluble in the sodium chloride solution can be separated by centrifugation. 130 g. of the crude edestin (vacuum-dried) were obtained from 450 g. of the sifted meal. Semi-normal sodium salicylate solution was also tried instead of the benzoate. A violet coloured product is in this case generally obtained, owing to the action of the salicylate on the metal of the press. This colour is retained in an obstinate manner by the globulin even after recrystallisation.

Preparation of excelsin from Brazil-nuts.

The shelled nuts were passed through a hand mincing machine, and the fat was removed by extraction with light petroleum. 250 g. of fat-free powder were extracted with 250 cc. of semi-normal solution of sodium benzoate, the mass was then mixed with blotting paper, and the liquid expressed in the same manner as that described in the case of edestin. About 145 cc. of extract were obtained. The residue was then extracted twice more, each time with 250 cc., by benzoate solution (with addition of the blotting paper etc.), each extraction yielding after pressure about 250 cc. of

liquid. The combined extracts were then thrown into 10 litres of water, and the excelsin was separated and treated by the method described in the case of edestin (centrifugalisation, washing, etc.). A yield of about 20 % of the sifted meal was obtained. In another experiment the crude excelsin was dissolved when still moist after washing with water on the centrifuge, in a 6 % solution of ammonium sulphate, and this solution was then centrifuged for about 10 minutes to get rid of small amounts of insoluble matter. After 3 days' dialysis, excelsin separated from the solution, and was centrifuged off, and washed successively with 95 % alcohol, absolute alcohol, and ether, and then vacuum dried. A perfectly white powder was obtained. Yield from 250 g. of meal about 30 g.

By a similar process, a mixture of legumin and vicillin was obtained from horse-beans, and these were separated by the method described by Osborne.

REFERENCES.

- Osborne (1909). *Abderhalden's Biochemische Arbeitsmethoden*, 2, 270
Schryver (1910). *Proc. Roy. Soc. B*, 83, 96.