

by centrifuging; the supernatant was saturated with ammonium sulphate. After standing for 12 hr. at +4° C., the precipitate was collected; it was dissolved and dialysed with distilled water until the ammonium ions completely disappeared.

The dialysate was then set at pH 4.65 by acetic acid-sodium acetate buffer (final concentration 0.02 M). The molarity was brought to 0.06 by adding sodium chloride. One volume of cooled absolute ethanol was slowly added, the temperature being kept at 0° C. Once the precipitate was removed by centrifuging, the supernatant was brought to 80 per cent of ethanol (3 volumes).

The α_1 -acid seromucoid was collected, successively washed with cooled absolute ethanol and by anhydrous ether, then dried in a vacuum.

By this process, 300–400 mgm. of seromucoid acid were collected for every litre of plasma.

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¹ Welmer, H. E., Mohl, J. W., and Winzler, R. J., *J. Biol. Chem.*, **185**, 569 (1950).

² Michon, J. (unpublished results, 1961).

Preparation of Urease Crystal from Jack Bean

A PREVIOUS communication¹ reported a method for high-yield crystallization of urease from jack bean. Some improvement of this method was later made to secure a constant yield and purity of the resultant enzyme preparations in any circumstances and this procedure is reported here.

(1) The enzyme fraction obtained by ammonium sulphate fractionation was dialysed overnight at room temperature of 15°–25° C. The dialysate was divided into about 500-ml. portions. Each portion was heated to 50°–52° C. in a hot water bath and allowed to remain at this temperature for 15 min. The solution was then dipped in an ice-water bath to be cooled to room temperature. Constant mechanical stirring was maintained throughout the heating and cooling treatment. The bulk of denatured precipitate which formed during the heat treatment was centrifuged and discarded. Loss of activity in this treatment was confined to approximately 10 per cent. Two litres of the supernatant (from 500 gm. of jack bean meal) were mixed with 1.1 litre of calcium phosphate gel and the enzyme was adsorbed by the gel at pH 5.0–5.5 as previously reported. Urease was eluted three times with 300 ml. of M/12 phosphate buffer of pH 7.5. To 900 ml. of the eluate, 337 gm. of ammonium sulphate was added for the second fractionation.

In the previous method, the amount of denatured precipitate which formed by dialysis was affected a great deal by room temperature and this had considerable effect on the result of the following procedures. To provide for sufficient removal of impure proteins from the dialysate, this heat treatment was again added.

(2) After treatment with acetone, the resulting precipitate, insoluble in water from 500 gm. of the starting material, was treated with 30 ml. of mixed solution of 9 vol. of M/30 citrate buffer (pH 6) and 1 vol. of 10 per cent aqueous solution of 2-mercaptoethanol. Citrate buffer used here was prepared by dilution of 0.5 M citrate buffer (pH 6.0) used in

recrystallization of urease by Dounce^{2,3}. After separation of the supernatant, 10 ml. of the mixed solution was added to the remaining insoluble portion and this was treated again as before. To the combined supernatant containing urease in highly purified state, 0.3 volume of acetone was added without any adjustment of the pH and the mixture was placed in an ice chamber overnight to allow crystallization.

It was noticed that the quantity of impure protein which transferred to the supernatant together with urease increased as pH value of the buffer became higher.

The yield of urease crystals thus prepared with additional steps was approximately 25 per cent.

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¹ Hanabusa, K., *Nature*, **189**, 551 (1961).

² Dounce, A. L., *J. Biol. Chem.*, **140**, 307 (1941).

³ Sumner, J. B., *The Enzymes*, 1, Pt. 2, 876 (Academic Press, Inc., New York, 1951).

α, γ -Diaminobutyric Acid in Seeds of Twelve Species of *Lathyrus* and Identification of a New Natural Amino-Acid, L-Homoarginine, in Seeds of Other Species Toxic to Man and Domestic Animals

THE term 'lathyrism' has been applied somewhat uncritically to the toxic effects produced in many creatures by the ingestion of the seeds of various species of *Lathyrus* or by the injection of extracts of these seeds.

Selye¹ has pointed out that there are at least two distinct manifestations of 'lathyrism'. The first, induced by the ingestion of seeds containing β -(N- γ -L-glutamyl)-aminopropionitrile, he calls 'osteolathyrism' because of the skeletal changes seen in rats. The second, induced by the ingestion of the seeds of a greater range of species, he calls 'neurolathyrism' because of a nervous derangement seen in animals and man.

The isolation of a neurotoxic compound, α, γ -diaminobutyric acid, from the seeds of *L. latifolius*, a species inducing 'neurolathyrism', has recently been described². Using α, γ -diaminobutyric acid as a 'marker', it has now been shown (by paper chromatography in 5 solvent systems and high-voltage paper ionophoresis at pH 3.6 and 1.9) that α, γ -diaminobutyric acid is identical with the basic amino-acid designated B_2 by Bell³ and found as a free amino-acid in concentrations of the order of 1 per cent dry weight in the seeds of *Lathyrus aurantius*, *L. cirrhosus*, *L. gorgoni*, *L. grandiflora*, *L. heterophyllus*, *L. laevigatus* ssp. *aureus*, *L. luteus*, *L. multiflora*, *L. rotundifolius*, *L. sylvestris*, *L. tuberosus*, and *L. latifolius* itself.

This neurotoxic amino-acid has not been detected in the seeds of *L. sativus*, *L. cicera*, or *L. clymenum*³, those species associated with lathyrism (a 'neurolathyrism') in man, horses and cattle¹. It is of interest, however, that the seeds of these three species, unlike those in which α, γ -diaminobutyric acid has been detected, contain major concentrations of a new natural amino-acid which has been identified chromatographically as homoarginine (α -amino- ϵ -guanidino-caproic acid)³. It is now reported that this amino-