

action with the butyric acid test indicates either the absence of syphilitic infection or a successful cure of the disease. There is no necessary relation between the Wassermann test and the quantity of globulins in the luetic serum.

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**The quantitative separation of leucin from valin.**

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Of the known amino-acids determined in semi-quantitative estimations of final proteolytic products, leucin and its relatives, isoleucin and valin, have proven unusually difficult to prepare pure in even approximately quantitative amounts. The separation of these substances, because of their close physical and chemical similarity, has offered almost insurmountable difficulties to previous investigators. The acids form isomorphous mixtures which are absolutely inseparable by crystallization; and their esters have so nearly the same boiling points that they cannot be fractionated by distillation. Because of these difficulties, most investigators have not attempted to separate the mixture, but have reported the entire mass as leucin. Fischer<sup>1</sup> states that all the figures reported from his laboratory for leucin in protein hydrolyses refer to this mixture. Ehrlich<sup>2</sup> has recently reported a method for separating the three substances, but it involves a long process, large losses, and the racemization of the isoleucin and valin.

We have been able to separate the leucin isomers readily from valin in quantitative amounts. The method, which is very simple, rests on the fact that if a molecular lead acetate solution is added to an ammoniacal solution of the leucin-valin mixture, the leucins are precipitated as analytically pure  $\text{Pb}(\text{C}_6\text{H}_{12}\text{O}_2\text{N})_2$ . If too great an excess of lead acetate is added, a portion of the valin may also be precipitated. Consequently, the mixture is first analyzed, an estimate of the proportion of leucin calculated from the carbon content, and 20 per cent. excess of the theoretical amount of lead

<sup>1</sup> Fischer: Unters. über Aminos., Polypeptide, und Proteine, p. 67.

<sup>2</sup> Ehrlich: *Bioch. Zeitschr.*, 8, 399, 1908.

acetate used for precipitation. The valin is obtained analytically pure by freeing the filtrate from lead with  $H_2S$ , evaporating to dryness, and washing with absolute alcohol. A slight amount of valin dissolves, but is regained by evaporating the washings.

The following is a typical separation, the material being a portion obtained by tryptic digestion of casein and fractional distillation of the amino-acid esters. 12.546 g. of the mixture was used. Analysis showed 52.79 per cent. C, 9.55 per cent. H. The mixture was suspended in 80 c.c. of boiling water. The flask was removed from the flame and 20 c.c. of concentrated aqueous ammonia added. The flask was loosely stoppered, and shaken gently until the acids were dissolved. The leucin was then precipitated with 25 c.c. of M/1 lead acetate. The cooled solution was filtered, and the precipitate washed with 50 c.c. of dilute ammonia. 8.955 g. of lead salt, equivalent to 5.025 g. of leucin, and 7.322 g. of valin were obtained analytically pure, making 12.347 g. from the original 12.546 g. Analytic data:

Lead salt: (1) 44.25 per cent. Pb; (2) 44.36 per cent. Pb. Calculated for  $Pb(C_6H_{12}O_2N)_2$ , 44.29 per cent. Pb.

Valin: 51.44 per cent. C; 9.42 per cent. H. Calculated for  $C_5H_{11}O_2N$ , 51.24 per cent. C; 9.47 per cent. H.

The specific rotation of the valin was  $[\alpha]^{20}_D + 26.51^\circ$ . The pure active substance has the rotation  $+ 28.8^\circ$ . The product was partially racemized, the usual result of long tryptic digestion.

The lead-leucin salt contains a mixture of leucin and isoleucin. Levene and Jacobs<sup>1</sup> have shown that these isomers can be readily separated in the absence of valin. The complete separation of the two leucins from valin, therefore, renders the systematic separation of all three comparatively easy of accomplishment. This is of importance, not only for protein analysis, but also for the preparation of pure active valin and isoleucin, a task which has hitherto been extremely difficult.

The work will be reported in full in the *Biochemische Zeitschrift*.

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<sup>1</sup> Levene and Jacobs: *Bioch. Zeitschr.*, 9, 231, 1908.