LETTERS TO THE EDITORS

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NOTES ON POINTS IN SOME OF THIS WEEK'S LETTERS APPEAR ON P. 119.

CORRESPONDENTS ARE INVITED TO ATTACH SIMILAR SUMMARIES TO THEIR COMMUNICATIONS.

The Glutamic Acid of Tumour Proteins

IN analysing tumour proteins, we[1] have isolated without exception partially racemized glutamic acid. However, Chibnall and co-workers[2] recently reported the isolation of optically pure *l*(+)-glutamic acid in four cases. These authors did not use the same method which we employed, but a different procedure which is excellent for the rapid and efficient isolation of natural glutamic acid from standard proteins.

Prof. Chibnall kindly sent us a copy of his preliminary note so that we could immediately carry out corresponding experiments.

The essential difference in the method of the British authors lies in a preliminary purification of the dicarboxylic amino-acids by precipitating them twice as the calcium salts[3] from 90 per cent alcohol. We have investigated the solubility of the calcium salts and found that the calcium-*d*,*l*-glutaminate is at least ten times as soluble as the calcium salt of the *l*-glutamic acid in 90 per cent alcohol. Although the pure racemic salt is relatively insoluble, the above-mentioned difference can become very important through the tremendous influence of contaminating material in crude extracts, which is well known to all those who have isolated active principles from natural sources.

In our laboratory in recent months, Mr. A. M. Akkerman has investigated the 'ternary' system of *d*- and *l*-glutamic acid hydrochlorides in 20 per cent hydrochloric acid at 0° C. It was found that the *d*,*l*-hydrochloride is not a racemic compound but a racemic mixture (conglomerate), the solubility of which is exactly twice as great as that of the hydrochloride of *l*(+)-glutamic acid. Since we precipitate the crude glutamic acid by saturating the aqueous solution with hydrochloric acid at 0° C., we have also studied the solubilities in this concentration and found that, although both forms are less soluble, they showed quite the same difference in solubility.

In order to test the effects of these solubility differences upon the isolation, we have fractionated a mixture containing 75·5 per cent of pure *l*- and 24·5 per cent of pure *d*-glutamic acid according to the method of the British authors. We obtained 66 per cent of the *l*-glutamic acid originally present in the mixture in pure state ($[\alpha]_D = +31{\cdot}4°$).

The influence of accompanying substances is shown in the following experiment in which we used the hydrolysate from dried calf's embryo from which we had previously been able to isolate optically pure *l*-glutamic acid by our method. 600 mgm. of *d*,*l*-glutamic acid were added to the hydrolysate from 20 gm. of embryo tissue and the mixture fractionated according to the method of Chibnall *et al.* We obtained 1·16 gm. of pure *l*-glutamic acid hydrochloride ($[\alpha]_D = +31{\cdot}2°$). In a second experiment 700 mgm. of *d*,*l*-glutamic acid were added to 7·8 gm. of an alcohol precipitate which was prepared from the same embryo tissue in a previously described manner[1]. The fractionation, when done according to the method of the British authors, yielded 506 mgm. of pure *l*-glutamic acid hydrochloride ($[\alpha]_D = +31{\cdot}4°$).

Since the criticism might be raised that the depressions of optical rotation in our preparations of glutamic acid from tumours could be caused by analytically undetectable impurities having opposite rotation, we have attempted the isolation of the *d*(−)-glutamic acid. A resolution of the partially racemic glutamic acid by E. Fischer's[4] procedure was not practicable since benzoylation itself produces a partial racemization. We have therefore treated a partially racemic glutamic acid from Brown-Pearce tumours with fermenting yeast according to the method of F. Ehrlich[5] and were indeed able to isolate pure *d*(−)-glutamic acid ($[\alpha]_D = -31{\cdot}2°$).

In conclusion, it can be said that the method as it is employed by Chibnall *et al.*, in spite of very good yields obtained in the isolation of *natural* glutamic acid, appears not to be applicable for detecting the *partial racemization* of glutamic acid. In employing the method which we used, we recommend the inoculation with crystals of both enantiomorphs after saturation with hydrochloric acid, and further the recrystallization with the least possible loss of material. Details of our experiments will appear in the *Zeitschrift für physiologische Chemie*.

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[1] *Z. physiol. Chem.*, **258**, 57 (1939); *Klinische Wochenschrift*, **18**, 801 (1939).
[2] NATURE, **144**, 72 (1939).
[3] Foreman, *Biochem. J.*, **8**, 463 (1914).
[4] Fischer, E., *Ber. deutsche chem. Ges.*, **32**, 2464 (1899).
[5] *Biochem. Z.*, **63**, 379 (1914).

Dissociation of the Hæmocyanin Molecule

SEVERAL proteins are dissociated by a change of the hydrogen ion concentration, by adding amino compounds or salts to the solution [1, 2, 3, 4] and by exposure to various kinds of radiation[5].

The hæmocyanin molecule of *Helix pomatia* (molecular weight 6,700,000) may, upon a change of *p*H, dissociate into halves, eighths and sixteenths[6]. The reaction is reversible: the dissociation fragments recombine, if we bring the solution back to the original *p*H[6]. Certain amino compounds may also cause dissociation[1].

By means of the ultracentrifuge[1,2] we have investigated the influence of different types of salts and of a few non-electrolytes in acetate (*p*H 5·2) and in phosphate (*p*H 6·0) buffers of molarity 0·08. Well-defined sub-multiples ($\frac{1}{2}$, $\frac{1}{8}$, and $\frac{1}{16}$ of the original molecule) are obtained. The dissociation effect increases with the valence of the ions. The 1–1 valent sodium and ammonium chlorides both have the same effect: no dissociation occurs in 0·2 molar solutions, while in 1·0 molar 78 per cent of the hæmocyanin is dissociated into half-molecules. Nearly