ON GLYCYLPROLIN ANHYDRIDE OBTAINED ON TRYPTIC DIGESTION OF GELATINE.

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In a previous communication on tryptic digestion of gelatine one of us (Levene) reported the finding of a substance having the composition $C_7H_{10}N_2O_2$.[1] The mode of separation of this substance was fully described in an article published by G. Wallace and one of us (Levene [2]). In the previous publications the supposition was expressed that the substance might be a precursor of prolin. Indeed on further cleavage with mineral acid the substance $C_7H_{10}N_2O_2$ decomposed into prolin and glycocoll, thus showing that it is a dipeptid, prolylglycin anhydride—$C_5H_9NO_2 + C_2H_5NO_2 - 2H_2O = C_7H_{10}N_2O_2$.

One and five tenths grams of the air dry substance was heated for five hours with strong hydrochloric acid in a sealed tube at 150° C. The product of the reaction was taken up in water and evaporated to dryness under diminished pressure, and the residue again dissolved in water, and evaporated. This operation was repeated three times. The remaining hydrochloric acid was removed by means of silver sulphate, and the sulphuric acid by means of barium hydroxide, and the filtrate from the barium sulphate was evaporated to dryness. The residue was crystalline in nature, possessed a very sweet taste, formed with copper oxide a salt, gave rise to pyrrol on sublimation, and, on heating, gave the odor of pyrolidin. Thus the presence of prolin in the residue was made very probable. The presence of glycocoll was demonstrated by the formation of the typical picrate described by one of us (Levene).

The separation of the two aminoacids was accomplished by means of boiling methyl alcohol. The mixture was repeatedly extracted with methyl alcohol until the residue no longer gave on

[1] *Journal of Exper. Med.*, 1906, viii, 180.
[2] *Zeitschr. f. physiol Chem.*, 1906, xlvii., 143.

sublimation the pyrrol test. The substance gave a bright pink sublimate which is characteristic for glycocoll formed a picrate resembling that of glycocoll, and possessed a very sweet taste. On rapid heating it turned brown and began to melt at 240° C., and decomposed at 250° C. (An Anschutz thermometer was used for the experiment.) The substance had the following composition:

0.0881 gram of the substance gave on combustion 14.4 c.c. nitrogen (over 50% KOH) at T° = 18.5° C and p = 747 m.m.

For $C_2H_5NO_2$ the quantities are as follows:

Calculated.	Found.
N = 18.66%	18.87%

Thus the substance was glycocoll. The yield of it was 0.350 gr.

The methyl alcohol extract was concentrated under diminished pressure and then evaporated nearly to dryness on a water bath. It was then taken up in absolute alcohol, filtered, and dried. The substance had all the properties of prolin. Its melting point was 205° C.

0.185 gram of the substance gave on combustion 20.8 c.c. nitrogen (over 50% KOH) at T° = 22° C. and p = 760 m.m.: N = 13.03%.

For $C_5H_9NO_2$ the quantities are as follows:

Calculated.	Found.
N = 12.17%	13.03%

Thus this sample consisted of prolin still containing some glycocoll.

The alcoholic mother liquid was allowed to evaporate over sulphuric acid in vacuum desiccator. A crystalline deposit was formed. It was filtered, washed with alcohol and ether, dried, and analyzed:

0.1356 gr. of the substance gave on combustion 0.2600 gr. CO_2. The water absorption tube was lost through an accident. For $C_5H_9NO_2$:

Calculated.	Found.
C = 52.17%	52.28%

0.1037 gr. of the substance gave on combustion 0.1995 gr. CO_2 and 0.740 gr. H_2O. For $C_5H_9NO_2$:

Calculated.	Found.
C = 52.17%	52.46%
H = 7.82%	7.92%

This finding furnishes conclusive evidence, lacking up to now, that prolin is a primary component of the proteid molecule.