barium hydrate, from barium hydrate with carbon dioxide, and at the end, an *exact* equivalent of sulphuric acid. This leaves only the mono-amino acids in solution. It has been found that all the known acids of this class react neutrally to indicators changing color at $H^+ = 10^{-7}$, except the dicarboxylic acids, glutaminic and aspartic, and these can be titrated accurately as monobasic acids, even in the presence of the others, with rosalic acid as indicator. The amino acids not precipitated by phosphotungstic are, therefore, divided as follows:

Phosphotungstate filtrate { Non-amino nitrogen, *prolin, oxyprolin, ½ tryptophane,* and possibly unknown acids. / Amino nitrogen { *Dicarboxylic acids (glutaminic and aspartic).* / *Mono-carboxylic acids (leucin, alanin, etc.).*

The method is being applied, among other things, to a study of the fibrinoses in progress with Drs. Levene and Birchard in this laboratory.

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The determination of amino nitrogen as a measure of the rate and extent of proteolysis.

By **DONALD D. VAN SLYKE.**

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

Amino acids in proteins, as shown by Emil Fischer's work, are undoubtedly bound together by peptid (-CO-NH-) linkings, the nitrogen in these linkings being in the *imino* form. When hydrolysis occurs, the peptid linkings are split, yielding -COOH and $-NH_2$ derivations. The hydrolysis of each peptid linking, therefore, frees an *amino* group. Consequently, determination of the amino nitrogen should afford direct chemical measure of the rate and extent to which a protein is hydrolyzed, whether by action of acids, alkalies or enzymes. It should also indicate the relative molecular size of isolated intermediate products, such as albumoses, peptones and peptids, as the larger molecules have relatively more nitrogen in the peptide linkings, and less amino nitrogen. Results have been obtained which indicate that the amino determination fulfills the above requirements of the theory of protein structure, and that it will be of practical value in the study of enzyme action and of the products of partial hydrolysis.

It has been found that every known amino acid obtained by proteolysis, except prolin and oxyprolin, gives off one or more atoms of nitrogen when treated with nitrous acid.[1] The dipeptids, leucyl-leucin and leucyl-glycin, give off to nitrous acid only one atom of nitrogen each, the atom in the peptid linking not being removed. Glycin anhydride, containing two atoms of nitrogen, both in peptid form, gives off no nitrogen at all. Peptides having serin or glycin on the end of the chain containing the free amino group may yield somewhat more than the theoretical one atom of nitrogen, for reasons which will be shown later. With the exception of such peptides, which cannot be yielded by most proteins in sufficient amount to interfere appreciably with determinations, agreement between theory and results appears absolute. Native proteins show but minimal amounts of amino nitrogen. The proportion is greater in the primary albumoses, and still greater in the deutero. (The albumose results will be published shortly with Drs. Levene and Birchard.) The progressive increase of amino nitrogen during hydrolysis is shown by the following series: egg albumen in 2 per cent. solution was submitted to the action of 5 per cent. sodium hydrate at 60°, 5 cubic centimeter samples being drawn at intervals for amino determination. Addition of an occasional drop of amyl alcohol during the earlier determinations is necessary to prevent foaming of the viscous solutions.

Time in hours.	Per cent. of nitrogen in amino form.
0	3.0
0.5	7.15
1.5	10.31
4.5	19.45
12.0	28.22
24.0	39.02
48.0	46.62
72.0	54.02
96.0	61.10
144.0	68.42

Completely hydrolized albumen contains 8.5 per cent. of its nitrogen in amino form.

A similar, but much more rapid increase was obtained when

[1] *Proc. of the Soc. for Exper. Biol. and Med.*, 1909, vii, 46. The apparatus in improved form is manufactured by Machlett & Sons, New York.

casein was heated at 80° with 20 per cent. hydrochloric acid. In this case, hydrolysis was complete in ten hours, the amino nitrogen having reached its maximum, 71 per cent. of the total.

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A demonstration of Krogh's micro-tonometer for the determination of gas tensions in fluids.

By **M. M. SCARBROUGH.** (By invitation.)

[*From the Physiological Laboratory, Department of Medicine, Yale University.*]

The advantages of Krogh's apparatus are, first, the small amount of fluid required; second, the large specific surface obtained; third, the rapidity and accuracy of determinations. The bubble of gas used is about 2 mm. in diameter, having a specific surface of 30 as compared with a specific surface of 3.3 in Pflüger's aërotonometer, 5.2 in Bohr's hemataërometer and 3.7 in Fredericq's. A fine spray of the fluid to be examined is played on the bubble for a few minutes; the bubble is then drawn into a capillary burette where it is measured before and after the absorption of oxygen and carbon dioxide. For carbon dioxide alone in fluids, a very simple vessel is used; the tonometry is accomplished by shaking a small bubble of air with a relatively large amount of fluid. The bubble is then transferred to the capillary burette and analyzed. The limit of error can be brought down to + 0.2 per cent.[1]

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Breeding experiments in poultry.

By **H. D. GOODALE.** (By invitation.)

A. *Note on the Results of a White Leghorn by White Plymouth Rock Cross.*

The cross was made in only one way, viz., white Leghorn females × White Plymouth Rock males. The chicks were white with sometimes a few black spots. They developed into white birds, with a few black spots. Later the surface color became

[1]For full description of apparatus and methods see Krogh's articles in *Skandinavisches Archiv für Physiologie*, 1908, xx, 259–288.