The response of a muscle to chloroform or saponin after treatment with a strongly sensitizing solution, as $m/8$ NaI, $m/8$ $NaClO_3$, $m/8$ $NaNO_3$, or $m/8$ NaCNS, approaches closely in character to the normal contraction, *i. e.*, the upstroke is rapid and accompanied by often vigorous twitching. There is, however, *no relaxation;* and the associated coagulative change in the tissue-colloids is more pronounced than in the unsensitized muscle similarly treated. In other words, *there is a correlation between the vigor of the contraction and the degree of the coagulation*, indicating that the fundamental change in contraction is of the same nature as in colloid coagulation, *i. e.*, that contraction is the expression of a coalescence of colloidal particles in the fibrillæ, due presumably to increased surface-tension of these particles. These experiments also indicate that rigor contraction is of the same essential nature as normal contraction.

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## A new method for the analysis of proteins.

By **DONALD D. VAN SLYKE.**

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The method outlined will serve to supplement the ester method, and to characterize proteins when relatively small amounts of material are available. Two and a half grams of protein are hydrolyzed by 15 to 18 hours boiling with 20 per cent. hydrochloric acid. The solution is concentrated to a syrup, then transferred to a one-liter Claissen distilling flask with 200 cubic centimeters of water. Saturated barium hydrate solution to 25 cubic centimeters excess is added and the *ammonia* is distilled *in vacuo* into $N/10$ sulphuric acid from a bath at 45° C. The residual solution is acidified with sulphuric acid and boiled while silver sulphate is added until all the hydrochloric acid is precipitated. The precipitate, which carries the *melanine* with it, is Kjeldahled to determine the melanine nitrogen. The filtrate is brought to 100 cubic centimeters and 5 cubic centimeter duplicates taken for total and amino nitrogen determinations.[1]

[1]Method for Determination of Amino Nitrogen, *Proc. of the Soc. for Exper. Biol. and Med.*, 1909, vii, 46.

The remaining 80 cubic centimeters are precipitated with phosphotungstic acid as described by Osborne and Harris,[2] except that twice the volume of solution is employed and the bases are given forty-eight hours to precipitate. It has been found that cystine is precipitated quantitatively with the hexone bases. The phosphotungstates are washed with suction, and decomposed in the cold with a slight excess of barium hydrate solution. The excess barium is precipitated as carbonate. The solution is then concentrated at a low temperature and brought to 50 cubic centimeters; 10 cubic centimeter samples are taken for total and amino nitrogen determinations. The remaining 30 cubic centimeters are washed in a copper Kjeldahl flask with 20 cubic centimeters of water and 15 grams of solid reagent sodium hydrate. The mixture is boiled six hours under a reflux condenser, the top of which is closed by a Folin 3-bulb tube containing 20 cubic centimeters of $N/10$ sulphuric acid. One-half the *arginine* is split off as ammonia,[3] the reaction being quantitative under the conditions here outlined. 90 to 98 per cent. of the ammonia is caught and titrated in the acid of the Folin tube. The remaining 2 to 10 per cent. is boiled off on a Kjeldahl still, after the alkaline solution has been diluted. The bases, other than arginine, give off no ammonia. To the solution used for arginine determination one adds 3 grams of potassium nitrate, concentrates, fuses and determines the sulphur, from which the *cystine* is calculated. By these determinations the phosphotungstate precipitate is divided as follows:

- Phosphotungstate precipitate
  - Non-amino N
    - *Arginine* (¾ of nitrogen, non-amino), determined by decomposing with sodium hydroxide.
    - *Histidine* (⅛ of nitrogen, non-amino) by difference from the arginine.
  - Amino N
    - *Cystine* (all of nitrogen, amino), determined by sulphur.
    - *Lysine* (all of nitrogen, amino), by difference from cystine.

The amino and non-amino nitrogen in the phosphotungstic filtrate are determined by difference, avoiding the necessity of Kjeldahling solutions containing phosphotungstic acid. The filtrate is freed from phosphotungstic and sulphuric acids with

[2] *Jour. of the Amer. Chem. Soc.*, 1903, xxv, 323.

[3] Osborne, Leavenworth, and Brautlecht, *American Jour. of Physiol.*, 1908, xxiii, 180.

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.

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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.