be determined in the nitrometer. This is easily accomplished by slowly adding a weighed sample (*e. g.*, 1 g.) of the nitrate to 20 cc. conc. sulfuric acid cooled to 0° and kept at this temperature until all the nitrate is dissolved. This converts the nitrate into the nitrocompound, which may then be introduced into the nitrometer, the cup washed with 10 cc. additional sulfuric acid, and the reaction completed in the usual manner. This method will yield practically theoretical results for the nitrates.

Summary.

1. The nitrometer has been used for a great variety of analytical operations, in which a definite quantity of a gas is evolved, not soluble to a very considerable extent in the liquid from which it is evolved, and not acting upon mercury.

2. The characteristic nitrometer reaction has been used only for nitrous and nitric acid, their salts and esters.

3. Further evidence is given that the nitrometer reaction does not liberate NO from true nitrocompounds in which the nitro group is linked directly to the carbon atom.

4. Characteristic reactions for nitrocompounds are given and evidence is produced that nitrocompounds in which the nitro group is attached to carbon through nitrogen give most of these reactions.

5. The application of the true nitrometer reaction is extended for the determination of nitrogen of the NO_2 and NO groups in nitroamines and nitrosoamines in which the respective groups are attached to carbon through nitrogen.

6. The nitrometer reaction may be used as a means of ascertaining whether the NO_2 and NO group is attached directly to the carbon or through a nitrogen atom.

7. An anomaly is noted in which the nitrates of urea and guanidine cannot be determined in the nitrometer in the usual manner. They must be converted into their respective nitrocompounds first, after which they may be readily determined.

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THE COMPOSITION OF NEUROKERATIN.

BY BURT E. NELSON.

Received September 9, 1916.

Neurokeratin, which was first isolated by Kühne, is supposed to be the chief characteristic constituent of the neuroglia cells of the brain, and of the axilemma and outer sheath of the medullary substance of the nerve trunks. It is classed chemically with the general group of keratins,

which form the characteristic constituents of the hair, hoofs, horn, nails, shells, scales and other tissues of epiblastic origin, and whose chief characteristics among the other classes of proteins are their insolubility in water, salt solutions, acid and weak alkali solutions, their indigestibility in pepsin-hydrochloric acid, and their relatively high content of sulfur in the molecule.

Widely varying analytical figures have been reported by other observers[1] for the elementary composition of this substance, the discrepancies doubtless arising from different methods used in isolating it, and the analysis of impure products. It was with a view of obtaining more trustworthy results that the following analysis was undertaken:

The material used was the total protein residue from several lots of brains of patients diagnosed as having general paresis, senile dementia (with placques), and dementia praecox, which had been previously extracted with alcohol, ether, and warm water, for the lipoid and extractive matters.

Previous to these extractions the minced brain tissues had been allowed to remain in 85% alcohol for two months, to insure thorough coagulation and hardening of the proteins. This final dried protein residue was finely ground and digested in an incubator at blood heat, six times with one hundred times its amount of pepsin-salt-hydrochloric acid mixture, for twenty-four hours each, or three times after the cessation of the biuret reaction. It was then similarly digested five times with 0.1% solution of sodium hydroxide to dissolve nucleins and finally extracted again with alcohol and ether and dried at a gentle heat.

It formed a pale yellowish, light powder, showing no further changes on treatment with solvents (which removed at most only traces of soluble matters) and had a mineral ash content of 0.22 to 0.25%.

Elementary analysis for carbon and hydrogen, by combustion of one-half gram lots gave:

C, 54.86%, 54.73%, 54.95%, and 54.95%; H, 7.33%, 7.30%, 7.18%, and 7.33%.

Nitrogen determinations by the Kjeldahl method, on half-gram lots, using no catalyzing agent, and continuing the digestions for three hours after the solutions were colorless, gave

N, 13.12%, 13.24%, 13.16%, and 13.16%.

Similarly, sulfur determinations on one-gram lots, by combustion in oxygen gas in a closed calorimeter bomb, gave

Sulfur, 1.37%, and 1.40%.

Phosphorus was entirely absent.

The average elementary analysis of our material is therefore

C, 54.87%; H, 7.28%; N, 13.17%; S, 1.38%; O, 23.07%; and ash, 0.23%.

[1] Hammersten, *Physiol. Chemie*, 6; Argiris, *Z. physiol. Chem.*, **54**, 86–94 and ourselves, *Psychiatric Bull. New York State Hospitals*, **9**, No. 3.

This is an unusually low nitrogen, and especially sulfur, content for a keratin, although closely similar to the figures obtained in our previous analyses. The other elementary analyses of this substance have varied as follows:

C, 56 to 58.5%; H, 7.25 to 9.00%; N, 11.5 to 14.0%; and S, 1.63 to 2.31%.

In an attempt to further learn the structural make-up of this protein molecule, a single partition of the nitrogen content among the several amino acids, etc., was made, using a slight modification of Van Slyke's method.

2.715 g. of the neurokeratin were hydrolyzed by heating for sixty hours with diluted hydrochloric acid, or until the total amino nitrogen was constant. The resulting solution was then analyzed and gave:

Total hydrolyzed nitrogen 0.3570 g. (100%), ammonia nitrogen 0.01875 g., uncorrected arginine nitrogen 0.01344 g., melanin nitrogen 0.0518 g., total nitrogen precipitated by phosphotungstic acid 0.0896 g., total nitrogen not precipitated by phosphotungstic acid 0.18984 g., amino nitrogen precipitated by phosphotungstic acid 0.0800 g., total amino nitrogen not precipitated by phosphotungstic acid 0.0900 g., and total sulfur precipitated by phosphotungstic acid 0.0378 gram.

By calculation we arrive at figures for the various products of hydrolysis as follows:

Ammonia nitrogen	5.24%
Melanin nitrogen	14.51%
Arginine nitrogen	2.692%
Cystine nitrogen	4.40%
Histidine nitrogen	6.279%
Lysine nitrogen	11.729%
Nitrogen from one or all of pyroline, oxypyroline, or tryptophane	27.95%
Nitrogen from one or all of glutamic acid, aspartic acid, tyrosine, leucine, isoleucine, alanine, or glycocoll	25.21%
Total	98.01%
Error and loss	1.99%

The results of this single fractionation cannot, however, be accepted as final. They do indicate, however, that most of the sulfur exists in the form of cystine, which is characteristic of keratins as a class.

All of the above figures representing the ultimate elementary analysis of this material are in fairly close accord with some previously obtained by us in a similar manner, so the reasons for the discrepancies in published results must, as previously surmised, lie in variations in the purity of the neurokeratins analyzed. The indigestibility of our protein, its insolubility in dilute alkali, and its freedom from phosphorus, would seem to surely eliminate the chance for an admixture with brain globulins, other proteins, or nucleins. Some elastic tissue from the finer cerebral vessels might possibly, however, be present.

The low nitrogen content, as before stated, is peculiar for any protein

of this class, being indeed more nearly like that in the case of some mucin substances, while the sulfur found more nearly coincides with the amount found in most globulins, rather than in keratins.

A. Argiris[1] obtained on analysis of his neurokeratin:

C, 56.60%; H, 7.15 and 7.40%; N, 14.16%; and S, 2.24 to 2.31%, calculated to the ash-free material.

His analysis of the hydrolytic products also gave different results, but was performed after the older methods. On the other hand, one of the analyses quoted by Hammersten gives nitrogen as low as 11.5%.

Whether or not the long standing of the tissues in 85% alcohol and the subsequent extraction with boiling alcohol, might have permanently rendered other of the proteins indigestible and insoluble, or what portion, if any, of similar indigestible proteins might possibly have been left from the cerebral vessels, we are unable to say definitely; but these last then must probably also have been present in preparations of neurokeratin obtained by Kühne and Chittenden's method.

Conclusions.

Neurokeratin, as obtained by us, is a protein of unusually low nitrogen and sulfur content, as compared with more commonly known keratins, and appears to differ from these latter also in the structural make up of its molecule.

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[CONTRIBUTION FROM THE CHEMISTRY SECTION OF THE IOWA AGRICULTURAL EXPERIMENT STATION.]

AN ACCURATE AERATION METHOD FOR THE DETERMINATION OF ALCOHOL IN FERMENTATION MIXTURES.

BY ARTHUR W. DOX AND A. R. LAMB.

Received August 28, 1916.

Introduction.

A great deal of work has been done upon methods for the determination of ethyl alcohol by physical means, principally the specific gravity, the index of refraction, or the boiling point of alcoholic solutions. Physical methods, however, are all open to the same objection, *viz.*, that the presence of any other substance than ethyl alcohol in the solution tested will always cause incorrect—usually high—results. The production of alcohol in fermentation processes, in whose products it is generally desired to estimate the alcohol, is invariably accompanied by the formation of by-products in greater or less amount, even in pure yeast cultures. Many of these other products are volatile, and cannot be separated from the alcohol by distillation, especially in dilute solutions. The estimation

[1] *Loc. cit.*