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ON THE ANALYSIS OF PEPTONES.

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At the last meeting of the Association I had the opportunity, gentlemen, of drawing your attention to the analogies of reaction which peptone and gelatine present; and I insisted on the fact that in the analysis of the peptones of commerce, the gelatine, which in the meat peptones is due to the transformation of the tendinous parts of the

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muscular tissue, was precipitated by phospho-tungstic acid, as well as the real peptone of albumen and the albumose. The gelatine contains nitrogen, and therefore in the application of the König process for the determination of peptones, the gelatine is inevitably precipitated as well as the peptone, and the estimation of nitrogen under these conditions gives an important excess of peptone.

M. Bruylants said in the last discussion we had on the question, that the double iodide of potassium and mercury which I showed to be distinctive of albumose, peptone, and gelatine, was perhaps the basis of a system of separation of these bodies. The late hour of the meeting prevented my presenting to you the whole results of my researches on this point, and the application which I had already made of the reagent in the separation of these compounds. Keeping in mind the ideas I have expressed as to basing the operations of estimation on the rational preliminary separation of gelatine, albumose, and peptone, I use for the analysis of preparations of peptones the technical processes which I am about to show you. They are different from those suggested by König, but I think that elements for discussion and experiment will be found in them which will lead, perhaps, to new improvements.

METHOD FOR THE ANALYSIS OF PEPTONE PREPARATIONS.

The composition of these preparations always approximates to that of the albuminoids from which they have been derived by the action of the gastric juice. The constituent elements which it is necessary to determine in the analysis of peptones are the following:—1. Water. 2. Ash. 3. Insoluble matters, if any. 4. Gelatine. 5. Albumose. 6. Peptone. 7. Definite compounds. Among these elements we must remark that insoluble gelatine and the defined compounds may be absent.

Water.—The proportion per cent. is determined by evaporating in a water-bath some grammes of peptone. The complete desiccation is continued in an oven at 100° C. The residue is weighed after standing in vacuo over concentrated sulphuric acid for twenty-four hours.

Ash.—The residue previously dried is ignited and weighed accurately.

Insoluble matter.—The peptone is diluted with water, the insoluble residue is collected on a tared filter, washed with water, dried in the oven at 100° C., and weighed after cooling under the desiccator.

Gelatine.—The estimation is founded on its precipitation by ammonium sulphate; but as the albumose is also precipitated by this reagent, it is necessary, as a preliminary, to separate this compound. For this purpose I employ iodide of potassium and mercury, using Mayer's formula:—

Iodide of potassium	49·801 grammes.
Chloride of mercury	13·546 „
Distilled water	1,000·000 „

A known weight of peptone, 1 or 2 grammes, dissolved in water, is precipitated by an excess of the reagent. After filtration and washing, the liquors are evaporated to a few cubic centimetres, then an excess of ammonium sulphate in saturated solution is added. The mixture is brought to the boiling point, which causes the coagulation of the gelatine against the sides of the vessel. When quite cold, the liquid is decanted, and the gelatine

washed first with ammonium sulphate, then rapidly with cold water, and finally with boiling alcohol, which removes the double iodide of potassium and mercury entangled by the precipitate. The gelatine remains adhering to the glass; the vessel is then dried in the oven and weighed. The gelatine still retains some ammonium sulphate, *which it is necessary to deduct from the weight obtained.* For this purpose the coagulum is dissolved in warm water, an excess of hydrochloric acid is added, it is filtered, and the filter washed with as little distilled water as possible. The ammonium sulphate is titrated in solution by a normal solution of chloride of barium, the end of the reaction being ascertained by filtering small portions to which drops of barium chloride are added.

One c.c. of normal chloride of barium containing $\frac{120}{1000}$ of crystallised salt represents 0.08 gramme of ammonium sulphate.

Albumose.—One or two grammes of peptone are taken and dissolved in 5 c.c. of distilled water. An excess of ammonium sulphate is added to the clear solution. This reagent precipitates the albumose and gelatine if any are present. The precipitate is collected on a tarred filter. This is washed with solution of ammonium sulphate, dried and weighed. The precipitate is then dissolved in a sufficient quantity of hot water, and after filtration the ammonium sulphate is estimated by the process described above. From the weight of the precipitate obtained is deducted (1) the weight of the gelatine; (2) the weight of the ammonium sulphate; the difference giving the weight of the albumose.

Peptone.—One or two grammes of peptone are taken as before. The peptone is then dried in vacuo at 60° C., which produces great inflation of the mass. The porous residue is washed with alcohol at 95° in order to eliminate creatine, creatinine urea, sarcolactic, formic, acetic and butyric acids, which are especially found in peptones prepared directly from meat. These compounds can be estimated in mass by evaporation of the alcohol. The residue is taken up by some grammes of water, and an excess of a solution of phosphotungstate of soda thus prepared is added

Tungstate of soda	50 grammes.
Boiling distilled water	1000 "
Phosphoric acid (medicinal)	100 "
Pure hydrochloric acid	150 "

This last acid must be added after the solution is cold. The liquid is filtered after standing for twenty-four hours.

This reagent precipitates the gelatine, the albumose and the peptone. After settlement a small portion of the clear liquid is tested, then the greater part of the solution is decanted and the precipitate is thrown on a Schleicher's filter carefully tared. The precipitate is dried in the oven, weighed after cooling in the desiccator, and the weight noted.

The precipitate is then introduced into a porcelain capsule and ignited in a gas muffle to total incineration of the organic matter and filter. This last residue is weighed and its weight, minus the weight of the filter ash, is deducted from that of the dry precipitate. The difference represents the weight of the peptonic matter precipitated. From this is deducted (1) the weight of the gelatine; (2) the weight of the albumose. The last difference gives the weight of the peptone.

This method gives results as accurate as those based on the determination of peptone by the estimation of nitrogen, and it has the advantage of being much quicker. Comparative analyses of eight samples of different peptones have given results differing but slightly from those obtained by calcination.

Definite Compounds.—Under this denomination are included the nitrogenous bases and the fatty and amido acids of muscular tissue.

The definite compounds are divided into nitrogenous and non-nitrogenous substances. Some are soluble and others insoluble in alcohol. Taking this classification I group them in the following order: Substances soluble in alcohol:

Nitrogenous: Creatine, creatinine urea.

Non-nitrogenous: Sarcosine, formic, butyric, and acetic acids.

These substances may easily be determined collectively by evaporation of the alcoholic solution obtained by washing the product yielded by evaporation to dryness in vacuo before the precipitation of the peptone by phosphotungstate of soda. Substances insoluble in alcohol:

Nitrogenous: Taurine.

Non-nitrogenous: Inosine, dextrin, glycogen.

The proportion of these elements in the preparation of the peptone analysed is determined by difference.

I have applied my method of analysis to several commercial preparations of peptone as well as to the peptones specially prepared by myself. It is executed with perfect regularity. The following are three results selected from among the numerous applications which I have made of the method. They bear on the principal types of peptone preparations.

Designation.	Peptone of purified meat-albumen.	Peptone of egg-albumen.	Peptone direct from meat.
Peptone.. .. .	37·675	34·700	25·857
Albumose	31·300	53·350	15·964
Gelatine	none	none	9·826
Definite Compounds	5·525	5·930	29·972
Mineral Salts	8·285	1·025	18·386
Water	10·250	4·625	none
Insoluble	9·965	3·980	none

These figures mark profound differences of composition in the relative proportions of peptone and albumose. The peptone of meat albumen and the direct peptone of meat do not offer a constant ratio in regard to the proportional figures of albumose.

This anomaly may be imputed to the mode of preparation employed, which is more efficacious for the peptone of meat albumen than for that of coagulated albumen, which last is less easily peptonisable than albumen in solution in the preparation of meat peptone properly so called.