lack of experimental detail, Buchner and Antoni imagined that in our comparative experiments the concentration of glucose and of enzyme had not been kept constant, and ascribed part of the increase produced by boiled juice to the favourable effect of a diminution in the concentration of the sugar and of the alcohol, which is always present, by dilution with the added boiled juice. The details given above show that neither of these influences had any share in the effects observed by us.

The Quantitative Estimation of Small Quantities of Nickel in Organic Substances.

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In the course of an investigation into the toxic action of certain nickel compounds, it was found necessary to devise a method of detecting and estimating nickel, when included in animal tissue, in quantities not exceeding a few milligrammes per cent.

A method has therefore been worked out, which, although in many respects only differing from the usual methods in virtue of slight alterations of detail, is capable of demonstrating extremely small quantities of nickel accurately. The method may be divided into three stages: (1) The Ashing; (2) The Separating; and (3) The Estimating stages.

1. *Ashing.*—The substance to be examined must be placed in a porcelain crucible (platinum is unsuitable, as a considerable loss of nickel takes place, probably by an alloy of platinum and nickel being formed) and evaporated to dryness over a water bath. If the substance be solid, it should be cut up into small pieces. The crucible is then heated carefully with a Bunsen flame, but it may be wise to further dry in a hot air oven or on a sand bath before this. Then it is burned over a Fletcher burner, and lastly fully incinerated in the blow-pipe flame. With some care, it is possible, as a rule, to oxidise fully all the carbon, without recourse to any foreign material. The crucible is then placed on the water bath, and 10 c.c. of pure hydrochloric acid are added and allowed to evaporate to dryness, this process being repeated. The residue is then extracted with water to which a small quantity of hydro-

chloric acid is added. For this purpose, 2 c.c. of a four times normal acid are generally employed. The extract is then filtered. The ash so obtained is practically completely soluble.

Ashing by Kjeldahl's method, or better, with sulphuric and nitric acids, can also be employed, but has two disadvantages over the simple incineration method: Firstly, it takes longer; and secondly, it introduces foreign salts, which should, if possible, be avoided.

2. *Separation.*—Firstly, it is necessary to get rid of the iron and at the same time of the phosphates. Those tissues, which contain iron in excess, *e.g.*, blood, may be treated by precipitation with excess of ammonia and filtration. The process should be repeated three times, the precipitate being redissolved each time with the same quantity of acid as was used for the extraction. If some of the iron separates out from the filtrate on being evaporated, it may be necessary to refilter before dryness is reached. When the substance contains little or no iron, it is necessary to add a sufficiency to combine with the quantity of phosphates present. If the phosphates are present in excess, the following method is employed. The cold solution is made neutral to litmus or very faintly acid with ammonia. An excess of ammonium acetate is then added (as a rule 8 to 10 c.c. of a 10-per-cent. solution suffices), and sufficient ferric chloride to colour the supernatant fluid yellowish-red. The mixture is then boiled, when all the iron separates out as phosphate and basic acetate. For those tissues yielding large quantities of phosphates, *e.g.*, liver, the amount of ferric chloride necessary is comparatively large, and some difficulty may be experienced with the filtration. The only possible help is obtained by using two or more filters. The washing of the precipitate must be carried out carefully, in spite of the considerable loss of time. Precipitation by ammonia may be carried out for the three subsequent repetitions. The precipitate is each time dissolved in the smallest possible quantity of acid.

After the united filtrates have been evaporated to dryness, the residue is again dissolved in water, and dilute hydrochloric acid added, about 6 c.c. of four times normal acid being usually sufficient. Sulphuretted hydrogen is then passed through the hot solution for at least half an hour, and it is then allowed to stand for a time, as the sulphides, which form in acid solution do not always readily separate out. The liquid is then filtered and the precipitate well washed with sulphuretted hydrogen water. The filtrate is again evaporated to dryness, re-dissolved in a little water on the water bath and then a solution of pure sodium hydrate is added in successive portions to the hot liquid, until no more ammonia comes off. Care should be taken to use as little sodium hydrate solution as possible, as every sample in the market

contains small traces of iron. The nickel is thus precipitated in the form of the hydrate, and this is converted into nickel sesquioxide by the addition of 1 or 2 c.c. of bromine to the cold mixture. The nickel oxide is then collected by filtration, and after having been well washed, is dissolved in hydrochloric acid, and the solution evaporated to dryness to remove the excess of acid, and the residue re-dissolved in water with a faint trace of acid, in order to prevent the formation of basic salts. The solution is finally made up to a definite volume.

In the process of separation, no especial difficulties save the management of the voluminous iron precipitate, are met with as a rule. At times an insoluble residue is found on the filter paper when the oxide is dissolved. This is a trace of a sulphide of copper or another metal of this group, which has escaped precipitation by sulphuretted hydrogen in acid solution.

3. *Estimation.*—The usual method of quantitative estimation of nickel colorimetrically is carried out with ammonium sulphide, but it has been found that sharper results can be obtained by employing *a*-dimethylglyoxime $CH_3.C(N.OH).C(N.OH).CH_3$, which was recently shown by Tschugaeff to form a scarlet red compound with nickel in the presence of ammonia.*

For this purpose, a saturated solution of the reagent in absolute alcohol is prepared, this is diluted with water until a little of the compound separates out, and alcohol is then added until complete solution takes place. The fluid to be tested and a standard solution of nickel sulphate are placed in burettes. A measured quantity of the fluid is then run into a Nessler tube and to this 0·5 c.c. of a 10-per-cent. solution of ammonia and the same quantity of the dimethylglyoxime solution are added and the whole made up to 30 c.c. It is better first to add the ammonia to the nickel solution, then the dimethylglyoxime, and then allow the colour to develop before diluting up to the 30 c.c. mark. All the solutions must be cold. The fluid becomes coloured pinkish red, the depth of the coloration depending on the quantity of nickel present. The most convenient quantity to work with is about 0·08 to 0·01 milligramme. The colour is then compared with that produced by varying quantities of nickel from the standard solution. The determination is not complete until a quantity has been found, which gives a colour which is just too pink, and a second quantity the colour of which is just appreciably less pink, than the fluid to be tested. The quantity of nickel contained is then calculated as the amount midway between the two tubes. With a little practice, it is quite easy quickly to determine very small differences of colour. The estimation should be concluded as rapidly as is compatible with accuracy,

* 'Deut. Chem. Ges. Ber.,' 1905, vol. 38, p. 2520.

as, after a short time, the nickel compound with dimethylglyoxime separates out of the coloured solution as a precipitate.

The advantages of this method over the ammonium sulphide method are: (1) small traces of iron do not interfere with the final colour, nor with the sharpness of the method; (2) smaller quantities of nickel can be accurately estimated; and (3) it is easier to work in a bad light with the pink than with the brown colorimetric determination.

Dealing with the colorimetric test alone, with solutions of pure nickel sulphate, the smallest quantity which gives the reaction is 1/1000 milligramme. To detect such a small amount, the solution must be placed in the Nessler tube, then the ammonia and the solution of dimethylglyoxime added, when one can recognise the characteristic pink colour, and, lastly, the fluid is made up to 30 c.c.; on comparing this with distilled water, a faint but distinct difference is seen. 3/1000 milligramme gives a recognisable pink colour in 30 c.c. of fluid. Working with 0·07 milligramme, differences of 1/1000 milligramme can be recognised with a little practice. This represents a potential error of + or −0·7 per cent.

In test analyses, serum or blood with nickel sulphate added, the experimental error was kept as low as 2 per cent., using about 1 milligramme of nickel. For example, about 30 grammes of blood were placed in a crucible and 0·9 milligramme of nickel, in the form of the dissolved sulphate, was added. After ashing, extracting, removing the copper and iron groups, and precipitating the nickel in the form of the sesquioxide, etc., the final fluid was made up to 30 c.c.; 2 c.c. of this fluid were compared with varying quantities of a solution containing 0·01 milligramme of nickel per cubic centimetre. It was found that 5·9 c.c. gave a colour, which was just too pink, and 5·8 c.c., a colour, which was not pink enough, so that the 2 c.c. contained 0·0585 milligramme of nickel, and the whole solution 0·88 milligramme. This represents a loss of 0·02 in 0·9, or about 2 per cent.

Electrolysis is only to be preferred when large quantities of nickel are to be measured, while the method described above is intended for the recognition and measuring of quantities of nickel not exceeding a few milligrammes.