XXIII. A STUDY OF THE WATER-SOLUBLE ACCESSORY GROWTH PROMOTING SUB- STANCE IN YEAST. I.

BY JACK CECIL DRUMMOND.

*(From the Biochemical Laboratory of the Cancer Hospital
Research Institute, London.)*

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The researches of Stepp [1909, 1912, 1913], Hopkins [1912] and Funk [1913, 1, 2, 3] have demonstrated the importance of certain constituents of a normal dietary which for want of better names have been variously termed "vitamines," growth substances, and accessory growth hormones. It is now known that unless an adequate supply of these as yet unidentified substances is present in the food supply of an animal, serious consequences affecting the general nutrition of the subject will arise. Recently McCollum and Davis [1915, 1, 2, 3] have carried out some researches which have yielded results indicating that two distinct types of accessory substance exist. These by reason of their properties have been conveniently designated the "fat-soluble A" and the "water-soluble B." The view held by these authors that both substances are equally important components of a normal dietary has received experimental confirmation by Drummond [1916], and has been accepted by the majority of other investigators [Macallum, 1916; Osborne and Mendel, 1916]. Following up his studies along these lines McCollum, in collaboration with Kennedy [1916], has arrived at the conclusion that the production of polyneuritis in birds by exclusive rice feeding [Eykmann, 1897; Funk, 1913, 1, 2, 3], or exclusive feeding of a ration made up of purified foodstuffs [Funk, 1914, 1] is a specific result of the absence of the water-soluble factor B. In other words it is proposed to regard the so-called "antineuritic vitamine" as being identical with the water-soluble factor B. McCollum and Kennedy have based this suggestion upon the curative value possessed by many extracts, known to contain the water-soluble substance, when administered to birds which have developed the typical symptoms of polyneuritis gallinarum (Eykmann), as a result of feeding solely upon a diet lacking in that substance.

Our knowledge of the chemistry of these accessory substances or "vitamines" is as yet lamentably small, so that should further experimental evidence be forthcoming in support of the proposal advanced by McCollum and Kennedy it would mark a distinct advance in this field of research.

For some time past an investigation has been in progress in this laboratory aiming at the isolation and identification of the dietary factor *B*.

Viewed from this standpoint alone the results of the research have been disappointing, but on the other hand many observations have been made during its progress which help to throw a certain amount of light upon the nature of this elusive substance.

Taken collectively these observations tend to uphold the view advanced by McCollum and Kennedy, since it is found that the chemical properties of the water-soluble growth-promoting factor *B* are, as far as they have been studied, very similar to those which have been described for the antineuritic principle. Ever since Eykmann demonstrated that the condition induced in fowls by a diet of polished rice was analogous to human beri-beri, and that there existed a substance in the layers of the rice grain removed during polishing which would cure the condition, numerous investigators have attempted to isolate the curative agent in a pure state.

From the mass of literature which records the results of these many attempts it is difficult to abstract that which may be regarded as sound. Various properties of the curative substance have been described at one time or another. Chamberlain and Vedder [1911] showed that it was soluble in alcohol and dialysable, whilst Cooper and Funk [1911] demonstrated that it would withstand drastic acid hydrolysis and that it was precipitated by phosphotungstic acid.

Since these facts seemed to point to the active substance being of the nature of a comparatively simply constituted nitrogenous base, several investigators attempted to isolate the pure substance along the lines usually adopted in such cases. Funk [1911] confirmed that the substance was precipitated by phosphotungstic acid, and made the further observation that upon sub-fractionation of the substances precipitated by this reagent by the silver process it was to be found in association with the pyrimidine bases. These results were repeated and confirmed by him [1912, 1], and upon these facts he based his reasons for assigning to the product the name "vitamine" [1912, 2]. At about the same time several isolations of the active substance were reported [Schaumann, 1914; Edie, Evans, Moore, Simpson and Webster, 1912; Susuki, Shinamura and Odake, 1912].

The last three claims, however, have now been shown to be unfounded [Funk, 1913, 1], whilst the crystalline derivatives isolated by Funk himself from the pyrimidine fraction of an alcoholic extract of rice polishings, and thought by him to be active [1912, 1], were shown to consist of impure specimens of nicotinic acid and betaine [Drummond and Funk, 1914]. Pol [1917] has recently described the isolation of a crystalline acid from *Phaseolus radiatus* which he states has cured polyneuritis in birds and beri-beri in man. The details of his process have, unfortunately, not been available, but it is improbable that the crystalline substance he has isolated is the curative substance in a pure condition, because the author mentions that the preparation loses its activity as purification progresses. It is conceivably the same type of product as was isolated by Funk, where a definite crystalline body (nicotinic acid) in some cases contained the active substance as a contamination.

We are therefore practically without knowledge regarding the chemical nature of the antineuritic substance and cannot claim that much progress has been made in obtaining such information during the past few years.

With regard to the properties of the substance itself we are in possession of a number of scattered observations which have been made from time to time, and which generally speaking are in agreement.

The antineuritic substance is apparently only partially soluble in alcohol. This solubility is very small in absolute alcohol, but becomes greater as more dilute alcohol is used [Funk, 1912, 1; Cooper, 1913, 1; Vedder and Williams, 1913; Sullivan and Voegtlin, 1916].

It is usually described as being insoluble in ether, chloroform and other lipoid solvents [Cooper, 1913, 1]. It is adsorbed by animal charcoal [Chamberlain and Vedder, 1911; Cooper, 1913, 1] whilst a colloidal aluminium silicate (Lloyd's reagent) has recently been used for quantitatively adsorbing the antineuritic substance [Seidell, 1916; Eddy, 1916].

Most authors agree that the substance is unstable under certain conditions to alkali. Cooper [1913, 1] reported its destruction by that agency, and this observation has been confirmed [Chamberlain, Vedder and Williams, 1912; Fraser and Stanton, 1915; Funk, 1915]. On the other hand Williams and Seidell [1916], are inclined to doubt whether cold alkali does exert an adverse influence upon the substance, whilst Steenbock [1917] definitely states that only hot alkali has such an effect.

With regard to the effect of acids, most authors agree that it is stable even when they are applied hot and in a relatively concentrated condition. Cooper

and Funk [1911] found the curative value of yeast extracts unaffected by hydrolysis for twenty-four hours with 20 % sulphuric acid, and this stability to acid is confirmed in papers by Funk [1911], Vedder and Williams [1913], Williams and Seidell [1916], and Steenbock [1917]. The degree of thermostability possessed by the substance has been noted by several investigators who appear to agree that temperatures below 100° do not exert a deleterious effect [Cooper and Funk, 1911; Steenbock, 1917]. Higher temperatures, however, are usually considered to injure its activity.

The value of yeast as an antineuritic has been recorded upon several occasions [Cooper and Funk, 1911; Funk, 1912, 1; Cooper, 1913, 2; Schumann, 1914; Willcox, 1916; Seidell, 1916].

As has already been remarked the first named authors showed that acid hydrolysis failed to lower the antineuritic value of yeast, whilst Cooper [1914] has shown that autolysed yeast is as active as was the original yeast.

Barsickow [1913] found yeast itself to be curative, but that heated yeast and "cerolin" (a yeast extract) possessed no such value. He believed that the curative action was due to the action of nuclein or certain salts.

Funk [1913, 1] investigated the curative value of yeast- and thymus-nucleic acids and some of their degradation products when administered to polyneuritic pigeons, and found that certain of these substances exerted a beneficial influence upon the progress of the disease. Some of the many attempts to isolate the active substance from yeast preparations have already been alluded to. More recent ones are those described by Seidell [1916], and Williams and Seidell [1916].

Attention must now be directed to a consideration of the recorded properties of the water-soluble growth promoting accessory factor. That yeast possessed a rich supply of a growth promoting factor was noted by Hopkins [1912] and by Funk [1913, 2; 1914, 2]. The latter author has since then made several attempts to isolate the substance along lines such as were employed by him in his efforts to isolate the antineuritic substance from the same source.

These attempts have failed to achieve their object, but his results show that the active substance possesses several properties, also possessed by what he terms the "anti-beri-beri vitamine." Funk and Macallum [1915] confirmed the presence of the growth promoting substance in dried brewer's yeast, and in a later paper [1916] they described its presence in autolysed yeast and recorded that it is precipitated by phosphotungstic acid. Attempts at further fractionation of the resulting precipitate failed, for no growth

promoting powers were possessed by the purine or pyrimidine fractions obtained from it. Funk [1915, 1916] has stated that the growth promoting accessory from yeast forms a phosphotungstate insoluble in acetone.

With regard to the solubility of the growth promoting accessory *B* it is known that it is extractable by alcohol [Stepp, 1909; Hopkins, 1912; and others]. It is stable to temperatures below 100° [Drummond, 1916], but is apparently to some extent adversely affected by autoclaving at higher temperatures [Hogan, 1917]. From this brief summary, which is scarcely a complete review of the literature describing the two substances, it will be seen that many of the properties by which the antineuritic substance is described are also possessed by the water-soluble growth accessory factor.

As has already been remarked McCollum and Kennedy have suggested the identity of the two substances [1916], and the same view is apparently held by Macallum [1916].

The experiments which are reported in this paper entirely support this view and make it almost certain that the two substances are either identical or are individually closely related members of the same class of substance.

EXPERIMENTAL¹.

For the purposes of this research young rats from five to six weeks old and weighing approximately 50 g. were utilised. They were drawn from healthy pure-bred stock reared under careful observation and belonged to the black variety of *Mus norvegicus* [Donaldson, 1915]. This particular strain has been found eminently satisfactory for the studies in growth and nutrition which are being made in this laboratory.

The experimental animals were kept in wooden boxes with sawdust covered floors and provided with warm enclosed sleeping quarters.

They were fed twice daily, unused food from the previous meal being removed from the cage upon each occasion. The artificial dietaries were made up into the convenient form of firm balls weighing approximately 5 g. This forms a rough and ready method of estimating the food consumption of the animals. Fresh drinking water is at all times an essential to the health and welfare of the rat, and was in these experiments renewed at least twice daily, the earthenware drinking pans being scalded out on each occasion.

A basal artificial diet was employed which contained all the necessary

¹ I wish to express my gratitude to Mr A. Chaston Chapman for his kindness in supplying me with the highly pure yeast-nucleic acid used in this work.

components of a normal diet with the exception of the water-soluble accessory substance. This diet was made up as follows:

Caseinogen	18 parts	Salt mixture	5 parts
Starch	40 „	Sucrose	17 „
Agar	5 „	Butter-fat	15 „

The various components were carefully purified before use by extractions designed to remove all trace of the water-soluble growth factor. The composition of the salt mixture together with full details of the preparation and purification of the various components of the basal diet are described in a recent communication by Halliburton and Drummond [1917].

That this diet was for all intents and purposes free from the water-soluble accessory may be seen from Chart I, which may be compared with Charts II and III in which are represented the growth curves of rats fed upon a diet such as the above to which had been added the missing accessory substance in the form of yeast preparations. The animals fed upon these diets showed normal growth and reproduction upon the completed ration.

1. *Presence of the water-soluble growth promoting accessory substance in dried yeast and commercial yeast extract.*

Chart II shows the influence upon the growth of young rats exerted by the addition of 3–12 % of dried yeast powder to the basal diet. The rats were able to complete their life cycle without any difficulty, and the animals reared solely upon this diet appeared as healthy and as resistant to disease as those which had enjoyed a normal mixed ration.

Chart III indicates that “marmite,” a commercial yeast extract, is rich in the water-soluble accessory factor. The addition of 6 % of this preparation to the basal diet satisfactorily makes good the inadequacy it previously

possessed. Smaller additions of "marmite" were less efficient in this respect, whilst greater percentages were apparently not well tolerated by the animals. These results confirm the findings of the authors that have been referred to with regard to the presence of the water-soluble accessory factor in yeast and its aqueous extract.

Chart II. Curves 1-4. Growth of rats on basal diet + 6 % dried yeast. Curves 5-7. Growth of rats on basal diet + 3 % dried yeast. Curves 8-11. Growth of rats on basal diet + 12 % dried yeast. Y indicates birth of young. These were all reared satisfactorily by the mothers.

Chart III. Curves 1-3. Growth of rats on basal diet + 6 % "marmite." Curves 4-6. Growth of rats on basal diet + 3 % "marmite." Curves 7-10. Growth of rats on basal diet + 1 % "marmite." Curves 11-13. Growth of rats on basal diet + 12 % "marmite." Y indicates birth of young. All were reared satisfactorily by the mother.

2. Solubility of the water-soluble accessory substance in alcohol and ether.

The accessory factor is not removed from dried yeast by extraction with absolute alcohol, so that it appears that the substance is insoluble in pure

alcohol (Chart IV). The dried yeast for this experiment was extracted with several changes of boiling alcohol under reflux. Similarly from the same chart it can also be seen that prolonged ether extraction removes little or none of the active substance.

Chart IV. Curves 1-4. Growth of rats fed on basal diet + 6 % alcohol-extracted dried yeast. Curves 5-7. Basal diet + 6 % ether extracted yeast. Curves 8-11. Basal diet + alcohol extract equivalent to 6 % of dried yeast. Y marks birth of young.

It may be mentioned here that dried yeast contains but negligible traces of the fat-soluble growth promoting accessory substance.

Extraction of "marmite" by grinding up with absolute alcohol and repeatedly shaking removes but a small proportion of the accessory factor (Chart V), but when extracted with a more dilute alcohol (70 %) the proportion that goes into solution is greatly increased.

Chart V. Curves 1-4. Growth of rats fed on basal diet + 6 % alcohol-extracted "marmite." Curves 5-7. Ditto on basal diet + 6 % "marmite" extracted by 70 % alcohol. Curves 8-11. Ditto fed on basal diet + alcohol extract equivalent to 6 % "marmite." Y indicates birth of young. All were reared.

3. *Dialysis of yeast extract.*

A strong solution of "marmite" in distilled water was dialysed against running distilled water for forty-eight hours. The combined dialysates were evaporated to small bulk *in vacuo* at low temperature and tested for the presence of the accessory substance under consideration. Chart VI shows that the substance is dialysable through parchment paper, although it was not completely removed from the "marmite" in the time during which the dialysis was carried out. This dialysate contained large amounts of inorganic salts chiefly potassium phosphate and sodium chloride and considerable amounts of leucine and adenine.

Chart VI. Curves 1-4. Rats fed upon basal diet + dialysate representing 6 % "marmite." Curves 5-8. Rats fed upon basal diet + 6 % residue remaining after dialysis.

Chart VII. Curves 1-4. Basal diet + 6 % dried yeast heated to 100° for 30 mins. Curves 5-7. Basal diet + 6 % dried yeast autoclaved at 120° for 30 mins. Curves 8 and 9. Basal diet + 6 % "marmite" heated at 100° for 30 mins. Curves 10-13. Basal diet + 6 % "marmite" autoclaved at 120° for 30 mins. Y indicates birth of young. x indicates the time at which the addition of 6 % "marmite" was made to the diet.

4. *Effect of temperature upon the water-soluble growth promoting accessory substance.*

Chart VII demonstrates the growth promoting value of yeast and yeast extracts that have either been subjected to a temperature of 100° or have been autoclaved at 120° for thirty minutes.

The activity of these preparations was practically unaffected by the first treatment, whilst injury but not complete destruction resulted in the second case.

5. *Influence of acids.*

Chart VIII indicates the behaviour of the accessory factor in the presence of acids. One sample of "marmite" was subjected to the influence of dilute hydrochloric acid (1 %) at boiling point for twelve hours, the resulting fluid being carefully neutralised and concentrated to the original bulk. This preparation was not as valuable in promoting growth when added to the basal diet as was a corresponding amount of the untreated marmite, but the activity was not greatly diminished.

Chart VIII. Curves 1-4. Basal diet + 6 % "marmite" after dilute hydrochloric acid treatment. Curves 5-9. Basal diet + 6 % "marmite" after sulphuric acid hydrolysis. x indicates the time at which the addition of 6% "marmite" to the diet was made.

A second quantity of "marmite" was subjected to hydrolysis with 20 % sulphuric acid at boiling point for ten hours. The sulphuric acid was then quantitatively removed from the diluted liquid by means of baryta, and the neutral filtrate, together with the washings of the bulky precipitate, was evaporated to a thick syrup at a low temperature under diminished pressure.

This preparation possessed a greatly decreased power to promote growth in the experimental animals. In view of the observations which are given later in this paper, this result is regarded as being due not so much to an actual destruction of the accessory substance as to loss occurring by adsorption on the bulky precipitate of barium sulphate and pigmented matter.

6. *Influence of alkali.*

Chart IX displays the growth curves of rats fed upon the basal diet together with additions of treated yeast preparations submitted to various treatments with alkali. One set of curves demonstrates that treatment of "marmite" for twenty-four hours at room temperature with 5 % sodium hydroxide results in very little depreciation in its growth promoting value.

Chart IX. Curves 1-4. Basal diet + 6 % "marmite" after treatment with alkali at room temperature. Curves 5-8. Basal diet + 6 % "marmite" after hot alkali treatment. x indicates the time at which the addition of 6 % "marmite" to the diet was made.

On the other hand treatment with hot 5 % sodium hydroxide for five hours tends to destroy its activity. In both these cases after treatment with alkali the fluid was neutralised and carefully evaporated *in vacuo* at low temperature to its original bulk.

ATTEMPTED SEPARATION OF THE ACTIVE SUBSTANCE.

1. *Precipitation from extracts by phosphotungstic acid.*

A preparation consisting of the concentrated dialysate from 200 g. of commercial yeast extract, prepared as already described, was diluted to a volume of 2000 cc., acidified to the extent of 5 % with sulphuric acid, and precipitated by the addition of a 30 % aqueous solution of phosphotungstic acid. The flocculent, buff-coloured precipitate was allowed to stand overnight at ice-room temperature and filtered off next morning, the precipitate being thoroughly washed with 5 % sulphuric acid. When dry the precipitated phosphotungstates weighed 87 g. Since the majority of such salts contain phosphotungstic acid to the average extent of 85 % of their weight, this yield represents some 12 g. of substances removed from the extract. The precipitated phosphotungstates were decomposed by treatment with

hydrochloric acid and extraction with the amyl alcohol-ether mixture recommended by van Slyke [1915]. Very incomplete decomposition was effected by this method, for a bulky flocculent precipitate, probably representing precipitated pigment, resisted decomposition. The aqueous fraction was separated after repeated extraction, filtered, neutralised and evaporated to small bulk at reduced temperature and pressure.

Chart X. Curves 1-4. Basal diet + phosphotungstic acid precipitate fraction corresponding to 18 % "marmite." Curves 5-8. Basal diet + phosphotungstic acid filtrate fraction corresponding to 18 % "marmite."

Chart X demonstrates that this fraction possessed a small growth promoting activity when administered in doses representing 6-18 g. of the original "marmite." Great loss had, however, occurred during the process. The filtrate from the phosphotungstic acid precipitation was freed from sulphuric acid and the excess of the precipitant by means of baryta and after concentration tested for activity. None however was observed (Chart X).

Whether the active substance is completely precipitated by phosphotungstic acid is uncertain, for any which was unprecipitated would be adsorbed and lost during the removal of the sulphuric and phosphotungstic acids by baryta, owing to the very bulky precipitate which results. Great loss also occurred without a doubt due to the adsorption of the active body by the flocculent residue of undecomposed phosphotungstates which has been already noted.

A further preparation of the phosphotungstates from 200 g. of "marmite" was made, and weighed in the dry condition 90 g. This was treated when quite dry by shaking up with repeated changes of acetone [Funk, 1916]. A large proportion (69 g.) of the precipitate remained undissolved. It would seem probable from studies upon the solubilities of the phosphotungstates in acetone (not yet published), that the inference to be drawn from this fact is that the chief extractives of yeast precipitated by phosphotungstic acid are purine bases or the inorganic bases potassium and ammonium. Both fractions of the phosphotungstic acid precipitate were freed from acetone, decomposed by the amyl alcohol-ether process and worked up for inclusion in the diets

as in the former case. A large proportion (40 %) of the acetone-insoluble phosphotungstate was not decomposed by this process, and again formed a flocculent precipitate which much interfered with the separation.

The curves shown in Chart XI demonstrate that the water-soluble growth accessory factor is mainly present in the acetone-insoluble fraction of the phosphotungstic acid precipitate, although great loss has occurred during the separation.

Chart XI. Curves 1-4. Basal diet + acetone-insoluble phosphotungstic acid precipitate fraction corresponding to 10 % "marmite." Curves 5-8. Basal diet + acetone-soluble phosphotungstic acid precipitate fraction corresponding to 10 % "marmite."

2. Basic lead acetate precipitation of yeast extracts.

A 10 % solution of "marmite" gave an exceedingly bulky flocculent precipitate upon the addition of basic lead acetate solution. After removing this precipitate and subjecting it to a thorough washing, both precipitate and filtrate were decomposed by sulphuretted hydrogen gas, the lead-free solutions being neutralised and concentrated at low temperature and diminished pressure.

Little growth promoting power was observed in either fraction (Chart XII), it being considered probable that the heavy and flocculent nature of the lead sulphide precipitates had resulted in practically complete adsorption and loss of the active substance.

Chart XII. Curves 1-3. Basal diet + lead acetate filtrate fraction corresponding to 6 % "marmite." Curves 4-6. Basal diet + lead acetate precipitate fraction corresponding to 6 % "marmite." Curves 7-9. Basal diet + lead acetate filtrate fraction representing 6 % dialysed "marmite." Curves 10 and 11. Basal diet + lead acetate precipitate fraction representing 6 % dialysed "marmite." x indicates the time at which the addition of 6 % "marmite" to the diet was made.

A sample of the dialysate from the "marmite" was precipitated in a similar manner with basic lead acetate and little evidence of the presence of the active substance in either fraction was obtained, and again great loss had occurred.

3. *Silver nitrate precipitation.*

To the forty-eight hours' dialysate from 250 g. yeast extract silver nitrate was added until no further precipitation occurred. The silver-free filtrate after neutralisation and concentration showed little or no evidence of the presence of the growth accessory substance under investigation (Chart XIII).

Chart XIII. Curves 1-4. Basal diet + purine fraction from dialysed "marmite" corresponding to 6% "marmite." Curves 5-8. Basal diet + corresponding pyrimidine fraction. x indicates the time at which the addition of 6% "marmite" to the diet was made.

The silver precipitate was decomposed by sulphuretted hydrogen gas and the filtrate concentrated with care after neutralisation. Small traces of the active substance appeared to be present. Chemical examination of the fraction demonstrated that the majority of the nitrogen was present in the form of adenine.

4. *Relation of adenine to the water-soluble accessory substance.*

Adenine itself was found to possess no growth promoting properties when added to a diet deficient in the water-soluble factor *B* (Chart XIV). This is of interest in view of the fact that the recent work of Williams and Seidell [1916] has not received confirmation [Voegtlin and White, 1916; Steenbock, 1917; Harden and Zilva, 1917]. Williams and Seidell attributed an anti-neuritic action to a supposed isomeric form of adenine. Funk [1913, 1] found a distinct improvement in the condition of polyneuritic pigeons following the administration of thymus-nucleic acid, guanosin, adenosin and cytidine, and slightly less satisfactory results when certain purine and pyrimidine bases were given.

The influence upon growth of pure yeast-nucleic acid and of a nucleic acid prepared from the spleen in a case of splenic leucaemia was therefore studied, but neither preparation in any way improved the growth value of the basal diet (Chart XIV).

Commercial meat extract ("Lemco") does not show evidence of the presence of the water-soluble accessory factor (Chart XV), so that the identity of the latter substance with any of the meat bases is rendered doubtful. Commercial meat extracts have been found to possess no power to improve the condition of polyneuritic birds [Chick and Hume, 1917].

Chart XIV. Curves 1-4. Basal diet + 1 % yeast nucleic acid. Curves 5-8. Basal diet + 1 % spleen nucleic acid. Curves 9 and 10. Basal diet + 0.5 % adenine. Curves 11 and 12. Basal diet + 0.2 % adenine.

Chart XV. Basal diet + 6 % "Lemco."

DISCUSSION.

The results of this investigation, in so far as they are complete, tend to support the view held by McCollum and Kennedy that it is a deficiency of the water-soluble accessory substance in the dietary of birds that is responsible for the incidence of the typical symptoms which are held to represent a pathological condition analogous to beri-beri in man.

Attempts have been made along many lines to isolate the accessory substance from yeast extracts. All of them have however failed to achieve their object, and the only useful result of the investigation which has as yet been obtained has been that a knowledge of a few of the properties of the elusive substance has been gained.

Many workers have lamented the difficulties which are encountered in research in this particular field, and have drawn attention to the way in which the active substances disappear as the fractionation proceeds.

The usual explanation of this has been in the past to regard the substance as unstable. Thus Funk [1913, 3] believed that he had found evidence of the frequent occurrence of nicotinic acid in fractions which had once possessed, but had later lost, curative power, and on these grounds he was inclined to regard that acid as a derivation product of the active "vitamine." Similarly more recent writers have described the existence of isomeric modifications of adenine [Williams and Seidell, 1916], and of certain betaines [Williams, 1917], which possess curative properties. This latter work has not, however, received the support of other workers [Voegtlin and White, 1916; Harden and Zilva, 1917], and therefore stands in need of confirmation before it can be accepted.

It seems far more probable that the correct explanation of the loss which occurs during fractionation of extracts containing the antineuritic substance or the water-soluble growth promoting substance is to be found in the readiness with which these substances, whether identical with one another or not, are adsorbed from solution by precipitates.

As has already been mentioned this property was observed several years ago by Chamberlain and Vedder [1911], and Cooper [1913, 1], whilst certain modern work confirms the observation. Thus Seidell [1916] has based a process for separating the substance from autolysed yeast upon its adsorption by fullers' earth, and Voegtlin and White [1916] record that mastic emulsion and colloidal arsenious sulphide will carry down the antineuritic principle in the adsorbed condition.

Further proof that this is probably the explanation of the great loss which is so often encountered in the isolation of the substance is to be found in the fact, that whenever in the course of the fractionation recourse to precipitation is made, the precipitate, particularly if bulky and flocculent in nature, will usually carry down the majority, if not all, of the active substance from solution.

It is naturally a very difficult matter to trace the growth promoting principle during fractionation, because minute traces of that substance do not always demonstrate their presence by causing an appreciable increase in the body weight of the experimental animal, whereas much smaller quantities of such extracts are often sufficient to cause a decided improvement in the condition of a polyneuritic pigeon. Unfortunately neither the experimental results recorded in this paper nor this discussion can throw much light upon

the chemical identity of the class of substance under consideration. One point remains clear, namely, that there is now less evidence than ever to support the continued use of the word "vitamine" coined by Funk. Williams and Seidell [1916] have quite recently supported the adoption of the term, but McCollum and Kennedy [1916] have clearly pointed out that the arguments against its inclusion in biochemical nomenclature far outweigh those advanced by the supporters of Funk, and will continue to do so until our knowledge of the chemical nature of these substances justifies the adoption of the appellation.

SUMMARY.

1. Experimental evidence is given which demonstrates that certain of the properties possessed by the antineuritic substance and the water-soluble accessory growth factor *B*, are very similar.
2. This fact supports the view that the two substances are identical.
3. The repeated failures to isolate substances of this class in the pure condition are attributed more to the fact that they are readily removed from solution in the adsorbed condition by precipitates, than to their reputed instability.

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