THE PREPARATION FROM YEAST AND CERTAIN FOODSTUFFS OF THE SUBSTANCE THE DEFICIENCY OF WHICH IN DIET OCCASIONS POLYNEURITIS IN BIRDS.

By CASIMIR FUNK.

(From the Bio-chemical Department, Lister Institute of Preventive Medicine.)

THIS substance has recently been isolated(1) in what appears to be pure condition from rice-polishings. It crystallises in colourless needles which melt at 233° and the results of the single analysis, which the amount of material at my disposal permitted, indicated the formula $C_{17}H_{20}N_2O_7$. The administration of this substance (about 0·02 grm.) to pigeons suffering from polyneuritis effected a rapid cure. The small yield obtained did not however allow of many such curing experiments being performed and as the substance was not recrystallised, doubts about its purity might be entertained. A confirmation of these facts was therefore absolutely necessary and in the first instance yeast which is known to be curative was chosen as the source of the material since it promised to give a better yield than rice-polishings. It was of great interest to see whether yeast contained the same substance as rice-polishings or only an analogous compound. The yield obtained did not however come up to expectation.

In spite of the facts that a few grams of pressed yeast are sufficient to cure a pigeon suffering from polyneuritis and, as Eykman recently showed(2), the curative substance can be extracted by means of alcohol, the yield was even smaller than in the case of rice-polishings. Before Eykman's paper appeared the author tried to extract yeast with alcohol but only a small part of the active substance could thus be removed. The bulk of the substance remained in the yeast, even when the latter was boiled with alcohol for several hours. This suggests that the substance is to a great extent present in the yeast in a combined state. Schaumann(3) in a recent publication has confirmed the results previously published by the author, but thinks that besides the curative

substance another substance is required. He founds this opinion upon the ground that the yield of curative substance obtained from yeast or rice-polishings compared with the quantity of original yeast or polishings necessary for cure is so exceedingly small. As just stated however the extraction is far from being complete, and during the fractionation the yield of the curative substance becomes smaller and smaller.

The yeast was worked up by the two following different methods. In the first case the alcoholic extract was simply evaporated and the residue hydrolysed with sulphuric acid, the solution being then treated as in the case of rice-polishings(1). By precipitating first with phosphotungstic acid and then with silver nitrate and baryta, half a gram of the curative substance was obtained.

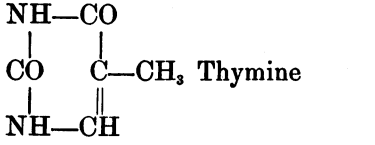
In the second case the alcoholic extract of yeast after evaporation was extracted with water. On precipitation with silver nitrate and baryta however only a minute quantity of the curative substance could be detected. The filtrate from the silver precipitate contained the bulk of it and it was necessary to hydrolyse this with acid before the precipitation could be effected. The resulting fraction unfortunately contained all the pyramidine bases which are known to be constituents of yeast nucleic acid and of these uracil and thymine could be isolated in a pure state. From this mixture it was found that the reagents ordinarily employed for the separation of the curative substance also precipitated more or less of the pyramidine bases. After a long and troublesome fractionation however a small quantity of the pure curative substance was obtained which showed the characteristic properties.

A large number of cures was effected on pigeons and there can be no doubt that this substance is the sole curative agent, the dose necessary being 2–4 cgr., smaller doses were not tried.

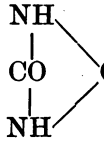
Nature and mode of combination of the curative substance. In the first place it is necessary to correct the previous statement that the curative substance was isolated in form of a nitrate. This is not the case, the substance which was analysed having been found to be the free base. In the meantime(4) I have suggested the name *vitamine* for it as being one of those nitrogenous substances, minute quantities of which are essential in the diet of birds, man and some other animals. The curative substance must be regarded as a base, probably belonging to the pyramidine group. The aqueous solution is of neutral reaction and does not react with acids. By boiling with copper oxide no copper salt is formed and therefore it is not an aminoacid. Recrystallised from

dilute alcohol the substance melts at 233°, at the same temperature as the curative substance from rice. It gives the same reactions and both substances must therefore be considered as identical. It is precipitated in a pure state by mercuric acetate as well as by silver nitrate, but not by mercuric sulphate or nitrate.

All these properties suggest that the curative substance is a pyramidine base, analogous to uracil and thymine and that it probably is a constituent of nucleic acid. On this view the two nitrogens would be combined as in other pyramidine bases to form a ureid.



Thymine



$C_{16}H_{18}O_6$ Vitamine.

Only a constitution of this kind would explain the neutral reaction of the substance and the great analogy to the other pyramidine bases.

The curative substance was also isolated by analogous methods from milk (this fact being very important for our knowledge of infantile scurvy) and brain. Everything suggests that in all these cases the curative substance is identically the same. Further, a substance curing avian polyneuritis was found in lime-juice and is at present being more closely investigated.

EXPERIMENTAL.

Investigation of yeast.

Extraction of yeast followed by hydrolysis of the extract. 75 kg. of air-dried and pulverised yeast were extracted in portions of 2½ kg. each with 4 litres alcohol on the shaking machine for 2 hours. The yeast was then filtered off and the filtrate evaporated in vacuo. The residue, corresponding to 12½ kg. of yeast in each case, was hydrolysed with 1 litre of 10 % sulphuric acid for 5 hours. The fatty acids were filtered off and the filtrate diluted with water to obtain a 5 % solution of sulphuric acid, and precipitated with phosphotungstic acid. In this way 927 grm. of dry phosphotungstate were obtained from 75 kg. of yeast. The precipitate was decomposed with 2500 grm. of baryta in the usual way. In the filtrate the excess of baryta was eliminated carefully with sulphuric acid and the filtrate, after neutralisation with nitric acid, was evaporated in vacuo to a volume of 1 litre. To the liquid a silver nitrate solution was added until a drop gave with a cold solution of baryta a brown precipitate of Ag_2O. A bulky precipitate, consisting of

purine bases, separated which was filtered and to the filtrate pulverised baryta was added until a drop of the solution gave with silver nitrate and ammonia only a trace of a white precipitate. The precipitate thus formed was filtered off, thoroughly washed with water and decomposed with H_2S. From the filtrate the last traces of baryta were eliminated carefully with a very dilute solution of sulphuric acid. Alcohol was added and the solution evaporated in a vacuum desiccator, 0·6 grm. of a crystalline substance being obtained which was recrystallised from hot dilute alcohol. On cooling 0·45 grm. of colourless needles separated which melted after drying at 233°, the same melting point as that of the substance from rice-polishings. The substance was precipitated by mercuric acetate but not by mercuric nitrate and sulphate. When its solution was boiled with cupric oxide no copper salt was formed. The substance gives no precipitate with nitron and cannot therefore be a nitrate. Seven pigeons were cured with this substance, 2–4 cgr. being employed. The filtrate from the silver nitrate precipitate, freed from silver and baryta, was ineffective in curing pigeons.

Extraction of yeast followed by exhaustion of the extract with water. The alcoholic extract from the second lot of yeast (100 kg.), obtained as above, was extracted on a water bath with water, filtered and the aqueous solution precipitated with phosphotungstic acid in 5 % sulphuric acid solution. In this way 2800 grm. of a dry phosphotungstate were obtained which were decomposed with 5500 grm. of baryta. The filtrate freed from an excess of baryta, was neutralised with HNO_3 and evaporated in vacuo. The residue was precipitated with silver nitrate in the manner previously described, and the fraction obtained with $AgNO_3$ and baryta decomposed, alcohol added and the solution evaporated in a vacuum desiccator. In a few days 2·1 grm. of crystals separated which presented however a different appearance from the substance previously obtained and which were less soluble and could therefore be recrystallised from water. 1·8 grm. of substance were obtained on cooling which crystallised in form of rosettes. The substance, after drying at 110° in vacuo, melted at 330° (corr.).

·1507 substance gave ·489 CO_2 and ·0238 H_2O; C : 43·09; H : 3·60.

·2054 (by Kjeldahl's method) required 36·4 c.c. N/10 H_2SO_4; N : 24·81.

These figures correspond to uracil which has been found in the autolysis products of the yeast nucleic acid.

Calculated for $C_4H_4N_2O_2$; C : 42·82; H : 3·59; N : 25·05.

The filtrate from the uracil was hydrolysed with 5 % sulphuric acid for 5 hours. As uracil is precipitated by mercuric nitrate whilst the curative

substance is not, mercuric nitrate was added and 24·8 grm. of dried mercury salt were obtained. In both precipitate and filtrate from the precipitation with $Hg(NO_3)_2$ only traces of the curative substance could be detected.

Investigation of the silver nitrate filtrate. Although in the case of the hydrolysed extract the silver nitrate filtrate was found to be completely inactive, this filtrate in the second case was found to be strongly active. It was thought therefore that the curative substance being in combination did not come down with silver nitrate. The filtrate freed from silver and baryta was precipitated again with phosphotungstic acid. In this way 820 grm. of precipitate were obtained which were decomposed with 2000 grm. of baryta. The filtrate was made up to 5 % sulphuric acid and hydrolysed for 5 hours. After a careful elimination of sulphuric acid the liquid was concentrated in vacuo to about 200 c.c. and precipitated with mercuric sulphate. After standing for 24 hours the liquid was filtered off, the precipitate well washed and decomposed with H_2S. The filtrate from the HgS was freed from H_2SO_4 by means of baryta and was concentrated in vacuo. The mercuric sulphate however also carries down the bulk of the curative substance. Besides uracil 0·25 grm. thymine could be isolated in this fraction. It was obtained in form of plates which melted at above 300° and gave the following figures on analysis:

·1609 substance (by Kjeldahl's method) required 25·6 c.c. N/10 H_2SO_4. Found 22·27 % N, $C_5H_6O_2N_2$ requires 22·22 % N.

The filtrate from the mercuric sulphate, treated with silver nitrate and baryta, gave a silver salt which after decomposing yielded a small quantity of crystals (0·05 gr.), which melted at 233° and possessed curative properties. Thus a separation of the curative substance was effected, even in this case.

Isolation of allantoin from rice-polishings.

51 kg. rice-polishings were treated in portions 1½ kg. with 4 litres of absolute alcohol on the shaking machine for 1 hour. The polishings were filtered off and the alcoholic extract corresponding to 10½ kg. of polishings in each case was melted on the water bath with 1 litre of water and the aqueous extract was evaporated in vacuo. The aqueous solution was precipitated with basic lead acetate and the filtrate, freed from lead, was precipitated with silver nitrate and baryta. The silver salt formed was filtered off, well washed and decomposed with H_2S. The filtrate

from the Ag_2S was freed from the last traces of baryta by an addition of very dilute sulphuric acid and the final filtrate evaporated in vacuo to a small volume and the residue mixed with alcohol and transferred into a vacuum desiccator. In a few days the residue began to crystallise. The crystals (yield 0·6 grm.) were filtered off and recrystallised from 5 % hydrogen peroxide. The substance melted with decomposition at 231°. When mixed with synthetical allantoin the melting point was not depressed.

0·1282 substance by Kjeldahl's method required 32·4 c.c. N/10 H_2SO_4. Found 35·39 % N; calculated for allantoin $C_4H_6N_4O_3$ 35·49 % N.

The substance was entirely different from the curative substance. When mixed with an equal weight of the latter the melting point was depressed 21°. Allantoin is not precipitated by phosphotungstic acid but is precipitated by mercuric sulphate. These properties can be used for the separation of the two substances. Synthetical allantoin (prepared from uric acid) given to seven pigeons suffering from polyneuritis did not cure them, but seemed to prolong the life of the birds. This point however requires further investigation.

Detection of the curative substance in other foodstuffs.

Milk. 1398 grm. of a commercial dried milk preparation known under the name of 'Trumilk,' was extracted with 3 litres of alcohol on the shaking machine for 12 hours. The residue was filtered off and extracted in a Soxhlet apparatus with ether for 12 hours. The combined alcohol and ether extracts were evaporated in vacuo and the residue hydrolysed with 2 litres of 10 % H_2SO_4 for 5 hours. On cooling the solid fatty acids were separated off and the aqueous extract was precipitated with phosphotungstic acid. The precipitate was washed with 5 % H_2SO_4 and when dry weighed 51 grm. This was decomposed with 125 grm. of baryta and the liquid freed from baryta was then found to be effective in curing pigeons suffering from polyneuritis. After the solution had been neutralised with HNO_3, $AgNO_3$ was added, the precipitate formed was separated and to the filtrate baryta was added. The precipitate obtained was filtered and decomposed with H_2S. By evaporating this solution in a vacuum desiccator a small quantity of a crystalline substance in the form of needles was obtained which melted at 230° and possessed the characteristic curative properties.

Ox-brain. 2180 grm. of dried ox-brain were shaken out with 4 litres of alcohol. This was filtered off and the residue extracted again

with ether and finally with alcohol. The extracts were evaporated to dryness in vacuo and hydrolysed with 10 % H_2SO_4 for 5 hours. The solution gave on precipitation with phosphotungstic acid 220 grm. of a dry precipitate which was decomposed in the usual way with 600 grm. of baryta. In the filtrate the excess of baryta was eliminated with weak H_2SO_4 and the filtrate, neutralised with HNO_3, was evaporated in vacuo to about 100 c.c. This solution was found to be curative and was treated with $AgNO_3$ and baryta as before. The silver fraction after decomposition yielded on evaporation a little of a crystalline substance, which was apparently not quite pure, it melted at about 203° but possessed the curative properties.

Lime-juice. 42 litres of commercial lime-juice (nitrogen content 0·35 %) were made up to the content of 5 % H_2SO_4 and were precipitated with a solution of phosphotungstic acid. The precipitate, which weighed when dry 1200 grm., was treated in a mortar with 2500 grm. of baryta, shaken on the shaking machine, filtered off, suspended in water and shaken again. In the combined filtrates the excess of baryta was removed with dilute H_2SO_4. The filtrate from the $BaSO_4$ was neutralised with HNO_3 and the solution, which was found to be curative, was evaporated in vacuo. After the precipitation of the purine bases with $AgNO_3$, a saturated solution of baryta was added to the filtrate so long as the fluid gave a white precipitate with $AgNO_3$ and ammonia. The silver precipitate obtained in this way amounted to 5·9 grm.; it was decomposed with H_2S and the solution evaporated in a desiccator. No appreciable quantity of crystals separated out but the solution was found to be curative for pigeons. Further investigation of this material is in progress.

SUMMARY.

1. The substance preventing beri-beri was isolated from a number of foodstuffs and the previous statements of the author fully confirmed.

2. The base obtained from all these foodstuffs seems to be the same. For curing pigeons (*per os*) 0·02–0·04 grm. were employed.

3. The chemical properties of the curative substance suggest that it is a pyramidine base, forming a constituent of a nucleic acid.

REFERENCES.

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