LX. THE CHEMICAL INVESTIGATION OF THE PHOSPHOTUNGSTATE PRECIPITATE FROM RICE-POLISHINGS.

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The chemistry of rice-polishings has attracted many workers ever since it was shown that there occurred in the polishings a substance, apparently of a relatively simple chemical constitution, which had a powerful curative effect when administered in cases of beri-beri, or experimental beri-beri, produced by a diet of polished rice.

Practically all the communications which have appeared on the subject have, however, concerned themselves chiefly with that particular fraction of the polishings which contained the curative substance and have to a greater or less extent neglected other fractions which were obtained.

The objects of this investigation were, primarily, to repeat the attempt to isolate the curative substance in a pure condition, and, at the same time, to carry out as exhaustive an examination of the other simple nitrogenous substances contained in the phosphotungstic acid precipitate as possible, in the hope that some new substance might be isolated which could be regarded as a degradation product of the unstable curative substance.

We will not give a detailed account of the findings of previous workers, but will refer to and discuss them in the course of the description of our work.

EXPERIMENTAL.

For the purpose of this investigation 620 kilograms of rice-polishings¹ were rendered practically free from fatty substances by petroleum ether extraction, after which they were well extracted three times with alcohol in

¹ This fractionation was begun in 1913 [Funk, 1913, 1], but only the silver nitrate-baryta fraction was thus investigated.

a large extractor. The combined alcoholic extracts, which possessed a deep golden colour, were then freed from alcohol under diminished pressure at 30°, and yielded a thick, dark brown syrup.

This residue was now extracted with 10 per cent. sulphuric acid and the extract diluted with an equal volume of water. On the addition of a 50 per cent. solution of phosphotungstic acid a heavy precipitate was thrown down which was filtered off, washed with 5 per cent. sulphuric acid, and dried.

The dry phosphotungstates, which were to be investigated, were now decomposed by excess of baryta, and, after removal of the excess of baryta, the filtrate was neutralised with hydrochloric acid and concentrated to a thick syrup at low temperature and under diminished pressure.

Extraction of this syrup with alcohol removed a certain amount of inorganic salts, which remained behind insoluble and which for the most part consisted of potassium salts, and the alcoholic extract, after diluting with an equal volume of water, was precipitated by alcoholic mercuric chloride solution.

The heavy white sublimate fraction was filtered off, and, after removal of the excess of mercury from the filtrate and evaporation in vacuo, the purines present were precipitated together with silver chloride by the addition of 20 % silver nitrate in dilute nitric acid solution.

The combined precipitate of silver purine compounds and silver chloride was removed by filtration, and to the filtrate sufficient excess of silver nitrate solution was added to ensure that all compounds present which were capable of forming silver compounds had done so, when they were precipitated as such by the addition of an excess of baryta.

The filtrate from the silver nitrate-baryta fraction was now freed from excess of silver by means of hydrochloric acid, and from excess of baryta by sulphuric acid and the remaining basic substances were precipitated by the addition of phosphotungstic acid.

Four fractions were thus obtained:

1. Sublimate fraction;
2. Purine fraction;
3. Silver nitrate-baryta fraction;
4. Residual phosphotungstic acid fraction;

and these were investigated separately.

1. INVESTIGATION OF THE SUBLIMATE FRACTION.

This fraction, which weighed, when dry, 1505 g., was decomposed in aqueous suspension by means of sulphuretted hydrogen. The filtrate from the mercury sulphide possessed a bright blue colour which, however, rapidly faded on standing. It was decided to investigate this filtrate by two different methods, and accordingly it was divided into two equal portions.

Method I.

This half of the filtrate, in which the basic substances were present in the form of their hydrochlorides, was treated with silver nitrate and silver nitrate and baryta so as to obtain a purine fraction, a histidine fraction, and an arginine fraction. The final filtrate from these separations being freed from excess of baryta and silver was reprecipitated by phosphotungstic acid.

The four sub-fractions of the sublimate fraction were then investigated individually.

Purine sub-fraction of the sublimate fraction. This weighed 220 g. and consisted for the most part of silver chloride. It was ground up with water and the silver removed by sulphuretted hydrogen.

The filtrate from the silver sulphide, on concentration in vacuo at 30° C. to dryness, yielded a small, dark-coloured semi-crystalline residue. On recrystallisation from dilute alcohol, a very small amount of a slightly dirty white, granular substance was obtained. This compound on heating darkened at 220°, but did not show a melting point below 290°. Micro-analysis was attempted, using the method of Pregl, but gave variable figures, and it was found that the substance was not sufficiently pure for an elementary analysis to be made.

It was attempted to make a derivative which could be sufficiently purified to analyse, but the only one which could be isolated with readiness was the picrate, of which, however, we did not obtain sufficient to carry out an analysis.

The substance was a purine, gave a positive reaction with the xanthine test and the diazobenzenesulphonic acid test, a negative result with Wheeler and Johnson's reaction, and a doubtful positive reaction with Capranica's test for guanine.

The picrate was rather insoluble in cold water but more readily soluble in hot, insoluble in alcohol and showed no melting point below 290°.

From the bulk of the evidence we considered that we had isolated guanine, but in the absence of analytical data we could not be certain of this point.

The filtrate from the guanine picrate yielded about a milligram of a crystalline picrate melting without further purification at 145° , but owing to the extremely small amount at our disposal we were unable to make any further examination of this substance.

No other substances were isolated from this fraction.

Histidine sub-fraction of the sublimate fraction. A small fraction which weighed 9 g. It was decomposed in the usual manner in aqueous suspension by means of sulphuretted hydrogen, and the filtrate from the silver sulphide on concentration in vacuo at 30° yielded a very small amount of a residue which was obviously very impure.

This residue was taken up in 200 cc. of water and precipitated by alcoholic mercuric chloride. The white mercuric chloride salt was decomposed, and yielded on concentration of the filtrate from the mercury sulphide a small residue from which a crystalline platinum chloride salt was prepared.

The platinum double salt, after one recrystallisation from dilute alcohol, crystallised in fine needles, melted at $239-240^{\circ}$, and gave on analysis, after drying at 100° C. in vacuo, the figures:

7.643 mgms.; 2.439 mgms. platinum; 31.91 % Pt.

Calculated for choline platinichloride; 31.73 % Pt.

No substance could be isolated from this fraction other than this small amount of choline which was probably in this fraction as an impurity.

Arginine sub-fraction of the sublimate fraction. This fraction, which only weighed 0.4 g., did not give us a sufficient residue on decomposition to enable us to identify or isolate anything from it, other than a small trace of inorganic salts.

Phosphotungstic acid sub-fraction of the sublimate fraction. This final sub-fraction was decomposed by means of lead acetate and the filtrate from the lead phosphotungstate freed from lead, acidified with hydrochloric acid and concentrated at low temperature and under diminished pressure to a thick syrup. Extraction of this syrup with absolute alcohol left a small amount of insoluble inorganic salts, and the alcoholic extract was then precipitated with alcoholic mercury chloride.

The heavy sublimate salt was recrystallised twice from water and then decomposed. The filtrate from the mercury sulphide was concentrated as far as possible and the thick syrup thus obtained allowed to stand in an

evacuated desiccator over potash and calcium chloride. After some weeks there was obtained a crop of fine needle-shaped crystals which were extremely hygroscopic and closely resembled choline hydrochloride in appearance. A platinum double chloride was prepared, and, after one recrystallisation from dilute alcohol, melted at 240° .

Analysis (substance dried at 100° in vacuo).

22.155 mgms.; 7.023 mgms. platinum; 31.71 % Pt.

Calculated for choline platinichloride; 31.73 % Pt.

A fine crystalline picrate was also obtained, which on recrystallisation from water separated in fine clusters of golden yellow prisms which melted sharply at 240° .

The presence of comparatively large amounts of choline in rice-polishings has been noticed by many previous workers [Funk, 1911; Suzuki, Shimamura and Otake, 1912; etc.].

The mother liquors from the recrystallisation of the choline mercury chloride were decomposed by means of sulphuretted hydrogen and the filtrate from the mercury sulphide concentrated at low pressure and temperature to a thick syrup. Extraction of this syrup with cold alcohol yielded a substance which was rather insoluble in alcohol and which was not inorganic in nature. It was removed by filtration and after washing with alcohol examined. Without further purification it melted at 242° and gave a fine double salt with platinic chloride.

This salt, after one recrystallisation from dilute alcohol, melted at 242° .

Analysis (substance dried at 100° in vacuo).

6.412 mgms.; 1.869 mgms. platinum; 30.43 % Pt.

6.927 mgms.; 2.110 mgms. platinum; 30.46 % Pt.

Calculated for betaine platinichloride (mp. 242°); 30.28 % Pt.

The occurrence of the well-known and widely distributed plant base betaine, although not previously observed in rice-polishings, did not surprise us, and we subsequently obtained some interesting results on this point which will be described later.

The alcoholic solution after separation of the betaine hydrochloride was fractionated with platinum chloride but nothing further than small quantities of choline and betaine was isolated.

Method II. (Sublimate Fraction.)

The other half of the filtrate from the decomposition of the sublimate precipitate was acidified with sulphuric acid until it contained 5 % of the acid and was then precipitated with phosphotungstic acid. The precipitated phosphotungstates were then treated by a method which we are now using to a considerable extent in our investigations, namely extraction with acetone¹.

It has already been shown that the phosphotungstates vary a great deal in their solubilities in acetone or dilute acetone and advantage has been taken to some extent of this fact by Wechsler [1911]. So far as we know, however, there is no published record of any attempt to employ the different solubilities of the phosphotungstates as a means of separation.

We are, at the present time, making an extensive examination of these solubilities in the hope that by this means a new and useful process may be added to biochemical methods. Preliminary results, a few of which are contained in this communication, lead us to believe that the method may, when fully worked out, be of considerable value, but we cannot pass a verdict until we have completed our investigation of the subject.

In this particular case the phosphotungstates were ground up in a mortar in the cold with acetone until the extract ceased to possess a brown colour, when the insoluble residue was removed by filtration and washed with acetone. We find that extraction in the warm is undesirable, as it generally results in the formation of an extremely sticky mass which is very difficult to deal with, and we have always found that a far better separation is effected when the extraction is carried out at room temperature.

By this means, we obtained a deep brown solution of acetone-soluble phosphotungstates and a small residue insoluble in acetone.

Acetone-soluble phosphotungstates from the sublimate fraction. The deep brown solution was decomposed by shaking well with an excess of a saturated aqueous solution of lead acetate. After standing, the precipitated lead phosphotungstate was filtered off and the filtrate freed from excess of lead by means of sulphuretted hydrogen. The clear solution thus obtained was rendered free from sulphuretted hydrogen and fractionated, as in Method I, by means of silver nitrate, and silver nitrate and baryta into a purine fraction, a histidine fraction, an arginine fraction, and then, after removal of the excess of baryta and silver, into a final phosphotungstic acid fraction.

¹ This method was used for the first time in the fractionation of yeast and cod-liver oil by Funk, shortly to be published.

The purine fraction on decomposition yielded the same purine that we had previously encountered and which we believe was guanine, but here, again, insufficient was obtained to render the identification absolute.

Nothing definite could be isolated from the histidine and arginine fractions, which were very small.

The final phosphotungstic acid precipitate yielded on decomposition practically pure choline, and no betaine could be recognised or isolated from this fraction.

Acetone-insoluble phosphotungstates from the sublimate fraction. This residue, weighing 40 grams, was decomposed by lead acetate, the filtrate freed from excess of lead by sulphuretted hydrogen, acidified with hydrochloric acid and evaporated to a thick syrup under diminished pressure at 30°.

As no crystalline derivative could be isolated directly from this syrup, a sublimate salt was made and recrystallised from water. On decomposition it yielded a hydrochloride, which was soluble in alcohol and melted sharply at 263°.

A platinum double salt was prepared, which, after one recrystallisation from dilute alcohol, melted at 253° and after drying at 100° C. for two hours in vacuo gave the following figures on analysis:

14.896 mgms.; 4.478 mgms. platinum; 30.07 % Pt.

10.826 mgms.; 3.245 mgms. platinum; 29.98 % Pt.

Calculated for nicotinic acid platinichloride (mp. 253°); 29.72 % Pt.

The mother liquor from the mercurichloride precipitation on decomposition yielded a platinichloride which was readily identified as betaine platinichloride.

Apparently, in the investigation of this fraction by utilising the separation of the phosphotungstates by means of acetone, into an acetone-soluble and an acetone-insoluble portion, we have been able to separate the choline practically quantitatively from the betaine and nicotinic acid, thereby rendering the identification of the small quantity of the latter possible by removing the choline into another fraction.

We have therefore found the Sublimate Fraction to contain mainly choline together with small quantities of betaine, nicotinic acid and probably traces of guanine. It is of interest to note that nicotinic acid has been isolated from this fraction, which it has already been shown contains a small amount of the curative substance. The parallel occurrence of nicotinic acid

and the curative substance has already given rise to the suggestion made by one of us [Funk, 1913, 2] that nicotinic acid may be a degradation product of the curative substance. This point will, however, be referred to again later.

2. INVESTIGATION OF THE PURINE FRACTION.

This fraction, weighing 47 g., was decomposed by means of sulphuretted hydrogen and the clear filtrate from the silver sulphide concentrated under diminished pressure at 30°. After concentration to half bulk, the excess of nitric acid in the solution was quantitatively removed by means of nitron and after removal of the nitron nitrate the concentration continued to dryness. During the concentration two crops of a sandy solid were obtained which were removed by filtration. This substance, which was impure, weighed 0.3 g. and was recrystallised from water, in which it is very insoluble. Thus purified, it showed all the reactions of adenine, but did not give very concordant figures on analysis by the micro-combustion method of Pregl.

The picrate closely corresponded with adenine picrate.

Analysis (dried at 100° C. in vacuo for two hours).

2.740 mgms.; 0.717 cc. N. at 21° C. and 770 mm.—30.62 % N.

Calculated for adenine picrate 30.77 % N.

From the tarry residue left after the separation of the adenine and further concentration of the mother liquor, nothing very definite could be isolated except a very small quantity of a crystalline picrate melting, without further purification, at 280°. The amount, however, was far too small to admit of any examination.

The purine fraction consists mainly of adenine together with possible traces of other purines.

3. INVESTIGATION OF THE SILVER NITRATE-BARYTA FRACTION.

This fraction, which, as originally shown by Funk, contains practically the whole of the curative substance, has already been very exhaustively investigated and the results of the investigation published elsewhere [Funk, 1913, 2]. In that case 2.5 g. of a highly active substance were obtained, which after one recrystallisation from dilute alcohol gave a white substance crystallising in small needles which melted at 233°.

This substance did not give the Millon test nor the uric acid or phenol

tests, it was sparingly soluble in water and on analysis, using the micro-combustion method of Pregl, the following figures were obtained:

	C.	H.	N.
(1)	58.80 %	3.93 %	10.58 %
(2)	58.89 %	3.89 %	10.64 %

From these figures the formula $C_{26}H_{20}O_9N_4$ was arrived at to represent this substance.

Calculated figures for $C_{26}H_{20}O_9N_4$:

	C.	H.	N.
	58.62 %	3.79 %	10.53 %

The substance did not yield all its nitrogen by Kjeldahl's method.

From the mother liquor of this substance another crop of crystals was obtained which were somewhat less coloured and apparently more soluble in water.

After recrystallisation from dilute alcohol, this substance melted at 234° and no depression of the melting point was observed when melted together with a sample of synthetic nicotinic acid.

Analysis gave the following figures:

	C.	H.	N.
(1)	58.37 %	3.93 %	11.11 %
(2)	58.45 %	4.06 %	10.97 %

Calculation for nicotinic acid:

	C.	H.	N.
	58.30 %	4.08 %	11.34 %

The identity of the product from rice-polishings was further established by the melting point and analysis of the picrate.

Barger [1914] has remarked that the substance described with melting point 233° to which the provisional formula $C_{26}H_{20}O_9N_4$ had been given, showed a very close resemblance to nicotinic acid both as regards its melting point and its elementary composition, and he suggested that the possibility was not excluded that this body might in reality be merely nicotinic acid contaminated with a small quantity of a highly active substance rich in carbon.

It must be remembered that the substance $C_{26}H_{20}O_9N_4$ was not described as being curative, and, in fact, it was found that it has no greater curative action than nicotinic acid. With regard to the suggestion that the compound $C_{26}H_{20}O_9N_4$ is identical with nicotinic acid, our work goes to show that it is quite correct.

Both substances crystallise in the same form and the crystals melt at 232–234°. A mixed melting point determination gave the figure 232°.

The two picrates were prepared and both presented the same crystalline appearance. They both melted at 215°, and a mixed sample also melted at 215°. Their copper salts were also prepared and were identical in appearance.

Analysis of copper salt of $C_{26}H_{20}O_9N_4$ dried at 100° C. in vacuo:

(1) 19.100 mgms.; 4.180 mgms. CuO; 20.13 % Cu.

(2) 19.060 mgms.; 4.93 mgms. CuO; 20.65 % Cu.

Calculated for the copper salt of nicotinic acid; 20.67 % Cu.

The identity of the substance previously described as $C_{26}H_{20}O_9N_4$ with nicotinic acid could now be doubted no longer, and a careful examination of the mother liquid of these two crops of crystals did not result in the isolation of any other substance than nicotinic acid. All trace of the curative substance had disappeared, and a fraction, which originally had shown very marked curative properties, now consisted of nothing other than nicotinic acid, which possesses very slight action.

All attempts which we have made to isolate the elusive curative substance from this fraction, in which it originally occurs, have failed, and we can only hope that one or other of the new methods which we are testing at the present time, will give a process by which it can be isolated before it has time to become inactive.

4. INVESTIGATION OF THE PHOSPHOTUNGSTIC ACID FRACTION.

This fraction, weighing 195 g., was treated by grinding in a mortar with acetone, as already described, until the acetone extract ceases to be coloured.

The two fractions thus obtained were then investigated separately.

Acetone-insoluble sub-fraction of fraction 4. This weighed 25 g. and was decomposed by means of lead acetate. The filtrate, after being freed from lead, was acidified with hydrochloric acid and concentrated at low temperature and pressure to a syrup. As no definite crystalline substance could be obtained from this syrup, alcoholic mercuric chloride was added, and the precipitated mercury chloride salt removed by filtration.

On decomposition the mercurichloride yielded a small amount of a rather hygroscopic hydrochloride from which a well-defined platinichloride was prepared.

After one recrystallisation from dilute alcohol the platinum salt melted at 232°.

Analysis (dried at 100° C. in vacuo).

- (1) 11.670 mgms.; 4.024 mgms. platinum; 34.48 % Pt.
- (2) 7.155 mgms.; 2.463 mgms. platinum; 34.42 % Pt.

After another recrystallisation from dilute alcohol it melted at 234°.

- (3) 4.235 mgms.; 1.453 mgms. platinum; 34.39 % Pt.

The small quantity of this salt which we obtained prevented us from making any further examination of it, and we have so far been unable to identify it.

The filtrate from the platinum double salt, together with the mother liquors from its recrystallisation, were decomposed and attempts made to prepare a gold salt from the residue, but without success.

The filtrate from the mercuric chloride precipitation was decomposed and concentrated at low temperature and pressure to dryness. A very small residue of impure crystalline hydrochlorides was obtained which, however, contained inorganic substances. Alcohol extraction removed the organic portion, which yielded a small quantity of a platinichloride melting with decomposition at 280°, but the quantity obtained was far too small to admit of purification or examination.

Acetone-soluble sub-fraction of fraction 4. This weighed 175 g. and was decomposed in the usual manner by means of lead acetate. The filtrate, after freeing from lead, was concentrated at low temperature and pressure to a thick syrup. Nothing crystallised from this residue on standing, so saturated aqueous picric acid was added. A small amount of a brownish yellow semi-crystalline picrate separated out and was removed by filtration. This picrate was very insoluble in cold water and very little more soluble in hot. It was recrystallised from water and separated as a golden brown powder. It darkened on heating at 200° but showed no melting point below 289°. Determinations of its content of picric acid were made by the nitron method on a sample dried at 100° in vacuo for two hours. The figures obtained were:

- (1) 0.0749 g.; 0.1388 g. nitron picrate; 78.45 % picric acid.
- (2) 0.0395 g.; 0.0732 g. nitron picrate; 78.43 % picric acid.

Portions of the picrate and of the mother liquid from its recrystallisation were decomposed with ether and hydrochloric acid, and yielded upon

concentration to dryness a very small residue of the base in the form of its hydrochloride.

It was itself insufficiently pure to examine, and attempts to prepare a platinichloride, a gold salt or a picrolonate were all unsuccessful.

Guanidine picrate melts at 315° , exhibits similar solubilities to the compound we isolated from rice, and contains 79.10 % of picric acid. It is possible that we were here dealing with guanidine picrate, but we were unable to be sure of this point because of the very small quantity which was at our disposal.

The filtrate from the precipitation of this picrate was rendered free from picric acid by means of acidification with hydrochloric acid and shaking out with ether, and was then concentrated at 37° under diminished pressure. During the concentration a crystalline substance separated out and was filtered off, after which the concentration was continued to dryness.

The crystalline substance, which had separated out during the concentration, was recrystallised from dilute alcohol and separated out from that solvent in the form of small needles. Without further purification it melted sharply at 235° .

The isolation of this substance was of considerable interest to us, as we have encountered it several times in the investigation of rice-polishings. If the total phosphotungstate precipitate from rice-polishings is decomposed by means of lead acetate, and the filtrate, after freeing from lead and acidifying with hydrochloric acid, concentrated to dryness, a semi-crystalline residue of hydrochlorides is obtained. If now, this residue be extracted and recrystallised from dilute alcohol, the same crystalline substance melting at 235° is obtained.

On several occasions we have isolated this substance, and, after having collected some few grams, we decided to investigate it. It was recrystallised once again from alcohol and then melted sharply at 234° .

It was noticed that on drying, the crystals often—although not in every case—developed a fine blue colour. Some crystals apparently developed the colour to a far greater extent than others, and we found that the production of the colour was due to a photochemical action. The colour disappears when the crystals are dissolved, and when they separate out again they are perfectly white, so long as they are covered by the solvent. When they are removed from the solution and placed in the light they quickly develop the colouration again, and we have found them to develop this colouration after as many as six recrystallisations.

We have not yet satisfied ourselves as to the cause of the colouration, but believe it is in some way the result of the action of light upon minute traces of tungsten compounds which have been persistently retained from the phosphotungstic acid decomposition. This however was never observed in other fractionations.

The substance which we had now obtained in an apparently pure condition was analysed by micro-combustion. Concordant figures could not, however, be obtained on duplicate analyses, despite further purification of the substance by recrystallisation from dilute alcohol. The substance did not give all its nitrogen when estimated by Kjeldahl, and in many ways resembled nicotinic acid hydrochloride, which, however, as prepared from nicotinic acid, always melts at 263°.

A well-defined platinum double salt was prepared, which after one recrystallisation from dilute alcohol melted sharply at 253°, the theoretical melting point for the platinichloride of nicotinic acid, and no depression of the melting point was observed when a mixed sample of the two platinichlorides was melted.

Analysis (dried at 100° C. for two hours in vacuo).

- (1) 7.746 mgms. ; 2.318 mgms. platinum ; 29.86 %.
- (2) 10.479 mgms. ; 3.136 mgms. platinum ; 29.93 %.

Calculated for platinichloride of nicotinic acid ; 29.72 %.

The free base was liberated from the hydrochloride by means of silver acetate, and from the product thus obtained pure nicotinic acid was isolated, which was in every respect identical with the synthetic acid.

We were unable to explain the difference in the melting points of what we imagined were two distinct modifications of the hydrochloride of nicotinic acid, and could not account for the inconstant figures obtained on analysis of the hydrochloride melting at 235°, until an attempt to recrystallise the latter compound from absolute alcohol provided us with a clue, which, after investigation, resulted in the clearing up of the whole matter.

It was found that the compound melting at 235° was not all equally soluble in absolute alcohol and that a portion of it was very insoluble in cold alcohol.

This less soluble portion was fractionated with cold alcohol, and eventually a white crystalline substance was obtained which was very insoluble in cold alcohol and which after one recrystallisation from dilute alcohol melted sharply at 245°. A platinichloride was prepared and formed a fine,

orange-coloured, needle-shaped, crystalline compound which after one recrystallisation from dilute alcohol melted at 242° .

Analysis. 13.133 mgms.; 3.998 mgms. platinum; 30.44 % Pt.

Calculated for betaine platinichloride (mp. 242°); 30.28 % Pt.

It also gave a picrate which melted at 182° and was identical in every respect with betaine picrate.

We then realised that in the compound melting at 235° we were dealing with some complex, probably of the nature of mixed crystals, of nicotinic acid hydrochloride and betaine hydrochloride.

We therefore decided to effect a separation of the two constituents, and after several attempts found that this could be quantitatively carried out by utilising the insolubility of the copper salt of nicotinic acid.

The hydrochloride was dissolved in water and was warmed on the water bath at 60° for several hours with excess of a saturated solution of copper acetate until no further deposition of any copper salt occurred. The precipitated copper salt and the clear blue mother liquor were then separated, and decomposed individually by means of sulphuretted hydrogen.

The pale green, amorphous copper salt on decomposition and concentration of the filtrate from the copper sulphide yielded a crystalline hydrochloride, which after one crystallisation from dilute alcohol was shown to be identical with nicotinic acid hydrochloride. It melted at 264° , its picrate at 214° , and none of these derivatives showed any depression in its melting point when melted with the corresponding derivative of nicotinic acid.

The bright blue-green filtrate from the insoluble copper salt of nicotinic acid was decomposed in a similar manner, and yielded a hydrochloride, very insoluble in cold alcohol, which after a single recrystallisation from alcohol melted at 245° and was identical with betaine hydrochloride. Its picrate melted at 181° , and no depression of this melting point was observed on melting it together with a sample of betaine picrate. Its gold salt melted at 209° but on recrystallisation from 1 % hydrochloric acid, the other form melting at 247° crystallised out.

Analysis. 11.884 mgms. gold salt; 5.119 mgms. gold; 43.09 % Au.

Calculated for betaine aurichloride (mp. 247°); 43.11 % Au.

We also tried other methods of separating the two constituents of this complex, but found no method so useful as that which we have described in which we used copper acetate.

Alcohol fractionation removed the betaine hydrochloride gradually, but

we were not able to prepare a pure specimen of nicotinic acid by this process. Fractionation of the picrate of the complex also gave us fairly pure betaine picrate in the less soluble fractions, but we were unable to obtain nicotinic acid picrate free from betaine picrate.

It was of interest to us to try the curative action of the complex hydrochloride, and accordingly doses were administered by intra-muscular injection into pigeons suffering from experimental beri-beri, but we did not observe any arrest of the symptoms or of the onset of death. Betaine salts were likewise examined and found to be entirely without curative action.

The residue left after concentration of the mother liquors, from which the complex hydrochloride melting at 235° had separated out, was then examined, but only yielded a mercurichloride. This was decomposed and found to consist of choline as identified by its platinichloride, melting at 242° .

Analysis (dried at 100° C. in vacuo).

7.643 mgms.; 2.439 mgms. platinum; 31.91 % Pt.

Required for choline platinichloride; 31.73 % Pt.

The filtrate from this small quantity of choline mercurichloride on decomposition yielded a small residue which gave a platinichloride. After a single recrystallisation it melted at 237° .

Analysis (dried at 100° C. for two hours in vacuo).

(1) 8.418 mgms.; 2.906 mgms. platinum; 34.57 % Pt.

(2) 15.092 mgms.; 5.199 mgms. platinum; 34.45 % Pt.

This platinichloride seemed, despite its somewhat higher melting point, identical with that which had been isolated in the acetone-insoluble sub-fraction of this fraction. Here again we obtained insufficient to render much further examination possible. A small quantity was decomposed and the free hydrochloride prepared. No gold salt, picrate or picrolonate could be obtained, and consequently we have been unable to prepare any other derivative by means of which it might be identified.

There is the bare possibility that it might be the platinichloride of choline contaminated with traces of potassium platinichloride, which it is known are persistently retained by the choline salt, but it did not give the periodide test for choline.

No other substance could be isolated from this fraction, from which we have separated betaine, choline, nicotinic acid, a picrate that resembles guanidine picrate, and the platinichloride of a base which we have been unable to identify.

We may now summarise our findings, and it is here that a convenient point is reached at which to discuss some of the results of previous workers.

From an exhaustive fractionation of the phosphotungstic acid precipitate from the alcoholic extract of fat-free rice-polishings, we have isolated comparatively large amounts of choline, nicotinic acid and betaine, together with small amounts of the purines, guanine and adenine and traces of a substance which may be possibly guanidine.

Choline and nicotinic acid have been found by the majority of the previous workers, because they occur in larger amounts than any of the other constituents. We have, whilst carrying out this work, paid particular attention to the published work of Suzuki, Shimamura and Odake [1912] in the hopes that we might be able to find some corroboration of their results. We have, however, with a few exceptions, been entirely unable to do so. By decomposition of the phosphotungstic acid precipitate from rice-polishings with baryta and subsequent concentration of the filtrate, they obtained a product which was highly curative in action and which they designated "Rohoryzanin I."

In spite of the fact that it is quite obvious that "Rohoryzanin I" is a complex mixture of basic substances, they boiled it with 3 % hydrochloric acid for two hours and then looked on the products they isolated from the fluid as hydrolytic products of Rohoryzanin.

From this hydrolysis they obtained two acids, which they termed α and β acids, choline, nicotinic acid, and glucose. Thus they said that from 100 parts of Rohoryzanin there were obtained on hydrolysis 10 parts of the α and β acids, 30 parts of choline and nicotinic acid and 23 parts of glucose.

The two acids were obtained by allowing the hydrolysed solution of Rohoryzanin to cool, when golden brown coloured crystals separated out. These were separated, washed with a small quantity of cold water, and recrystallised from alcohol, when two different products, separated by their different solubilities in alcohol, were obtained, and from the elementary analyses the formulae $C_{10}H_8O_4N$ and $C_{18}H_{16}O_9N_2$ were given to the two acids.

Funk (1913, 1) tried to prepare these two bodies, following exactly the method described by Suzuki and his co-workers, but has never yet succeeded in obtaining them. Whenever the product which these authors termed "Rohoryzanin" was hydrolysed a clear solution was obtained which did not deposit any crystalline substance whatever on cooling. From this solution on concentration we have now separated the hydrochlorides of nicotinic acid and betaine by their different solubility in alcohol, but we can hardly believe

that these were the substances described as the acids $C_{10}H_8O_4N$ and $C_{18}H_{16}O_9N_2$. Unfortunately, beyond giving a few solubilities and reactions the authors do not give much information about these two products.

The Japanese workers, from their descriptions of "Rohoryzanin," were apparently under the delusion that they were dealing with a single substance and give several reactions for it. They even claim that a characteristic reaction for "Rohoryzanin" is the *p*-diazobenzenesulphonic acid test.

It was found that the substance described as "Rohoryzanin" does give the "diaz" reaction, but we believe that the purine content of this crude mixture of bases is quite sufficient to account for a positive reaction being obtained.

Suzuki, Shimamura and Odake also describe nicotinic acid, choline and glucose amongst what they term "die Spaltungsprodukte des Rohoryzanins," obtained by hydrolysis with 3 % hydrochloric acid.

With regard to nicotinic acid, we ourselves lean towards the belief that its occurrence in rice-polishings has some relationship to the occurrence of the curative substance, and that it may possibly even be a degradation product of the active body, but in the case of choline we firmly believe that it exists as such, and in comparatively large amounts, in the polishings.

The workers, whose work we are criticising so fully, purified their "Rohoryzanin I" by means of precipitation with tannin, and thereby obtained another product which they called "Rohoryzanin II," and which was three times more active than the original substance. The yield, however, was very small.

From a concentrated aqueous solution of "Rohoryzanin II," they obtained on addition of picric acid a precipitate of a golden-brown crystalline picrate. They separated it and found that it contained as an impurity nicotinic acid picrate, due to "Rohoryzanin II" containing impurities of nicotinic acid.

They succeeded in preparing this picrate, "Oryzanin picrate," free from nicotinic acid picrate and further purified it by recrystallisation from acetone.

Thus prepared, it was described as a golden-brown picrate crystallising in microscopic needles. It is insoluble in ether and petroleum ether, rather insoluble in water, more soluble in hot water, alcohol or ether. The yield was very poor, and very little detail is given beyond the few solubilities given above. It was highly active when administered to pigeons suffering from experimental beri-beri and was regarded by the discoverers as the curative substance of rice-polishings in the form of its picrate.

We have attempted to prepare this substance but have not yet succeeded

in doing so. Among the substances which we isolated from rice-polishings was one picrate which corresponded in many respects with the picrate described by the Japanese authors. This picrate, already described in a previous portion of the paper, we believe to have been guanidine picrate. Whether this is so or not we are not sure, but we certainly know that it possesses no curative action whatever.

Not one of the picrates which we isolated demonstrated any such action, and we have been quite unable to confirm the isolation of the curative substance in the form of its picrate as described by Suzuki and his collaborators.

SUMMARY.

1. We have carried out an exhaustive fractionation of the phosphotungstic acid precipitate from an alcoholic extract of rice-polishings.

2. We have once again confirmed the presence of considerable amounts of choline and nicotinic acid in the polishings, and have also detected the presence of betaine, adenine, guanine and possibly guanidine. Small amounts of other substances were isolated but in quantities too small to admit of identification.

3. We have repeated the work of the Japanese authors Suzuki, Shimamura and Otake, who claimed to have isolated the curative substance in the form of its picrate, but have been unable to confirm this finding, as well as some other portions of their work.

4. We have ourselves failed to isolate the curative substance which occurs in rice-polishings. It is apparently decomposed during the fractionation and all trace of it is lost.

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