Physiology. — *„On the Isolation of the anti-beri-beri Vitamin."* By B. C. P. JANSEN and W. F. DONATH. (Communicated by Prof. C. EYKMAN.)

(Communicated at the meeting of September 25, 1926).

The first attempts to isolate the substance that, according to EYKMAN's [1]) fundamental investigations, possess a prophylactic property against beriberi, were made by G. GRYNS [2]), but they were ineffectual. EYKMAN [3]) found still a number of properties of this substance, among which the very remarkable one that it is easily soluble in water and in 80° alcohol, and readily dialysable. This is of vital importance, as it goes to show that this substance has a comparatively small molecule, and on that account cannot be classed under the proteins, nucleoproteids, and the like. In view of its considerable physiological activity it probably belongs to the group that BARGER has included under the general name of the "simpler natural bases" and was termed by GUGGENHEIM "die biogenen Amine".

In 1911 C. FUNK [4]) published his first study on this substance which he designated by the name of "vitamin". In this publication he described a body, 20—40 mgrms. of which could cure a pigeon that had developed polyneuritis after a diet of polished rice. It appeared later on that this was not the "vitamin" sought for. Furthermore the symptoms that occurred on a diet of purified proteins + fats + carbohydrates + salts warranted the assumption of several of such "vitamins", which were differentiated by the designations A and B, afterwards also C, D and E, etc. vitamins. It is still a moot point, which may now be expected to be soon set at rest, whether the B-vitamin, described by the American and English observers, which was noted for its influence on the growth of young rats, is identical with the anti-beriberi- or anti-neuritic-vitamin, that protects man against beriberi and birds from polyneuritis [5]). This identity was discredited by authors as EYKMAN [6]) and MENDEL [7]). After FUNK a good many observers have tried

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1) C. EYKMAN, Geneesk. Tijdschr. voor Ned.-Indië, **36**, p. 214 (1896).

2) G. GRIJNS, Geneesk. Tijdschr. voor Ned.-Indië, **41**, p. 1 (1901).

3) C. EYKMAN, Arch. f. Hygiene, **58**, p. 150 (1906).

4) C. FUNK, Journ. physiology, **43**, p. 395 (1911).

5) See H. H. MITCHELL, Journ. biol. Chem., **40**, p. 399 (1919) and A. D. EMMETT and G. O. LUROS, Ibid. **43**, p. 265 (1920).

6) C. EYKMAN, C. J. C. VAN HOOGENHUIJZE and T. J. G. DERKS, Journ. biol. Chem. **50**, p. 311 (1921).

7) CROLL and L. B. MENDEL, Americ. Journ. Physiol. **74**, p. 675 (1925).

Fig. 1.
Ricebird (munia maja) with polyneuritis; about $1^1/_2 \times$ natural size.

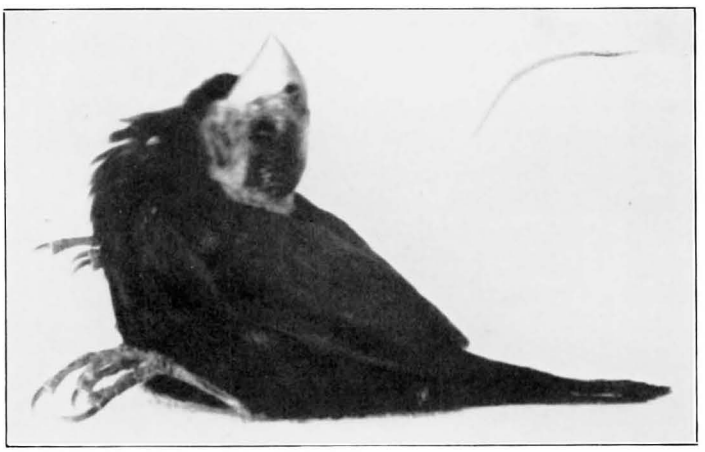

Fig. 2.
Ricebird (munia maja) with polyneuritis; about natural size.

to isolate the B-vitamin, and especially the anti-beriberi-vitamin, without any positive results, so far as we know. A review of their work can be found in the modern books on vitamins, see e.g. "The Vitamins" by H. C.SHERMAN, and L. L. SMITH, New York 1922 pp. 18—48. Last year two articles appeared in the "Chemisch Weekblad" by American authors [1]), which contained a short survey of the work on the isolation of the antineuritic, resp. B-vitamin. This prompted us to publish in the "Chemisch Weekblad" a paper on the state at that time of our attempts to isolate the antineuritic vitamin [2]). At the same time we could set our mind more deliberately to the investigations that had occupied us for more than nine years already in the intervals of other work. To our great satisfaction we can say that we have succeeded in isolating the anti-beriberi-vitamin in the same laboratory where EYKMAN and GRYNS have worked.

For our earlier work we refer to the above-named article in the "Chemisch Weekblad" and to the various Annual Reports of the "Geneeskundig Laboratorium" of Weltevreden. In this paper we intend to describe the method that led to our ultimate success in isolating the anti-beriberi-vitamin.

The material we started with were the fine rice-polishings (dedek) that can be procured in India at a very low price. An extract of it was fractionated in different ways. In order to determine the vitamin-content of the various fractions, we worked with small ricebirds called bondols (munia maja). Groups of ten of these birds, caged together, were fed with polished rice, to which was added a known quantity of the fraction under examination. The polished rice had previously been washed in running water for 2 $\times$ 24 hours, while the deficiency of mineral salts and A-vitamin was compensated by addition of 2 % of a salt-mixture, about equal to that used by OSBORNE and MENDEL, and of $\frac{1}{4}$ % codliver oil. These ricebirds are very fit reagents to testify the presence of the antineuritic vitamin, and to determine its content [3]). With a rare exception (no more than 1 or 2 % of the total number) all the ricebirds fed on washed, polished rice develop polyneuritis in from 9 to 13 days. (See Plate Fig. 1 and 2.) If 5 % of a definite sort of dedek is added to the rice, polyneuritis reveals itself only after from 15 to 23 days.

We now determined every time the quantity of the different that had to be added to the washed, polished rice, so that the 10 rice-birds fed with this mixture contracted polyneuritis within from 15 to 23 days. As we found that a ricebird ingests on an average 2 grms of rice a day, we could compute the quantity of a definite fraction per bird and per day that had to be added to the rice, to guard the animals from polyneuritis for 15—23 days.

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[1]) A. SEIDELL, Chem. Weekblad **22**, p. 353 (1925) and P. A. LEVENE and B. VAN DER HOEVEN, Chem. Weekblad **22**, p. 575 (1925).

[2]) B. C. P. JANSEN and W. F. DONATH, Chem. Weekblad **23**, p. 201 (1926).

[3]) Vide B. C. P. JANSEN, Mededeelingen Geneeskundig Laboratorium te Weltevreden 1920, p. 40.

For some kinds of dedek a quantity of 100 mgrms per ricebird and per day sufficed; however, with most commercial sorts this quantity amounted to 140 mgrs. Finely ground rice-polishings (dedek) were now extracted with tap-water. (The Batavian tapwater countains only about 130 mgrs of dissolved salts per Litre), to which 3½ cc of sulphuric acid had been added per Litre, to obtain the precise acidity (p_H ultimately $= \pm 4.5$), and 200 cc of spiritus fortior to neutralize the action of micro-organisms. It seemed to us that formol was a less favourable disinfectant. The extraction was performed in a row of 4 wooden casks of $\pm$ 40 Litres each in accordance with the principle of the counter-current. The bottom and the lid of each cask was provided with an opening resp. for the affluence and the effluence of the liquid. A little above the bottom a second loose bottom had been applied for the greater part consisting of copper-gauze. On this gauze rests a layer of mineral grit, and on top of this the dedek (16 kg per cask). Every day one of the casks was refreshed, and the other three were moved up, so that the fresh dedek came in contact with the liquid that has already extracted the dedek in the other three casks, and the most extracted dedek was extracted with the fresh water-alcohol-sulphuric acid mixture. In this way almost 100 kg of dedek could be extracted every week. This extract contained $\pm$ 20 kg of solid matter and approximately all the vitamin contained in the dedek. SEIDELL showed in 1916 that the vitamin is absorbed selectively by Lloyd's reagent, a kind of fuller's earth. After a prolonged enquiry we discovered in the "acid clay" put on the market by the BÜNING concern of Cheribon, a variety of fuller's earth that possessed the same absorbing properties with respect to the vitamin as Lloyd's reagent.

The extract from 100 kg of dedek was now diluted to 300 Litres (the p_H was if necessary raised to 4.5) and mixed with 300 kg of very finely ground acid clay. After some hours' stirring of this mixture by means of an electromotor, the acid clay was allowed to subside and the supernatant fluid was pipetted off. Finally the activated acid clay was sucked off in a filter of a surface of 45 $\times$ 60 cm acid, washed repreatedly with small amounts of water acidulated to $p_H = 4$—5, the residue was removed from the filter and treated with so much baryta that a paper, soaked with tropaeolin 0, is turned reddish brown, when immersed in the fluid; the p_H then amounts to $\pm$ 12 or 13. This baryta-extract, which contains the vitamin, was again sucked off on the filter and then washed, first with baryta, subsequently with distilled water; to the filtrate sulphuric acid was added to an amount that made the acid red on congopaper. That treatment with baryta should pass off as rapidly as possible, because the vitamin is being deteriorated in alkaline solution. However, the solution, acidulated with sulphuric acid, may safely be left to stand for one or two days, after which the barium-sulphate will have subsided. Most of the supernatant fluid was then decanted off, and the remaining fluid was filtered off on a hardened paper filter. This filtrate still contains $\pm$ 100 grms of solid matter, and 80 % of the original vitamin present in the dedek. Now the acid clay-extract

was again fractionated after KOSSEL and KUTSCHER [1]) with silver sulphate and baryta; their method was, however, refined by determining the p_H of the liquid. To this end the acid-clay-extract obtained from 100 kg of dedek was evaporated in a large porcelain dish placed in a heated water-bath. A very strong current of air was blown over the liquid by means of an exhauster, so that at a temperature of from 30° to 40° C. about 1½ to 2 Litres of water was evaporated per hour. The liquid was evaporated to a volume of ± 4 Litres. This was transmitted to a thick walled glass beaker of Pyrexglass of ± 10 Litres, and was subsequently treated with silversulphate or with silvernitrate. In our first experiments we used silver-sulphate partly in solution, partly as a solid. However, as it took some days of incessant stirring with an electromotor to dissolve a sufficient quantity of silversulphate, we used silvernitrate later on. Most often a quantity of ± 50 grms of silvernitrate was required. As known, the sufficiency of the addition will be attained when a sample of the liquid gives a brown precipitate directly on the addition of an excess of baryta. The addition of the silvernitrate to the acidulated acid-clay-extract yields a thick precipitate, not containing any vitamin. By violent stirring with an electromotor baryta was now allowed to flow in slowly from a burette or pipette. Then the p_H of the liquid (at the beginning only 2 or 3) rises slowly. The p_H was mostly determined with the aid of the capillator procured by the British Drug House, which gave us great satisfaction.

We are now able to supply so much baryta that the p_H rises to ± 4.5. In the resulting precipitate there is no appreciable quantity of vitamin. Now the liquid was filtered off and an amount of baryta was allowed to flow into it until the p_H became ± 6.5. In this second silverfraction there is rather more than 50 % of the vitamin contained in the acid-clay-extract. After filtering this deposit baryta is again added to the filtrate, until the $p_H = \pm 8.0$. In this 3rd silverfraction there is still a considerable amount of vitamin, but it is mixed with a relatively much larger quantity of inactive substances than in the 2nd silverfraction. Therefore, this 3rd silverfraction was not worked up directly, but the 3rd fraction of different preparations together, was again fractionated with silvernitrate and baryta after analysis with hydrochloric acid. In the filtrate of the 3rd silverfraction there is still a large amount of solid matter but practically no vitamin.

The 2nd silverfraction was decomposed with an excess of hydrochlorid acid, and filtered off from the consequent silverchlorid. This filtrate still included ± 20 grms of solid matter. The quantity required daily for a rice-bird, amounts to ± 0.080 mgrm = 80 γ. In a testing sample of the filtrate the excess of hydrochloric acid was determined by titration with alkali and an amount of sulphuric acid was then still added to raise the total acid-concentration, computed on sulphuric acid, to 5 %. Now a solution of phosphotungstic acid in 5 % sulphuric acid was added until a new addition

[1]) A. KOSSEL and P. KUTSCHER, Zeitschr. f. physiol. Chem. **31**, p. 165 (1900).

no longer yielded a precipitate. The phosphotungstic acid was first purified after WINTERSTEIN [1]), by shaking up the aqueous solution with sulphuric acid and ether, the phosphotungstic acid passing into the latter fluid. This treatment with phosphotungstic acid may also precede the fractionation with silver and baryta, with the same favourable result. Then, however, much more phosphotungstic acid is required, and our store of this acid was limited, whereas silver nitrate could be procured in sufficient quantity. When the fractionation with silver + baryta is carried out before the treatment with phosphotungstic acid, no more than ± 40 grms of the latter is wanted per 100 kg of dedek. The phosphotungstic acid-precipitate was left to subside during 34 hours ; it was subsequently filtered off on a suction-filter, and washed with 5 % sulphuric acid. Then the residue was sucked off completely and after this, for further purification, it was dissolved in acetone + water. This acetone-solution was, if need be, filtered and then poured into a large excess of 5 % sulphuric acid. Most of the phosphotungstic acid will then precipitate. A portion of it, however, remains in solution, but this no longer contains any vitamin worth mentioning.

After filtration this new precipitate of the phosphotungstic acid was decomposed with baryta. Once we tried to decompose it with ether and hydrochlorid acid after WINTERSTEIN, but we were not more successful, while the procedure was much more time-consuming.

When we decomposed with baryta, the precipitate was first dissolved again in acetone + water, concentrated baryta being added until, also after prolonged stirring, phenolphthalein-paper turned deep red. Rapid filtration of the barium-phosphotungstic acid precipitate followed, and a quantity of sulphuric acid was added to the filtrate, enough to throw down all the excess of baryta. A fortuitous surplus of the added sulphuric acid was removed with barium chlorid. Lastly the liquid was acidulated with hydrochloric acid. The solution still contains ± 5 grms of dry residue, while per ricebird and per day ± 30 γ is required to guard animals from polyneuritis for from 15 to 23 days.

It appears then that with this treatment with phosphotungstic acid no considerable purification is attained, but neither has a large loss of vitamin been caused, while substances have been removed, that are not eliminated in the succeeding procedure. This hydrochlorid solution was now evaporated to dryness, first on the waterbath, subsequently in vacuo over quick lime.

The sticky residue was now taken up in absolute alcohol, by which it was almost completely dissolved. A small quantity of insoluble material was filtered off and to the filtrate was added an alcoholic solution of platinic-chlorid. This yields a thick deposit of an orange tint. Virtually this deposit contains all the vitamin and only about ¼ of the solid matter present in the original alcoholic solution.

[1]) E. WINTERSTEIN, Chemiker Zeitung, 1898, p. 539.

After 24 hours the platinum precipitate was filtered off, washed with absolute alcohol, taken up in water and decomposed with hydrogen-sulphid. Also after an excess of hydrogen sulphid is passed through the liquid one has to wait at the very least another 24 hours, before all the platinum-sulphid is thrown down. The filtrate of platinum-sulphid was evaporated first on the waterbath, then in vacuo over lime, a considerable portion of the dry residue resulting from it, is crystalline. Of the original quantity of solid matter in 100 kg. of dedek only about 1.4 gramme is left. The vitamin-content of the dedek used, was such as to necessitate the addition of ± 7 % dedek to the washed polished rice to protect the rice-birds against polyneuritis for from 15 to 30 days. It follows then that for 2 grms of rice i.e. the quantity ingested daily by a ricebird, 140 mgrms is required. So in 100 kg of dedek there is enough for about 700.000 ricebirds per day.

Of the platinum-precipitate analyzed with hydrogen sulphid ± 8 γ is required for a rice-bird. So in the 1.4 gr. there is enough for ± 175.000 ricebirds. It will be seen then that after all the above operations as much as ± ¼ is left of the vitamin contained in the dedek.

Although it took us long before we got so far, the knowledge obtained facilitates further experimentation. The substance thus isolated, is comparatively pure, as we shall see lower down that of the pure vitamin ± 3 γ per ricebird is wanted daily to guard these animals from polyneuritis for from 15 to 23 days. As about 8 γ of the decomposed platinum-precipitate is required per bird daily, this precipitate appears to consist for ± ¼ of the vitamin we are trying to isolate. However, further purification takes up a good deal of time and great loss results from it, as besides vitamin at least two more substances are to be found in this decomposed platinum-precipitate. This is evidenced by what follows: When we evaporate the solution that is obtained by treating the platinum-precipitate with hydrogen-sulphid after filtering off the PtS_2, subsequently evaporate it to dryness over quick lime, and finally dissolve the residue in absolute alcohol, part of it remains undissolved. The animal experiment shows that it is not the vitamin we are in quest of.

Treatment of the alcoholic solution with acetone produces a milky cloudiness from which after one or two days a partly viscous, partly crystalline deposit is set free against the bottom and the wall of the cask. Now this deposit is much richer in vitamin than the solution which, however, contains still a great part of the solid matter. So in the platinum precipitate we find anyhow: 1°. a substance insoluble in absolute alcohol; 2°. a substance that dissolves in absolute alcohol but is insoluble in acetone (this appeared to be the vitamin) and 3°. a substance soluble in alcohol as well as in acetone. When we dissolve the precipitate with acetone in alcohol, part of it again remains undissolved: this then, is the substance insoluble in alcohol, which at the first treatment with alcohol remained in solution, in consequence of the presence of a large quantity of substances soluble in alcohol. We are now in a position to crystallize fractionally by dissolving

as much of the decomposed platinum-salt as passes into solution in absolute alcohol. To this a little acetone is added, some of the precipitate adhering to the wall of the flask is decanted off and once more acetone is added to the liquid. Another precipitate appears and again some of it is decanted off, etc. Each of the precipitates is dissolved in absolute alcohol, which has been filtered off from the undissolved portion, and precipitated every time with renewed quantities of acetone, etc. By repeating this process several times we get at length the pure vitamin in the form of hydrochloric acid salt. However, after addition of the acetone it generally takes 24 hours, sometimes many days, before the initial milky cloudiness has completely subsided, so that the whole procedure takes months and months. When the vitamin is approximately pure, the operation progresses better, because during the treatment of the alcoholic solution with acetone a crystalline precipitate ensues directly, which does not cling to the wall.

We have endeavoured to correct this lengthy and sparingly productive process, but to little purpose so far. Picrolonic-acid gives a yellow-coloured precipitate, consisting especially of picrolonates of the vitamin, and of the substance insoluble in alcohol, the latter being most difficult of solution in water. Through recrystallization from diluted alcohol here also a separation can be attained, but not in a much easier way than through the alcohol-acetone fractionation, described above. With this picrolonic-acid fractionation the fractions can be recognized by their melting-point. The optimum-temperature of the picrolonate of the constituent insoluble in alcohol is 340° C.; that of the picrolonate of the vitamin is 165° (uncorrected).

The several picrolonic-acid-fractions (mostly no more than some tens of milligrammes) were filtered off to advantage in Gooch's mugs with porous bottom of sintered glass, from which the precipitate can be scraped off almost quantitatively with a platinum spatula without causing any pollutions through paper fibres.

By treatment with ether or ethylacetate and hydrochloric acid it is easy to separate the hydrochlorid from the picrolonate. Via the picrolonic-acid as well as with fractionation with alcohol and acetone we have ultimately obtained from 300 kg of dedek ± 100 mgrm of a crystalline substance, which also after recrystallization had a melting point of 250° C., as determined with the Anschütz thermometer: the so-called corrected melting-point. Already at rather more than 200° C. the substance begins to turn brown, but then melts rather sharply at 250° C. A portion of this substance was transferred by means of gold-chlorid to a beautifully crystallizing double-salt.

Now for the evidence that these crystals are, indeed, the antiberiberi vitamin hydrochlorid.

Of some hundreds of groups of 10 ricebirds each, fed on a mixture of washed, polished rice and inactive fractions, there was not one group of which all the birds or even most of them kept healthy for more than 12 days, while

only very few individuals could hold out longer than 14 days. We now conducted the following experiments with the vitamin-hydrochlorid, besides a number of preliminary tests (all of which gave the same result as the experiments proper) : Every time ten ricebirds were fed during ± 3 weeks with washed polished rice, to which different quantities of the vitamin-hydrochlorid had been added.

A. with vitamin purified through crystallization from absolute alcohol and acetone :

Cage I : 1 part vitamin-hydrochlorid to 1 million parts washed, polished rice ; since the average daily diet of a ricebird is two grms of rice, this comes to 2 γ per bird and per day. One of the ricebirds contracts polyneuritis after 18 days, two after 23 days, the others are still in good health after 23 days.

Cage II : 1½ parts vitamin-hydrochlorid to 1 million parts washed, polished rice, i.e. 3 γ per bird and per day. After 3 weeks all the animals are healthy.

B. with vitamin-hydrochlorid purified via picrolonic acid :

Cage III : 1 part vitamin-hydrochlorid to 1 million parts washed polished rice ; i.e. 2 γ per bird and per day ; three ricebirds polyneuritis after resp. 13, 18 and 20 days. The others still healthy after 3 weeks.

Cage IV : 1½ parts vitamin-hydrochlorid to one million parts washed, polished rice ; i.e. 3 γ per ricebird and per day. After 3 weeks all are healthy.

C. with vitamin-hydrochlorid obtained through decomposition of the double salt with hydrogen-sulphid :

Cage V : 1 part vitamin-hydrochlorid to 1 million parts washed polished rice ; i.e. 2 γ per ricebird and per day. This experiment has been in progress for 14 days now, but all the animals still keep in good health.

Cage VI : 2 parts vitamin-hydrochlorid to 1 million parts washed, polished rice ; i.e. 4 γ per ricebird and per day. After 3 weeks all the animals are quite well.

The foregoing justifies us in assuming 2 γ per ricebird and per day to be the critical limit ; of the 30 ricebirds, fed with it, only one develops polyneuritis inside of 15 days ; the majority keep in good health even for more than 3 weeks. Of the ricebirds that received 3 γ or 4 γ hydrochlorid daily not one developed polyneuritis.

We have repeated these experiments with pigeons :

A. with hydrochlorid purified via the picrolonate.

Pigeon 1 and 2 : 1 part vitamin-hydrochlorid to 400.000 parts washed, polished rice. For a pigeon that ingests ± 12 grms a day, this comes to 30 γ a day. After 5 weeks the foodstuff was finished ; the pigeons looked quite well, but their bodyweight was reduced resp. from 267 to 222 and from 270 to 235 gms.

Pigeon 3 and 4 : 1 part vitamin-hydrochlorid to 200.000 parts washed, polished rice ; i.e. 60 γ per pigeon per day. After 6 weeks the food was

finished; the pigeons still looked quite well; the weight of the one pigeon had remained quite the same, that of the other had been slightly lowered, viz. from 270 to 213 gms.

In these pigeon-experiments the ratio of vitamin to rice was taken somewhat liberally, as we do not know beforehand whether ricebirds and pigeons require a relatively equal quantity of vitamin per day. However, the results being so favourable, we afterwards took he same ratio for both birds in the preparation purified by absolute alcohol and acetone.

Pigeon 5 and 6: 1 part vitamin-hydrochlorid purified with alcohol and acetone, to 1 million parts washed, polished rice, i.e. ± 12 γ per pigeon and per day. The test is proceeding for 4 weeks only, but the animals look well, only the weight is lessened a little, viz. from 271 to 245 grms, and from 292 to 235 grms.

Pigeon 7 and 8: 2 parts vitamin-hydrochlorid purified with alcohol and acetone to 1 million parts washed polished rice, i.e. ± 24 γ per pigeon and per day. This test is also in progress only 4 weeks, but the animals look thriving while their weight is hardly lessened, viz. from 322 to 314 grms and from 302 to 291 grms. Since ± 1 % proteins is lost in the twice 24 hours' washing of the polished rice, so that the protein-content of the washed rice amounted to only 5.5 %, it is not impossible that a deficiency in protein was responsible for the decrease in the weight of some animals. For this reason we added in the last two experiments 3½ % thoroughly minced meat, extracted repeatedly with boiling water, which meat, as appeared from advisedly arranged experiments, contained no appreciable quantity of antineuritic vitamin.

Now if we consider that the pigeons fed only on washed, polished rice develop polyneuritis most often within 2 or 3 weeks, mostly with a marked loss in bodyweight if not forcibly fed, the great influence of the slight addition of the vitamin-hydrochlorid becomes very evident. To make assurance double sure, we also administered to two pigeons the same washed polished rice, to which, as with the last pigeons, had been added 2 % salts + 2½ % extracted minced meat, but no vitamin chlorid. The one developed polyneuritis after 24 days, the other after 25 days, while their weight decreased resp. from 312 to 207 gms and from 289 also to 207 gms.

The pigeons to which an admixture of vitamin-hydrochlorid was administered, also behaved differently from those that had washed polished-rice alone. The latter practically cease eating spontaneously already after a few days, while it looks as if they are rummaging for the few grains to which a trace of silverlayer still adheres, and throw away the other grains, thus making a mess of their diet; the former, including the pigeons on only 1 part vitamin-hydrochlorid to 1 million parts washed polished-rice, finish their allowance with great relish.

From these experiments, therefore, we may safely conclude that the hydrochlorid, detected by us, with a melting point at 250° C., is in a high degree instrumental in warding off polyneuritis. But the question may

be asked whether this salt is indeed the vitamin-hydrochlorid, or whether perhaps the salt is contaminated with a small quantity of a still more active component that may be the vitamin that we endeavour to isolate. We believe that the latter supposition is altogether erroneous, if we consider that the salt has been purified through recrystallization, while the mother-lye appeared to be much less active. If the supposition were right, we should have to assume that the crystals had absorbed by selection the active component from the liquid, and had detained it after recrystallization, and transference to goldsalt and back again to hydrochlorid. To be sure this idea must really be precluded.

So a proportion of 1 or 2 parts vitamin to 1.000.000 parts rice would accordingly create in a man, who consumes ± 500 gms of rice a day, a want of ½ or 1 mgrm of vitamin. This is of the same order of magnitude as what we know of the consumption of other substances with great physiological activity, such as thyroxin, adrenalin, and the like.

Until it is quite purified the vitamin-hydrochlorid crystallizes in bundles of needles. The pure salt consists of rosettes of bundled rodlets. The hydrochlorid in a pure state is not hygroscopic ; but it is readily soluble in very little water ; it is also soluble in ethyl-, and methyl-alcohol, the solution in ethyl-alcohol gives a precipitate with an admixture of amyl-alcohol, acetone, ether, chloroform, benzol, petroleum-ether or ethyl-acetate.

A 2 %-solution of the hydrochlorid in water produces the following reactions : Sublimate yields a slight precipitate that becomes stronger by adding sodium-acetate ; from a solution of mercuric-sulphate in dilute sulphuric acid a thick precipitate ensues ; with iodin potassium-iodid a fine black precipitate originates ; with picric acid a cloudiness arises ; styphninic-acid yields a precipitate ; Dragendorff's reagent, a solution of bismuth-iodid in potassium-iodid solution, gives a thick, reddish precipitate; with zinc-chlorid, cadmium-chlorid, lead-acetate, copper-acetate, potassium-chromate, potassium-salphocyanate, and with perchloric acid, no precipitate comes forward. As is evident from the preparation, phosphotungstic acid, picrolonic acid and gold chlorid give a precipitate, the last two consisting of needle-shaped crystals. The aqueous solution does not give a precipitate with platinic-chlorid, the solution in absolute alcohol with an alcoholic solution of platinum-chlorid, does. By treating with sulphanilic acid and nitric-acid, and subsequently with soda, we observe an intensely red colour (diazo-reaction of Pauly).

In the hydrochlorid no other elements appeared to occur than C, N, H, O and Cl. After transference of the hydrochlorid to the nitrate we could demonstrate that no halogens occur in the vitamin itself.

Of the hydrochlorid and of the gold-double salt we made some elementary-analyses by means of PREGL—MÜLLER—WILLENBERG's micro-apparatus with quartz-tube and ground-in absorption tubes. We publish these results only provisionally. Within a few months we hope to be able to repeat these analyses with larger quantities of material.

We found:

Of the gold-salt:

13.775 mgrms give 2.864 mgrms H_2O, 7.447 mgrms CO_2 and 5.852 mgrms Au;

found: 2.31 % H, 14.74 % C. and 42.48 % Au.

Duplicate: 14.876 mgms give 3.505 mgms H_2O, 8.072 mgms CO_2 and 6.278 mgms Au;

found: 2.07 % H, 14.80 % C. and 42.20 % Au.

10.589 mgms gives with micro-Kjeldahl 0.461 cc^3 $\frac{n}{10}$ NH_3;

found: 6.1 % N.

8.991 mgms give 0.50 cc (measured over water) N of 760 mm and 32° C.; found 6.08 % N 7.293 mgms give 8.901 mgms AgCl,

found: 30.21 % Cl.

Computed for $C_6H_{10}ON_2 . HCl . AuCl_3$:

2.36 % H, 15.45 % C, 42.48 % Au, 6.02 % N and 30.54 % Cl.

Of the hydrochlorid:

7.198 mgms give 4.515 mgms H_2O; carbon was lost;

found: 6.97 % H.

3.289 mgms give 2.894 mgms AgCl;

found: 21.77 % Cl.

Computed for $C_6H_{10}ON_2 . HCl$:

6.77 % H, 21.84 % Cl.

We see, then, that analyses correspond (not very beautifully though) to the formula $C_6H_{10}ON_2 . HCl$ for the hydrochlorid. Now, if we consider that fractionation with silver and baryta causes the body to precipitate in the histidingroup, whereas it gives a very intense red-coloration with the diazo-reaction of PAULY [1]), the suggestion is obvious that possibly there may be an imidazol-nucleus in the body, that also occurs in other substances essential to life, such as histidin, histamin and carnosin. The formula may then be imagined to be:

$$(C_3H_7O) - C\begin{matrix} \diagup NH-C-H \\ \diagdown N-C-H \end{matrix}$$

But this has to be substantiated by later inquiry.

In conclusion we would take this opportunity of tendering our thanks to our analysts, especially to Raden SOEDARSONO, for their valuable assistance in the experimental work.

Weltevreden (Batavia), Medical Laboratory.
August 1926.

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